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Genetic evaluation of the Baja California rock squirrel *Otospermophilus atricapillus* (Rodentia: Sciuridae)

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Abstract

The Baja California rock squirrel (*Otospermophilus atricapillus* Bryant) is endemic to the Baja California Peninsula, Mexico and known from only five localities. *O. atricapillus* is considered as the sister species of *O. beecheyi* (Richardson) and both have been considered different species mainly by its colorations. In an attempt to better understand the relationship within *O. atricapillus* and with its sister species *O. beecheyi*, we used genetic information gathered from *O. atricapillus*, aiming to investigate the phylogenetic and phylogeography of *O. atricapillus* - *O. beecheyi*. We analyzed two sets of mitochondrial cytochrome *b* gene (Cyt *b*) data: One 800 (bp) fragments of 118 individuals and a second one of 1140 (bp) for the 32 haplotypes found. Our own hypothesis is that specific characteristics used to distinguish *O. artricapillus* from *O. beecheyi* are at the same level than the differences among the recognized subspecies of *O beecheyi*. Under that condition, a genetic analysis will show that *O. atricapillus* are within the genetic variation of *O. beecheyi*, and the current diagnostic characters of *O. atricapillus* could be considered only at subspecific level. The analysis of molecular variance revealed that *O. atricapillus* is not significantly different from *O. beecheyi*, and that, apart from highland populations in the Sierra Nevada, the sampled populations of *O. beecheyi* and *O. atricapillus* are not significantly different among all of them.

Keywords: cytochrome *b*, México, genetic variation, *Otospermophilus*, Rodentia, *Spermophilus*, taxonomic

Introduction

Two species of ground squirrels of the genus *Otospermophilus* are currently recognized (e.g., Thorington & Hoffmann, 2005) in the Southern Pacific Coast of North America: the California ground squirrel (*Otospermophilus beecheyi* Richardson) ranges north of 29°N, with records in Baja California (Mexico), California, Oregon, Washington, and in a small area in northwestern Utah (United States), while and the Baja California rock squirrel (*O. atricapillus* Bryant) occurs south of 28°N in the Baja California Peninsula (Mexico), with records from only five localities in the Sierra de San Francisco-Santa Marta, Sierra de La Giganta, and La Purísima region (Álvarez-Castañeda *et al*., 1996; Yensen & Valdés-Alarcon, 1999). *O. atricapillus* is uncommon across the Baja California Peninsula, where it is found in areas with black basalt rock and mesic habitats (Álvarez-Castañeda *et al*., 1996; Yensen & Valdés-Alarcon, 1999), and associated with a variety of Sonoran desert vegetation with a high diversity of co-dominant life forms (Peinado *et al*., 1994). On the other hand, *O. beecheyi* is a common inhabitant of grasslands, meadow-woodland complexes, agricultural areas, and woodlands at lower elevations from central Washington to the state of Baja California (Hubbart, 2001). *O. beecheyi* is also found in the California highlands, in areas of mixed conifer forest; squirrel nests are associated with *Pinus ponderosa* (ponderosa pine) and *Abies concolor* (white fir) that prevail at mid elevations (Schoenherr, 1992).

The Baja California rock squirrel *O. atricapillus* is recognized as distinct species from *O. beecheyi* because of its distinctive external morphology, especially its dark face, which is used as a diagnostic feature (Howell, 1938; Hall, 1981; Yensen & Valdés-Alarcon, 1999; Hubbart, 2001; Helgen *et al*., 2009). Observations during several years of field research (Álvarez-Castañeda, personal observation), however, have shown substantial variation in coat color among individuals of *O. atricapillus* in Comondú, its type locality, and in the San Pedro de la Presa

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areas, with the prevalence of individuals with dark face, but with a significant number of specimens with pale faces. Both facial color morphs occur regardless of the season of the year. Hence, we collected specimens along the species' color gradient throughout its distribution range, including its type locality. After reviewing specimens of all the *O. beecheyi* subspecies, we concluded that the color variation that is diagnostic for *O. atricapillus* lies within the variation found in *O. beecheyi*.

In an attempt to better understand the relationship within *O. atricapillus*, and with its sister species *O. beecheyi*, we used genetic information gathered from the five areas from which *O. atricapillus* is known to occur in Baja California Sur, aiming to investigate the phylogenetic and phylogeography of *O. atricapillus* - *O. beecheyi* and to evaluate the relationship between the two species. Our hypothesis is that the color characteristics used to distinguish *O. atricapillus* from *O. beecheyi* are at the same level as the differences among the recognized subspecies of *O beecheyi*.

Material and methods

In their review of the genus *Spermophilus*, Helgen *et al.* (2009) elevated the former subgenus *Otospermophilus*, with three species (*O. atricapillus*, *O. beecheyi*, and *O. variegatus* Erxleben) to full generic status. The other two species formerly included within the subgenus *Otospermophilus* were included by Helgen *et al.* (2009) within the genus *Notocitellus* (*N. annulatus* and *N. adocetus*). We restricted our analyses to the sister species *O. atricapillus* and *O. beecheyi* throughout the Californias. The third species of the genus *Otospermophilus* is *O. variegatus*, whose distribution is from Nevada and Utah to Southwestern Texas in the United States through Michoacan and Puebla in Mexico (Hall, 1981; Oaks *et al.*, 1987).

Surveys were conducted from 1993 to 2009 throughout the Baja California Peninsula, Mexico in different seasons, including locations where *Otospermophilus* might be found (Fig. 1). Voucher specimens and tissues collected are housed in the mammal collections at the Centro de Investigaciones Biológicas del Noroeste (CIB) and the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), following the recommendations for handling specimens of the American Society of Mammalogists (Sikes *et al*., 2011).

The phylogenetic relationships among specimens of *O. beecheyi* and *O. atricapillus* were assessed by analyzing 800 base pairs (bps) of the mitochondrial cytochrome *b* gene (Cyt *b*). In total, 118 individuals from 20 geographic groups (localities within 20 km of distance) were assembled from the Baja California Peninsula and California (Fig. 1, Table 1), and studied for stability of putative diagnostic characteristics (Howell, 1938; Yensen & Valdés-Alarcon, 1999).

Laboratory procedures. Genomic DNA was extracted in the laboratory from liver tissue preserved in 95 % ethanol using the DNeasy Kit (Qiagen, Inc., Valencia, CA). The first Cyt *b* fragment (~800 bp) was amplified using primers MVZ05 (CGA AGC TTG ATA TGA AAA ACC ATC GTT)/spermo 06 (TAT GGG TGA AAG GGR AYT TTA TCT); afterwards, primers MVZ127 (TRY TAC CAT GAG GAC AAA TAT C)/MVZ 14 (GGT CTT CAT CTY HGG YTT ACA AGA) were used to complete Cyt *b* gene amplification (1140 bp).

The following conditions were used for initial double-stranded amplifications: 12.5 µl template (10 ng), 4.4 µl ddH$_2$O, 2.5 µl of each primer (10 nM), 0.474 µl (0.4 nM) dNTPs, 0.5 µl (3 mM) MgCl$_2$, 0.125 µl Taq polymerase (platinum, Invitrogen, Carlsbad, CA), and 1× Taq buffer to a final volume of 25 µl.

Amplification conditions consisted of the following sequence: an initial 3-min denaturation at 94 °C followed by 37 45-sec denaturation cycles at 94 °C for, 1-min annealing at 50 °C, and 1-min extension at 72 °C. The amplified products were purified using the QIAquick PCR purification kit (Qiagen). This template was cycle-sequenced with MVZ05 using the Taq FS Kit and analyzed on an ABI 377 automated sequencer.

Sequence alignment and haplotype determination. Nucleotide sequences were aligned using the Sequencher ver. 3.1 software (Gene Codes Corp., Ann Arbor, Michigan), visually inspected, and translated into amino acids for alignment confirmation. Initially, we obtained sequences for an 800 bp fragment of Cyt *b* for all 118 samples; these were used as basis to identify 32 unique haplotypes with the Collapse software (ver. 1.1, Posada, 2004, available at http://darwin.uvigo.es). In order to increase the number of characters available for phylogeographic analysis, we sequenced the complete Cyt *b* gene (1140 bp) for these 32 samples. Representative haplotypes generated for this study were deposited in GenBank (accession numbers: AF157918, AF157945, JF925283 to JF925313).

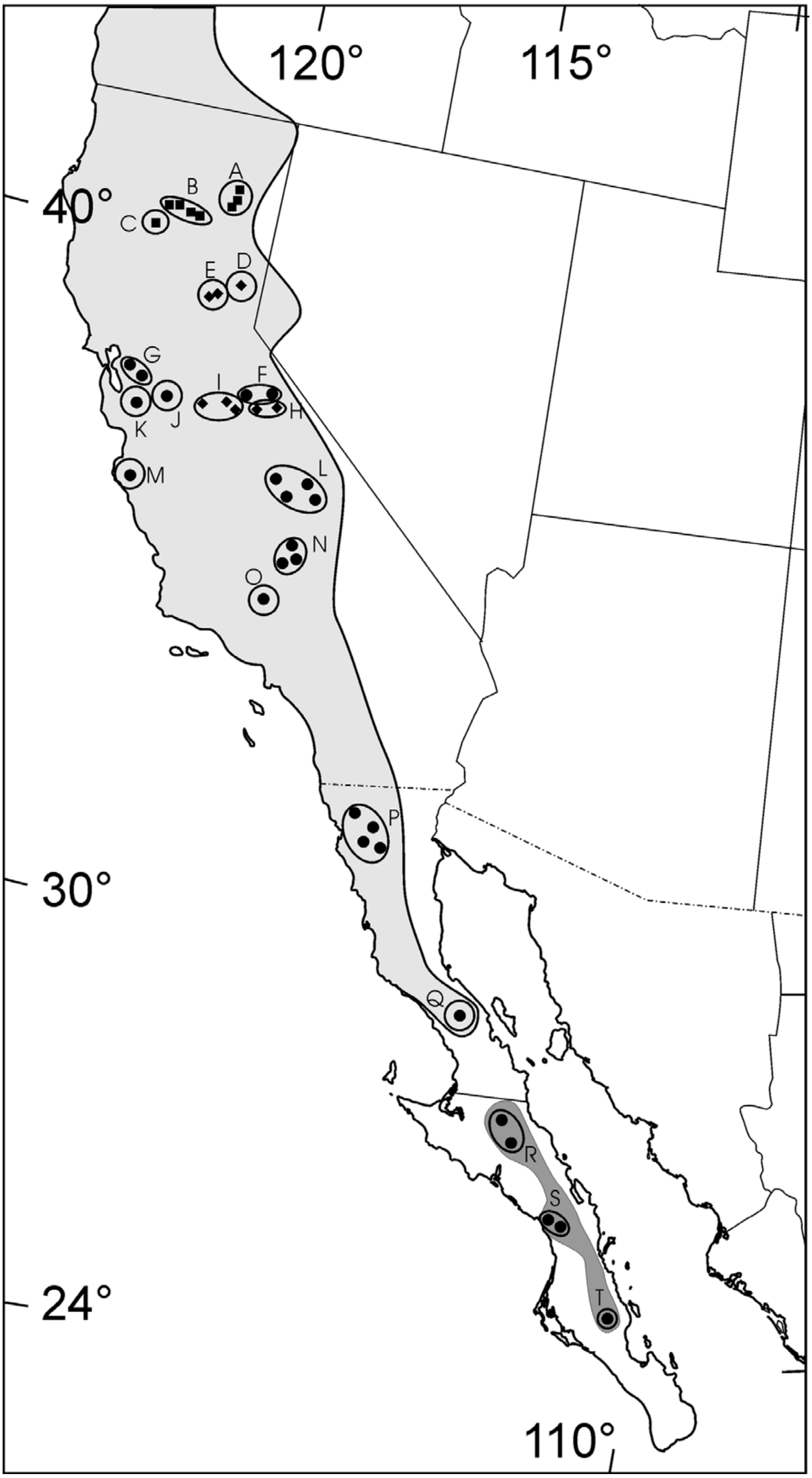

FIGURE 1. Map of California and the Baja California Peninsula showing 20 geographic groups (A to T) from which specimens for the genetic analysis were collected. Localities are listed in Table 1. Localities are clustered into geographical groups. The light gray area represents the distribution of *O. beecheyi* and dark gray that of *O. atricapillus*. Solid circles are specimens from Clade A; solid squares from Clade B; and solid rhomboids from Clade C.

TABLE 1. Geographic groups ($n = 20$) of *Otospermophilus atricapillus*- *O. beecheyi.* The columns indicate geographic group (G), haplotype number (H), number of specimens with the haplotype (N), current subspecies assignation (SSP), state (St), specific locality (locality), geographical coordinates (latitude and longitude, in decimal degrees), museum where the specimens are housed (Mus), museum catalogue number (Catalog), and the accession key to the GenBank number (Genbank). * no catalog numbers in Genbank.

G	H	N	SSP	St	Localities	Latitud	Longitud	MUS	No. Cat.	GenBank
A	2	1	D	CA	Brockman Lava Beds, Eagle Lake	40.587	-120.839	MVZ	217741	JF925284
A	2	1	D	CA	Pine Creek, Eagle Lake	40.664	-120.793	MVZ	217734	JF925284
A	1	1	D	CA	Slate Creek	40.848	-120.774	MVZ	220495	JF925283
B	5	1	D	CA	Lassen National Forest	40.345	-121.432	MVZ	218054	JF925287
B	3	1	D	CA	Highway 89, Mineral	40.349	-121.591	MVZ	218053	JF925285
B	3	2	D	CA	Reading Island	40.390	-122.197	MVZ	219576, 219577	JF925285
B	4	1	D	CA	Manton	40.435	-121.870	MVZ	219139	JF925286
B	3	1	D	CA	Manton	40.439	-121.866	MVZ	219138	JF925285
C	6	2	D	CA	Red Bluff Diversion Dam	40.156	-122.204	MVZ	219659, 219660	JF925288
D	7	2	F	CA	Independence Lake	39.447	-120.309	MVZ	222761, 222762	JF925289
E	8	2	F	CA	Dutch Flat	39.198	-120.833	MVZ	221758, 221759	JF925290
E	7	1	F	CA	Blue Canyon Airport	39.255	-120.713	MVZ	221749	JF925289
E	7	1	F	CA	Blue Canyon Airport	39.275	-120.709	MVZ	221750	JF925289
F	9	1	S	CA	Bohler Creek	37.900	-119.130	MVZ	208499	JF925291
F	9	1	S	CA	Warren Fork of Lee Vining Creek	37.954	-119.226	MVZ	208500	JF925291
G	10	2	B	CA	Camp Ohlone	37.833	-121.750	MVZ		AF157918, AF157919
G	10	1	B	CA	Walnut Creek	37.927	-122.025	MVZ	218221	JF925292
H	12	1	S	CA	White Wolf, YNP	37.845	-119.589	MVZ	207156	JF925294
H	11	1	S	CA	Walker Creek	37.897	-119.130	MVZ	216232	JF925293
H	11	1	S	CA	Bohler Creek	37.900	-119.130	MVZ	216227	JF925293
I	7	6	S	CA	Yellow Pine, Yosemite Valley	37.732	-119.608	MVZ	201336 to 201341	JF925289
I	17	1	S	CA	Happy Isles Fen, Yosemite Valley	37.733	-119.558	MVZ	201348	JF925299
I	16	1	S	CA	Merced Lake, YNP	37.734	-119.393	MVZ	207160	JF925298
I	7	1	S	CA	Chapel, Yosemite Valley	37.741	-119.591	MVZ	201342	JF925289
I	11	1	S	CA	Chapel, Yosemite Valley	37.741	-119.591	MVZ	201343	JF925293
I	7	1	S	CA	Crane Flat, YNP	37.751	-119.791	MVZ	201346	JF925289
I	11	1	S	CA	Crane Flat, YNP	37.751	-119.791	MVZ	201344	JF925293
I	13	1	S	CA	Crane Flat, YNP	37.751	-119.791	MVZ	201345	JF925295
I	14	1	S	CA	Crane Flat, YNP	37.751	-119.791	MVZ	201347	JF925296
I	7	1	S	CA	Tamarack Flat, YNP	37.755	-119.743	MVZ	207166	JF925289
I	7	1	S	CA	Coulterville	37.756	-120.086	MVZ	207155	JF925289
I	11	1	S	CA	Coulterville	37.756	-120.086	MVZ	207154	JF925293
I	11	11	S	CA	Hodgdon Meadow, YNP	37.799	-119.868	MVZ	216229	JF925293
I	7	1	S	CA	Aspen Valley, YNP	37.825	-119.772	MVZ	207165	JF925289
I	7	1	S	CA	Merced Grove, YNP	37.524	-119.930	MVZ	216230	JF925289
I	15	1	S	CA	Merced Grove, YNP	37.524	-119.930	MVZ	216231	JF925297
J	18	1	F	CA	La Grange	37.625	-120.567	MVZ	207146	JF925300

continued next page

TABLE 1. (continued)

G	H	N	SSP	St	Localities	Latitud	Longitud	MUS	No. Cat.	GenBank
J	19	2	F	CA	La Grange	37.625	-120.567	MVZ	207147, 207148	JF925301
K	20	1	B	CA	San Jose	37.408	-121.734	MVZ	218715	JF925302
K	21	1	B	CA	San Jose	37.408	-121.734	MVZ	218718	JF925303
L	22	2	P	CA	Kings Canyon Natl. Park	36.486	-118.831	MVZ	224584	JF925304
L	23	1	P	CA	Kings Canyon Natl. Park	36.486	-118.831	MVZ	224585	JF925305
L	22	1	P	CA	Lone Pine	36.593	-118.122	MVZ	219214	JF925304
L	24	1	P	CA	Sequoia National Forest	36.786	-118.902	MVZ	223959	JF925306
L	22	3	P	CA	Little Onion Valley, Grays Meadow	36.800	-118.310	MVZ	219215 to 219217	JF925304
M	25	2	B	CA	Hastings Natural History Reservation	36.369	-121.555	MVZ	220336, 220337	JF925307
N	22	1	P	CA	Kern River Preserve, Weldon	35.588	-118.440	MVZ	223833	JF925304
N	22	1	P	CA	Bodfish Creek/Kern R. Confluence	35.599	-118.498	MVZ	222865	JF925304
N	26	1	P	CA	Bodfish Creek/Kern R. Confluence	35.599	-118.498	MVZ	222866	JF925308
N	22	1	P	CA	Fay Creek, Weldon, Kern County	35.717	-118.306	MVZ	223832	JF925304
N	22	2	P	CA	Taylor Meadow	35.831	-118.298	MVZ	222493, 222495	JF925304
N	22	2	P	CA	Cannell Meadow	35.834	-118.373	MVZ	222491, 222492	JF925304
N	22	1	P	CA	Erskine Creek	35.588	-118.300	MVZ	222867	JF925304
O	22	1	F	CA	Joaquin Flat, Tehachapi Mts.	35.027	-118.696	MVZ	200794	JF925304
P	18	1	N	BC	Carretera Ejido Erendira	31.208	-116.214	CIB	12931	JF925300
P	18	1	N	BC	Cañon San Francisquito	31.455	-116.264	CIB	12933	JF925300
P	18	1	N	BC	Carretera Piedras Gordas	31.554	-116.308	CIB	12932	JF925300
P	18	1	N	BC	Arroyo La Huerta	31.599	-116.089	CIB	12930	JF925300
P	18	1	N	BC	Rancho Santa Maria	32.191	-116.350	CIB	12934	JF925300
Q	27	2	R	BC	Cataviña	29.717	-114.732	CIB	18847, 18849	JF925309
Q	28	1	R	BC	Cataviña	29.717	-114.732	CIB	18845	JF925310
R	30	3	A	BCS	San Ignacio	27.287	-112.896	CIB	12914 to 12916	JF925312
R	31	2	A	BCS	San Ignacio	27.287	-112.896	CIB	12917, 12918	JF925313
R	29	1	A	BCS	Sierra de San Francisco	27.590	-113.092	CIB	12911	JF925311
R	30	1	A	BCS	Sierra de San Francisco	27.590	-113.092	CIB	12910	JF925312
R	31	4	A	BCS	Sierra de San Francisco	27.590	-113.092	CIB	12907, 12909, 12912, 12913	JF925313
S	29	9	A	BCS	San Miguel de Comondu	26.175	-112.064	CIB	1192, 1643, 12920 to 12926	JF925311
S	29	2	A	BCS	La Purísima	26.184	-112.074	CIB	7903, 12919	JF925311
T	29	5	A	BCS	San Pedro de la Presa	24.871	-111.055	CIB	4917, 12927 to 12929	JF925311
T	29	1	A	BCS	San Pedro de la Presa	24.871	-111.055	CIB		AF157944
T	32	1	A	BCS	San Pedro de la Presa	24.871	-111.055	CIB		AF157945

[A] A = *O. b. atricapillus*, B = *O. b. beecheyi*, D = *O. b. douglasii*, F = *O. b. fisheri*, N = *O. b. nudipes*, P = *O. b. parvulus*, R = *O. b. rupinarum*, and S = *O. b. sierrae*.

[B] CA = California; BC = Baja California; BCS = Baja California Sur

[C] CIB = Centro de Investigaciones Biológicas del Noroeste; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley.

YNP = Yosemite National Park

Two separate analyses were conducted. First, a minimum spanning network was performed based on the 800 bp fragment of Cyt *b* of 118 specimens. Second, a phylogenetic analysis was performed on the whole Cyt *b* gene (1140 bp). The phylogenetic analyses included the 32 haplotypes that corresponded to representative specimens, plus two specimens of *O. variegatus* (AF157860 and AF157878); and 3 members of the genus *Callospermophilus* to root the three, following Harrison *et al*. (2003): *C. madrensis* (AF157946), *C. lateralis* (AF157887), and *C. saturatus* (AF157916).

Phylogenetic analysis. For phylogenetic estimation, we employed maximum-parsimony (MP), maximum-likelihood (ML), and neighbor-joining (NJ) of PAUP version 4.0b10 (Swofford, 2002), and Bayesian analysis (BI) of MrBayes version 3.0b4 software (Ronquist & Huelsenbeck, 2003). For the MP analysis, all characters were equally weighted and a heuristic search was performed with 1,000 replicates, setting a random addition of sequences and implementing the tree-bisection reconnection (TBR) branch-swapping method. Genetic distances were calculated using the Jukes-Cantor (JC) and the Kimura two-parameter (K2P) models. The latter is the most commonly used model for comparing divergence levels among studies (Baker & Bradley, 2006). The Kimura two-parameter matrices among individuals were used to generate a neighbor-joining tree; the support for nodes was assessed with bootstrap analyses, including a fast heuristic procedure with 1,000 pseudo-replicates. The Hasegawa-Kishino-Yano model with a gamma-distributed among-site rate variation (HKY + G; Hasegawa *et al*., 1985) was deemed the most appropriate for our data, using the comparison model in MrModeltest version 2 (Nylander, 2004) under the Akaike Information Criterion (AIC). The HKY + G model was used for maximum-likelihood searches consisting of 100 random replicates with TBR branch swapping. Bootstrap value ≥50% was reported for branch support. The Bayesian analysis (BI) employed the Hasegawa-Kishino-Yano + G model and four separate runs implementing the Metropolis-Coupled Markov Chain Monte Carlo simulation starting from a random tree. Each run was conducted with 5×10^6 generations and sampled at intervals of 1,000 generations. The first 1,000 samples of each run were discarded as burn-in; all remaining trees sampled were analyzed to find the posterior probability of clades. A consensus tree was generated with the 50% majority-rule algorithm, and the percentage of samples recovered in a particular clade was the clade's posterior probability.

Network and population genetic analysis. Intraspecific Cyt *b* gene genealogies were inferred for 118 samples from 20 geographic groups using the statistical parsimony method (Templeton, 2001) implemented in the program TCS v1.18 (Clement *et al*., 2000). This program allows the estimation of phylogenetic relationships at low levels of divergence and provides a 95% plausible set for all haplotype connections. A minimum spanning network was obtained to depict the relationship among the 32 unique haplotypes identified.

The distribution of the genetic variance of population structure was obtained using AMOVA (Weir & Cockerham, 1984; Excoffier *et al*., 1992) conducted in ARLEQUIN v2.001 (Schneider *et al*., 2000). In AMOVA, the molecular groups are constructed in relation to groups of localities from which we wish to test a previous hypothesis. The genetic hypotheses tested are: 1) The *O. atricapillus* populations are genetically different from *O. beecheyi* to a sufficient extent to be considered as a different species, and 2) *O. atricapillus* will have larger genetic variation than the current variation within the recognized subspecies (based on geographical location) of *O. beecheyi* (*O. beecheyi beecheyi*, *O. b. douglasii*, *O. b. fisheri*, *O. b. nudipes*, *O. b. parvulus*, *O. b. rupinarum*, and *O. b. sierrae*). Variation percentages were obtained directly from the matrix of squared-distances between all pairs of haplotypes in each case (Excoffier *et al.*, 1992, and the genetic structure was estimated by means of F_{ST} (Weir & Cockerham, 1984).

The Tajima's D, Fu's *F* test, haplotype diversity, and nucleotide diversity were all performed in ARLEQUIN version 2.000 (Schneider *et al*., 2000). The former test was used to detect deviations from neutrality caused by evolutionary forces, such as hitchhiking, population size expansion, background selection, or selective sweep (Fu, 1997). The occurrence of population expansion was investigated with the frequency distribution of pairwise differences between Cyt *b* sequences (Mismatch distribution – Roger & Harpending, 1992); this analysis was performed in ARLEQUIN version 2.000 (Schneider *et al.*, 2000). The null hypothesis of an expanding population (Roger & Harpending, 1992) produces a unimodal distribution; a ragged distribution indicated a population in stable equilibrium. The confidence intervals for ? were calculated with 1000 bootstrap replicates for the alpha level of 0.05.

Color variation. The variation in dorsal coloration is very important in *O. beecheyi* for the description of the subspecies and it is one of the characteristics used to consider *O. atricapillus* as different species from *O. beecheyi,* whose shoulders, neck, and sides are lighter gray. The visual coloration analysis of the four regions showed a constant color among the specimens of each of the different subspecies and a larger variation among the specimens of different subspecies (Howell, 1938). The revision of 511 specimens of seven subspecies of *O. beecheyi* and 24 specimens of *O. atricapillus* showed a color pattern that includes four regions: (a) the dorsal area of the head from the nose to the ears; (b) the posterior area of the ears, including shoulder and part of the sides; (c) the posterior part of the neck to the upper area of the back; and (d) the central part of the back and the rump. Considering that color pattern is used as a diagnostic characteristic at a species level, direct comparisons of the specimens of *O. atricapillus* were made with the four regions of the dorsal color pattern found in the subspecies of *O. beecheyi* (Appendix I), which are summarized here. *Otospermophilus beecheyi douglasii* is similar in external characters to *O. b. beecheyi*, but with paler coloration; male's skull is larger, while female's skull is smaller; compared with *O. b. fisheri*, the back is darker, with a black triangle between the shoulders; tail is longer and deeper buff. *O. b. sierrae* is similar in size and coloration to *O. b. beecheyi*; however, head sides are grayish and the skull is slightly smaller, with great postorbital breadth; compared with *O. b. fisheri*, head and upper parts are darker, feet are whitish, and tail is darker. *O. b. fisheri* is similar in size to *O. b. beecheyi*, but with paler coloration (above and below); shoulders with clearer white coloration, and the hind feet shorter; skull is smaller, mainly in females, and zygomatic arch is less wide. *O. b. fisheri* is similar to *O. b. parvulus* in color, but larger in size. The skull of *O. b. parvulus*'s is similar to that of *O. b. fisheri*, but smaller. *O. b. nudipes* and *O. b. beecheyi* are similar in coloration, but the latter is smaller, and shows paler feet and under parts with conspicuous white shoulders; *O. b. nudipes* is similar in size to *O. b. parvulus* but darker. Skull is similar to *O. b. parvulus* but smaller than that of *O. b.beecheyi* and that of O. *b. fisheri*. *O. b. rupinarum* is similar in external character and skull to *O. b. nudipes* dorsal color is less brownish and without a darker area on head, nape, and shoulders. Skull is slightly smaller and relatively narrow across the zygomata. *O. b. nesioticus* is similar to *O. b. beecheyi*, but hind feet are larger, with grayish coloration, and light shoulder stripes are less evident; skull is larger; nasal branches of premaxillae are broader.

Results

Genetic variation. We detected 32 haplotypes within the geographic range of *Otospermophilus* in the Californias (Table 1). Only four haplotypes occur in the attributed *O. atricapillus* range (H29 to H 32), with a Nei´s diversity of h= 0.58 ± 0.084, mean number of pairwise differences of 1.69 ± 1.02, and nucleotide diversity of 0.001 ± 0.001. Haplotype H29 is widely distributed in all the species' range.

Phylogenetic analysis. All phylogenetic methods produced very similar results, differing only in nodes with poor support. The maximum parsimony analysis yielded 10 trees (length = 487, CI = 0.700, RI = 0.887; polytomy concentrated within the clades B and C; tree not shown), while maximum-likelihood analysis produced one tree (score –ln L = 2801.7). The HKY + G model parameters are: mean base composition was A = 28.8%, C = 25.2%, G = 12.4%, and T = 33.4; K = 9; gamma distribution = 0.2777.

All the phylogenetic analyses indicated (i) that *O. atricapillus* is well nested within the clade of *O. beecheyi* (Fig. 2), and (ii) there are three well supported main clades within *O. beecheyi*. Clade A (Southern Clade) included specimens from the California lowlands throughout the Baja California Peninsula, including *O. atricapillus, O. beecheyi beecheyi, O. b. fisheri, O. b. parvulus, and O. b. rupinarum*, including specimens close to the type locality of *O. beecheyi beecheyi* (H10); Clade B (Northern Clade) encompassed all specimens from the California highlands at El Dorado Forest (*O. b. douglasii)*, while Clade C (Central Clade) included all specimens from the California highlands at Yosemite (*O. b. fisheri* and *O. b. sierrae*; Fig. 2).

The average K2p sequence divergence values between *O. beecheyi* specimens of the lowlands (Clade A) and the two California highlands areas, El Dorado forest (Clade B) and Yosemite (Clade C) were 7.02 and 7.38%, respectively. The K2P sequence divergence within clade A within the Baja California Peninsula southern groups (*O. atricapillus* and *O. b. rupinarum* Clade AI) was 0.29% (Table 2).

The analysis of molecular variance (AMOVA) revealed no significant differences (P = 0.05) in F_{ST} (with in populations), F_{SC} (among population within groups), and in F_{CT} (among groups) values for any of the two hypotheses tested. Therefore 1) *O. atricapillus* is not significantly different (F_{CT} = 0.14) from *O. beecheyi*, and 2) the genetic differences among *O. atricapillus* and the seven subspecies sampled of *O. beecheyi* are not significantly different among all of them (F_{CT} = 0.22); under that condition *O. atricapillus* is within the genetic variation found in *O. beecheyi* (Table 3). However, in pair analyses, *O. b. douglasii* and *O. b. sierrae* are significantly different (F_{CT} = 0.001) from all the others. Tajima's D and Fu's F test values for all specimens showed a demographic contraction, whereas the two highland and the southern Baja California populations are under expansion (Table 4). The genetic analysis revealed higher values for pairwise mean diversity within all the specimens. Lower Nei´s diversity (*h* = 0.58) was noted in specimens from the southern Baja California Peninsula. Yosemite areas displayed the lowest mean gene diversity and nucleotide differences (Table 4).

TABLE 2. Genetic distances (%) obtained from the Cyt *b* gene (upper and lower matrices) and within clades (diagonal, bold) proposed for the examined geographical areas. Data on the upper-right and on the diagonal (bold) matrices were generated using the Kimura-two parameter model to allow comparison to traditional estimates of genetic distance in small mammals. Distances are in the lower-left Jukes-Cantor index.

	Lowlands			Highlands		
	Southern Clade			Northern Clade	Central Clade	
	Clade AI	Clade AII	Clade AIII	Clade B	Clade C	Outgroup
Clade AI	0.29	1.04	1.52	7.02	7.38	7.40
Clade AII	1.03	0.40	1.50	7.56	7.36	7.42
Clade AIII	1.52	1.50	0.45	7.52	7.70	7.71
Clade B	6.88	7.41	7.39	0.24	8.20	8.59
Clade C	7.24	7.22	7.56	8.05	0.38	7.40
Outgroup	7.26	7.28	7.56	8.40	7.25	0.75

TABLE 3. AMOVA results using different population arrangements in relation to the two hypotheses of genetic structure: 1) The *O. atricapillus* populations are genetically different to a sufficient extent to be considered as a different species from *O. beecheyi*; and 2) The seven currently recognized subspecies of *O. beecheyi* sampled in California and in the Baja California Peninsula and the specimens of *O. atricapillus* are conspecific. AG = among groups; AP = among populations; WP = within populations.

Model	Localities	Variance component	% variance
O. beecheyi versus *O. atricapillus*	1) A-Q 2) R-T	$\Phi_{ct} = 0.14$ $\Phi_{sc} = 0.49$ $\Phi_{st} = 0.57$	AG = 14.51 AP = 42.70 WP = 42.79
6 subspecies	1) A-C (*douglasii*) 2) D, E, J, O (*fisheri*) 3) F, H, I (*sierrae*) 4) G, K, M, (*beecheyi*) 5) L, N (*parvulus*) 6) P (*nudipes*) 7) Q (*rupinarum*) 8) R, S, T (*atricapillus*)	$\Phi_{ct} = 0.22$ $\Phi_{sc} = 0.40$ $\Phi_{st} = 0.53$	AG = 22.13 AP = 31.68 WP = 46.20

TABLE 4. Molecular diversity indexes for the main groups. Sample size (*n*), number of haplotypes (*k*), haplotypic or Nei´s diversity (*h*), Mean pairwise difference among the haplotypes (Md), nucleotide diversity (π), Tajima's D (Tajima's D) and Fu's *F* (fu) test for detect the deviations from neutrality caused by evolutionary forces. The parameters are from sampled populations of *Otospermophilus beecheyi* from California, U.S.A. and *O. atricapillus* from the Baja California Peninsula. Data are based on a 800 bp fragment of the Cyt *b* gene.

	All	Southern Clade	Northern Clade	Central Clade	*O. atricapillus*
N	118	67	10	40	28
K	32	17	6	9	4
H	0.91 ± 0.013	0.86 ± 0.025	0.88 ± 0.075	0.69 ± 0.049	0.58 ± 0.084
Md	33.10 ± 14.6	6.48 ± 3.1	1.86 ± 1.1	1.49 ± 0.9	1.66 ± 1.0
Π	0.041 ± 0.020	0.008 ± 0.004	0.002 ± 0.001	0.001 ± 0.001	0.002 ± 0.001
Tajima's D	1.83	-0.20	-1.04	-1.27	-1.27
Fu	10.25	-0.18	-2.01	-1.73	-2.73

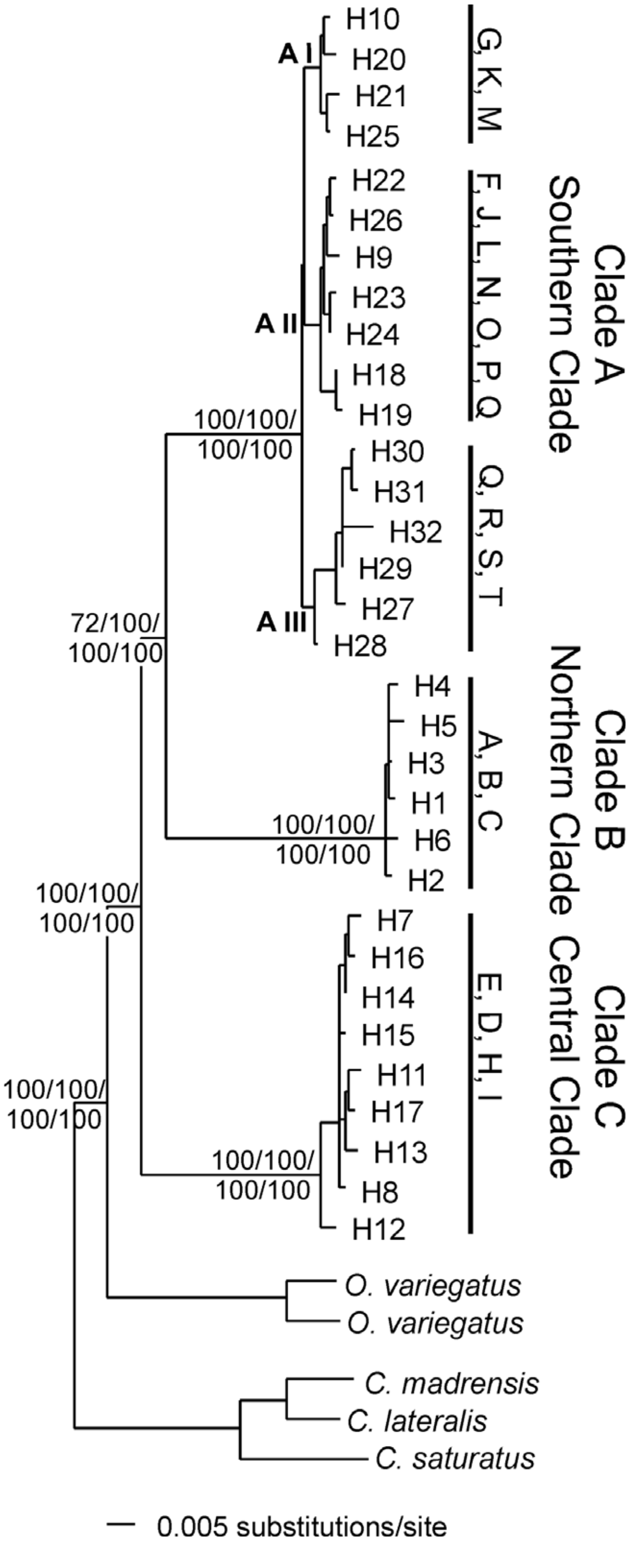

FIGURE 2. Topology of phylogenetic relationships among specimens of *O. atricapillus* and *O. beecheyi* from California and the Baja California Peninsula based on the complete Cyt *b* gene *Callospermophilus madrensis, C. lateralis, and C. saturatus* was used as outgroup. The main topology recovered from all the analyses shows that *O. atricapillus* (haplotypes 29 to 32) and *O. beecheyi* (haplotypes 1-28) are monophyletic. All the phylogenetic analyses revealed three major clades. The first clade groups all specimens from lowlands across California and Baja California Peninsula (Clade A, Southern Clade); the second one contains specimens from the California highlands in El Dorado Natural Forest (Clade B, Northern Clade); and the third contains specimens from the California highlands in Yosemite (Clade C, Central Clade). The nodal support values in each of the main nodes are in order: NJ bootstrap, maximum-likelihood bootstrap, Bayesian posterior probabilities (x100), and maximum-parsimony bootstrap. At the tip of each branch is the number of the haplotype.

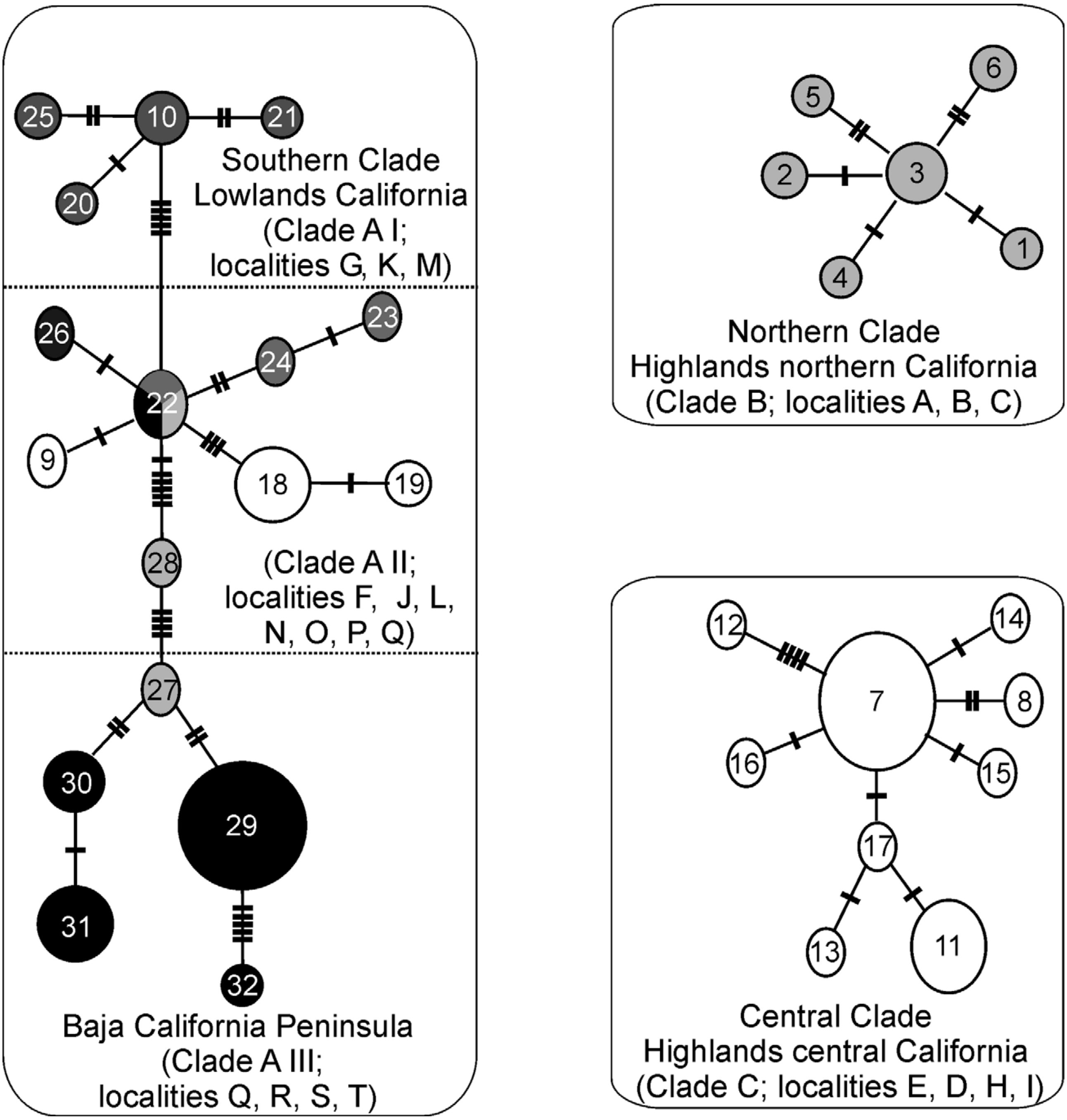

FIGURE 3. Haplotype network for the 800-bp Cyt *b* data set includes populations from California and the Baja California Peninsula. Each perpendicular hash mark across the line between adjacent haplotypes in the network represents a single-base substitution. The circle size is directly proportional to the number of specimens per haplotype; the key to the haplotype (Table 1) is adjacent to each circle; some haplotypes are present in more than one population. The clades are in boxes; Clade A (Southern Clade, lowlands across California and Baja California Peninsula); Clade B (Northern Clade, El Dorado Natural Forest); and Clade C (Central Clade, California highlands in Yosemite). Color for the haplotype circles are based on subspecies designation (see material and methods from subspecies allocation. Solid circle = *O. b. atricapillus*, dark gray circle *O. b. beecheyi*, light gray circle *O. b. douglasii*, open circle = *O. b. fisheri*, solid ellipse = *O. b. nudipes*, dark gray ellipse = *O. b. parvulus*, light gray ellipse = *O. b. rupinarum*, and open ellipse = *O. b. sierrae*.

Haplotype network. The haplotype network was divided into three geographic groups with different numbers of haplotypes (Fig. 3). Clade A (Southern Clade) haplotypes are found in the California-Baja California (lowlands), 17 haplotypes were present in 13 geographic groups (F, G, J through T) with a mean nucleotide pairwise difference between pairs of 6.48 (*SD* = 3.1). Three haplotypes (H18, H22, H29) occur in more than one locality. Haplotypes H22 and H29 are widespread in three different localities, including the northern part of the Baja California Peninsula through central California (Fig. 1; L, N, O) and in the southern Baja California Peninsula (R, S, T), respectively.

Clade B (Northern Clade) includes the haplotypes of El Dorado National Forest, northern Sierras of California (highlands). The clade has six haplotypes present in three geographic groups with a mean number of differences between pairs of 1.86 (*SD* = 1.1) and between haplotypes (Fig. 1; A through C). Each of the six haplotypes is found in more than one locality.

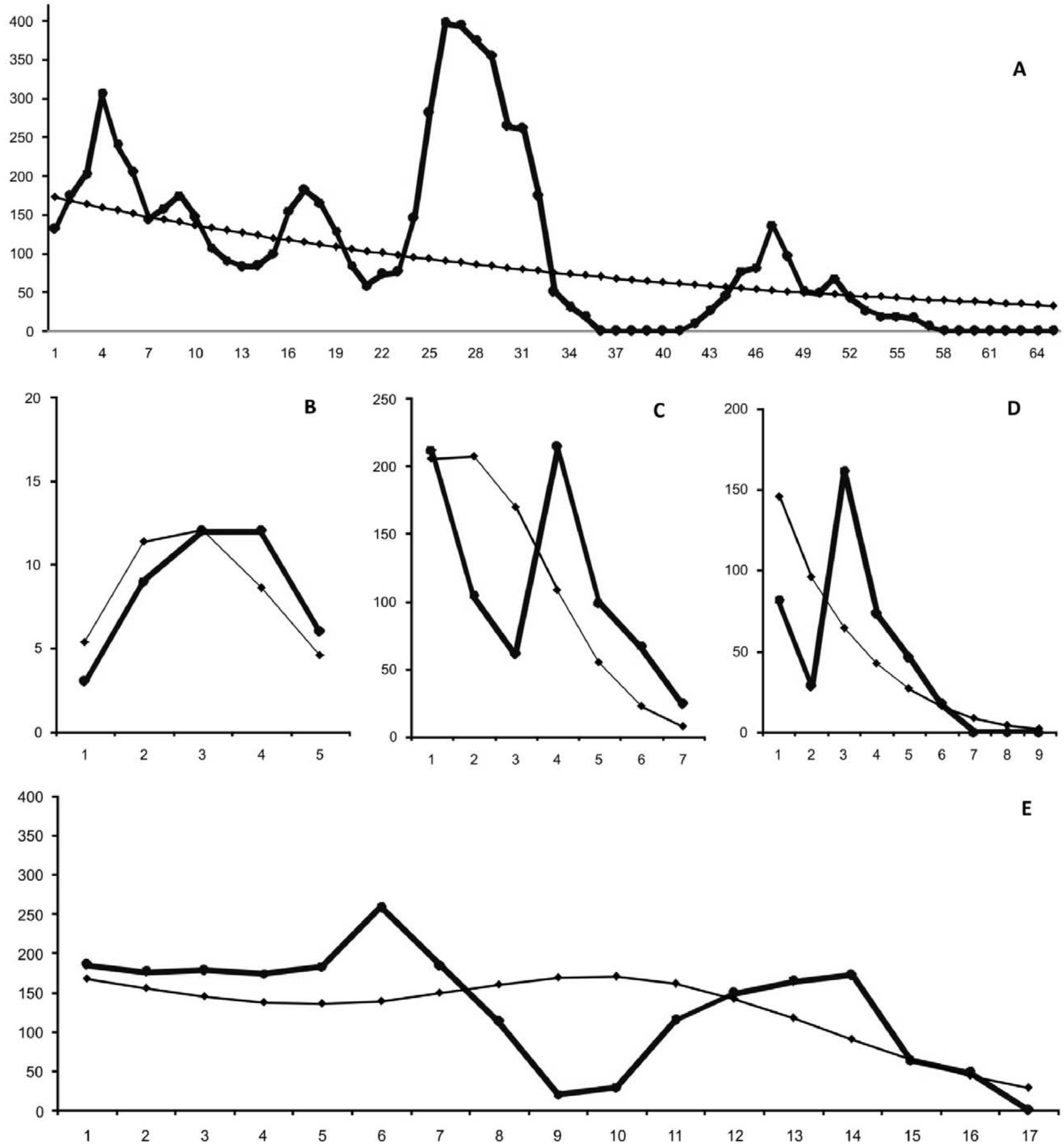

FIGURE 4. Observed (wide line) and expected (thin line) mismatch distributions for 800 *cyt b* gene, including *Otospermophilus atricapillus* and *O. beecheyi*. A) All the sample specimens from the seven subspecies of *O. beecheyi* and *O. atricapillus*. B) Specimens from Northern Clade. C) The specimens from Central Clade. D) Specimens from *O. atricapillus*, and E) All specimens Southern Clade.

Clade C (Central Clade) includes the haplotypes of Yosemite National Forest (highlands). The clade has nine haplotypes present in four geographic groups with a mean number of differences between pairs of 1.49 (*SD* = 0.9) between haplotypes (Fig. 1, D, E, H, I). Haplotypes H7 and H11 are widespread in more than one locality.

Otospermophilus atricapillus specimens from the geographical localities R through T have only four haplotypes (H29 to H32) throughout their entire range (~500-km wide), with an average of 1.66 (*SD* = 1.0**)** base-pair differences. For *O. beecheyi* from the rest of the range (~1500-km wide), there are 28 haplotypes in three clades (Fig. 3), with an average of 33.10 (*SD* = 14.6) base-pair differences. *O. atricapillus* and *O. beecheyi* do not share haplotypes; however, haplotypes of both species are in the same clade (AIII) with only two mutation steps between two *O. atricapillus* haplotypes (H29 and H30) and an *O. beecheyi* haplotype (H27).

The mismatch distribution (*sensu* Roger & Harpending, 1992) of all the specimens was multimodal, and bimodal in l Southern Clade (Fig. 4a-e). El Dorado, Yosemite, and Baja California Sur have one clear peak in the pairwise differences between haplotypes, suggesting colonization and further structuring within the lineage. The Southern Clade shows two peaks, and for the all the sampled specimens at least four (Fig. 4). The Baja California Sur (*O. atricapillus*) specimen could be considered in equilibrium and originating from bottleneck populations.

Color variation. High variation was found among specimens of *O. atricapillus*, equivalent to that variation among many subspecies of *O. beecheyi*, with a very strong black coloration in the population associated with black soils as San Miguel de Comondú and La Purisima (Fig. 1S), and light brown with those not associated with black soils as San Ignacio, Sierra de San Francisco, San Miguel de Comondú, and San Pedro de la Presa (Fig. 1 R to T). The revision of the specimens of the three known geographic groups (Fig. 1, R to T) for *O. atricapillus* showed that the black color face is not present in all the areas and is more common in the type locality.

Considering specimens of *O. beecheyi* and *O. atricapillus* altogether, the two integumental regions that have the greatest color variation were: the post-auricular region, from white in *O. b. nudipes* to salt-pepper in *O. b. nesioticus*; and the dorsal part of the neck, from salt-pepper in *O. b. fisheri* and in *O. atricapillus* to dark brown in *O. b. douglasii* and in *O. atricapillus*. We found that the coloration of *atricapillus* specimens is within the intraspecific variation of *O. beecheyi*.

Discussion

Otospermophilus atricapillus (Bryant, 1889) was described as a subspecies of *Spermophilus grammurus* (Say, 1823); the latter is currently considered a junior synonym of *O. variegatus* (see Thorington & Hoffmann, 2005). One of the characteristics used to consider *O. atricapillus* as a separate species was the darker coloration of the back and dorsal part of the neck and head (Howell, 1938). However, the examination of 50 specimens of *O. atricapillus* at the Centro de Investigaciones Biológicas del Noroeste mammal collection, together with field observations, revealed that the black-face trait is not present in all areas and is more common in Comondú, the type locality. Nevertheless, *O. atricapillus* specimens with a lighter dorsum closely resembling the dorsal surface of *O. beecheyi* can also be found there. The other diagnostic characteristics of *O. atricapillus* were a longer and lighter tail and a smaller skull (Howell, 1938). Recent morphological analyses reported between *O. beecheyi* and *O. atricapillus* showed that both species are very similar (Helgen *et al*., 2009). However, a genetic study (Harrison *et al*., 2003) confirmed the separation of both species. The possible explanation to the result of the genetic studies of Harrison *et al*. (2003) is that they used only one specimen near from the type locality (or from the Sierra populations), which does not explain genetic variation within *O. beecheyi*. Our phylogenetic analysis showed that all *O. atricapillus* specimens are clustered within the monophyletic *O. beecheyi* group, and would be considered conspecific with the latter following the phylogenetic species concept (Cracraft, 1997).

The AMOVA indicated that the two hypotheses (*O. atricapillus* is genetically different from *O. beecheyi* and *O. atricapillus* will have larger genetic variation that the current one within the recognized subspecies of *O. beecheyi*) were rejected (*P* = 0.05), and the combination of between-populations within groups or within-population components explains more than 95% of the overall pooled variation. Importantly, regardless of the hierarchical grouping of samples, the largest variation component is found among individuals within a population (~50% in each case), except for between populations in the highlands and lowlands (see below). Hence, we do not have data to support the specific status of *O. atricapillus* in relation to *O. beecheyi*, and our findings suggest that the two are conspecific. The genetic analyses suggested that the subspecies currently recognized are not significantly different. The three genetic groups have a number of pair-wise differences among them that fail to reach statistical significance ($F_{CT} > 0.05$). Therefore we considered the specimens from all localities as *O. beecheyi*.

Tajima's *D* and Fu's *F* test showed that the populations of the three clades are expanding, the mismatch distribution that comes from bottleneck origin, the population of Northern Clade with a recent expansion, and Central Clade in equilibrium. Both highland populations could have expanded from a Pleistocene relict population in relation to the reduction of the permanent snow-covered areas in the higher part of the Sierras. The populations in California and in the Baja California Peninsula are stabler although the Baja California one could have derived from a bottleneck. However, the desertification process experienced recently across the Baja California Peninsula (Well, 1986; 2000) has affected the populations living in this area, with very recent geographical fragmentation, restricted gene flow among populations, and the restriction of populations to the most mesic locations in the oasis and highest areas of the Sierras; this effect, however, is much too recent to be detected using Cyt *b* for the population genetic structure.

Our own genetic analysis showed a high difference among the populations of the highlands in relation to those of the lowlands. The level of differences could be considered at species level. However, to support this idea we need to make detailed morphological and nuclear gene analyses, analyses that are doing now (Phoung in lit).

The genetic analyses among *O. atricapillus* and *O. beecheyi* show only two mutational steps; both are a monophyletic group and *atricapillus* are within the genetic variation among the population considered as *O. beecheyi*. Under these conditions and following the phylogenetic species concept (Cracraft, 1997), low genetic variation, the morphologic differences used to distinguish *O. atricapillus* and *O. beecheyi* that are similar to those used among the subspecies within *O. beecheyi*, and a continuous distribution of both species, make us conclude that *O. atricapillus* and *O. beecheyi* could be considered as the same species, and that the morphological traits previously used to differentiate them *O. atricapillus* from *O. beecheyi* (Bryant, 1889; Howell, 1938) should be used instead to distinguish them at subspecies level.

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APPENDIX I

List of the specimens reviewed for the analysis of the coloration pattern among the subspecies. The data contain the name of the subspecies, latitude, longitude (in decimal degrees), and number of specimens from the specific locality, catalog number, and the abbreviation of the museum (MVZ, Museum of Vertebrate Zoology of the University of California; CIB, Centro de Investigaciones Biológicas del Noroeste).

Otospermophilus beecheyi atricapillus (24): Baja California Sur: Arroyo Comondú, 6.5 mi SW San Miguel de Comondú 500 ft (25.97000 -111.90000, 1, 110900 MVZ); San José de Comondú 700 ft (26.05667 -111.81722, 1, 110901 MVZ); Comondú 1000 ft (26.06250 -111.81250, 15, 49531-49547, 50224 MVZ); Santa Agueda (27.25944 -112.34972, 2, 159728 MVZ); San Ignacio 500 ft (27.30000 -112.90000, 5, 38202, 49545-49547, 15929 MVZ).

Otospermophilus beecheyi beecheyi (78): California: 2 mi W Beaumont (-117.00672 2414.01600, 5, 99900-99902, 90649-90650, MVZ); 3 mi E Banning (33.92979 -116.80466, 4, 99906-99909 MVZ); 0.5 mi W Beaumont (33.93121 -117.00014, 3, 99903-99905 MVZ); Arroyo Seco Canyon near Pasadena (34.16000 -118.16000, 6, 6376-6378, 6386, 6387,7030 MVZ); 1.25 mi S Chalk Peak (35.96988 -121.42276, 1, 30283 MVZ); lab area, Hastings Natural History Reservation (36.38659 -121.54931, 20, 177440-177442, 177444-177460 MVZ); Monterey (36.59962 -121.89747, 6, 3438-3443 MVZ); 1 mi NE Seaside (36.62027 -121.82225, 10, 29374, 29376, 29378, 29379, 29382, 29384-29387 MVZ); 7 mi SE Livermore (37.62590 -121.67560, 8, 102300-102307 MVZ); 2 mi SW Walnut Creek (37.87857 -122.08666, 1, 17847 MVZ); 3 mi NW Walnut Creek (37.93023 -122.01967, 14, 28023-28033, 28039-28041 MVZ).

Otospermophilus beecheyi douglasii (66): California: Millerton Gulch, 2.25 mi NE Inverness (38.11928 -122.82132, 4, 101333-101336 MVZ); 7 mi W Cazadero (38.52674 -123.17684, 4, 20322-20325 MVZ); E side Ukiah Valley (39.18842 -123.20644, 11, 29901-29911 MVZ); Winslow, 5 mi W Fruto (39.58918 -122.54394, 4, 17835-17838 MVZ); near Laytonville (39.68634 -123.48519, 5, 28736-28740 MVZ); Butte Creek, 4 mi SE Chico (39.68867 -121.77333, 4, 17827-17830 MVZ); Mill Creek, 2 mi NE Tehama (40.04512 -122.09565, 5, 17831-17834, 18551 MVZ); Dale's Ranch on Payne's Creek (40.31526 -122.06915, 6, 34506-34511 MVZ); McCloud River, near Baird Station (40.78469 -122.29559, 5, 3390-3395 MVZ); Castle Lake (41.22756 -122.38206, 3, 13026-13028 MVZ); Scott River, 6 mi NW Callahan (41.37830 -122.82550, 7, 13019-13024, 16392 MVZ); 1 mi S Weed (41.40811 -122.37893, 4, 65441, 67074-67076 MVZ); 18.5 mi NW Portland (45.71300 -122.94450, 4, 94211-94214 MVZ).

Otospermophilus beecheyi fisheri (116): California: 8 mi S Susanville (40.30385 -120.64870, 1, 30469 MVZ); Butt Creek, 8 mi S Chester 4300 ft (40.20199 -121.22839, 1, 121894 MVZ); 1 mi NNW Milford 4200 ft (40.18277 -120.37776, 1, 96425 MVZ); 1.5 mi WSW McKessick Peak 6000 ft (40.08633 -120.27633, 1, 96426 MVZ); 1 mi SW Pyramid Lake 3950 ft (40.05225 -119.57584, 1, 88250 MVZ); 1 mi N Oroville, NW side River (39.52681 -121.55535, 1, 74399 MVZ); near Feather River Station on Northern Electric Railway (39.46793 -121.64929, 2, 29917, 29918 MVZ); 5 mi WSW Fallon (39.44587 -118.86279, 1, 100730 MVZ); Cottonwood Creek, Virginia Mts. 3400 ft (39.44083 -119.77472, 1, 95291 MVZ); 1 mi N Virginia City (39.32422 -119.64861, 1, 85185 MVZ); river bottom, 1.5 mi E Marysville (39.14880 -121.56800, 3, 138961-138963 MVZ); 4 mi SE Marysville (39.10553 -121.53687, 2, 138959, 138960 MVZ); 0.5 mi E Zephyr Cove, Lake Tahoe (39.00222 -119.94571, 2, 85184, 85186 MVZ); 5 mi SE Minden 4900 ft (38.90292 -119.69908, 1, 86595 MVZ); Carson River, 5 mi SE Minden 4900 ft (38.90292 -119.69908, 2, 88068, 88069 MVZ); 6 mi S Minden 4850 ft (38.86719 -119.76472, 1, 93698 MVZ); 6.5 mi S Minden 4900 ft (38.85994 -119.76472, 1, 88251 MVZ); Bridgeport (38.25590 -119.22669, 1, 114574 MVZ); 0.25 mi E Bellota 125 ft (38.05227 -121.00416, 1, 160572 MVZ); Bohler Creek 7265 ft (37.90028 -119.12977, 2, 208499, 216227 MVZ); Hodgdon Meadow Campground, Yosemite National Park 4760 ft (37.79880 -119.86750, 1, 216229 MVZ); Smith Creek, 6.9 mi NE Coulterville 2946 ft (37.75606 -120.08596, 2, 207154, 207155 MVZ); Crane Flat, Yosemite National Park 6311 ft (37.75139 -119.79149, 5, 201344-201347, 216228 MVZ); Big Oak Flat Rd., Yosemite Valley, Yosemite National Park 4800 ft (37.74633 -119.77586, 1, 21853 MVZ); 3 mi NE Coulterville 3200 ft (37.74276 -120.15718, 2, 22959, 22961 MVZ); forest S of Chapel, Yosemite Valley 1220 m (37.74078 -119.59071, 2, 201342, 201343 MVZ); Happy Isles Fen, Yosemite Valley 1237 m (37.73267 -119.55807, 1, 201348 MVZ); Yellow Pine Campground, Sentinel Beach, Yosemite Valley 1236 m (37.73230 -119.60800, 6, 201336-201341 MVZ); Claribel Station 57 m (37.71472 -120.82408, 1, 27855 MVZ); El Portal 2000 ft (37.67435 -119.78117, 2, 21851, 21852 MVZ); 6.5 mi SW La Grange 230 ft (37.62480 -120.56688, 1, 207153 MVZ); 6.3 mi SW La Grange on Lake Rd. 230 ft (37.62480 -120.56688, 7, 207146-207152 MVZ); 7 mi E Mariposa (37.45201 -119.86518, 1, 67162 MVZ); 0.375 mi N Charles Green Ranch Houses, 2 mi N Friant 350 ft (37.01781 -119.70854, 1, 93614 MVZ); 3 mi SE Friant (36.95177 -119.69211, 1, 13856 MVZ); Cedar Grove, S Fork Kings River 4600 ft (36.78750 -118.66040, 2, 99337, 99338 MVZ); 1.5 mi N Mendota (36.78100 -120.37960, 2, 28666, 28667 MVZ); 15 mi SW Los Banos (36.74165 -120.89474, 1, 99805 MVZ); 12 mi W Fresno (36.73880 -119.95310, 1, 28665 MVZ); 3 mi W Miramonte 2900 ft (36.70550 -119.07410, 1, 55033 MVZ); 2 mi NNE New Idria 1900 ft (36.44430 -120.67240, 2, 72540, 72540 MVZ); 3 mi NW Tipton (36.09545 -119.35664, 2, 99803, 99804 MVZ); Tipton 260 ft (36.05952 -119.31074, 5, 13859-13863 MVZ); Trout Creek 6000 ft (35.97336 -118.23899, 3, 15151-15153 MVZ); 1 mi N Earlimart (35.90505 -119.27857, 2, 28663, 28664 MVZ); Taylor Meadow, Sierra Nevada 7000 ft (35.83066 -118.29175, 5, 15146-15150 MVZ); Cannell Meadow, Sierra Nevada 7500-7800 ft (35.82508 -118.36717, 1, 15143 MVZ); Fay Creek, 6 mi N Weldon 4100-4300 ft (35.69000 -118.29810, 2, 15144, 15145 MVZ); W slope Walker Pass 4600 ft (35.66920 -118.03710, 3, 15136-15139 MVZ); Kern River at Isabella 2500 ft (35.61000 -118.49000, 2, 15140, 15140 MVZ); French Gulch, Piute Mts. 6700 ft (35.47933 -

118.34507, 1, 59975 MVZ); 8 mi NE Bakersfield (35.45038 -118.90920, 1, 13864 MVZ); Carrizo Plains, 7 mi SE Simmler (35.27897 -119.90177, 1, 13865 MVZ); E side Levee, Buena Vista Lake 290 ft (35.19396 -119.29954, 2, 51418-51419 MVZ); San Emigdio Creek 1500 ft (35.07567 -119.18050, 2, 28661, 28662 MVZ); Maricopa (35.05881 -119.39983, 4, 29763-29766 MVZ); 9 mi SW Tehachapi (35.03650 -118.33391, 2, 77588, 77589 MVZ); Joaquin Flat, Tehachapi Mts. 2330 ft (35.02651 -118.69642, 1, 200794 MVZ); 3 mi SE Rose Station 1300 ft (34.93096 -118.87953, 1, 44348 MVZ); Fort Tejon (34.87600 -118.89392, 5, 6380-6391 MVZ); Mt. Pinos (34.81200 -119.14600, 3, 6388, 6389, 7031 MVZ); 1.5 mi ESE Mt. Pinos (34.80772 -119.11623, 1, 42105 MVZ).

Otospermophilus beecheyi nesioticus (6): CALIFORNIA: Avalon, Santa Catalina Island (33.34228 -118.32671, 6, 31192-31196, 181729 MVZ).

Otospermophilus beecheyi nudipes (90): CALIFORNIA: Mann Ranch, 0.5 mi NW Oak Grove (33.38934 -116.79220, 1, 136127 MVZ); Warner Pass (33.21000 -116.60000, 3, 7568, 7569, 7580 MVZ); Grapevine Spring (33.17565 -116.52355, 1, 3192 MVZ); 1 mi SE Wynola (33.08745 -116.63338, 1, 124191 MVZ); Witch Creek (33.08060 -116.71390, 8, 3184-3191 MVZ); Julian (33.07000 -116.60000, 8, 3172-3179 MVZ); Cuyamaca Mts. (32.94961 -116.59951, 4, 3180-3183 MVZ); 2 mi N, 1 mi W Lakeside on El Cajon road (32.88450 -116.87780, 2, 31324, 31325 MVZ); Point Loma (32.68874 -117.24324, 10, 3158-3167 MVZ); Mt. Spring (32.67000 -116.10000, 4, 7645-7648 MVZ); Chula Vista (32.64000 -117.08300, 3, 3154-3156 MVZ); Dulzura (32.64000 -116.78000, 8, 3148-3152, 7657, 7658, 94710 MVZ); Jacumba (32.61800 -116.18900, 1, 7649 MVZ); Campo (32.60516 -116.46926, 1, 3153 MVZ); near mouth of Tia Juana River (32.55530 -117.12830, 1, 3193 MVZ). BAJA CALIFORNIA: 18 mi W of Alaska [=La Rumerosa] (32.53333 -116.33000, 1, 149538 MVZ); Laguna Hanson, Sierra Juarez (32.05333 -115.90722, 4, 37963-37965, 149539 MVZ); San Antonio Mine, 10 mi SE San Jose (32.00306 -116.67028, 2, 112807, 112808 MVZ); San Jose (30.98450 -115.68200, 2, 35455, 35456 MVZ); Aguaje del Sauce, 6 mi NW Valladares (30.89389 -115.76778, 1, 35908 MVZ); La Grulla, Sierra San Pedro Martir (30.88333 -115.45000, 12, 35457-35461, 36018, 36019, 112802-112806 MVZ); La Encantada, Sierra San Pedro Martir (30.79417 -115.29167, 1, 36147 MVZ); El Cajon Canyon, E base of San Pedro Martir Mts. (30.79417 -115.29167, 6, 37632-37637 MVZ); Vallecitos, Sierra San Pedro Martir (30.75000 -115.21667, 3, 35462, 35463, 35909 MVZ); San Ramon, mouth of RÃo Santo Domingo (30.71972 -116.01750, 2, 35454, 36171 MVZ).

Otospermophilus beecheyi parvulus (108): CALIFORNIA: Strawberry Valley, San Jacinto Mts. 6000 ft (33.72238 -116.76049, 9, 2078-2086 MVZ); Tahquitz Valley, San Jacinto Mts. 8000 ft (33.76334 -116.66470, 2, 2154, 2155 MVZ); Schain's Ranch, San Jacinto Mts. 4900 ft (33.79126 -116.67606, 4, 1635-1637, 2706 MVZ); 1 mi S Palm Springs (33.80125 -116.56591, 4, 39937-39940 MVZ); near Palm Springs (33.82836 -116.53341, 8, 31304-31311 MVZ); Blaisdell Canyon, 2.25 mi S Whitewater 1100 ft (33.87769 -116.62166, 2, 99940, 99941 MVZ); 1.5 mi S by W Cabazon 1700 ft (33.89310 -116.79123, 2, 89626, 89627 MVZ); 0.5 mi W, 0.25 mi S Cabazon 1800 ft (33.91152 -116.79644, 17, 99913-99929 MVZ); Cabazon (33.91507 -116.78444, 12, 1405-1413, 99910-99912 MVZ); 1.5 mi W Whitewater 1175 ft (33.92279 -116.66329, 4, 99930-99933 MVZ); 1 mi W Whitewater 1200 ft (33.92510 -116.68140, 6, 99934-99939 MVZ); 2 mi E, 2 mi N Beaumont 2850 ft (33.96225 -116.92919, 4, 85015-58021 MVZ); Lower Covington Flat, Joshua Tree National Monument 5000 ft (34.03046 -116.30386, 2, 113993-113993 MVZ); Redlands (34.05600 -117.18200, 3, 77047, 77048, 77050 MVZ); Bluff Lake, San Bernardino Mts. (34.21944 -116.96972, 2, 6252-6253 MVZ); 2.5 mi NE Arrowbear Lake, Snow Valley II (plot) - off plot, San Bernardino National Forest, T2N R2W SW1/4 Sec. 25 6800 ft (34.22655 -117.04560, 2, 176005-176006 MVZ); 1 mi WSW Green Valley Lake, Green Valley Creek (plot) - off plot, San Bernardino National Forest, T2N R2W N1/2 Sec. 28 6400 ft (34.23366 -117.09336, 4, 176008-176011 MVZ); Victorville (34.51040 -117.31228, 2, 6094, 6095 MVZ); Mt. Springs Canyon, Argus Mts. 5500 ft (35.93440 -117.60110, 2, 92560, 92561 MVZ); Little Lake 3100 ft (35.93672 -117.90566, 2, 28658-28659 MVZ); 0.25 mi W Junction Ranch, 16 mi SSE Darwin 5725 ft (36.07525 -117.51650, 2, 64983, 64983 MVZ); 4 mi SW Olancha, Walker Creek 5200 ft (36.26500 -118.04750, 5, 98968-98972 MVZ); 3 mi NE Jackass Spring, Panamint Mts. 6300-6400 ft (36.57316 -117.47980, 7, 27506-27511, 27516 MVZ); 3 mi E Jackass Spring, Panamint Mts., Death Valley National Monument 6200-6500 ft (36.58766 -117.46593, 3, 27513-27515 MVZ); 4 mi W Lone Pine, Lone Pine Creek (36.59554 -118.13023, 1, 27502 MVZ); Lone Pine Creek 4500 ft (36.59614 -118.11225, 1, 17755 MVZ); Little Onion Valley, Sierra Nevada Mts. 7500 ft (36.81322 -118.31914, 1, 17756 MVZ); Carl Walter's Ranch, 2 mi N Independence 3900 ft (36.82607 -118.21480, 4, 27503-27505, 27512 MVZ); 5 mi W, 1.25 mi S Independence 6000 ft (37.12006 -118.35662, 5, 98962-98967 MVZ).

Otospermophilus beecheyi sierrae (47): CALIFORNIA: Chinquapin, Yosemite National Park 6200-6300 ft (37.65240 -119.70272, 1, 22657 MVZ); 1.5 mi SE Merced Lake, Yosemite National Park 7345 ft (37.73393 -119.39339, 1, 207160 MVZ); Gentry's, Big Oak Flat Rd., Yosemite National Park 5800 ft (37.73524 -119.70349, 1, 23511 MVZ); 1 mi E Merced Lake, Yosemite National Park 7500 ft (37.73948 -119.39723, 2, 23272-23274 MVZ); Merced Lake, Yosemite National Park 7234 ft (37.74090 -119.40804, 4, 207161-207164 MVZ); Merced Grove Big Trees, Yosemite National Park 5400 ft (37.74806 -119.83516, 1, 22659 MVZ); Tamarack Flat, Yosemite National Park 6604 ft (37.75456 -119.74298, 2, 207166-207167 MVZ); Smith Creek, 6.9 mi NE Coulterville 2946 ft (37.75606 -120.08596, 5, 208291-208295 MVZ); Indian Creek, Yosemite National Park 7100 ft (37.75643 -119.58206, 1, 22660 MVZ); Crane Flat, Yosemite National Park 6300 ft (37.75686 -119.79919, 1, 22658 MVZ); Aspen Valley, Yosemite National Park 6400 ft (37.83588 -119.77733, 2, 23274, 207165 MVZ); 3.2 mi E of White Wolf Campground Entrance via Hwy. 120, Yosemite National Park 8490 ft (37.84486 -119.58945, 1, 207156 MVZ); road near Crabtree Cow Camp, 5 mi SW Pine crest 6000 ft (38.15106 -120.01368, 1, 128575 MVZ); Kyburz Station (38.77513 -120.29514, 2, 19033-19034 MVZ); 1.5 mi E Woodfords 5500 ft (38.77571 -119.79492, 2, 32377, 32379 MVZ); 2 mi NE Woodfords 5600 ft (38.79619 -119.79784, 1, 32378 MVZ); Brun's Ranch, Fredericksburg 5100 ft (38.82870 -119.78574, 1, 32380 MVZ); Fallen Leaf Lake (38.90268 -120.06140, 1, 11886 MVZ); 0.5 mi NE Dutch Flat 4300 ft (39.20000 -120.83000, 2, 88174-88175 MVZ); Dutch Flat 3400 ft (39.20593 -120.83880, 1, 18660 MVZ); Blue Canyon 4800 ft (39.25708 -120.71244, 1, 18661 MVZ); Cisco 6000 ft (39.30000 -120.54000, 3, 19347, 19348, 89410 MVZ); Sagehen Creek, 1 mi SE end of Independence Lake (39.42131 -120.31332, 1, 116600 MVZ); Sagehen Creek, 3 mi NW Hobart Mills 6400-6500 ft (39.43800 -120.21117, 3, 128081, 129069, 144269 MVZ); Campbell's Hot Springs, near Sierraville 5000 ft (39.57534 -120.34680, 1, 27959 MVZ); Meadow Valley (39.93241 -121.05985, 2, 138964-198965 MVZ); near summit Red Hill, 13 mi W, 7.5 mi N Quincy 6300 ft (40.04622 -121.19137, 1, 101508 MVZ); Rich Gulch, 11 mi W, 8 mi N Quincy 3850 ft (40.04662 -121.14514, 1, 107351 MVZ); near Mono Meadow, Yosemite National Park 7300-7400 ft (no data, 1, 22661 MVZ).