2013

ON THE EDGE: A GENETIC ASSESSMENT OF *APLODONTIA RUFA* FROM THE EDGE OF THEIR DISTRIBUTION

Antoinette J. Piaggio
USDA/APHIS/WS National Wildlife Research Center, Toni.J.Piaggio@aphis.usda.gov

Jennifer Jeffers
Nevada Department of Wildlife

Western North American Naturalist 73(4), © 2013, pp. 485–496

ON THE EDGE: A GENETIC ASSESSMENT OF *APLODONTIA RUFA* FROM THE EDGE OF THEIR DISTRIBUTION

Antoinette J. Piaggio[1] and Jennifer Jeffers[2]

ABSTRACT.—*Aplodontia rufa* (mountain beaver) is considered the sole remaining extant genus and species of an ancient lineage that once broadly inhabited the Great Basin and is now restricted to the Pacific Northwest and portions of California and Nevada. *Aplodontia rufa californica* in Nevada is distributed patchily at the edge of mountain beaver distribution. Due to concern over the status of these populations this subspecies is listed in Nevada as sensitive. The Nevada Department of Wildlife is concerned about the status of *Aplodontia rufa californica* populations scattered across areas of central western Nevada and has worked to gain an understanding of the subspecies' current distribution and numbers. Because there is a lack of a thorough genetic analysis of these populations in Nevada, this study aims to assess the evolutionary relationships and connectivity of populations within Nevada and California. Therefore, we sampled each of the 9 known localities of *Aplodontia rufa* in Nevada, as well as 4 sites from the type locality region of the central Sierra Nevada Mountains of California for comparison, using mitochondrial DNA for phylogenetic and network analyses. Additionally, we used microsatellite markers to assess connectivity of populations within Nevada and to proximate California populations. We found that Nevada populations share mitochondrial DNA haplotypes with California populations and therefore belong to the same subspecies. Furthermore, we found evidence of gene flow between Nevada and California populations. Within Nevada, we detected population differentiation that suggested fragmented populations with restricted connectivity. The results of this study will allow Nevada wildlife managers to develop targeted management strategies to enhance connectivity between populations where it is lacking, to protect connectivity that exists, and also to conserve habitat required by this species. This study increases our understanding of this unique and ancient rodent species at the edge of its distribution.

RESUMEN.—*Aplodontia rufa* (castor de montaña) es considerado el único género y especie aún existente de un linaje antiguo que habitó en la Gran Cuenca, en la actualidad, se limita al noroeste del Pacífico y a algunas partes de California y Nevada. *Aplodontia rufa californica* en Nevada tiene una distribución dispersa y está en disminución. Como el estado de estas poblaciones es preocupante, esta subespecie se encuentra entre las especies susceptibles de Nevada. Este estudio se originó debido a la falta de un análisis genético exhaustivo de estas poblaciones en Nevada y la necesidad de comprender mejor cómo viven para desarrollar estrategias de manejo efectivas. El Departamento de Vida Silvestre (*Department of Wildlife)* de Nevada tiene interés en la conservación de las poblaciones de *Aplodontia rufa californica* que se encuentran en las áreas centro oeste de Nevada y ha intentado obtener información sobre su actual distribución y cifras concretas. En este estudio, analizamos la conexión y las relaciones evolutivas de las poblaciones que se encuentran dentro de Nevada y California. Por lo tanto, tomamos muestras de cada una de las 9 áreas conocidas donde se encuentra *Aplodontia rufa* en Nevada y de 4 lugares de la región tipo de la parte central de las Montañas Sierra Nevada de California para compararlas utilizando ADN mitocondrial con el fin de llevar a cabo análisis filogenéticos y de relación entre las especies. Además, utilizamos microsatélites como marcadores para evaluar la conexión de las poblaciones dentro de Nevada y poblaciones cercanas de California. Encontramos que las poblaciones de Nevada comparten haplotipos de ADN mitocondrial con las poblaciones de California; por lo tanto, ambas pertenecen a la misma subespecie. Además, encontramos indicios de flujo génico entre ambas poblaciones. En Nevada detectamos que las poblaciones se diferenciaban, lo cual sugirió la existencia de poblaciones fragmentadas con conexión limitada. Los resultados de este estudio permitirán a los directores de los espacios de conservación de vida silvestre de Nevada desarrollar estrategias de control bien enfocadas para mejorar la conexión entre las poblaciones donde la especie no está presente, proteger la conexión ya existente y conservar el hábitat que estas especies necesitan. Este estudio nos permite un mejor entendimiento de esta especie de roedores única y antigua en el borde de su distribución.

The mountain beaver, *Aplodontia rufa*, is a unique species and the sole remaining genus (*Aplodontia*) within a formerly diverse, ancient lineage (family: Aplodontiidae). The genus *Aplodontia* is sister to all squirrels that form the superfamily Sciuridea (Huchon et al. 1999, Montgelard et al. 2002, Herron et al. 2004, Steppan et al. 2004) and is considered to be the most evolutionarily conservative in the class Mammalia (Steele 1989).

[1]USDA Wildlife Services, National Wildlife Research Center, Wildlife Genetics Lab, 4101 LaPorte Avenue, Fort Collins, CO 80521. E-mail: toni.j.piaggio@aphis.usda.gov
[2]Nevada Department of Wildlife, Wildlife Diversity Division, 380 West B Street, Fallon, NV 89406.

Aplodontia rufa occupies a limited geographic range in the North American Pacific Northwest region that includes isolated populations in portions of California and Nevada, broad distribution in Oregon and Washington, and a small distribution in southern British Columbia (Piaggio et al. 2013). Previously, *A. rufa* was considered a single extant species with 7 subspecies (Hall 1981); however, there is evidence that 2 species exist (Piaggio et al. 2013). *Aplodontia rufa nigra,* a subspecies with limited and isolated distribution along the Northern California coast, is a federally listed endangered species (50 FR 64716). The remaining subspecies are regarded either as populations of concern or as pest species, depending on the portion of their range that is considered. The resulting management strategies are diverse and at times conflicting. Some subspecies are protected as federally endangered species, whereas others are subjected to active control efforts (Borrecco and Anderson 1980).

The subspecies *A. r. californica* is the most broadly distributed subspecies in California (Peters 1864). This subspecies is primarily limited to riparian areas in moderate to high-elevation areas in the Sierra Nevada Mountains (Steele 1989). Isolated, disjunct populations of this subspecies are also found in the extreme western portions of Nevada (Beier 1989). Active colonies (area with multiple burrows) of *A. rufa* that occur in Nevada primarily occupy elevations between 1980 and 2700 m. Most colonies are found in aspen, willow, and fir communities that have a dense understory (Beier 1989). Prior to 2001, historical records from Nevada included only 3 records: 1932 at Marlette Lake, 1934 at Incline Village, and 1946 at Snow Valley (Nevada Natural Heritage). A colony located at the Incline Village locality now appears to be abandoned (based on surveys by J. Jeffers during this study), presumably due to extensive urban development. The other 2 historical localities have existing active mountain beaver colonies. A survey conducted by Nevada Department of Wildlife (NDOW) during 2002–2003 indicated a population estimate of <100 animals found in 9 distinct localities (Nevada Department of Wildlife 2003 Job Progress Report). Unsuitable habitat, water bodies, and/or mountain ranges separated localities with active colonies. Based on this survey, NDOW upgraded their state classification of this animal from a protected species to "sensitive" status in 2004. It is critical to the management of sensitive populations that taxonomy is clearly understood. Therefore, a goal of this study was to confirm or identify the subspecific status of Nevada populations by comparing known mitochondrial DNA haplotypes of *A. r. californica* from California to haplotypes collected from the 9 populations in Nevada.

Previous work determined that *A. r. californica* had a broad distribution in California but that populations (areas with multiple colonies) were uncommon, scattered, and disjunct (Steele 1989). Further, Steele (1989) found that many areas of suitable habitat were unoccupied. He therefore concluded that *A. r. californica* populations had declined in California and that the declines were in some cases attributed to genetic isolation. Steele identified a need for further investigation of genetic diversity in the small, isolated populations of *A. r. californica* and concluded that an understanding of population genetics would aid in the management of this subspecies. It was therefore recognized in Nevada, where this subspecies is listed as sensitive, that an understanding of the genetic relationships among the isolated populations in Nevada was critical to developing an effective management plan. Therefore, the second goal of the current study was to apply a population genetics approach by using microsatellite DNA and estimating indirect genetic measures of population structure and diversity. Information from this study will furnish a better understanding about population genetics and subspecific taxonomy of this species in Nevada and provide data that are critical for management decisions and conservation efforts.

METHODS

Sampling and DNA Extraction

Hairs or tissue samples were collected from individuals that were live-trapped in Nevada ($n = 25$) and California ($n = 11$). Individuals from 9 sampling localities in Nevada (Logan Creek, Hobart, Tunnel Creek, Galena Creek, Price Lake, Marlette Lake North, Marlette Lake South/Snow Valley, Mount Rose North, and Mount Rose South) were collected in 2001, 2002, 2004, and 2006 and from 4 localities in California between July and September

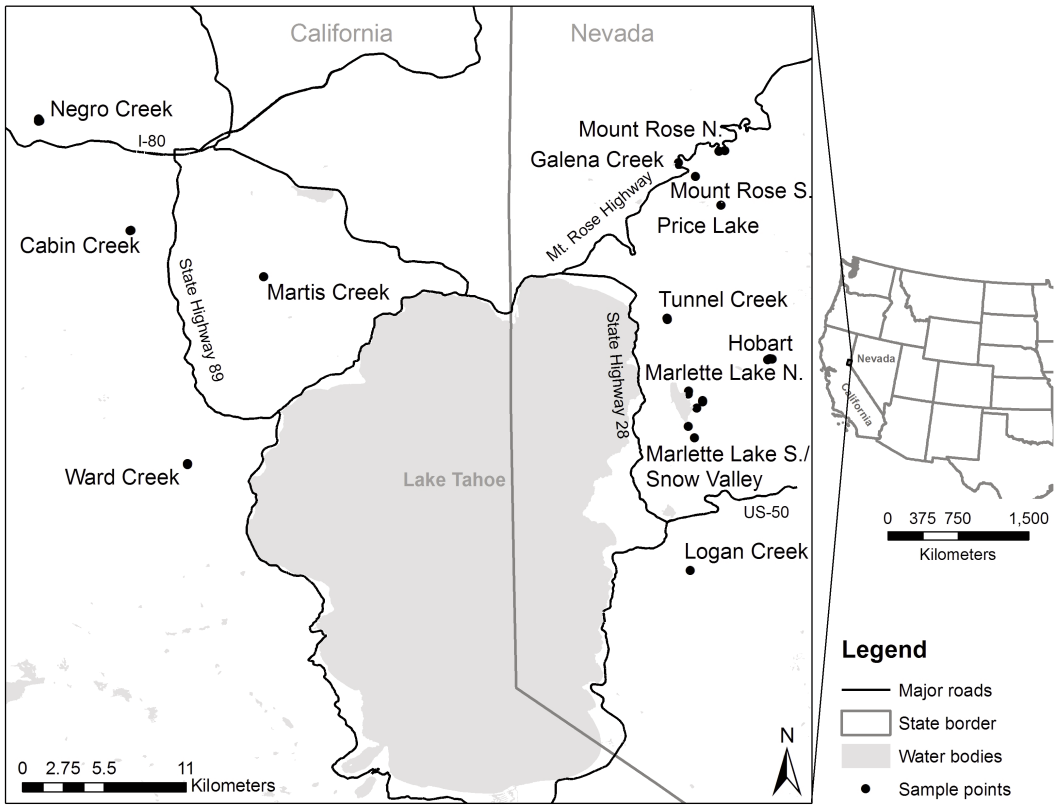

Fig. 1. Map of sampling localities (with place name) in California and Nevada. Each dot represents a captured individual.

2006 (Fig. 1). Individuals were live-trapped following methods described in Piaggio et al. (2013). Trapping was conducted only where water was present because *Aplodontia* have inefficient renal function and therefore require constant access to water (Pfeiffer et al. 1960). At each sampling locality, we sought to obtain as many individuals as possible. Hair samples were collected by plucking hairs in small bunches (5–6 hairs per bunch) from the neck and back area of the animal to obtain hair follicles. Hair samples were then placed in coin envelopes with silica beads and labeled. Tissue samples were collected as in Piaggio et al. (2013). All animals were handled following American Society of Mammalogy guidelines (Sikes et al. 2011) and under protocols set forth by the NDOW Scientific Collection permit process. Fourteen tissue samples from *A. r. olympica* from a population in Washington were also analyzed as outgroup taxa (Piaggio et al. 2013). Genomic DNA from hair was extracted as in Piaggio et al. (2013).

DNA Amplification, Sequencing, and Genotyping

The primers MVZ05 and MVZ04 (Smith and Patton 1993) were used to amplify a portion (460 base pairs [bp]) of the mtDNA cytochrome *b* (cyt *b*) gene following protocols detailed in Piaggio et al. (2013). To amplify DNA from hair samples, we modified the PCR by using a 25-µL reaction with PuReTaq Ready-To-Go PCR beads (GE Healthcare Bio-Sciences, Piscataway, NJ). The remaining amplification and sequencing process followed Piaggio et al. (2013).

Mountain beavers from Nevada and California were genotyped using 10 autosomal microsatellite loci (Piaggio et al. 2009; ArE04F_H05R, ArE12F_C06R, ArB07F_E04R, ArG05F_H06R, ArA08F_A08R, ArH04F_H04R, ArC09F_C09R, ArG08F_G08R, ArD10F_F05R, ArC10F_A05R). Microsatellite PCRs with DNA from tissue samples were performed following Piaggio et al. (2009) but required modifications for DNA extracted

from hair samples. These modifications included increased primer, increased *Taq* polymerase, and altered DNA quantities (2 μL of 1:10 dilution of extracted DNA) for panels A and C. For panel B, PuReTaq Ready-To-Go PCR beads (GE Healthcare Bio-Sciences) and an increase in primer and DNA quantities were required for amplification. Microsatellites were visualized on an ABI 3130 genetic analyzer and binned using Genemapper software (Applied Biosystems). Bins were independently confirmed manually by 2 separate technicians. The software packages GMCONVERT (Faircloth 2006) and CONVERT (Glaubitz 2004) were used to transform genotyping data for formats used in downstream analyses.

Mitochondrial DNA Statistical Analyses

The cyt *b* sequences were aligned in Sequencher (version 4.8 Gene Codes Corp., Ann Arbor, MI). GenBank accession numbers are JX419569–JX419570; JX419612–JX419613; JX 419622; JX419646–JX419647; JX419657–JX 4195670; JX419750–JX419763; JX419812; outgroup, JX419571–JX419583. Maximum likelihood (ML) phylogenetic analyses were performed using the web-based version of RAxML (Stamatakis 2006, Stamatakis et al. 2008) available through the CIPRES supercomputer (http://www.phylo.org). The general time reversible substitution model with proportion of invariable sites and gamma distributed rate variation estimation was employed through RAxML based on previous cyt *b* model analysis (Piaggio et al. 2013). Bootstrap analysis of nodal support was evaluated with the number of pseudoreplicates automatically generated by the program. Trees were visualized in FigTree ver. 1.1.2 (http://tree.bio.ed.ac.uk/software/figtree) and further edited for publication using Adobe Illustrator CS6. Phylogenetic trees do not resolve relationships well among recently derived haplotypes because phylogenetic assumptions such as extant ancestors are violated (Posada and Crandall 2001). Therefore, to further assess relationships among DNA sequences with low divergences, a median joining network (Bandelt et al. 1999) was generated in Network 4.6.1.0 (Fluxus Technology).

Genetic diversity within populations was described from cyt *b* sequences as the number of individuals sequenced (*n*), number of haplotypes (H), haplotype diversity (*h*), nucleotide diversity (π) (Nei 1987), and average pairwise differences within populations. These values were generated by use of Arlequin 3.1 (Excoffier et al. 2005) and PAUP* 4.0b10 (Swofford 2003).

Autosomal Microsatellite Statistical Analyses

Genotypes from hairs were tested for errors (allelic dropout or false alleles) through multiple runs of a subset (15%) of samples. We used Arlequin 3.1 (Excoffier et al. 2005) to test microsatellite loci for a significant departure from Hardy–Weinberg equilibrium (HWE) expressed as differences in expected heterozygosity (H_e) and observed heterozygosity (H_o) at each locus for *A. r. californica* in California and Nevada and *A. r. olympica* in Washington. Bonferroni correction was used to compute critical significance levels for multiple tests of HWE (Rice 1989). Genetic variability estimated from microsatellites is described as mean number of alleles (N_A), allelic richness (a), and number of private alleles (pa). These diversity measures were computed for each locus for *A. r. californica* in California and Nevada and *A. r. olympica* in Washington, across loci for each locality in Nevada, and for California and Nevada each as a group. Diversity measures across loci were not computed for California localities because we sampled only cursorily in these areas. Each locus was examined for null allele frequencies by using MICRO-CHECKER (Van Oosterhout et al. 2004) with a 95% confidence interval (Brookfield 1996). Significant differences between populations in average within-population diversity measures, N_A, a, H_e, and H_o were tested using Mann–Whitney *U* tests (Sokal and Rohlf 1995) with a significance level of 0.05. Inbreeding coefficients (F_{IS}) were calculated for each population with 1200 randomizations and significance at 0.05. Tests for significant pairwise linkage disequilibrium between loci were performed by hand or with FSTAT 2.9.3 (Goudet 2001).

Population differentiation was estimated from microsatellites by pairwise F_{ST} comparisons between populations (Weir and Cockerham 1984). Population differentiation significance was ascertained by producing an expected distribution based on randomizations generated with Monte Carlo simulations in Arlequin 3.1 (Excoffier at al. 2005). Bonferroni corrections were made to adjust for multiple

comparisons of these data (Rice 1989). Population differentiation was also appraised using an analysis of molecular variance (AMOVA) with 9000 permutations to examine nested levels of variation within individuals, among individuals within populations, and among populations (Excoffier et al. 1992). Populations were examined for an effect of isolation-by-distance (IBD) in Arlequin 3.1 by testing for correlation between linearized F_{ST} estimates and straight-line pairwise geographic distances in kilometers (Slatkin 1993, 1995) through Mantel tests with 10,000 iterations. Distances between Nevada populations and California were calculated with a mean center method for California, because samples in California were taken from a few easily located burrows and not with the goal of systematically sampling each population. Therefore, we grouped California as a single population to represent the genetic diversity of the type locality region of *A. r. californica.*

Assignment tests were performed using BayesAss ver. 1.3 (Wilson and Rannala 2003). This program uses a Bayesian approach to determine the likelihood that an individual came either from the population where it was collected or from a different population. The assignment test identifies individuals that are recent migrants and those that are offspring of recent migrants. This program was used to detect occurrence and directionality of migration among populations in Nevada and between Nevada and California. These assessments were run with 6 populations: all 5 Nevada populations and California as a single population. The number of iterations was 3,000,000, of which 999,999 were burnin, and the sampling frequency was 2000. Allele frequency, migration rate, and inbreeding rates were set to defaults.

BAPS ver 5.2 (Corander et al. 2008) is software that performs Bayesian analysis of population structure. The analysis estimated the number of genetic clusters (K) distributed among samples without *a priori* population information. Geographical locality data was incorporated in the analysis, which then identified the estimated number of genetic clusters as a graphic (Voroni tessellation) and showed their distribution across the landscape. The program was run as an assessment of population mixture with a spatial clustering of individuals and 5 replications of each estimation (K = 1–10).

Average *h,* or gene diversity, over all microsatellite loci (with confidence intervals) was calculated in Arlequin 3.1 and was used to calculate effective population size (N_e) of California and each population in Nevada. The equation $N_e = h/4m(1 - h)$ (Nei 1987) was used with a mutation rate of 10^{-3} (Weber and Wong 1993) and under the assumption of equilibrium.

To determine whether populations in Nevada or California have experienced recent or historical expansion, contraction, or stability, 2 tests were employed. The imbalance index β (Kimmel et al. 1998) is based on allele frequencies and variance in repeat numbers (β = 1, stable populations; β > 1, recent expansion or recovery from a previously reduced population; β < 1, recent expansion from stable population). The imbalance index was calculated along with 95% confidence intervals by using the SAS package and a program written and shared by T. Lehmann (Donnelly et al. 2001). To further check for evidence of a population contraction (i.e., population bottleneck), the program BOTTLENECK was implemented (Cornuet and Luikart 1996). This program tests for signs of a recent reduction in N_e based on allele frequencies calculated with number of alleles and adjusted for sample size. A significant reduction in N_e is identified by allelic modeshifts and heterozygosity alterations using a one-tailed Wilcoxon's signed-ranks test. An infinite alleles model was applied across 10,000 iterations.

Results

Sampling

There were 9 distinct localities identified and sampled (NDOW, 2003 Job Progress Report). Trapping efforts were intensive and averaged between 3 and 4 weeks per locality. The average number of individuals captured was 2 per active colony. We sampled a single individual from Galena Creek, but only mtDNA sequencing was completed while genotyping with microsatellites was not successful. Populations were defined based on geographical proximity (Figs. 1, 4). In some cases, sampling localities were grouped as a single population for further analyses: (1) Galena Creek (mtDNA analyses only), Price Lake, Mount Rose South, and Mount Rose North were combined into Mount Rose; and (2) Marlette Lake North and Marlette Lake South were

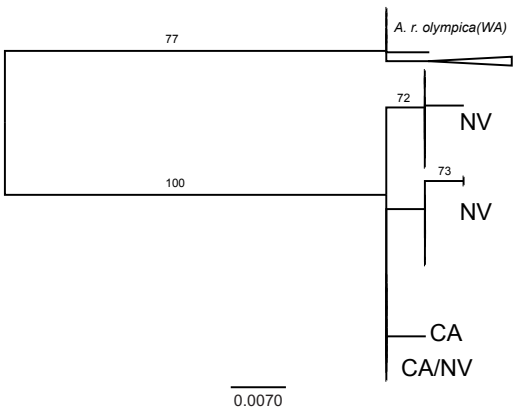

Fig. 2. Maximum likelihood phylogram with bootstrap support >65 at nodes. Outgroup samples are another subspecies, *Aplodontia rufa olympica*, from Washington. There are 6 haplotypes among all our samples from California and Nevada, with one shared between both, one unique to California, and the rest found only in Nevada.

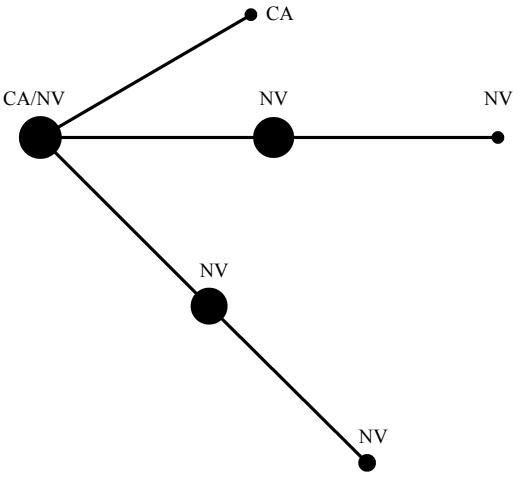

Fig. 3. Median joining network of mtDNA haplotypes. Circle size is proportional to haplotype frequency. Six haplotypes were identified in this study: one shared between California and Nevada, one found only in California, and 4 found only in Nevada.

combined into Marlette Lake. Genotypes were compared for combined populations, and in all cases >65% alleles were shared; thus, we believe these population combinations did not violate true population structure.

Mitochondrial DNA Sequences

Each of the 36 *A. rufa* individuals from Nevada and California and the 14 individuals from Washington were successfully sequenced for a portion of the mtDNA cyt *b* gene. Alignments without the outgroup resulted in 452 characters: 447 of these were constant and 5 were variable, with 2 characters being parsimony uninformative and the remaining 3 parsimony informative. The maximum likelihood tree—with the outgroup and a GTR+I+G model with bootstrap support >65%—is presented in Fig. 2. There is 4%–5% pairwise sequence divergence between *A. r. olympica* from Washington and known *A. r. californica* from California. Pairwise sequence divergences found between all samples from California and Nevada range from 0.20% to 0.60%, which includes populations separated by large geographic distances. For comparison, pairwise sequence divergence within the outgroup taxa, which were samples from a single sampling locality of *A. r. olympica*, ranged from 0.20% to 0.70%. There were 6 mtDNA haplotypes found between California and Nevada. There were 2 haplotypes identified among the California samples. One haplotype was from a single individual from Martis Creek, California, and the other was shared among all other California individuals and 2 individuals from Tunnel Creek, Nevada. In the median joining network (Fig. 3), the haplotype shared between California and Nevada was the node for all other haplotypes. The remaining 4 haplotypes were found only in Nevada. One was shared between 2 individuals, with one individual from Marlette Lake and the other the single individual sampled at Logan Creek (Fig. 1). Another haplotype was from a single individual from Marlette Lake, and the remaining haplotypes were shared among the populations (except Logan Creek). Diversity measures within populations and within each state are presented in Table 1. Overall cyt *b* sequence diversity within each Nevada population was low to moderate (0.00–0.67).

Autosomal Microsatellites

Thirty-four *A. r. californica* were successfully genotyped at all 10 loci. No genotyping error was detected across multiple runs of the hair samples tested. A HWE violation was detected in Nevada at one locus (ArE12F_C06R). The Nevada samples showed significant evidence of null alleles at the same locus (Table 2). Significant evidence of null alleles was also found in California at a single locus

TABLE 1. Mitochondrial DNA cytochrome *b* and microsatellite DNA estimates of genetic diversity measures per sampling locality in Nevada per population. California was grouped as a single population for comparison of genotypic diversity to Nevada populations. *n* = number of individuals that were sequenced/genotyped, H = number of haplotypes, *h* = haplotype diversity, π = nucleotide diversity, Pw diff = average pairwise differences between sequences, N_A = number of alleles across loci, a = mean allelic richness across loci, pa = private alleles across loci. Standard deviations are in parentheses for π and Pw diff.

		Mitochondrial DNA			Microsatellite DNA			
Population	*n*	H	*h*	π	Pw diff	N_A total	a	pa
Negro Creek, CA	4/4	1	0.00	0.000	0.00	—	—	—
Cabin Creek, CA	2/2	2	1.00	0.002 (0.003)	1.00 (1.00)	—	—	—
Martis Creek, CA	2/2	1	0.00	0.000	0.00	—	—	—
Ward Creek, CA	3/3	1	0.00	0.000	0.00	—	—	—
Mount Rose, NV	7/6	2	0.29	0.003 (0.002)	1.14 (0.84)	26	1.38	1
Tunnel Creek, NV	3/2	2	0.67	0.002 (0.002)	0.67 (0.67)	16	1.32	0
Hobart, NV	7/7	2	0.29	0.002 (0.002)	0.95 (0.73)	21	1.31	2
Marlette Lake, NV	7/7	4	0.57	0.004 (0.003)	1.71 (1.13)	25	1.40	1
Logan Creek, NV	1/1	1	n/a	n/a	n/a	16	1.60	0
TOTAL								
NEVADA	25/23	6	0.24	0.003 (0.002)	1.19 (0.79)	36	2.89	11
CALIFORNIA	11/11	1	0.00	0.000	0.00	35	3.41	10

(ArD10F_F05R), but there was no violation of HWE. Further, when Nevada populations were examined separately, no population violated HWE or showed evidence of null alleles at locus ArE12F_C06R after Bonferroni correction. Neither locus (ArE12F_C06R or ArD10F_F05R) was dropped from further analyses.

Genetic diversity estimates were compared within Nevada populations, between Nevada and California (Table 1), and per locus (Table 2). There were 11 private alleles found in Nevada and 10 in California (Table 1). Among populations of *A. r. californica* in Nevada, private alleles were identified within 3 of the 5 populations (Table 1). Allelic richness within Nevada populations ranged from 1.31 to 1.60. Allelic richness across loci ranged from 1.00 to 5.13 in Nevada and from 1.00 to 6.74 in California (Table 2). Private alleles were found in 50% of the loci in California and Nevada (Table 2). Across most loci (60%), a greater number of alleles were found in the samples from a single population of *A. r. olympica* from Washington than in Nevada or California populations (Table 2). Number of alleles per locus from both Nevada and California populations ranged from 1 to 7 (Table 2). Mann–Whitney *U* tests did not show a significantly greater number of alleles in Washington than in California ($P = 0.05$) or Nevada ($P = 0.11$). Observed heterozygosity in Nevada ranged from 0.22 to 0.83 and within California from 0.09 to 0.91 (Table 2). There were no significant differences in allelic diversity (N_A, a, H_e, and H_o) between Nevada or California samples nor between any populations in Nevada (Mann–Whitney *U* tests: $P > 0.05$). There was no evidence of significantly high F_{IS} in any population. There was no evidence of linkage disequilibrium between any pair of loci in any population.

All pairwise estimates of F_{ST} showed significant differentiation between Nevada and California populations and between 2 populations within Nevada (Mount Rose and Hobart) after Bonferroni correction. The AMOVA analysis showed that the greatest degree of variation among samples from California and Nevada is explained by variation among individuals (78.13%; $P < 0.01$). Some variation was also accounted for among populations (14.85%; $P < 0.01$). There was not a significant correlation between population differentiation and geographic distances ($R < 0.01$, correlation coefficient = 0.30, Mantel test $P > 0.05$; Table 3). Therefore, there is no evidence of IBD.

BayesAss ver. 1.3 (Wilson and Rannala 2003) assessments detected migrants between Nevada populations from Hobart into Mount Rose (migrants and offspring of migrants) and into Marlette Lake (migrants). Migrations were also detected as offspring of migrants from a Nevada population (Mount Rose) into a California population (Martis Creek). The proportions of nonmigrant individuals in Nevada populations were 0.75 for Mount Rose (95% CI: 0.67, 0.96), 0.76 for Marlette Lake (0.67,

TABLE 2. Estimates of various genetic diversity measures per microsatellite locus compared between *A. r. olympica* (WA) and the 2 regions of *A. r. californica* (NV and CA). N_A = number of alleles sampled per locus, a = allelic richness per locus, pa = private alleles per locus, H_o = observed heterozygosity, H_e = expected heterozygosity, WA = Washington population (adapted from Piaggio et al. 2013), NV = Nevada, CA =California. Null allele frequencies are based on Brookfield2 estimates from MICROCHECKER software. An asterisk (*) indicates significant evidence of null alleles with 95% confidence intervals. Null allele estimates for 2 loci in the Washington population were moderate (WA: ArE12F_C06R = 0.1375; ArG08F_G08R = 0.1969; NV: ArE12F_C06R = 0.2127; CA: ArD10F_F05R = 0.2727). Significant violation of HWE is denoted by a dagger (†).

Multiplex	Locus	Size range (bp)	N_A total	a	pa	H_o	H_e
Panel A	ArE04F_H05R	WA: 267–279	WA: 7	—	—	WA: 0.77	WA: 0.79
		NV: 263–271	NV: 5	3.99	0	NV: 0.70	NV: 0.67
		CA: 263–271	CA: 5	4.95	0	CA: 0.55	CA: 0.71
	ArE12F_C06R	WA: 253–259	WA: 4	—	—	WA: 0.47	WA: 0.71*
		NV: 241–251	NV: 5	3.74	2	NV: 0.22	NV: 0.56†*
		CA: 241–255	CA: 5	4.95	2	CA: 0.55	CA: 0.71
	ArB07F_E04R	WA: 372–380	WA: 5	—	—	WA: 0.67	WA: 0.66
		NV: 374	NV: 1	1.00	0	NV: n/a	NV: n/a
		CA: 374–376	CA: 2	1.81	1	CA: 0.09	CA: 0.09
	ArG05F_H06R	WA: 155–185	WA: 9	—	—	WA: 0.83	WA: 0.88
		NV: 165–183	NV: 6	5.13	3	NV: 0.83	NV: 0.77
		CA: 161–187	CA: 7	6.74	4	CA: 0.91	CA: 0.83
Panel B	ArA08F_A08R	WA: 252–258	WA: 4	—	—	WA: 0.57	WA: 0.59
		NV: 252–256	NV: 2	1.39	1	NV: 0.43	NV: 0.43
		CA: 252–260	CA: 3	2.97	2	CA: 0.36	CA: 0.60
	ArH04F_H04R	WA: 281–285	WA: 3	—	—	WA: 0.18	WA: 0.17
		NV: 289–295	NV: 3	2.39	0	NV: 0.43	NV: 0.52
		CA: 289–295	CA: 3	2.82	0	CA: 0.27	CA: 0.39
	ArC09F_C09R	WA: 339–355	WA: 6	—	—	WA: 0.71	WA: 0.71
		NV: 337	NV: 1	1.00	0	NV: n/a	NV: n/a
		CA: 337	CA: 1	1.00	0	CA: n/a	CA: n/a
Panel C	ArG08F_G08R	WA: 232–236	WA: 4	—	—	WA: 0.38	WA: 0.56*
		NV: 234–248	NV: 4	3.14	2	NV: 0.35	NV: 0.31
		CA: 234–244	CA: 3	2.82	1	CA: 0.64	CA: 0.54
	ArD10F_F05R	WA: 310–316	WA: 4	—	—	WA: 0.59	WA: 0.60
		NV: 312–316	NV: 2	2.00	0	NV: 0.48	NV: 0.48
		CA: 312–316	CA: 2	2.00	0	CA: 0.09	CA: 0.52*
	ArC10F_A05R	WA: 150–166	WA: 7	—	—	WA: 0.83	WA: 0.76
		NV: 154–172	NV: 7	5.13	3	NV: 0.70	NV: 0.71
		CA: 154–166	CA: 4	4.00	0	CA: 0.56	CA: 0.70

TABLE 3. Distance in kilometers between each *A. r. californica* population in Nevada and between the Nevada populations and California.

	Mount Rose, NV	Marlette Lake, NV	Hobart, NV	Tunnel Creek, NV	Logan Creek, NV	CA
Mount Rose, NV	00.00					
Marlette Lake, NV	15.92	00.00				
Hobart, NV	12.94	05.14	00.00			
Tunnel Creek, NV	10.13	06.20	6.00	00.00		
Logan Creek, NV	26.79	10.88	14.79	16.95	00.00	
CA	30.58	30.74	33.67	27.94	35.55	00.00

0.99), 0.91 for Hobart (0.67, 0.99), and 0.85 for California (0.68, 0.98).

BAPS version 5.2 (Corander et al. 2008) found K = 6 as the most probable (0.69) explanation of genetic clustering across all samples from California and Nevada. There were 4 clusters found in California (Fig. 4), each corresponding to a sampling locality (Negro Creek, Cabin Creek, Martis Creek, and Ward Creek). Only 2 clusters were identified in Nevada (neither being shared with California; Fig. 4): one includes 4 sampling localities (Mount Rose, Tunnel Creek, Marlette Lake, and Logan Creek) and the other is primarily one population (Hobart) and a single individual from the closest population to the east (Marlette Lake).

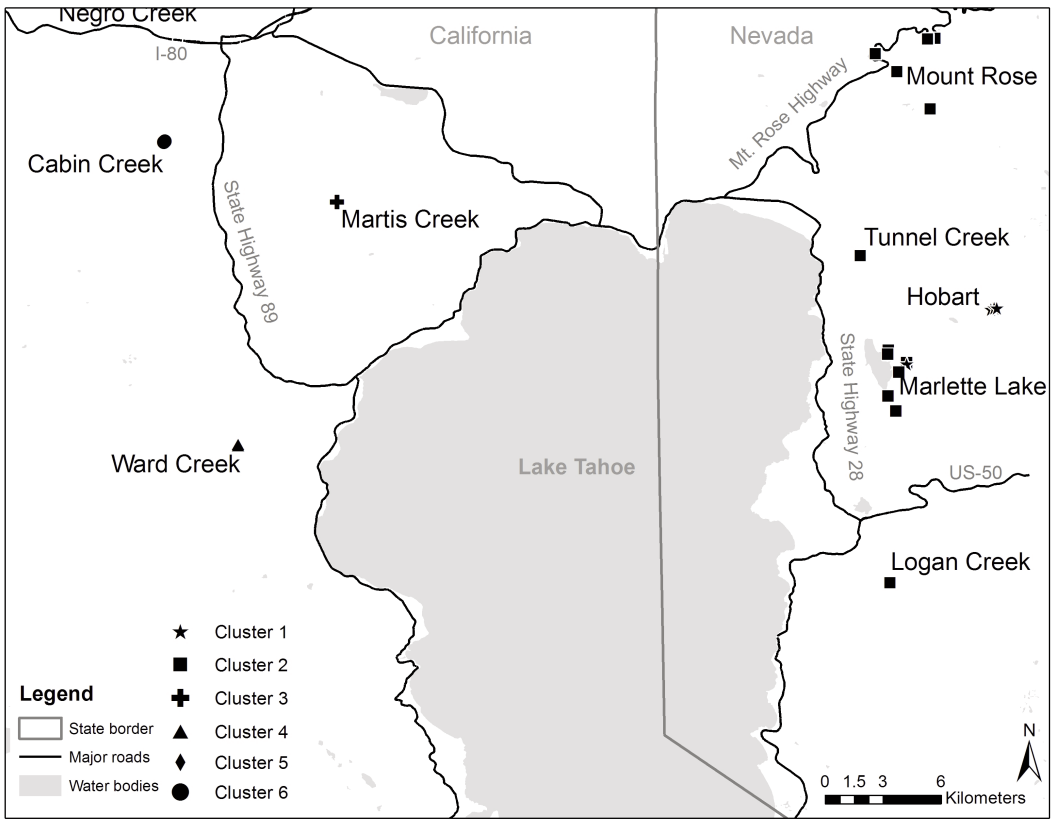

Fig. 4. Map of sampled area showing results of Bayesian cluster analyses. Individuals are represented by shapes, and each shape corresponds to membership in K = 6 genetic clusters.

The estimation of N_e for all California samples was 240.20 (95% CI: 66.46, 836.96). N_e was also estimated for 3 populations in Nevada with adequate sample size: Mount Rose, 153.23 (95% CI: 44.12, 391.03); Marlette Lake, 166.67 (53.33, 444.44); and Hobart, 112.32 (34.09, 250.00).

The imbalance index β (Kimmel et al. 1998) for Nevada overall was 2.28 (95% CI: 1.19, 4.08), suggesting a recent expansion or recovery from a previously reduced population. Beta for California overall was 1.77 (95% CI: 1.09, 2.24), which probably indicates a stable population. When Nevada populations were considered separately, Mount Rose (β = 1.02; 95% CI: 0.86, 1.82) and Tunnel Creek (β = 1.21; 95% CI: 0.24, 1.59) appear to reflect stable populations; whereas Marlette Lake (β = 2.48; 95% CI: 0.78, 2.95) and Hobart (β = 3.71; 95% CI: 2.00, 12.55) seem to be expanding or recovering from reduced populations. There was not sufficient sample size in Logan Creek to calculate β. The program BOTTLENECK (Cornuet and Luikart 1996) detected signals of a population bottleneck with a significant degree of heterozygosity excess in a one-tailed test ($P < 0.05$; $P = 0.04$) and a shifted mode in the Hobart population from Nevada. The Marlette Lake population had a significantly shifted mode. The remaining Nevada populations and California showed no evidence of significant population reduction.

DISCUSSION

Mitochondrial DNA cyt *b* sequence diversity within *A. r. californica* was low across all populations we sampled. In fact, sequence diversity among 14 *A. r. olympica* outgroup samples from a single population was equivalent. Furthermore, in our investigation of all other *A. rufa* subspecies (Piaggio et al. 2013), the low diversity found in *A. r. californica* is

atypical for the same fragment of the cyt *b* region. Therefore, we believe the low diversity within *A. r. californica* is a biological reality and not due to low molecular sampling (i.e., not a sufficient portion of the genome analyzed).

Significant effort was made in capturing and sampling as many *A. rufa* individuals as possible within each of the 9 distinct Nevada localities previously identified by the NDOW (NDOW, 2003 Job Progress Report). However, it is important to note limitations that we experienced in our efforts to trap live mountain beaver. One limiting factor was the availability of suitable trapping sites because surface water was required (see methods). Literature on the number of *Aplodontia* per burrow system for *A. r. californica* is limited but indicates an average of 1 or 2 animals per burrow system except during breeding and birthing seasons (Grinnell and Storer 1924, Pfeiffer 1954, Steele 1989). We trapped outside breeding and birthing seasons when sites could be accessed; therefore, we could not expect more than 2 *A. rufa* per runway. Furthermore, previous surveys document that *A. rufa* is in low abundance across Nevada (NDOW, 2003 Job Progress Report). Therefore, although active burrows can be located, there is low probability that trapping (even intensive trapping) will result in capture of more than a single individual. Thus, even though our sample sizes are low per locality, we feel that we successfully captured a representative genetic sampling from each population.

A single mtDNA haplotype that is shared between California and Nevada appears to be basal, although by only a single step (Figs. 2, 3). These data suggest that DNA haplotypes have been shared between both areas, but more recently there have been unique haplotypes derived in Nevada (unless they are in California but unsampled in this study). Furthermore, the occurrence of a shared haplotype between California and Nevada, as well as the low divergence of the other haplotypes in Nevada, make it clear that these individuals from Nevada belong to the subspecies *A. r. californica.*

Clearly California and Nevada share a recent common ancestor, as evidenced by the mtDNA sequence data (Figs. 2, 3). Furthermore, there is evidence from microsatellite data of recent migrants from a Nevada population into a California population and no IBD, suggesting some degree of gene flow between the two populations. However, differentiation was identified from microsatellite data, indicated by high number of private alleles, genetic cluster analysis (Fig. 4), F_{ST}, and the imbalance index from Nevada; thus, there has recently been increasing isolation resulting in differentiation between these areas.

Although populations in Nevada are separated by large land expanses (Table 3), water bodies, and unsuitable habitat, most belong to a single genetic cluster (except Hobart), and microsatellite genetic variability is primarily due to variation among individuals. Also, there was only one significant pairwise F_{ST} (Mount Rose to Hobart) and no correlation of geographic distance to population differentiation (no IBD). Therefore, it appears that most Nevada populations share allelic diversity, which implies gene flow. However, the only evidence of migrants between Nevada populations was from Hobart, the easternmost population and a separate genetic cluster, into Marlette Lake and Mount Rose. Furthermore, a single individual in Marlette Lake was assigned to the Hobart genetic cluster. Hobart and Marlette Lake are closest in proximity to one another, and therefore, an occasional migrant is not surprising. The migration detected from Hobart to Mount Rose was surprising especially because these populations were significantly differentiated and geographically quite distant (Table 3). Perhaps this is a reflection of occasional gene flow between Hobart and Marlette Lake and the subsequent lack of differentiation between Marlette Lake and Mount Rose.

Effective population size estimates (N_e) suggest that all populations of *A. r. californica* are fairly small (112–240). If the estimated range of N_e for each population in Nevada (112–166) can be considered a proxy for an overall *A. rufa* effective population estimate in Nevada, then N_e correlates well with NDOW's estimate of about 100 animals, generated by using an index of active burrows (Steele 1989). The lowest N_e was found in the Hobart population in Nevada, which had shown a signal of expansion or recovery from a reduced population based on its imbalance index. Interestingly, this population currently appears to contribute migrants to other Nevada populations. Marlette Lake also had an imbalance index suggesting recovery from a reduced

population, and both Marlette Lake and Hobart showed evidence of a population bottleneck in another analysis. Other indications of small populations are the measures of microsatellite genetic diversity. These measures were similar between Nevada and California, and although the samples came from a fairly broad geographical area relative to our samples from a single population in Washington, the diversity measures were essentially the same. Therefore, genetic diversity in Nevada, and possibly California populations (need more sampling to confirm), is relatively low (when compared to a single population in Washington). Together, these results suggest that populations in Nevada may have suffered population reduction and that some may have begun to recover.

Aplodontia rufa is important ecologically because many species use mountain beaver burrows (Maser et al. 1981). These species include shrews (*Sorex* spp.), moles (*Scapanus* spp. and *Neurotrichus gibbsii*), snowshoe hare (*Lepus americanus*), deer mouse (*Peromyscus maniculatus*), voles (*Microtus* spp.), mink (*Mustela vison*), long-tailed weasel (*Mustela frenata*), and spotted skunk (*Spilogale gracilis*). Further, many animals are predators of *A. rufa,* such as black bear (*Ursus americanus*), coyote (*Canis latrans*), foxes (*Vulpes* spp. and *Urocyon cinereoargenteus*), bobcat (*Lynx rufus*), mountain lion (*Puma concolor*), raptors, long-tailed weasel (*Mustela frenata*), and mink (*Mustela vison*). Microsatellite data revealed that population differentiation has occurred in the study area. This is most clearly reflected in the fact that, although the level of microsatellite diversity is the same in Nevada and California, private alleles (alleles unique to an area) have accumulated in each area. Gene flow probably occurs along riparian habitats where there is consistently available water, because *A. r. californica* depends on water for renal function (Pfeiffer et al. 1960). The differentiation might be attributed to the increase in urban/suburban development. Populations of *A. r. californica* in California have been negatively affected by water diversion, development projects, and urbanization (Steele 1989). During this study, we found these anthropogenic activities to be an ongoing issue in the Lake Tahoe region. These activities will not decrease, and therefore, the connection (gene flow) between California and Nevada *A. r. californica* populations will likely continue to decrease. Therefore, when water diversion projects and development are proposed in an area where mountain beavers occur, their habitat needs should be considered. Habitat protection and burrow conservation should be a priority for all populations of *A. r. californica* and particularly for the Hobart population in this study, as it is genetically differentiated from other Nevada populations. Appropriate management of corridors of connection (i.e., riparian areas) and burrow sites for *A. r. californica* populations in Nevada is critical to the evolutionary potential of this sensitive and ecologically important species.

Acknowledgments

We acknowledge the generosity of Tovi Lehmann at NIH NIAID for sharing a SAS program that calculates Kimmel's imbalance index. We also acknowledge Wendy Arjo for her contribution of samples from Washington and her guidance throughout, especially her advice on collecting hair and tissue samples from live mountain beavers. We thank Sue Fox, Wildlife Resource Consultants, for her exceptional ability to locate active *Aplodontia* colonies in California, and we also thank David Catalano, NDOW, for field assistance. We thank Carol Ritland for a review of an early draft of this manuscript.

Literature Cited

Bandelt, H.-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology Evolution 16:37–48.

Beier, P. 1989. Use of habitat by mountain beaver in the Sierra Nevada. Journal of Wildlife Management 53: 649–654.

Borrecco, J.E., and R.J. Anderson. 1980. Mountain beaver problems in the forests of California, Oregon, and Washington. Proceedings of the Vertebrate Pest Conference 9:135–142.

Brookfield, J.F.Y. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5:453–455.

Corander, J., P. Marttinen, J. Sirén, and J. Tang. 2008. Enhanced Bayesian modeling in BAPS software for learning genetic structures of populations. BMC Bioinformatics 9:539.

Cornuet, J.M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Donnelly, M.J., M.C. Licht, and T. Lehmann. 2001. Evidence for recent population expansion in the evolutionary history of the malaria vectors *Anopheles*

arabiensis and *Anopheles gambiae.* Molecular Biology and Evolution 18:1353–1364.

EXCOFFIER, L., G. LAVAL, AND S. SCHNEIDER. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50.

EXCOFFIER, L., P.E. SMOUSE, AND J.M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

FAIRCLOTH, B.C. 2006. gmconvert: file conversion for genemapper output files. Molecular Ecology Notes 6: 968–970.

GLAUBITZ, J.C. 2004. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes 4:309–310.

GOUDET, J. 2001. FSTAT, version 2.9.3, a program to estimate and test gene diversities and fixation indices. Updated from Goudet (1995). University of Lausanne, Lausanne, Switzerland.

GRINNELL, J., AND T. STORER. 1924. Animal life in Yosemite. University of California Press, Berkeley, CA.

HALL, E.R. 1981. The mammals of North America. 2nd edition. John Wiley & Sons, Inc., New York, NY. Volume 2, pages 601–1181 + 90.

HERRON, M.D., T.A. CASTOE, AND C.L. PARKINSON. 2004. Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (*Spermophilus*). Molecular Phylogenetics and Evolution 31:1015–1030.

HUCHON, D., F.M. CATZEFLIS, AND E.J. DOUZERY. 1999. Molecular evolution of the nuclear von Willebrand factor gene in mammals and the phylogeny of rodents. Molecular Biology and Evolution 16:577–589.

KIMMEL, M., R. CHAKRABORTY, J.P. KING, M. BAMSHAD, W.S. WATKINS, AND L.B. JORDE. 1998. Signatures of population expansion in microsatellite repeat data. Genetics 148:1921–1930.

MASER, C., B.R. MATE, J.F. FRANKLIN, AND C.T. DYRNESS. 1981. Natural history of Oregon Coast mammals. General Technical Report PNW-133, Pacific Northwest Forest and Range Experimental Station, USDA Forest Service.

MONTGELARD, C., S. BENTZ, C. TIRARD, O. VERNEAU, AND F.M. CATZEFLIS. 2002. Molecular systematics of Sciurognathi (Rodentia): the mitochondrial cytochrome *b* and 12S rRNA genes support the Anomaluroidea (Pedetidae and Anomaluridae). Molecular Phylogenetics and Evolution 22:220–233.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, NY.

PETERS, W.C. 1864. Ueber neue Arten der Säugethiergattungen *Geomys, Haplodon,* und *Dasypus.* Monatsbericht der Königlich Preussischen Akademie der Wissenschaften zu Berlin 17:177–179.

PIAGGIO A.J., B.A. COGHLAN, A.E. MISCAMPBELL, W.M. ARJO, D.B. RANSOME, AND C.E. RITLAND. 2013. Molecular phylogeny of an ancient rodent family (Aplodontiidae). Journal of Mammalogy 94:529–543.

PIAGGIO A.J., M.A. NEUBAUM, H. YUEH, C.E. RITLAND, J.J. JOHNSTON, AND S.L. PERKINS. 2009. Development of 10 polymorphic microsatellite loci isolated from the mountain beaver (*Aplodontia rufa rufa*). Molecular Ecology Resources 9:323–325.

PFEIFFER, E.W. 1954. Reproduction in a primitive, *Aplodontia rufa.* Doctoral dissertation, University of California, Berkeley, CA.

PFEIFFER, E.W., W.C. NUNGESSER, D.A. IVERSON, AND J.F. WALLERIUS. 1960. The renal anatomy of the primitive rodent, *Aplodontia rufa,* and a consideration of its functional significance. Anatomical Record 137: 227–236.

POSADA, D., AND K.A. CRANDALL. 2001. Intraspecific gene genealogies: trees grafting to networks. Trends in Ecology and Evolution 16:37–45.

RICE, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

SIKES, R.S., W.L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

SLATKIN, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

_____. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457–462.

SMITH, M.F., AND J.L. PATTON. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society 50: 149–177.

SOKAL, R.R., AND F.J. ROHLF. 1995. Biometry. Freeman, New York, NY.

STAMATAKIS, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

STAMATAKIS, A., P. HOOVER, AND J. ROUGEMONT. 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57:758–771.

STEELE, D.T. 1989. An ecological survey of endemic mountain beaver (*Aplodontia rufa*) in California 1979–83. Wildlife Management Division, California Department of Fish and Game, Sacramento, CA.

STEPPAN, S.J., B.L. STORZ, AND R.S. HOFFMANN. 2004. Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1. Molecular Phylogenetics and Evolution 30:703–719.

SWOFFORD, D.L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Inc., Sunderland, MA.

VAN OOSTERHOUT, C., W.F. HUTCHINSON, D.P.M. WILLS, AND P. SHIPLEY. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535.

WEBER, J.L., AND C. WONG. 1993. Mutation of human short tandem repeats. Human Molecular Genetics 2:1123–1128.

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

WILSON, G.A., AND B. RANNALA. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191.

Received 8 January 2013
Accepted 12 September 2013