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33. PHYLOGEOGRAPHY OF THE DESERT WOODRAT, *NEOTOMA LEPIDA*, WITH COMMENTS ON SYSTEMATICS AND BIOGEOGRAPHIC HISTORY

JAMES L. PATTON[1] AND SERGIO TICUL ÁLVAREZ-CASTAÑEDA[2]

[1]Museum of Vertebrate Zoology, University of California
[2] Centro de Investigaciones Biológicas del Noroeste, S.C.

Abstract

We analyze sequences of the cytochrome-*b* mitochondrial gene to examine the phylogeographic structure of woodrats of the *Neotoma lepida* complex in western North America. Complete sequences (1140bp) are available for 118 individuals from 41 separate populations, and 801bp sequences are available for a total of 311 individuals from 111 populations. Samples encompass the entire range of the species complex, from throughout the Baja California peninsula, including several islands on each side, California, Nevada, Utah, and Arizona. Phylogenetic analyses identify two major geographic clades that differ by nearly 9% Kimura 2-parameter distance, on average. These clades separate populations from coastal California and Baja California from those of the deserts of California, Nevada, Utah, and Arizona. This distinction matches Goldman's (1910) original division of the complex into two separate groups. Each of these clades is in turn subdivided into three subunits that are well supported by bootstrap analysis. Subclades from Baja California and coastal California have relatively deep coalescent histories and mostly exhibit evidence of temporal population stability. One broadly distributed clade from the Colorado, Mojave, and Great Basin deserts of California, Nevada, and western Utah, and a second from southern Utah and northwestern Arizona, on the other hand, have had recent coalescent histories, with evidence

Resumen

Se analizó las secuencias del gene del citocromo-*b* mitochondrial para examinar la filogeografía de ratas del complejo *Neotoma lepida* en el oeste de Norteamérica. Secuencias completas (1140 pb) estan disponibles para 118 individuos de 41 poblaciones separadas y, 801 pb están disponibles para 311 individuos de 111 poblaciones. Las muestras incluyeron toda la distribución del complejo, de La Península de Baja California —incluyendo algunos islas a ambos lados— hasa California, Nevada y Arizona. Análisis filogenéticos identificaron dos clados geográficos que difieren casi en 9%, en promedio, del Kimura 2. parámetro de distancia. Estoas caldos separan poblaciones de la costa de California y Baja California de aquellas del desierto de California, Nevada, Utah y Arizona. Esta distinción coincide con la división original propuesta por Goldman (1910) de separar el complejo en dos caldos. Cada uno de los caldos se subdivide en tres subunidades apoyados por un análisis de bootstrap. Los subclados de Baja California y de la costa de California tiene historias relativamente coalescentes y muestran evidencia de estabilidad poblacional temporal. Un clado ampliamente distribuido en Colorado, Mojave, el desierto de la Gran Meseta de California, Nevada y el oeste de Utah y, un segundo clado del sur de Utah y noroeste de Arizona, han tenido una historia coalescente reciente, con evidencia de una expansión poblacional reciente. Final-

of recent population expansion. Finally, populations of desert woodrats on the east side of the Colorado River in Arizona and Sonora exhibit marked, if shallow, geographic structure despite a rather limited geographic range. We discuss the possible taxonomic and biogeographic consequences of these data, but await the results on on-going analyses of contact zones before defining the number of specific and infraspecific units within the *Neotoma lepida* complex.

Key words: *Neotoma lepida*, Desert woodrat, phylogeography, cytochrome-*b*, systematics, biogeography

mente, poblaciones de ratas en el este del Río Colorado en Arizona y Sonora exhiben marcadas estructuras geográficas, no obstante su distribución restringida. Se discute las posibles consecuencias taxonómicas y biogeográficas de estos datos, aunque se esperan análisis en curso en zonas de contacto, para definir el número de unidades específicas y subespecíficas dentro del complejo *Neotoma lepida*.

Palabras clave: e.g., *Neotoma lepida*, rata del desierto, filogeografía, cytochromo-*b*, sistemática, biogeografía.

We examine the molecular phylogeographic structure of the Desert woodrat, *Neotoma lepida*, based on sequence data from the mitochondrial cytochrome-*b* (cyt-*b*) gene for 111 populations representing 23 of the 31 subspecies recognized by Hall (1981; Fig. 1) as well as *Neotoma bryanti* (from Isla Cedros, Baja California). Only 5 of the 11 insular forms from both sides of the Baja California peninsula (including three listed as separate species by Hall: *anthonyi*, *bunkeri*, and *martinensis*) and three peripheral taxa with small ranges in Utah (*marshalli*), Arizona (*auripila*), and Sonora (*aureotunicata*) have not been examined. The analyses presented here anticipate a systematic revision of the species complex, using nuclear genes and morphological characters in addition to those of the mitochondrial genome. Hence, while we suggest likely taxonomic changes here, including the elevation to species' status of some geographic segments, we defer formal taxonomic decisions until the documentation of character variation at all levels and on-going analyses of contact zones between differentiated mitochondrial DNA clades are completed.

The Desert woodrat has one of the largest ranges of any species in the genus (Hall 1981), extending from southeastern Oregon and southwestern Idaho in the United States to Cabo San Lucas in Baja California Sur, Mexico, and from the central coastal ranges of California near San Francisco east throughout the Great Basin and Mojave deserts, and through the basin of the Colorado River in western Colorado, eastern Utah, and northwestern and western Arizona south into northwestern Sonora, Mexico (Fig. 1).

The taxonomic history of the *Neotoma lepida* group, as defined by Goldman (1932, and most subsequent authors), has been complex, perhaps not unexpectedly for a taxon containing such a large number of named forms. In Goldman's (1910) revision of the genus *Neotoma* taxa currently included within the Desert woodrat complex were allocated to two separate groups, a western "*intermedia*-group" and a more easterly distributed "*desertorum*-group." However, Grinnell and Swarth (1913) documented intergradation between what they believed to be races of these two groups across San Gorgonio Pass in southern California, and allocated all members of Goldman's two groups to the single species *Neotoma intermedia* Rhoads 1894. Goldman (1927) concurred with this opinion in a paper describing *N. intermedia devia* from northern Arizona. Subsequently, Goldman (1932) realized that *Neotoma lepida* Thomas 1893 was the earliest name available for this complex of woodrats, and affirmed the type locality to be in western Utah or eastern Nevada. He then allocated 12 subspecies to *Neotoma lepida* and listed another eight taxa as separate species within his *Neotoma lepida* group. Six of these are endemic to islands on the Pacific or Gulf sides of the Baja California peninsula, four of which have retained species' status in more recent compilations (Hall 1981; Musser and Carleton 1993; Alvarez-Castañeda and Cortés-Calva 1999). All six of these island forms, however, are undoubtedly either only insular races of *N. lepida* or closely related to this species. On the other hand, the phylogenetic linkage of the remaining two species of Goldman's *lepida* group, *N. stephensi* and *N. goldmani*, to *N. lepida* has yet to be established (*e.g.*, Mascarello 1978; Koop *et al.* 1985). *Neotoma goldmani*, in fact, appears to be part of a complex of species that include *N.*

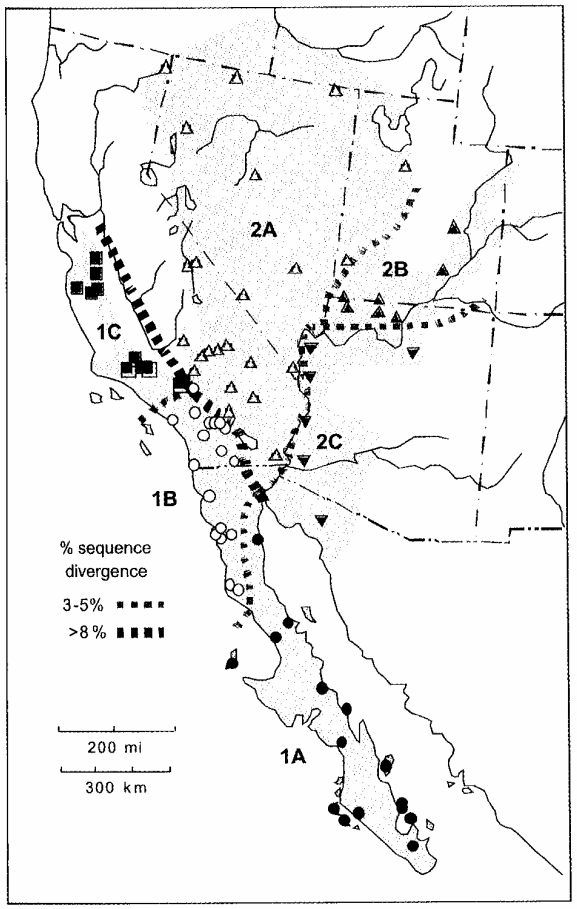

Fig. 1. Geographic distribution of the Desert woodrat, *Neotoma lepida*, (stippling), localities sampled for the mitochondrial cytochrome-*b* gene, and major geographic clade boundaries apparent from the parsimony trees generated from both the complete (1140 bp) and partial (801 bp) data sets. Clades are identified uniquely by a number and letter combination as in the tree (Fig. 2) and are mapped with separate symbols: Subclade 1A (solid circles); Subclade 1B (open circles); Subclade 1C (solid squares); Subclade 2A (open triangles); Subclade 2B (solid triangles); and Subclade 2C (inverted solid triangles). The approximate geographic positions of clade boundaries are indicated by dashed lines, the width of which is scaled to the average amount of genetic differentiation between adjacent pairs. This scale identifies clades between 3-5% different (subclades) and those nearly 9% divergent (Clades 1 and 2).

albigula, *N. micropus*, and *N. floridanus* (Edwards *et al.* 2001).

Mascarello (1978), using a combination of protein electrophoretic characters, chromosomes, and soft anatomical features of the glans penis, suggested that *Neotoma lepida*, as encapsulated by Goldman (1932) and Hall (1981), was composite. He elevated populations in Arizona to the south and east of the Colorado River to species status, employing *Neotoma devia* Goldman 1927 as the earliest available name. The substantial differentiation that exists among samples of Desert woodrats on both sides of the lower Colorado River, the area where Mascarello concentrated his sampling protocol, has been widely recognized, although subsequent authors have disagreed as to the meaning of this variation. Hoffmeister (1986), in his lengthy treatise on Arizona mammals, summarized patterns of variation in color and cranial morphometrics, as well as penile characters, and argued that all Arizona populations were merely part of a highly polytypic single species, *N. lepida*. On the other hand, Musser and Carleton (1993) agreed with Mascarello and listed *N. devia* as a separate species. Mascarello (1978) also noted that samples of *N. lepida* from southwestern California and northern Baja California were equally divergent from those of the California deserts as was Arizona *N. devia* in allozymic and penile characters, and were even more differentiated in cranial morphometrics. Mascarello's samples of this coastal form were limited in number and geographic extent, and he made no taxonomic conclusions. Finally, Planz (1992), using RFLP analysis of mitochondrial DNA, supported both Mascarello's separation of *N. devia* from *N. lepida* and recognized the division between coastal and desert populations of the latter in southern California and northern Baja California (see also Riddle *et al.* 2000).

Methods and Materials

Molecular techniques. Genomic DNA was extracted from liver either preserved originally in 95% ethanol or frozen in liquid nitrogen in the field and maintained at -80 °C in the lab, using either Chelex® (Walsh *et al.* 1991) or salt (Sambrook *et al.* 1989). We used the primer MVZ05 in combination with MVZ16 to amplify the initial approximately 850 base pair (bp) fragment of the mitochondrial cytochrome-*b* gene, or MVZ05-MVZ14 to amplify an approximately 1200 bp fragment, including the entire cyt-*b* gene. Double-stranded DNA was purified using the QIAquick PCR Purification kit (Qiagen, Valencia, CA), and this template was cycle-sequenced with MVZ05 and MVZ103 using the Taq FS kit and run on an ABI 377 automated sequencer.

Primer sequences and amplification conditions are described in Smith and Patton (1993, 1999). Sequences were edited using the Sequence Navigator software (Applied Biosystems, Inc.). The entire 1140 bp gene was obtained from 118 individuals representing 41 separate populations of *N. lepida*; the first 801 bp fragment was examined an additional 193 individuals. In total, therefore, data are available for 311 individuals from 111 separate localities sampled from throughout the range of the *N. lepida* complex (see Specimens Examined, Appendix). Nine other species of woodrats were used as outgroups in all phylogenetic analyses (listed in the Appendix). MacClade 3.5 (Maddison and Maddison 1992) was used to identify unique sequences for both datasets.

Data analysis. We examined the hierarchical relationship among unique haplotypes by the construction of minimum length trees, using the maximum parsimony criterion as implemented in PAUP* 4.0b10 (Swofford 2002). We treated all sites as equal and unordered, and we employed a heuristic search option with stepwise addition of taxa and tree bisection-reconnection (TBR) branch-swapping. We used 10 random input orders of taxa to ensure that the final tree corresponded to a global optimum, and represent the topology of relationships with the strict consensus of all equally minimal length trees obtained. Finally, we used bootstrap re-sampling, with 5000 replicates using the same settings as for the heuristic search, to assess the robustness of the resulting tree topology. The 1140bp dataset of the complete cytochrome-*b* gene for 118 individuals from 41 populations was analyzed initially to establish the major clade structure for this complex of woodrats. A separate analysis that included all 311 individuals for which a minimum of 801bp was available was then performed to place the 111 sampled localities into a phylogeographic context. Because the large size of the reduced dataset precluded a parsimony analysis, we used the neighbor-joining algorithm based on a Kimura 2-parameter distance matrix, as implemented in PAUP* 4.0b10. The robustness of the nodes in this tree was estimated by 1000 bootstrap re-samplings.

We used the software package Arlequin (version 2.001; Schneider *et al.* 2000) to calculate gene and nucleotide diversities, the mean pair-wise differences between all unique haplotypes, Tajima's D (to test for deviations from neutrality), and histograms of the total number of pair-wise differences among all 311 individuals for the 801bp dataset. Data were analyzed separately for each clade identified with bootstrap values greater than 80%. We then compared the histograms of pair-wise differences, or "mismatch distributions", to the distribution expected in an expanding population (Slatkin and Hudson 1991; Rogers and Harpending 1992). Approximate 95% confidence intervals for this distribution were obtained by a parametric bootstrap approach (Schneider and Excoffier 1999). Finally, we obtained the "raggedness index" of Harpending (1994) for each distribution, a measure of the "stationarity" of population history. Large values for this index characterize multimodal distributions commonly found in populations that have been stable for long periods of time or that are mixtures of regionally differentiated groups; lower indices characterize unimodal and smoother distributions typical of expanding populations.

Results and Discussion

Measures of molecular diversity. Ninety-five unique complete cytochrome-*b* sequences were obtained from the 118 individuals of *N. lepida* for which the entire gene was obtained, and 205 unique haplotypes were found among all 311 individuals for which at least 801 bp of sequence were examined. Most haplotypes in either dataset are found only in a single population, and where a given haplotype is found in more than one sample, these are usually geographically nearby. The only exceptions to this are samples from the deserts of California and Nevada where two haplotypes are found in populations separated by 200 to 300 miles (Pancake Range and Delamar Mountains, Nevada, to Kern River Plateau, California; and Kern River Plateau to Cargo Muchacho Mountains, southeastern California).

Table 1 provides standard measures of the number of pairwise differences, nucleotide diversity, and haplotype diversity that characterize each of the geographic clades that we identify below. Populations from central and coastal California exhibit the fewest number of differences among their included haplotypes (slightly less than 5 substitutions, on average, between any pair of haplotypes). Samples from most of peninsular Baja California and Arizona east of the Colorado River have the highest number of differences among their respective haplotypes (12.8 and 14.6 substitutions, respec-

Table 1. Measure of the number of pairwise differences, nucleotide diversity, and haplotype diversity for each of the six geographic clades of the Desert woodrat, *Neotoma lepida*. The clades are mapped in Fig. 1 based on the phylogenetic analysis of the complete cytochrome-*b* dataset (Fig. 2). Data given are means and one standard deviation. N_p = number of populations; N_i = number of individuals; N_h = number of unique haplotypes.

Clade	N_p	N_i	N_h	Mean pairwise difference	Nucleotide diversity	Haplotype diversity
1A (Baja California)	22	30	27	12.8092 ± 5.9382	0.01599 ± 0.00825	0.9931 ± 0.0105
1B (NW Baja/SW CA)	28	94	54	8.3532 ± 4.7748	0.01312 ± 0.00684	0.9812 ± 0.0046
1C (central CA)	17	33	21	4.9886 ± 2.4885	0.00683 ± 0.00379	0.9432 ± 0.0274
2A (CA/NV/UT deserts)	32	94	67	6.8691 ± 3.2662	0.00857 ± 0.00452	0.9849 ± 0.0058
2B (SE UT/ NW AZ)	7	27	24	6.0313 ± 2.9659	0.00753 ± 0.00412	0.9915 ± 0.0125
2C (western AZ)	5	33	12	14.5949 ± 6.7048	0.01822 ± 0.00931	0.8598 ± 0.0392

tively). Samples from the interior deserts of California, Nevada, and Utah are somewhat intermediate between these two extremes (6.0 and 6.9, respectively). Haplotype diversity is similar among all geographic regions, except in Arizona east of the Colorado River where this measure is notably lower (range 0.95-0.99 versus 0.86, respectively). Finally nucleotide diversities are generally lower among samples from coastal California, the Mojave and Great Basin deserts, and southern Utah and northwestern Arizona (range, 0.007-0.008) compared to those from elsewhere within the range of the Desert woodrat (range, 0.010-0.018).

Kimura 2-parameter distances among all pairs of haplotypes for which the complete cyt-*b* gene was sequenced average 6.07%, with the maximum difference between any two haplotypes 10.39. Table 2 summarizes Kimura 2-parameter distances, averaged among each geographic clade identified immediately below. Values among individuals within clades exhibit an average range from less than 1% to more than 2%, while averages among clades range from 3.5 to 9.5%. By any measure, the divergence among populations and geographic regions of the Desert woodrat are substantial, certainly equivalent to the high levels of differentiation observed for this gene in other species groups of woodrats (Edwards *et al.* 2001; Edwards and Bradley 2001).

Phylogenetic and phylogeographic structure. Figure 2 provides the strict consensus tree (of 153 equal minimum length trees) generated by a parsimony analysis of the 95 unique haplotypes of the 1140 bp dataset, 303 sites of which are phylogenetically informative. The tree illustrated is pruned to 32 haplotypes, excluding outgroups and many terminal branches within each clade, for visual simplicity. Bootstrap values based on the entire analysis of 118 total cyt-*b* sequences are given above internal nodes. The Neighbor-Joining analysis involving the 311 specimens for which at least 801bp were available had the same topology and identified the same nodes, each generally with strong bootstrap support (indicated below each node in the tree in Fig. 2).

Our samples of the Desert woodrat divide into two very well supported clades, each with bootstrap support of 100%, regardless of whether the analysis is limited to the complete cyt-*b* gene or includes all individuals examined. These two clades differ by an average of 8.8% Kimura 2-parameter corrected distance. Both analyses also identify the same substructure within each of these major clades, although the level of bootstrap support is always higher for the complete cyt-*b* analysis (Fig. 2). Clade 1, which encompasses all samples from coastal and central California south throughout the Baja California peninsula (Fig. 1), is divided into three subclades, each with bootstrap support of 86% or higher. Subclades 1A and 1B appear most closely related, although the bootstrap support for this node is weak (53% for the parsimony analysis, 0% for the neighbor-joining analysis). Unless additional samples provide further resolution, these three subclades are best considered an unresolved trichotomy. Each subclade differs from the others by an average of 4.5%. One of these (Subclade 1A) is found throughout most of Baja California, except the northwestern coast. This clade includes the insular species, *Neotoma bryanti*, which is only marginally differentiated from our closest mainland sample (Punta Prieta, 1.26% Kimura 2-parameter distance; 801bp data set). The

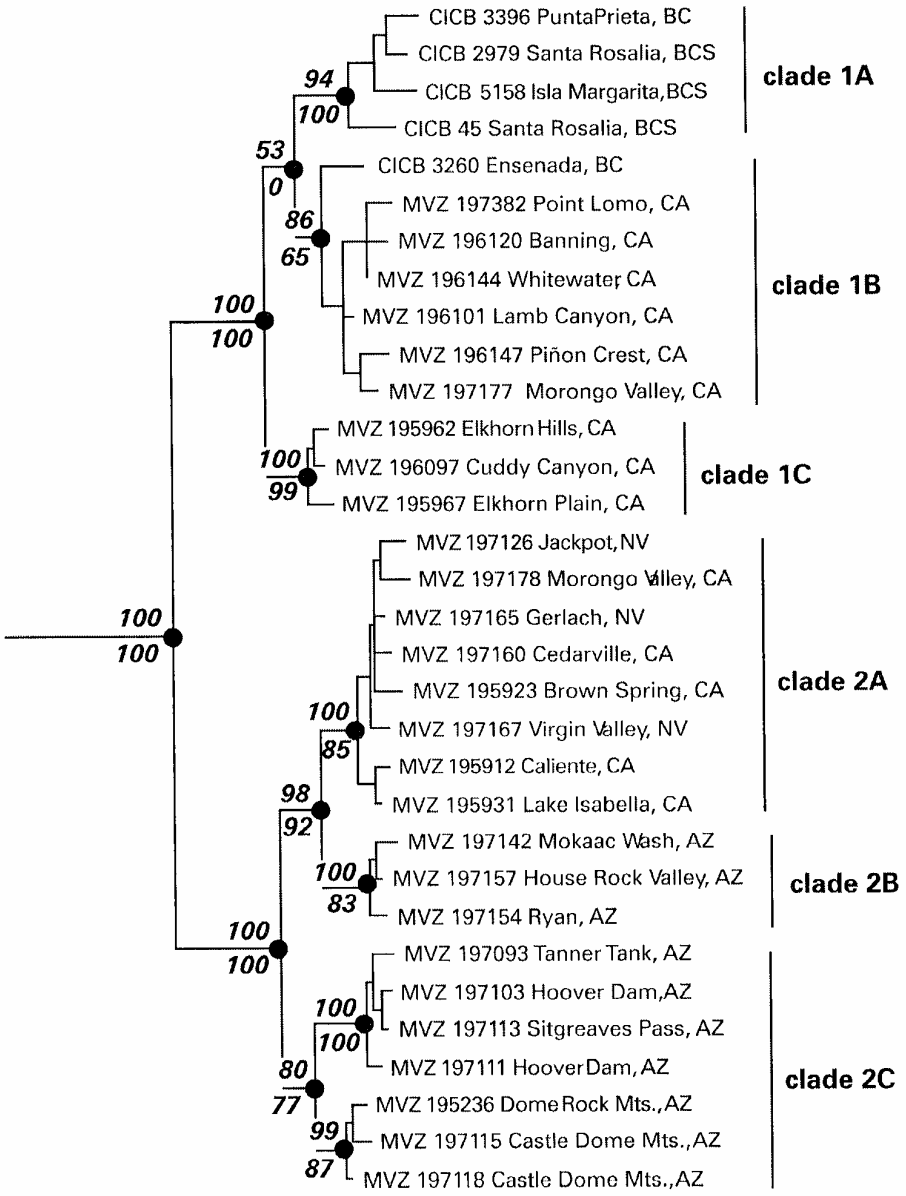

Fig. 2. Strict consensus of 153 equally minimum length maximum parsimony trees based on 118 complete cytochrome-*b* sequences (1140bp) of *Neotoma lepida*. Because of the size of the data set, only representative haplotypes of each clade are illustrated. Bold numbers above each internal node are bootstrap percentages (based on 5000 iterations) for the parsimony analysis. Bold numbers below each node are bootstrap values (1000 iterations) generated from a neighbor joining analysis that included all 311 individuals examined, and for which the same clade structure was obtained. The tree is based on 303 parsimony informative characters (717 characters were constant and 120 were parsimony uninformative). Length = 1324 steps; CI = 0.419; RI = 0.659, HI = 0.581.

second (Subclade 1B) occurs from northwestern Baja through southern California as far north as the western margins of the Tehachapi Mountains in Kern Co., California. And the third (Subclade 1C) is found from the Tehachapi Mountains west and north through the inner coast ranges of California to south of the San Francisco Bay area.

The interior, or desert Clade 2 is also divisible into at least three geographic units (Fig. 2). The first, Subclade 2A, is distributed from southeastern California west of the Colorado River north throughout the Mojave and Great Basin deserts through California, Nevada, and western Utah. Subclade 2B is distributed through the upper Colorado River drainage on its northern side, from southwestern Utah and adjacent Arizona to eastern Utah. Finally, Subclade 2C is found east of the Colorado River in Arizona and adjacent northwestern Sonora. Subclades 2A and 2B comprise a well-supported sister pair (bootstrap support 98%), differing from each other by an average of 3.5% (Table 2). This pair, in turn, groups with Subclade 2C from east of the Colorado River, at an average Kimura 2-parameter distance of 4.3%. In contrast to Subclades 2A and 2B, Subclade 2C also exhibits marked internal phylogeographic structure, with samples from north and south of the Bill Williams River each forming highly supported groups (bootstrap values of 100 and 99, respectively). Although these differ by a more modest 2.8%, their level of bootstrap support in both parsimony and neighbor joining analyses is equivalent to that for Subclades 2A and 2B. We combine all populations in Subclade 2C in the summary analyses, primarily because so few localities (2 and 3, respectively) are represented for each geographic unit contained within.

Importantly, individuals belonging to the two major clades have been taken at the same, or nearby localities in two areas in southern California: on the western slope of the Tehachapi Mountains (Kern Co. – El Paso Creek and Joaquin Flat [Subclade 1C, n = 10] are approximately 0.2-0.5 mi distant from Tejon Creek [Subclade 2A, n = 1]) and in Morongo Valley (San Bernardino Co. – a total of 12 individuals of Subclade 1B and 13 of Subclade 2A have been obtained at three localities spaced from the eastern to western end of the valley). Within each major clade, only individuals within separate subclades in Clade 1 have been found in true sympatry (*i.e.*, in adjacent houses), at two localities on the western margin of the Tehachapi Mountains (Kern Co., 1.5 mi SE fort Tejon – 13 individuals of Subclade 1B and 9 of Subclade 1C; and Los Angeles Co., 4.5 mi E Gorman – 4 of Subclade 1B and 1 of Subclade 1C). Since mitochondrial DNA is maternally inherited, these data provide no insight into whether these respective gene pools are isolated, or whether interbreeding occurs. We are currently engaged in detailed studies of these contact areas using nuclear genes to establish the degree of genetic interaction, if any, between individuals with such differentiated mitochondrial genotypes. Our sample localities for individuals of the desert Subclades 2A and 2B are separated by the Virgin River, but it is possible that these two subclades may contact one another in southwestern Utah. Individuals of Subclade 2C, however, are effectively separated from those of either Subclades 2A or 2B by the lower and middle Colorado River, and are likely fully isolated geographically, and thus genetically.

Population history and mismatch distributions. The pooled haplotype sample from all populations

Table 2. Kimura 2-parameter distances (x100) among 118 haplotypes distributed in six geographic clades (see Figs. 1 and 2) for which 1140bp of the cytochrome-*b* gene for geographic clades of the Desert woodrat, *Neotoma lepida* are available. Data are given as mean ± standard deviation; within clade measures are given on the diagonal.

	Clade 1A	Clade 1B	Clade 1C	Clade 2A	Clade 2B	Clade 2C
Clade 1A	2.25 ± 0.57	4.28 ± 0.29	4.55 ± 0.17	9.45 ± 0.39	9.00 ± 0.46	8.73 ± 0.46
Clade 1B		1.57 ± 0.52	3.65 ± 0.41	9.41 ± 0.35	8.65 ± 38	8.18 ± 0.30
Clade 1C			0.94 ± 0.29	8.73 ± 0.21	8.11 ± 0.26	7.84 ± 0.18
Clade 2A				1.31 ± 0.39	3.46 ± 0.28	4.40 ± 0.66
Clade 2B					0.89 ± 0.24	4.18 ± 0.22
Clade 2C						1.95 ± 1.02

in each of the six geographic clade groups all exhibit negative Tajima's D-values (Table 3), although the values for two of the subclades of Clade 2 are significantly different from zero (Subclade 2A, $p < 0.01$; Subclade 2B, $p < 0.05$). Tajima's (1989) test is based on an infinite-sites model without recombination, and is thus appropriate for clonally inherited mtDNA sequences. Negative but non-significant D-values suggest that evolution within each region of the coastal Clade 1 has been relatively independent of selection, heterogeneity of mutation rates, or major population perturbations during the coalescent history of the cyt-*b* sequences examined. On the other hand, the significantly negative D-values of both Subclades 2A and 2B of the Mojave and Great Basin deserts suggest that these populations have experienced either a recent selective sweep or rapid recent expansion (see Aris-Brosou and Excoffier 1996; Tajima 1996).

Distributions of pairwise differences among all haplotypes within each clade (the "mismatch" distribution; Rogers and Harpending 1992; Slatkin and Hudson 1991) support the suggestion that the geographic regions of Clade 1 have had a history of relatively stable populations without major bottlenecks, allowing for the development of substantial substructure, at least over the coalescent time of their respective mitochondrial lineages. The mismatch distributions for all three subclades are multimodal, significantly different from that expected for an expanding population ($p < 0.05$ in each case), and all but that for Subclade 1C from coastal California are characterized by a high average (Table 1) and a broad distribution of pairwise differences (Fig. 3). These features are typical of "stationarity", a history of relative population stability. Each clade, including Subclade 1C, also exhibits relatively high "raggedness" indices (Harpending 1994) expected for populations that have not experienced sharp declines or recent expansions. The general lack of haplotype diversity yet multimodal signal, including the high raggedness index, in the mismatch distribution of Subclade 1C suggests that populations in this region have been rather independent over their relatively more recent coalescent history than have populations of other subclades in Clade 1, perhaps because these are smaller in size and more isolated from one another. Desert woodrats along the central California coastal ranges are very patchily distributed, confined to favorable microhabitats (rock outcrops and patches of *Yucca whipplei*) on xeric slopes within the oak woodland and chaparral that otherwise primarily supports the Dusky-footed woodrat, *Neotoma fuscipes*, or large eared woodrat, *Neotoma macrotis*.

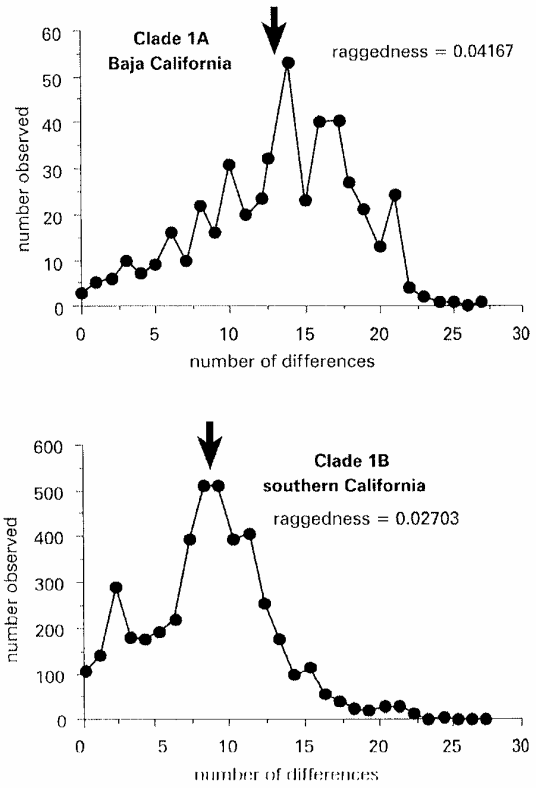

Fig. 3. Mismatch distributions for all haplotypes recovered from each of the two geographic subclades of mitochondrial Clade 1 of *Neotoma lepida* from Baja California and southern California. The "raggedness" index (Harpending, 1994) is given; vertical arrows indicate the mean pairwise difference.

Table 3. Tajima's D-values for each geographic clade of the *Neotoma lepida* complex, based on the 801bp haplotype data set. *Ns* = non-significantly different from zero; * = $p < 0.05$.

Clade	Tajima's D-value	*p*
1A	-1.19011	0.12131^{ns}
1B	-1.43368	0.06859^{ns}
1C	-1.55201	0.12908^{ns}
2A	-1.76904	0.02661*
2B	-1.77935	0.02751*
2C	-0.43009	0.35398^{ns}

In contrast to the multimodal mismatch patterns of all geographic regions of Clade 1 (Fig. 3), unimodal pairwise distributions characterize Subclades 2A and 2B from the interior deserts of California, Nevada, Utah, and northwestern Arizona (Fig. 4). In each case, the mean number of pairwise differences is small (approximately 6), the distributional range of differences is limited (0 to 16), and raggedness indices are low (30 to 100% less than those for the regions of Clade 1). Moreover, each distribution is not significantly different from one simulated by assuming a model of population expansion. These data, taken together with the significantly negative Tajima's D-values, support the hypothesis of recent coalescent histories followed by exponential population growth. The broad distributions of two haplotypes in Subclade 2A, occurring in distinct populations hundreds of miles distant, provides further support for the suggestion that the Desert woodrat has recently expanded to occupy its current range in the Colorado, Mojave, and Great Basin deserts west and north of the Colorado River. Taken together, therefore, a detailed molecular population perspective of the desert lineages of *Neotoma lepida* (Subclades 2A and 2B) may offer great insight into the recent history of the desert fauna and flora akin to that obtained from the middens left by this, and other species, of woodrats throughout the region (*e.g.*, Betancourt *et al.* 1990).

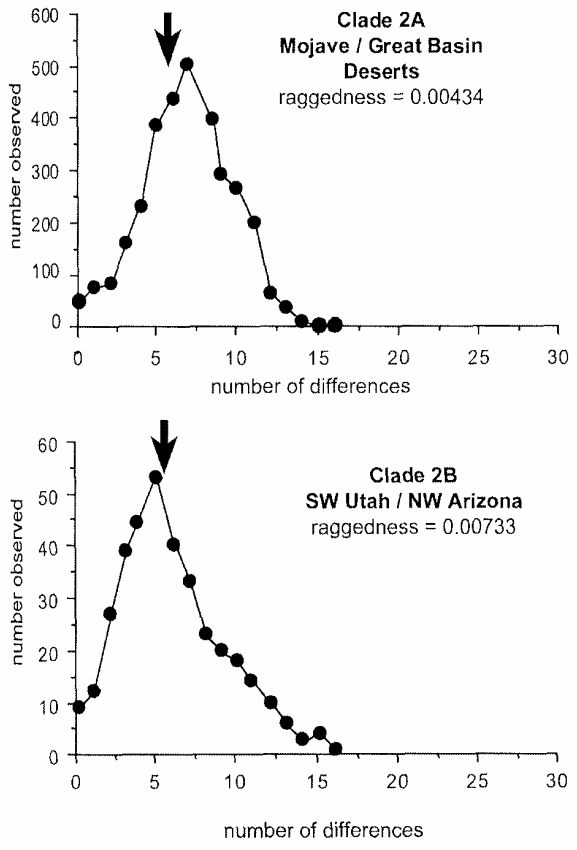

Fig. 4. Mismatch distributions for all haplotypes recovered from each of the two geographic subclades of mitochondrial Clade 2 of *Neotoma lepida* from the interior deserts of California, Nevada, and Utah. The "raggedness" index (Harpending, 1994) is given; vertical arrows indicate the mean pairwise difference.

Systematic implications. By any measure, be it simply the total amount of haplotype differentiation or the pattern of variance partitioning among hierarchical geographic units, molecular divergence within the *Neotoma lepida* complex is extensive, with maximum values well above those typical of many "good" species of New World murid rodents, including woodrats (*e.g.*, Bradley and Baker 2001; Edwards *et al.* 2001; Smith and Patton 1993, 1999). As mentioned above, it is not our intention here to make formal taxonomic changes, nor do we wish to establish the basis, as yet, for the definition of species' boundaries in this complex of woodrats. The systematic philosophy of the senior author (JLP) regarding the integration of molecular with morphological, and other, data in the definition of species boundaries, and in the recognition of infraspecific units, has been reiterated in numerous prior publications (*e.g.*, Patton and Smith 1990, 1994; Smith and Patton 1988). Rather, here we simply comment on the implications of the data we present as relevant to current taxonomy of the group (*e.g.*, Álvarez-Castañeda and Cortés-Calva 1999; Hall 1981).

The focus of taxonomic debates regarding species limits in the *Neotoma lepida* complex to date has been on the status of taxa on either side of the Colorado River in Arizona relative to those in California and Nevada (and presumably Utah). As stated above, Mascarello (1978) marshaled allozymic, chromosomal, and morphological evidence to support the species status of the Arizona populations, for which he used the earliest available name, *N. devia* Goldman 1927. Hoffmeister (1986), on the other hand, challenged the distinction of *N. devia* and concluded that Arizona populations did not warrant specific recognition. Hoffmeister could find

no differences between *devia*, south of the Grand Canyon, and *monstrabilis* Goldman 1932, on the north side, for example. Musser and Carleton (1993), in their compilation of muroid rodents of the world, followed Mascarello and listed *N. devia* as a species distinct from *N. lepida*. Our data clearly support the hypothesis of Mascarello, in that samples from east and south of the Colorado River in Arizona (Subclade 2C, including topotypic material of *devia* from Tanner Tank, Coconino Co., Arizona) are reciprocally monophyletic in their mitochondrial DNA relative to those to the west and north (Subclade 2B, including topotypes of *monstrabilis* from Ryan, Coconino Co.; figs. 1 and 2). Thus, Hoffmeister's hypothesis of intergradation between *devia* and *monstrabilis* across the Grand Canyon is not supported, although a careful examination of nuclear gene data remains relevant to this issue. Certainly, however, the mitochondrial sequence data are concordant with the allozymic, chromosomal, and morphologic data of Mascarello (1978) in distinguishing samples from opposite sides of the lower Colorado River in California and Arizona (Subclades 2A and 2C, respectively; figs. 1 and 2). The most reasonable hypothesis for these populations, therefore, is to follow Mascarello and recognize *N. devia* on the Arizona side as distinct from *N. lepida* on the California side. The role of the lower Colorado River as a barrier to small mammals, with species pairs often present on opposite sides, has been recognized since the early investigations of Joseph Grinnell (1914) and corroborated by modern molecular analyses (*e.g.*, Riddle *et al.* 2001).

While the differentiation of woodrat populations on opposite sides of the Colorado River was not unexpected, given the earlier analyses of Mascarello (1978), the extent of geographic structure across the range of *N. devia* in Arizona and Sonora was. As mentioned above, samples to the north and south of the Bill Williams River belong to reciprocally monophyletic groups, and the single individual from the Pinacate region in northwestern Sonora is equally divergent. The mitochondrial break north and south of the Bill Williams River is concordant with a morphological shift noted by Hoffmeister (1986), who allocated each group to separate subspecies (*devia* to the north and *auripila* Benson 1933 to the south). More thorough sampling across this region will be needed to determine if these forms meet, and if they interbreed where they do so.

The greatest surprise in our data, however, is in the trenchant distinctness between populations belonging to Clades 1 and 2 (nearly 9%, on average), with sympatry or near sympatry in two regions in southern California. This level of difference is well above that typical of conspecific populations of small mammals (Bradley and Baker 2001), and certainly suggests that members of each clade are specifically distinct. This hypothesis is supported by sympatry or near sympatry of individuals belonging to these two mitochondrial lineages at sites in southern California. Again, however, nuclear gene data will be all the more critical to determine if individuals of each different clade living adjacent to one another act as separate species, or interbreed freely. Mascarello (1978) did note both allozyme and morphological (glans penis) differences between the few populations belonging to each clade from southern California that he examined, and David Huckaby (pers. comm.) has documented sharp discordances in penile morphology between males of these two clades, differences that suggest reproductive incompatibility. It is interesting to note that all samples we obtained from across San Gorgonio Pass (from west to east: Lamb Canyon, near Beaumont; south of Banning; south of Cabazon; mouth of Whitewater Canyon; mouth of Blaisdell Canyon; Chino Canyon; Deep Springs; and Piñon Crest – $n = 29$ combined; see Appendix) were members of the coastal Subclade 1B, with none exhibiting a mitochondrial haplotype of the desert Subclade 2A. It is along this very same transect where Grinnell and Swarth (1913) thought intergradation was occurring between Goldman's interior *desertorum*-group (Clade 2) and coastal *intermedius*-group (Clade1). Animals do get smaller and paler from west to east across San Gorgonio Pass, but they retain the coastal mitochondrial genome. Once again, nuclear gene data will be necessary to determine if the smaller and paler animals to the east are simply convergent on the desert phenotype, or whether there is discordance in genes across this region. If the coastal Clade 1 were to be recognized as a species distinct from that of the interior desert, *intermedius* Rhodes 1894 would be the earliest available name (but see comment, below, about *bryanti* Merriam 1887). It is also important to point out that the amount of divergence in cytochrome-*b* between the coastal clades (4.3 to 4.5%; Table 2) is equivalent to that across the Colorado River that separates Subclades 2A or

2B from 2C. If one were to use sequence divergence as a metric for identifying species, then each of the major clades and most of the subclades we define here would enjoy species status. We do not, however, concur with suggestions that the degree of sequence divergence alone is a valid, or even useful, means upon which to define species boundaries (*e.g.*, Avise and Johns 1999).

A final comment on taxonomy of the *Neotoma lepida* group relates to some of the insular taxa off both coasts of Baja California. Of the eleven insular taxa, seven are commonly listed as subspecies of *N. lepida* (*abbreviata*, *insularis*, *latirostra*, *marcosensis*, *nudicauda*, *perpallida*, and *vicina*) and four remain separate species, as they were originally described (*anthonyi*, *bryanti*, *bunkeri*, and *martinensis*). Our sequence data for five island subspecies invariably show close identity between each and the nearest available mainland populations, with Kimura 2-parameter distances ranging between 1 and 2%. All sampled insular races are off the southern, gulf side of the peninsula (Fig. 1), and all belong to Subclade 1. Certainly, there is no reason to think that these are anything but subspecies, or even merely island variants not worthy of taxonomic recognition, of the widespread species of woodrat that inhabits the entire peninsula. We can conclude, however, that the derivation of each has been a relatively recent event in the history of the complex of Desert woodrats in Baja California. Whether the pattern of close similarity to, and thus recent histories with adjacent mainland populations characterizes all of the insular races of *Neotoma lepida* remains to be established. Such should not be an automatic expectation, especially given the mixture of recent and deeply divergent island taxa of *Peromyscus* delineated recently by Hafner *et al.* (2001). For example, *N. l. insularis* Townsend 1912 from Isla Angel de la Guarda has a markedly divergent male phallus (D. Huckaby, pers. comm.) and thus may represent an example of deeper divergence. We have not sampled this taxon as yet.

Finally, we emphasize that the substantial mitochondrial similarity of island populations relative to those of the adjacent mainland also characterizes our sample of *Neotoma bryanti* from Isla Cedros. This species differs by no more than 1-2% from any other individual of Clade 1A sequenced from along the length of the Baja California peninsula. Again, we hesitate to make a formal statement here, especially since the morphological distinctness of *bryanti* has never been evaluated beyond its initial description, but we would suggest that this taxon likely only warrants subspecific status, at best. If this were true, then *bryanti* Merriam 1887 would have priority as the name for Clade 1 of the *Neotoma lepida* complex as we define it here. As we have no data as yet on the other insular "species" in the *Neotoma lepida* complex (*anthonyi*, *bunkeri*, or *martinensis*), we can make no comment on their likely validity.

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Appendix

Specimens Examined

Provenance and Museum catalog number for each specimen used in the analyses are given below. Catalog numbers marked by an asterisk (*) identify those specimens for which the complete 1140bp cytochrome-*b* gene was sequenced; the initial 801bp fragment is available for all others. MVZ – Museum of Vertebrate Zoology, University of California, Berkeley; CIB – Centro de Investigaciones Biologicas, La Paz, Baja California Sur, Mexico. Uncataloged CIB specimens are identified by the initials of the field collector. Specimen numbers prefixed by DSR are the field catalog numbers of Duke S. Rogers and are deposited in the Monte L. Bean Museum of Brigham Young University, Provo, Utah.

Arizona: Coconino Co. – ca. 1 mi NE Tanner Tank (MVZ 197093I, 197094, 197095*-197097*, 197098, 197099*-197101*, 197102), east slope Kaibab Plateau, ca. 3 mi W House Rock Ranch (MVZ 197155*-197158*), Ryan (MVZ 197153, 197154*). La Paz Co. – west slope Dome Rock Mts. (MVZ 195235, 195236*). Mohave Co. – 1 mi E (by rd) Sitgreaves Pass, Black Mts. (MVZ 197113*-197114*), 22 mi SE Hoover Dam (on Hwy 93) (MVZ 197103*-197112*), Mokaac Wash (MVZ 197141, 197142*, 197143, 197144*, 197145-197147, 197148*-197152*). Yuma Co. – Castle Dome Mine, west slope Castle Dome Mts. (MVZ 197115*-197123*).

California: Alameda Co. – Del Puerto Canyon (MVZ 197371). Imperial Co. – Tumco Mines, Cargo Muchacha Mountains (MVZ 195259-195260). Inyo Co. – 3.5 mi E Big Pine (MVZ 195287, 195289), 6.4 mi S Big Pine (MVZ 195276, 195279). Kern Co. – 0.5 mi E Onyx (MVZ 195917*-195918), 0.7 mi E Weldon (MVZ 195919*, 195920*, 195921*, 195922), 1.3 mi NW Lake Isabella (MVZ 195930*, 195931*, 195932-195933), 2.8 mi NE Lake Isabella (MVZ 195934*-195935*), 1.5 mi SE Fort Tejon (MVZ 195771-195779, MVZ 196809-196821), 3 mi SE (by rd) Oak Creek Pass, Tehachapi Mts. (MVZ 197310-197313), 8.2 mi SE Inyokern (MVZ 195274-195275), Brown Spring, 3.5 mi S Weldon (MVZ 195923*-195926*, 195927-195929), Caliente Creek (MVZ 195912*-195914*, 195915-195916), Cuddy Canyon, 1 mi E Frazier Park (MVZ 196097*-106098*, 196099, 196100*), El Paso Creek, Tehachapi Mts. (MVZ 196768, 196770), Freeman Canyon (MVZ 195264-195265), Joaquin Flat, Tehachapi Mts. (MVZ 196822-196829), National Cement Plant, Tehachapi Mts. (MVZ 196764-196765), Pescadero Creek, Tehachapi Mts. (MVZ 196762-196763, MVZ 196837), Rancheria Creek, east end Walker Basin (MVZ 197308-197309), south side Tejon Creek, Tehachapi Mts. (MVZ 196830-196831). Los Angeles Co. – 0.4 mi W Gorman (MVZ 196832-196834, 198328-198330), 4.5 mi E (by rd) Gorman (MVZ 196766, 196835-196836, 198331-198332), 4 mi W Three Points (MVZ 198353-198354). Merced Co. – Romero Creek, Santa Nella (MVZ 195982-195985). Modoc Co. – 8 mi NE Cedarville (MVZ 197159*-197164*). Monterey Co. – 5.2 mi NE King City (MVZ 195214-195222), Arroyo Seco, 7 mi SW Greenfield (MVZ 195326-195327). Orange Co. – Dana Point (MVZ 195375*-195377*, 195378). Riverside Co. – 1 mi S and 1 me E Cabazon (MVZ 196132*-196137*), 1.5 mi S Banning (MVZ 196119*-196120*, 196122*-196123*), Aguanga (MVZ 198349-198352), Chino Canyon (MVZ 196141, 196142*-196142*), Deep Canyon (no voucher, ear biopsy only), Lamb Canyon, 2.5 mi S (by rd) Beaumont (MVZ 196101*, 196102, 196103*, 196104. 196105*), mouth Blaisdell Canyon (MVZ 196138*, 196139, 196140*), mouth Whitewater Canyon (MVZ 196144*), Piñon Crest, Santa Rosa Mts. (MVZ 196145*-196148*, 196149-196150, 196151*). San Benito Co. – 9.1 mi NE King City [Monterey Co.] (MVZ 195223-195234), Griswold Canyon (MVZ 196061-196065, 196072-196073). San Bernardino Co. – 1 mi W Amboy (MVZ 195320), east end Morongo Valley (MVZ 195321-195322, 198355-198364), mid Morongo Valley, south side (MVZ 195365-195370), west end Morongo Valley (MVZ 197174*-197178*, 198333-198334), Greenspot Pumping Station, ca 4.5 mi NE Redlands (MVZ 196052-196053), Halloran Spring (MVZ 195308-195309), Pisgah Lava Flow (MVZ 195316-195317). San Diego Co. – 198335-198348), Point Lomo (MVZ 197379, 197380*-197382*), south end San Felipe Valley (MVZ 195241-195242). San Luis Obispo Co. – 0.4 mi S Wells Ranch, Caliente Range (MVZ 196754), 13.3 mi NE (by rd) New Cuyama (MVZ 196759-196761), Beam Flat, Elkhorn Hills (MVZ 195966), Crocker Grade, Temblor Range (MVZ 195975-195976*), Elkhorn Hills (MVZ 196961-195962*), Elkhorn Plain Ecological Reserve (MVZ 195967*). Tulare Co. – 13.2 mi SSE Porterville (MVZ 196074-196076).

Nevada: Clark Co. – 5 mi E Searchlight (MVZ 195245-195246). Elko Co. – 3 mi S Jackpot (MVZ 197126*-197129*). Humboldt Co. – Virgin Valley, Sheldon National Wildlife Refuge (MVZ 197167*-197168*). Lincoln Co. – Delamar Mountains, 10 mi W Caliente (MVZ 197130*, 197131-197140). Nye Co. – (1.5 mi W Beatty (MVZ 195290-195291), Pancake Range (MVZ 197124). Pershing Co. – 8 mi NE Gerlach [Washoe Co.] (MVZ 197165*-197166*).

Utah: Beaver Co. – Indian Peak (DSR 5987*). Emery Co. – Huntington Canyon, 13.2 km NW (by rd) Huntington (DSR 5154*-5155*). Garfield Co. – Henry Mountains (DSR 5598*). Kane Co. – 59 km E & 25 km N Kanab (DSR 5662*). Utah Co. – 5.1 km S and 12.8 km W Lehi (DSR 3290*-3291*). Washington Co. – Fort Pierce Wash, Fort Pierce (DSR 5056*-5057*).

México: Baja California – Ensenada (CIB 3260*), 23 km W and 2 km E Punta Prieta (CIB 3396*), Paso a Punta Prieta, Isla Cedros (CIB 764), 1 km N and 16 km E El

Rosario (CIB 2785*), 1 km W San Felipe (CIB 3377), 14 km N and 16 km E Abreojoa (CIB [AGR 645-646]), 26 km WSW (by rd) Bahia de Los Angeles (MVZ 159790, 159792), 3 km N and 3 km W Bahia de Los Angeles (CIB [MDLPC 411]), 5 km N and 6 km E El Rosario (CIB 2781*, [AGR 794-795]), 5 mi W and 1.25 mi S San Telmo de Abajo (MVZ 148244-148245), 7 km S and 3 km W Colnet (CIB [MDLPC 462-463]), 9 km S and 7 km E San Vicente (CIB [AGR 870-871]), Melling Ranch (CIB 3706). Baja California Sur – 10 km N and 14 km W Santa Rosalia (CIB 2797*, 2798, 45*), 2 km N Puerto Alcatraz, Isla Margarita (CIB 5158*), 4 km NE Puerto Cortez, Isla Margarita (CIB [MFG 6, 33), 3 km N Puerto Magdalena, Isla Magdalena (CIB 5152), 5 km N Puerto Magdalena, Isla Magdalena (CIB [AGR 381-382]), 6 km SE Migriño (CIB ERM 566-567), El Triunfo (CIB [MDLPC 96]), Isla del Carmen (CIB 828), Isla Espiritu Santo (CIB 863), Isla San Francisco (CIB 773), Isla San José (CIB 831), Isla San Marcos (CIB 819), La Purisima (CIB [AGR 554]), Mina "Mulineña", El Triunfo (CIB 5341), Santa Rita (CIB [AGR 1047-1048). Sonora – Tanque Papagos, El Pinacate (CIB 4561).

Outgroups: *Neotoma albigula albigula* (Arizona: Coconino Co.; ca. 1 mi NE Tanner Tank [MVZ 197065]); *Neotoma cinerea alticola* (Nevada: Elko Co.; 1 mi WSW Contact [MVZ 197092]); *Neotoma macrotis simplex* (California: Kern Co.; 2.8 mi NE Lake Isabella [MVZ 195911]); *Neotoma stephensi stephensi* (Arizona: Coconino Co.; Woodhouse Mesa, south edge Wupatki National Monument [MVZ 197170]). In addition to these specimens, sequences of five other species were obtained from GenBank: *N. alleni* (AF 186802), *N. floridana* (AF 186822), *N. goldmani* (AF 186830), *N. mexicana* (AF 186821), and *N. micropus* (AF 186827).