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GENETIC SAMPLING OF PALMER'S CHIPMUNKS IN THE SPRING MOUNTAINS, NEVADA

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ABSTRACT.—Palmer's chipmunk (*Neotamias palmeri*) is a medium-sized chipmunk whose range is limited to the higher-elevation areas of the Spring Mountain Range, Nevada. A second chipmunk species, the Panamint chipmunk (*Neotamias panamintinus*), is more broadly distributed and lives in lower-elevation, primarily pinyon-juniper (*Pinus monophylla–Juniperus osteosperma)* habitat types. Panamint chipmunks are not closely related to Palmer's, but field identification of the 2 species is unreliable. Palmer's chipmunk is a species of concern in the state of Nevada and is listed by the International Union for Conservation of Nature (IUCN) as endangered. As such, conservation of Palmer's chipmunks is a priority in the Spring Mountains National Recreation Area. We sampled putative Palmer's chipmunks from 13 sites distributed across the Spring Mountains during 2010–2011. We removed Panamint chipmunks by using DNA-based identifications and then analyzed the genetic population structure of Palmer's chipmunks by using a panel of 9 microsatellites. Of the 228 samples that were genotyped, 186 were Palmer's; there was no evidence of hybridization between species. Four sites had exclusively Panamint chipmunks, 5 had exclusively Palmer's chipmunks, and 3 had a mixture of the 2 species. In this study, Palmer's chipmunks were exclusively captured at sites above 2400 m elevation, and Panamint chipmunks were exclusively captured at sites below 2200 m. Panamint chipmunks were trapped in areas typed as pinyon-juniper, but they were also trapped at sites typed as ponderosa pine (*Pinus ponderosa*) and mixed conifer. Both species were trapped at 3 sites; at all 3 sites, the lower-elevation traps contained Panamint chipmunks and the higher ones Palmer's chipmunks. Population structure within Palmer's chipmunks was minimal: heterozygosity was relatively high, and the populations displayed no signs of recent bottlenecks. Indications are that the distribution of Palmer's chipmunk is limited to higher-elevation areas in the Spring Mountains, but within this area, Palmer's chipmunk occurs as a single, large, well-connected, and stable population.

RESUMEN.—*Neotamias palmeri* es una ardilla de tamaño mediano cuyo hábitat se limita a las áreas más elevadas de *Spring Mountain Range*, Nevada. Una segunda especie de ardilla, *Neotamias panamintinus*, tiene una distribución más amplia y vive en áreas más bajas, principalmente en ambientes de pino piñonero (*Pinus monophylla–Juniperus osteosperma)*. Si bien *Neotamias panamintinus* no se relaciona con *Neotamias palmeri*, la identificación de ambas especies en el terreno no es confiable. *Neotamias palmeri* es una especie en riesgo en el estado de Nevada y está incluida en la lista de especies en peligro de extinción de la Unión Internacional para la Conservación de la Naturaleza (*IUCN*), por lo tanto, la conservación de esta especie es una prioridad en *Spring Mountains National Recreation Area*. Entre el 2010 y el 2011 tomamos muestras de supuestas *Neotamias palmeri* en 13 lugares ubicados en *Spring Mountains*. Eliminamos *Neotamias panamintinus* identificando con muestras de ADN y analizamos la estructura genética de la población de *Neotamias palmeri* utilizando un panel de 9 microsatélites. De los genotipos de las 228 muestras que tomamos, 186 correspondían a *Neotamias palmeri* y no se encontraron rastros de hibridación entre las especies. En cuatro lugares se encontraban exclusivamente *Neotamias panamintinus,* en cinco exclusivamente *Neotamias palmeri* y en tres se encontró una mezcla de las dos especies. En este estudio, se capturaron únicamente *Neotamias palmeri* en lugares con una elevación >2400 m y se capturaron únicamente *Neotamias panamintinus* en lugares con una elevación <2200 m. La especie *Neotamias panamintinus* se atrapó en ambientes de pino piñonero, en ambientes de pino ponderosa (*Pinus ponderosa*) y también en bosque mixto de coníferas. Ambas especies se encontraron en tres lugares, en los cuales las trampas ubicadas en zonas menos elevadas contenían *Neotamias panamintinus* y las ubicadas en zonas más elevadas contenían *Neotamias palmeri*. La estructura de la población en la especie *Neotamias palmeri* era mínima, la heterocigosidad era relativamente elevada y las poblaciones no mostraron signos de cuellos de botella recientes. Se observó que *Neotamias palmeri* se limita a áreas de mayor elevación en *Spring Mountains*, dentro de dicha área, esta especie constituye una población única, de tamaño considerable, estable y sin aislamiento entre sus miembros.

Palmer's chipmunk (*Neotamias palmeri*) is a medium-sized chipmunk in the "*Dorsalis*" clade (Banbury and Spicer 2007). This clade also contains the cliff chipmunk (*N. dorsalis*), Uinta chipmunk (*N. umbrinus*) and the grey-collared chipmunk (*N. cinereicollis*), which collectively are the Palmer's chipmunk's closest relatives (Piaggio and Spicer 2001). Several of

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these species are widely distributed in the western United States, yet the only chipmunk other than Palmer's found in the Spring Mountain Range, Clark County, Nevada, is the Panamint chipmunk (*N. panamintinus*), a smaller and distantly related (Piaggio and Spicer 2001) chipmunk. Few published studies discuss Palmer's chipmunk, but it has been reported as limited to higher elevation (2250–3600 m) areas in the Spring Mountains (Hall and Kelson 1959); Wilson and Ruff (1999) report the lower elevation limit as 2100 m. A recent unpublished study (Lowrey and Longshore 2011) reported the capture of Palmer's chipmunks at elevations ranging from 2080 m to 3290 m. Panamint chipmunks are associated with pinyon-juniper (*Pinus monophylla–Juniperus osteosperma*) forests (Lowrey and Longshore 2011) and, in the Spring Mountains, are associated with lower-elevation sites (1600–2400 m; Hall and Kelson 1959). Lowrey and Longshore (2011) caught Panamint chipmunks at elevations up to 2643 m. The marked differences in niche characteristics between these 2 species may be due to adaptive differentiation. For example, in laboratory experiments, Hirshfeld and Bradley (1977) found that Palmer's chipmunks exhibit faster growth than do Panamint chipmunks. The authors attributed this difference in growth to a longer period of hibernation and interpret this as an adaptation in Palmer's chipmunks to existence at higher elevations.

Palmer's chipmunk is a species of concern in the state of Nevada (http://www.ndow.org/wild/animals/facts/chipmunk_palmers.shtm) and is listed by the International Union for Conservation of Nature (IUCN) as endangered (http://www.iucnredlist.org/apps/redlist/details/21355/0). As such, conservation of Palmer's chipmunks is a priority within the Spring Mountains National Recreation Area, and the potential for negative effects on this species weighs heavily in the planning of management activities. Further, though the 2 species are only distantly related, field identification of Palmer's and Panamint chipmunks is difficult; it is not known whether they can co-exist in the same microsites. The potential for hybridization between these 2 species is unknown, but hybridization has been documented between other morphologically distinct chipmunk species (Good et al. 2003).

Genetic analyses provide efficient approaches to identifying morphologically cryptic organisms, describing hybridization patterns, evaluating population structure and connectivity, and evaluating the likelihood of recent population contractions (bottlenecks). Interpretation of genetic results is greatly simplified for isolated species where the organism's range is well understood. Thus, a species like Palmer's chipmunk that can be confused with a sympatric species and has likely been isolated for thousands of years is particularly amenable to genetic analyses.

Assuming that Palmer's chipmunk is endemic to the higher-elevation areas in the Spring Mountains, its vulnerability will largely be related to its population size and connectivity across the Spring Mountains. The area where Palmer's chipmunks are known to occur is extensive (>80 km^2) and contains potential barriers, such as alpine ridges. If local populations within specific canyons are isolated, then genetic drift and lack of gene flow will produce genetic structure at neutral loci when evaluated across the extent of the range. Even with moderate levels of connectivity, we would expect to see some genetic population structure and "isolation by distance," meaning that the population is not structured by geographic barriers, but rather simply by geographic distance (Wright 1943).

Because Palmer's chipmunk is isolated in a limited geographic area, its long term persistence will be affected by its ability to maintain genetic variability. Over time, alleles will drift to fixation, reducing heterozygosity and fitness (Hedrick and Kalinowski 2000). Alleles will also be created through mutation, thereby increasing heterozygosity and adaptive potential. The rates of both allele loss due to genetic drift and allele creation through mutation are controlled by effective population size (N_e), an idealized measure of the number of breeding individuals in a population (Wright 1931). If N_e is stable over time, then these 2 processes will equilibrate (Kimura and Crow 1964), leading to stable population heterozygosity. In general, for wild mammal populations, N_e is approximately 10% of the adult population size (Frankham 1995).

The primary goal of this study was to examine the genetic population structure of Palmer's chipmunks across the extent of the species' range, determine the species' approximate effective population size and stability, and determine the degree to which local areas are genetically connected. Additional goals were to genetically

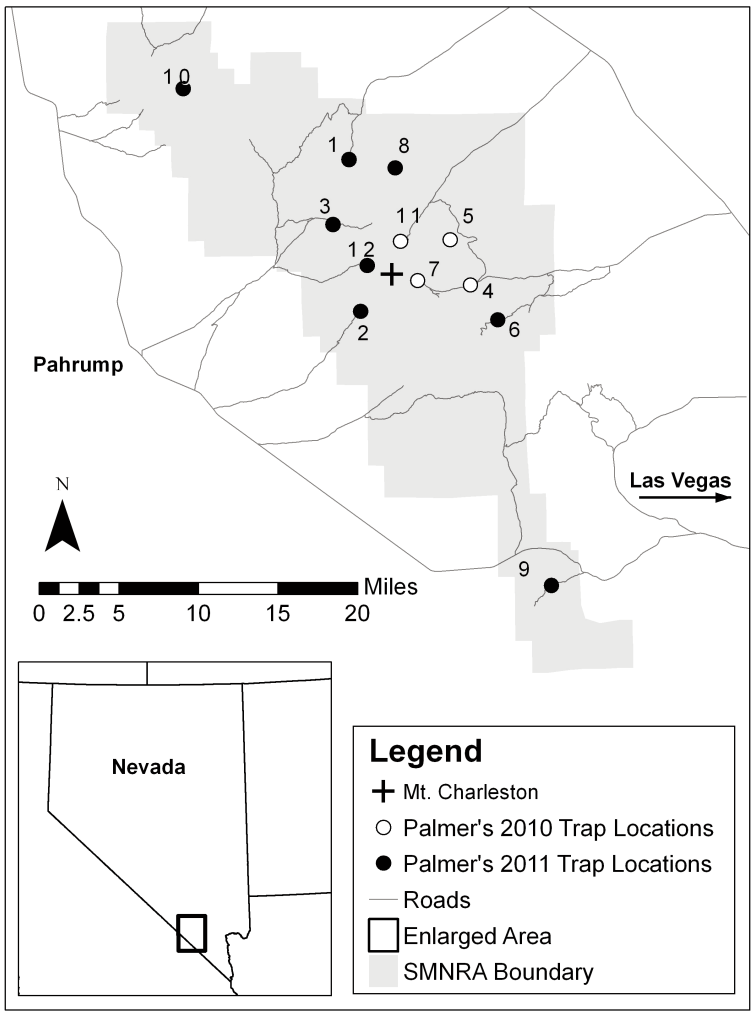

Fig. 1. Areas sampled for chipmunks in 2010 and 2011. Open circles indicate sampling during fall 2010. The "McWilliams and "Bristlecone" sites (0.79 km apart) are here collapsed into "Upper Lee." Numbering is 1 = Bonanza, 2 = Carpenter Canyon, 3 = Clark Canyon, 4 = Climate, 5 = Deer Creek, 6 = Harris Springs Road, 7 = Mary Jane, 8 = Mud Springs, 9 = Potosi, 10 = Stirling, 11 = Upper Lee, and 12 = Wallace Canyon. "Climate" locations were intentionally located in the pinyon-juniper forest to capture Panamint chipmunks. Palmer's chipmunks were the target species at all other sites.

identify all captures to species, determine whether Palmer's and Panamint chipmunks co-occur and hybridize, and ascertain whether Panamint chipmunks are found in areas exterior to pinyon-juniper forest types where they might be confused with Palmer's chipmunks.

METHODS

Study Area

The study area encompasses the Spring Mountain Range, Clark County, Nevada (Fig. 1). The area ranges in elevation from 1500 m to 3632 m. Lower-elevation areas are dry and largely composed of Mojave desert scrub, with Joshua trees (*Yucca brevifolia*) becoming common with increasing elevation. Above the desert lies a band of pinyon-juniper forest. With increasing elevation, forest types change to ponderosa pine (*Pinus ponderosa*), move through a mixed conifer forest composed of ponderosa pine and white fir (*Abies concolor*), and finally change to bristlecone pine (*Pinus longaeva*) at higher elevations. Climate varies greatly;

TABLE 1. Sites sampled for Palmer's and Panamint chipmunks in the Spring Mountains, Nevada. Tree species are *Abies concolor* (ABCO), *Juniperus osteosperma* (JUOS), *Juniperus scopulorum* (JUSC), *Pinus longaeva* (PILO), *Pinus monophylla* (PIMO), *Pinus ponderosa* (PIPO), and *Populus tremuloides* (POTR). Palmer's chipmunks were captured exclusively at all sites with elevation ranges above 2400 m. Panamint chipmunks were caught exclusively at sites with elevation ranges below 2200 m.

Site	Year sampled	Location	Elevation (m)	Tree species	Chipmunk species found
Bonanza Trail	2011	Bonanza Trail	2300–2370	ABCO, PIPO	Palmer's, Panamint
Bristlecone	2010	Upper Lee Creek	2630[a]	ABCO, PILO, PIPO	Palmer's
Carpenter Canyon	2011	Carpenter Canyon	2180–2290	ABCO, JUOS, PIMO, PIPO	Palmer's, Panamint
Clark Canyon	2011	Clark Canyon	2450–2520	ABCO, PIPO	Palmer's
Climate	2010	Lower Kyle Canyon	2120–2140	JUOS, PIMO	Panamint
Deer Creek	2010	Deer Creek	2590–2700	ABCO, PIPO, POTR	Palmer's
Harris Springs	2011	Harris Springs	1760–2170	ABCO, JUOS, PIMO, PIPO	Panamint
Mary Jane	2010	Upper Kyle Canyon	2420–2490	ABCO, PIPO, POTR	Palmer's
McWilliams	2010	Upper Lee Creek	2680[a]	ABCO, PIPO, POTR	Palmer's
Mud Springs	2011	Mack's Canyon	2340–2670	ABCO, JUOS, JUSC, PIPO	Palmer's, Panamint
Potosi	2011	Potosi pass	1890–1980	JUOS, PIMO	Panamint
Stirling	2011	Mt. Stirling WSA[b]	1980–2020	JUOS, PIMO, PIPO	Panamint
Wallace	2011	Wallace Canyon	2450–2520	ABCO, PIPO	Palmer's

[a]Bristlecone and McWilliams were the first areas trapped in 2010. In these areas a single GPS location was taken at the center of the grid. For all other sites GPS locations were taken at each trap.
[b]Wilderness Study Area.

foothills areas average <30 cm annual precipitation, with average summer high temperatures approaching 36 °C. Higher elevations have much cooler temperatures and abundant winter snowfall; a commercial ski resort in upper Lee Canyon reports receiving an average of 355 cm of snow annually.

Sampling Design

Sampling was designed to capture enough individuals at each site to characterize the allele frequencies at that site; trapping was not designed to estimate local abundance. Ideally, each trapping grid contained 30 large Sherman live traps in a 5 × 6 arrangement, with traps spaced approximately 30 m apart from each other, far enough that captures at traps would be largely independent, maximizing per-trap efficiency (Dean Pearson, USDA Forest Service, Missoula, MT, personal communication). However, given the terrain and habitat in the Spring Mountains, trap locations were often constrained by available habitat. In general, 3 of these trap grids were placed in close proximity (~0.3 km) and left for 4 days. Thus, standard effort to characterize each site was to sample for 360 trap-days. This protocol varied somewhat, particularly during initial trapping in 2010, which was exploratory.

Initial sampling in fall 2010 had 3 goals. First, we needed to test layout and effort levels to determine the efficacy of the trapping protocol. Second, we needed to capture both Palmer's and Panamint chipmunks to provide samples for genetic testing. Last, we wanted the trapping effort to contribute to the generalized trapping across the Spring Mountains scheduled to begin in 2011. In 2010, we therefore sampled 5 areas to capture chipmunks: 4 at higher elevations in the area around Mount Charleston and one at low elevation (Climate) in the pinyon-juniper forest (Table 1, Fig. 1).

To determine whether significant genetic structure occurs across the Spring Mountains, samples should be located near the edges of the range where sites are most distant from each other. Additionally, sites should be located in areas where peripheral island populations may exist (e.g., Mount Stirling; Fig. 1). If significant structure is found at this scale, additional sampling in intermediate areas can elucidate where barriers exist, and the nature

of those barriers can be inferred. In 2011, we therefore sampled an additional 8 sites, locating sites on both the east and west sides of the range, and extended the sampling north and south into isolated montane areas of Mount Stirling and Potosi Mountain, respectively (Table 1, Fig. 1). Prior to the trapping season, sites were initially located remotely via satellite imagery and later chosen after groundtruthing. All sites were chosen based on presence of characteristics typically associated with Palmer's chipmunk habitat (e.g., forest types other than pinyon-juniper, down wood, higher elevations, etc.). Within the trapping area, plant communities were determined based on Nachlinger and Reese (1996).

Tissue Sampling and Storage

We baited each trap with a mixture of rolled oats and peanut butter. To provide insulation and reduce trap stress, we placed upholsterer's cotton in each trap for use as bedding material. Traps were placed under cover to reduce daytime heating and nighttime cooling. Traps were checked once a day in the morning. If the trap contained a chipmunk, we released the chipmunk from the trap into a capture bag. Chipmunks were held while ~2 mm of tissue from the right ear was collected using sterile scissors; once the sample was collected, the chipmunks were immediately released. No other measurements or handling to identify species were performed; sampling was designed to minimize stress and was in accordance with established handling protocols for wild animals (Sikes et al. 2011). Sterile forceps were used to immediately place ear-tissue samples in airtight plastic vials containing silica-gel desiccant. Vials were labeled with the trapping area, grid number, trap location, and date. Lastly, we took a GPS waypoint at the trap location. All samples were shipped to the USFS Wildlife Genetics Laboratory in Missoula, Montana, for analysis.

DNA Analysis

DNA analyses were designed to allow species identification and to explore genetic structure. Species-level identification commonly uses mitochondrial DNA (mtDNA), where sequence data are compared to reference samples. Nuclear DNA, characterized by microsatellites, is commonly used for analyzing fine-scale population structure. Microsatellites are highly variable, and primers developed for one species often can be used to analyze related species (Primmer et al. 1996). Additionally, a panel of microsatellite markers is often effective for species-level identification, as microsatellite size generally differs between species. Lastly, if microsatellites differ in size between species, they provide an effective means to identify hybrids (e.g., Schwartz et al. 2004, Bingham et al. 2012).

DNA from tissue samples was extracted with the DNeasy Tissue Kit (Qiagen, Inc.) following the manufacturer's instructions. The reaction volume (10 μL) contained 1.0–3.0 μL DNA, 1X reaction buffer (Applied Biosystems), 2.0 mM $MgCl_2$, 200 μM of each dNTP, 1 μM reverse primer, 1 μM dye-labeled forward primer, 1.5 mg/mL BSA, and 1U Taq polymerase (Applied Biosystems). The PCR profile was 94 °C/5 min, [94 °C/1 min, 55 °C/1 min, 72 °C/30 s] × 36 cycles for tissue samples. PCR products were run in a 6.5% acrylamide gel for 2 h on a LI-COR DNA analyzer (LI-COR Biotechnology). Nine microsatellite loci developed for yellow-pine chipmunks (*N. amoenus;* Schulte-Hostedde et al. 2000) were analyzed: *EuAm26*, *EuAm35*, *EuAm37*, *EuAm41*, *EuAm94*, *EuAm108*, *EuAm114*, *EuAm138*, and *EuAm142*.

GenBank (http://www.ncbi.nlm.nih.gov/genbank/) contained reference sequences for both Palmer's and Panamint chipmunks analyzed at the cytochrome *c* oxidase 2 (COII) and cytochrome *b* (cyt *b*) regions of the mitome. We amplified the COII region using primers *L7600* and *H316* (Piaggio and Spicer 2001). The cyt *b* region was amplified in 2 segments using primers *L14724* and *H15230* and *L15060* and *H15906* (Piaggio and Spicer 2001). Reaction volumes of 50 μL contained 50–100 ng DNA, 1X reaction buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 200 μM each dNTP, 1 μM each primer, and 1 U Taq polymerase (Applied Biosystems). For both cyt *b* and COII, the PCR program was 94 °C/5 min, [94 °C/1 min, 55 °C/1 min, 72 °C/1 min 30 s] × 34 cycles, 72 °C/5 min. The quality and quantity of template DNA were determined by 1.6% agarose gel electrophoresis. PCR products were purified using ExoSap-IT (Affymetrix-USB Corporation, OH) according to manufacturer's instructions. DNA sequence data was obtained using the Big Dye kit and the 3700 DNA Analyzer (ABI; High

TABLE 2. Descriptive statistics for Palmer's chipmunk populations. Analyses were limited to sampling locations with 10 or more samples; *n* is the sample size, *A* the average number of alleles, H_o the average observed heterozygosity, H_e the average expected heterozygosity, and *F* the fixation index $1 - (H_o/H_e)$. H deficit and H excess p values are associated with Hardy–Weinberg exact tests for heterozygote deficit and excess. Self-assignment proportions indicate the proportion of samples that statistically were more likely to be drawn from their sample site than from other sites in the Spring Mountains. Standard errors are in parentheses.

Sampling site	*n*	*A*	H_o	H_e	*F*	*H* deficit (p values)	*H* excess (p values)	Self assignment (%)
Bonanza Trail	35	4.667 (0.764)	0.505 (0.100)	0.492 (0.095)	–0.022 (0.045)	0.851	0.141	62.9
Bristlecone	17	4.444 (0.801)	0.490 (0.099)	0.489 (0.100)	–0.023 (0.032)	0.255	0.761	23.5
Clark Canyon	15	4.222 (0.741)	0.504 (0.088)	0.520 (0.084)	0.019 (0.043)	0.326	0.693	66.7
Deer Creek	10	3.667 (0.782)	0.456 (0.118)	0.454 (0.111)	–0.013 (0.077)	0.325	0.699	40.0
Mary Jane	12	3.444 (0.648)	0.472 (0.105)	0.468 (0.104)	–0.049 (0.094)	0.123	0.882	58.3
McWilliams	21	4.556 (0.766)	0.513 (0.100)	0.490 (0.097)	–0.054 (0.020)	0.560	0.455	47.6
Mud Springs	16	4.111 (0.696)	0.521 (0.116)	0.503 (0.105)	–0.007 (0.049)	0.640	0.376	25.0
Wallace Canyon	55	5.333 (1.000)	0.509 (0.101)	0.513 (0.103)	0.007 (0.028)	0.015	0.986	38.2

Throughput Genomics Unit, Seattle, WA). DNA sequence data for COII and cyt *b* were generated using the given primers. Sequences were viewed and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI).

Species-level identification based on mtDNA was done by comparing our sequences to reference sequences of Palmer's and Panamint chipmunks available in GenBank. We used GenBank's BLAST search, which performs a proximity search, to determine which archived sequences were most similar.

Primary DNA analyses of chipmunk samples utilized Genalex 6.4 (Peakall and Smouse 2006). We performed principal coordinate analysis (PCoA) of genotype frequencies on all samples and, having removed all Panamint chipmunks (see results below), performed additional PCoA on Palmer's chipmunks. For those trapping locations where ≥10 Palmer's chipmunks were captured, we analyzed population structure using Genalex to evaluate pairwise Fst and Nei's D, sample-level Mantel tests, and AMOVA. We used the Mantel Non-parametric Test Calculator (Liedloff 1999) to compute site-level Mantel tests that compared pairwise Fst to Euclidian distance. We tested for site-level Hardy–Weinberg equilibrium with Genepop (Rousset 2008) using the default settings. We used Geneclass (Piry et al. 2004) to conduct assignment tests. We used LDNe (Waples and Do 2008) to estimate effective population size, using an allele frequency limit of 0.05 and assuming random mating. We used STRUCTURE (Pritchard et al. 2000) with K = 2 (burn-in = 50,000; run length = 100,000; "admixture" ancestry model; and no priors) to look for possible hybridization between Palmer's and Panamint chipmunks. We used program Bottleneck (Luikart and Cornuet 1998) to look for evidence of recent population bottlenecks. In Bottleneck (Luikart and Cornuet 1998), we evaluated the sign, Wilcoxon's, and mode tests.

We analyzed samples previously obtained from large, well-distributed populations of yellow-pine and red-tailed (*N. ruficaudus*) chipmunks captured in northern Idaho to compare heterozygosity, allelic richness, and effective population sizes for these species with Palmer's chipmunks. Idaho samples were collected at 1.61-km intervals across a 1290-km^2 area in Boundary County, Idaho (See Cushman et al. 2006 for maps of the study area).

RESULTS

Initial genetic analyses indicated that microsatellite markers were sufficiently variable to examine population structure in Palmer's chipmunks (Table 2), and PCoA appeared to diagnostically group Palmer's and Panamint chipmunks based on the first coordinate (Fig.

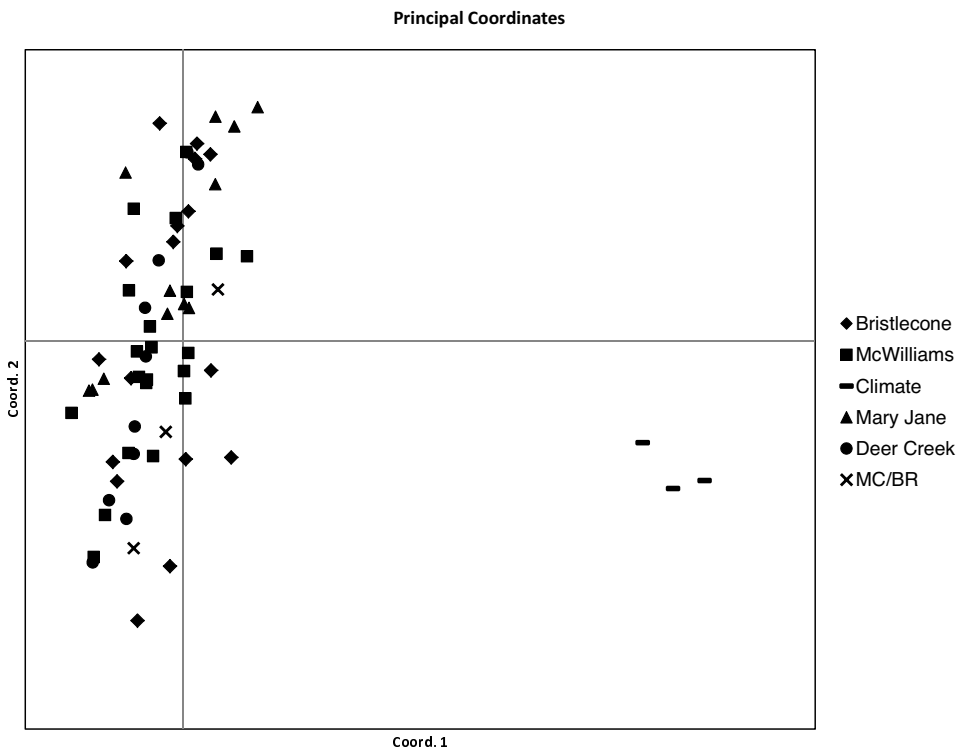

Fig. 2. A diagram of the first 2 coordinates in a principal coordinate analysis of 9 microsatellite loci from 66 chipmunk samples collected in the Spring Mountains, Nevada, in 2010. The location of "Climate" was chosen to intentionally capture Panamint chipmunks. Other sites were high-elevation areas with historical Palmer's chipmunk detections. MC/BR here refers to a group of 3 samples whose origins were either from the "McWilliams" or "Bristlecone" sites but were not identifiable due to smudging of the vial labels.

2). To confirm the diagnostic nature of the microsatellite analyses, we analyzed mtDNA for the 3 low-elevation "Climate" chipmunks and 2 chipmunks from each of the high-elevation sites (Bristlecone, Deer Creek, McWilliams, and Mary Jane; Table 1, Fig. 1). MtDNA sequences (cyt *b* and COII) identified the 3 chipmunks at Climate as being Panamint chipmunks, and all other samples as Palmer's (See Appendix for details). For all subsequent samples, we therefore relied on PCoA grouping based on microsatellites to separate Palmer's from Panamint chipmunks, with STRUCTURE (Pritchard et al. 2000) analyses serving as a secondary check (see below).

Across the 13 sites sampled in 2010–2011, we captured a total of 229 chipmunks. One sample from Stirling failed to amplify. Of those that amplified, 186 were identified as Palmer's chipmunks (Fig. 3; Fst between Palmer's and Panamint chipmunks = 0.222). Three samples from upper Lee Canyon could not be identified to either Bristlecone or McWilliams sampling sites due to label smudging. These samples, labeled MC/BR, were included in sample-level analyses but were removed from site-level population analyses because they lacked precise spatial coordinates.

Based on PCoA grouping, 2 samples, one from Carpenter Canyon and one from Harris Springs, appear to be outliers (Fig. 3). However, STRUCTURE (Pritchard et al. 2000) analyses with K = 2 showed no indications that these samples were hybrids; both samples grouped cleanly with Palmer's and Panamint chipmunks in Carpenter Canyon and Harris Springs, respectively. Additionally, no indications of hybridization were seen in any of the other 226 samples (Fig. 4).

Samples from higher-elevation sites, with minimum elevations >2400 m, were exclusively Palmer's chipmunks. Samples from

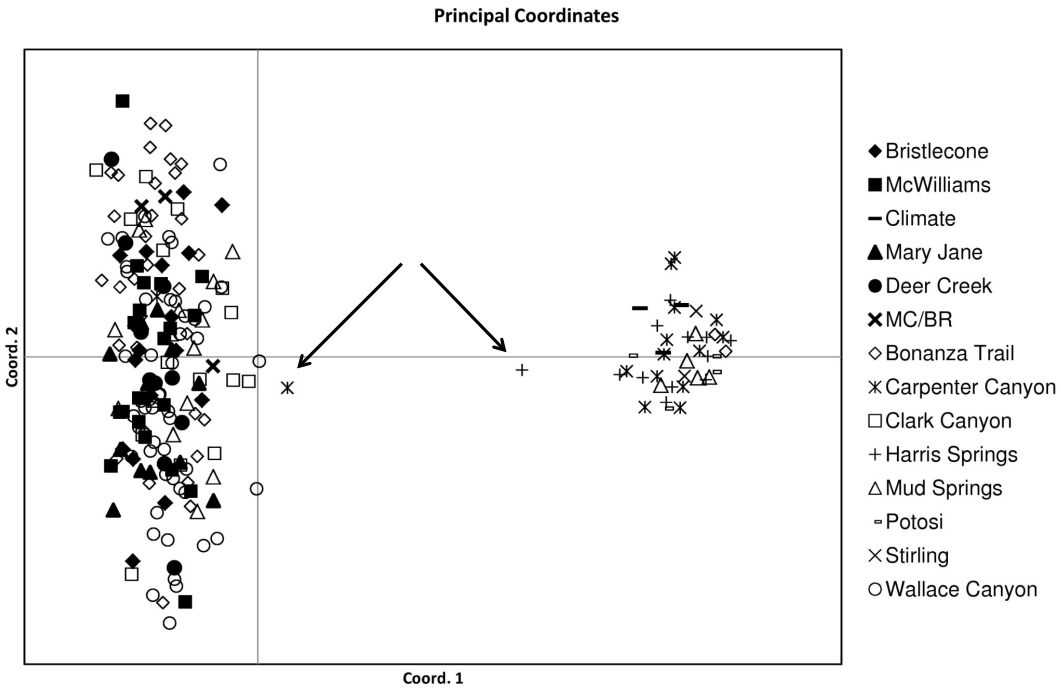

Fig. 3. A diagram of the first 2 coordinates in a principal coordinate analysis of 9 microsatellite loci from 228 chipmunk samples collected in the Spring Mountains, Nevada, in 2010–2011. Darker symbols are associated with sites collected in 2010. MC/BR here refers to a group of 3 samples whose origins were either from the "McWilliams" or "Bristlecone" sites but were not identifiable due to smudging of the vial labels. Arrows point to 2 samples that should be checked for hybridization.

lower-elevation sites, with maximum elevations <2200 m, were exclusively Panamint chipmunks (Table 1). Three sites at intermediate elevations had mixtures of the 2 species (Table 1). At all 3 sites, the lower-elevation traps contained Panamint chipmunks and the higher ones Palmer's chipmunks. Sites where Panamint chipmunks were captured mostly contained pinyon-juniper types, but Panamint chipmunks were caught in other forest types (Table 1). In some cases, pinyon-juniper forests were adjacent. For example, the Stirling site was located in a stand of ponderosa pine adjacent to a spring, but the site was surrounded by pinyon-juniper forests.

Descriptive Population Genetics Statistics for Palmer's Chipmunks

For all trapping site–level analyses of Palmer's chipmunks, we removed the 3 MC/BR samples whose precise location was unknown, and Carpenter Canyon, where only 2 Palmer's chipmunks were caught. This left 181 chipmunks from 8 sampling locations, all of which had ≥10 Palmer's chipmunk samples per location. Allelic richness, observed heterozygosity, and expected heterozygosity were similar across sampling locations. Allelic richness was highest in Wallace Canyon, which was also the largest sample, and lowest at Mary Jane (5.33–3.44). Observed heterozygosity ranged from a high of 0.52 at Mud Springs to 0.46 at Deer Creek. Expected heterozygosity varied from 0.52 in Clark Canyon to 0.45 at Deer Creek (Table 2). None of these differences were statistically significant. Hardy–Weinberg exact tests for heterozygote deficit and excess indicated that populations were in Hardy–Weinberg equilibrium. Wallace Canyon showed some signs of having heterozygote deficiency (Table 2) but, with Bonferroni-correction, the *P* value is not significant at the 0.05 significance level.

Population Structure of Palmer's Chipmunks

A microsatellite-based PCoA of the 186 Palmer's chipmunks indicated low levels of

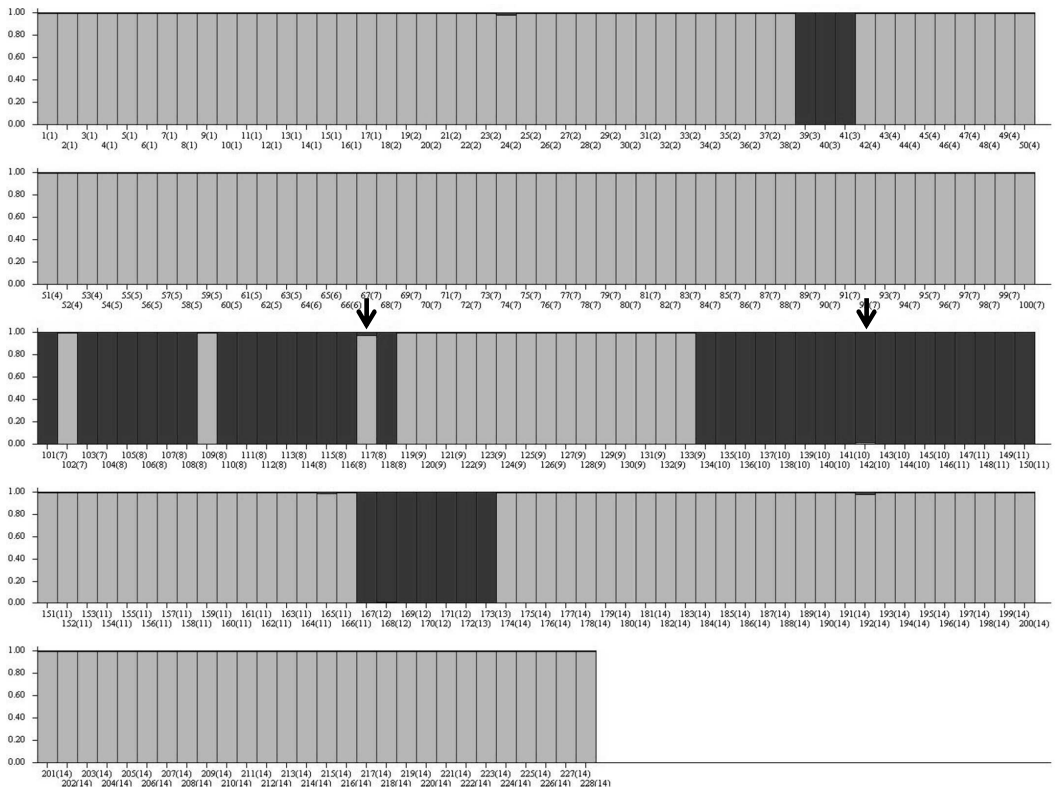

Fig. 4. "Q" plots from STRUCTURE (Prichard et al. 2000) with K = 2. Each bar represents a single sample, and the coloring of each bar indicates the probability of a sample being associated with group 1 or group 2. Ambiguous samples (in this case hybrids) would be indicated by bars that were part light gray and part dark gray. Arrows point to samples from Carpenter Canyon (left) and Harris Springs (right) which were slightly ambiguous based on principal coordinate analysis (Fig. 3). Dark gray indicates Panamint and light gray Palmer's chipmunks.

genetic divergence (Fig. 5). Among populations (excluding Carpenter Canyon and the 3 MC/BR samples), pairwise Fst and Nei's D were low. The highest pairwise Fst and Nei's D were between Clark Canyon and Mary Jane (0.054 and 0.107, respectively; Table 3). AMOVA indicated that 94% of variance occurred within populations and 6% between populations. Sample-level Mantel tests comparing genetic distance to geographic distance found a very slight positive trend, but little (r^2 = 0.0042) of the variance was related to Euclidian distance between samples. Site-level Mantel tests indicated modest correlation between relatedness and distance (Mantel r = 0.383, P = 0.053). Overall, 45.3% (SE = 3.7%) of the chipmunk genotypes were assigned to the sites where the chipmunks were captured (see Table 2 for site-level self-assignment rates).

Effective Population Size and Population Bottlenecks

Due to the low level of spatial structure in Palmer's chipmunks, we combined all sites to produce an effective population size estimate for the Spring Mountains by using a linkage disequilibrium approach as implemented in the program LDNe (Waples and Do 2008). This approach can be sensitive to low-frequency alleles and has been shown to be more robust if analysis is restricted to alleles with frequencies of >0.05 (Waples and Do 2008). Based on this threshold, we estimated an effective population size estimate of 93.3 (jackknife 95% CI = 67.4–135.1).

None of the tests performed with program Bottleneck (Luikart and Cornuet 1998) were significant, indicating no statistical evidence of recent short-term population declines (bottlenecks).

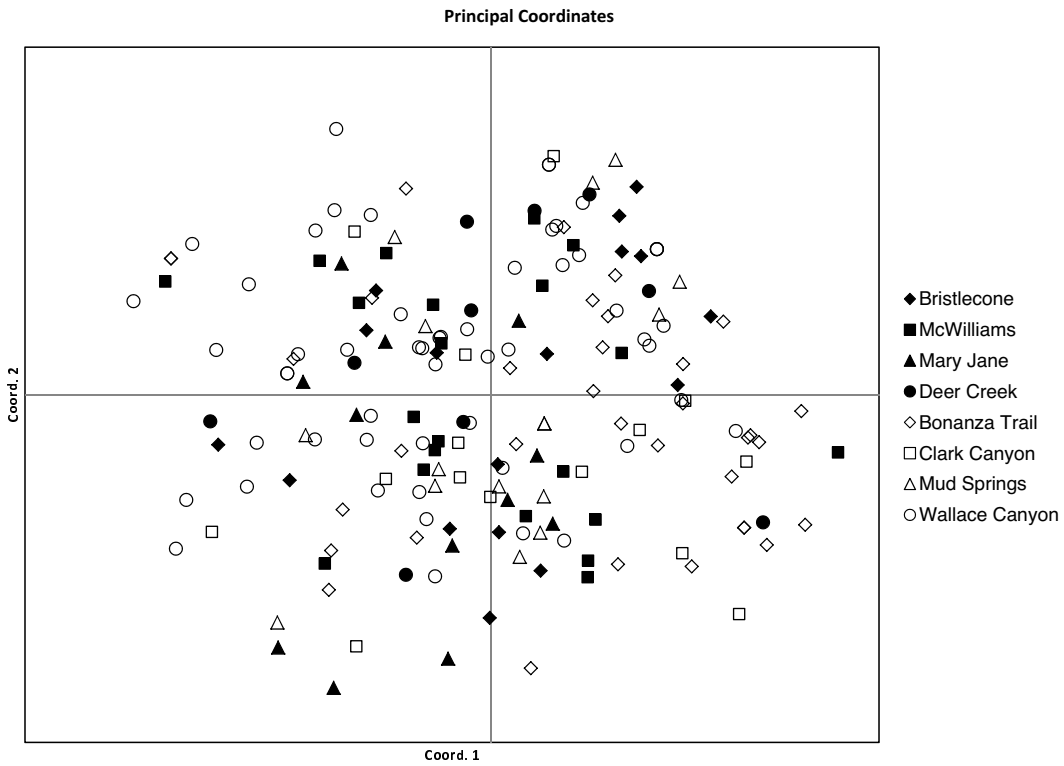

Fig. 5. Principal coordinate analysis of all genetically identified Palmer's chipmunks. Darker symbols are associated with sites collected in 2010. MC/BR here refers to a group of 3 samples whose origins were either from the "McWilliams" or "Bristlecone" sites but were not identifiable due to smudging of the vial labels. Sample site does separate sample locations in coordinate space.

TABLE 3. Pairwise Fst and Nei's D statistics for populations containing ≥10 Palmer's chipmunk samples. Values above the diagonal are pairwise Fst, and those below the diagonal are Nei's D. Geographically, Bristlecone and McWilliams are the closest, being 0.8 km apart and both located in upper Lee Creek Canyon. Bonanza Trail and Mary Jane Canyon are the most distant, being 13.7 km apart. Correlations between Euclidian distance and both Fst and Nei's D are low (r^2 = 0.117 and 0.093, respectively).

	Bonanza Trail	Bristlecone	Clark Canyon	Deer Creek	Mary Jane	McWilliams	Mud Springs	Wallace Canyon
Bonanza Trail		0.029	0.050	0.036	0.040	0.027	0.027	0.025
Bristlecone	0.062		0.042	0.028	0.030	0.012	0.020	0.015
Clark Canyon	0.102	0.102		0.051	0.054	0.037	0.047	0.043
Deer Creek	0.071	0.055	0.100		0.042	0.028	0.029	0.022
Mary Jane	0.073	0.052	0.107	0.084		0.029	0.027	0.024
McWilliams	0.049	0.030	0.094	0.060	0.050		0.030	0.024
Mud Springs	0.061	0.038	0.096	0.063	0.058	0.062		0.010
Wallace Canyon	0.064	0.031	0.089	0.043	0.050	0.052	0.024	

Comparisons between Palmer's and Common Chipmunk Species in Idaho

Based on the same 9 microsatellite loci used in this study, Palmer's chipmunks had lower allelic richness, heterozygosity, and effective population size than yellow-pine chipmunks collected in Idaho. Allelic richness and heterozygosity were similar when Palmer's chipmunks were compared to red-tailed chipmunks, also from Idaho, but effective population size was lower. All 3 populations showed slight heterozygote deficits (Table 4).

TABLE 4. Comparative genetic statistics for Palmer's chipmunk in the Spring Mountains, Nevada, with yellow-pine and red-tailed chipmunks sampled in northern Idaho. H_o is the observed heterozygosity, H_e the expected heterozygosity, F the fixation index $1 - (H_o/H_e)$, and N_e the estimated effective population size based on the LDN_e (Waples and Do 2008) with an allele frequency cutoff of 0.05 and random mating. Numbers in parentheses are standard errors except for N_e where they are the jackknife 95% confidence intervals around the estimates (Waples and Do 2008).

Species	n	Allelic richness	H_o	H_e	F	N_e
Palmer's chipmunk	181	5.667	0.502	0.522	0.046	93.3
		(1.000)	(0.097)	(0.101)	(0.022)	(67.4–135.1)
Yellow-Pine chipmunk	200	8.333	0.544	0.577	0.058	350.2
		(0.764)	(0.048)	(0.049)	(0.010)	(144.1–∞)
Red-tailed chipmunk	117	6.222	0.424	0.470	0.100	761.1
		(0.969)	(0.075)	(0.080)	(0.031)	(129.7–∞)

DISCUSSION

Palmer's chipmunks appear to be limited to higher-elevation areas in the central portion of the Spring Mountains. In this study, no Palmer's chipmunks were found at elevations below 2290 m, and for those 3 sites where both Panamint and Palmer's chipmunks were collected, the split between Palmer's and Panamint chipmunks was elevational, with Panamint chipmunks captured in those traps set at the lowest elevations. Though pinyon pine and Utah juniper forest types were present in most areas where Panamint chipmunks were found, Panamint chipmunks were captured in areas typed as ponderosa pine and mixed conifer forests, as well as in pure pinyon-juniper forests. We were using baited traps, and it is possible that Panamint chipmunks "pulled" from nearby pinyon-juniper areas. Alternatively, Panamint chipmunks may utilize lower-elevation forests other than pinyon-juniper. This finding is somewhat at odds with Lowrey and Longshore (2011), who found Panamint chipmunks exclusively in pinyon-juniper. However, both studies reinforce the understanding that Palmer's chipmunks are limited to higher-elevation areas in the central portion of the Spring Mountains above the pinyon-juniper areas, and Panamint chipmunks are largely associated with pinyon-juniper. The clear separation of Panamint and Palmer's samples within sites where both occurred, with Panamints always at lower elevations than Palmer's, indicates little niche overlap between these species.

Within the central high-elevation (>2300 m) area of the Spring Mountains, populations of Palmer's chipmunk appear to be well mixed, with low levels of spatial structuring. Self-assignment rates, though greater than random expectations, were modest and likely reflect local patterns of relatedness. Mantel tests, pairwise Fst, and Nei's D all indicated low levels of genetic structure; the population is not panmictic but also is not strongly subdivided. Neither topography nor distance strongly structured the population. None of the populations appears to be isolated; measures of genetic diversity are similar across all sites, and loci within each population are in Hardy–Weinberg equilibrium (Table 2). Given the presumed long isolation of Palmer's chipmunks from other closely related species, the well-mixed nature of the population, and the lack of any signs of recent bottlenecks, it is reasonable to assume that the population is relatively close to the drift-mutation equilibrium (Kimura and Crow 1964). The expected heterozygosities of approximately 0.5 indicate that Palmer's chipmunks have historically existed as a fairly large population within the Spring Mountains. Though our sampling was not designed to estimate abundance, Palmer's chipmunks appear to be common in many areas. Areas we sampled represent a tiny proportion of the area above 2300 m in the Spring Mountains, but we were able to collect nearly 200 individuals.

When compared to common, widespread species (yellow-pine and red-tailed chipmunks), Palmer's chipmunks have fewer alleles and a smaller effective population size. These results are not surprising, given the limited distribution of Palmer's chipmunk. However, the microsatellites used in this study were designed for yellow-pine chipmunks (Schulte-Hostedde et al. 2000) and are therefore expected to show greater variability in that species than they do in other species because of ascertainment bias (Hutter et al. 1998). When compared to red-tailed chipmunks, a species for which ascertainment bias is not expected,

Palmer's chipmunks are very similar for all statistics except for effective population size (Table 4).

CONCLUSIONS

Palmer's chipmunks are limited in geographic distribution but appear to be both common and well connected within their range. Assuming long-term isolation, this population has maintained considerable genetic diversity, such that allelic richness and heterozygosity are similar to those observed in common, widely distributed species. Relatively high genetic diversity, coupled with the lack of evidence of recent population bottlenecks, indicates that Palmer's chipmunks have historically been present in stable numbers. Given these characteristics, Palmer's chipmunk populations would not appear likely to be highly sensitive to local disturbance. Based on this sample, Palmer's chipmunks do not appear to co-occur with Panamint chipmunks and are therefore only likely to be confused in the field with Panamint chipmunks in a narrow zone where pinyon-juniper forests are interspersed with more mesic forest types.

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APPENDIX. Mitochondrial DNA tests of chipmunk samples from the Spring Mountains to verify species identification. Accession number refers to the GenBank (http://www.ncbi.nlm.nih.gov/genbank/) voucher specimen that most closely matched the sample sequence. Highest % identity refers to the sequence similarity as reported by GenBank's nucleotide BLAST search. Sample MC2 failed to amplify at COII but was a close match to a *Neotamias palmeri* voucher specimen at cyt *b*. Six novel haplotypes were identified, 2 for *N. palmeri* and 4 for *Neotamias panamintinus*. The related sequences have been submitted to GenBank.

Sample ID	Site	Highest % identity	Accession number	Associated species	Sequence base pairs	Haplotype ID
COII						
BR1	Bristlecone	99	AF147607	*N. palmeri*	561	P0017 COII
BR2	Bristlecone	99	AF147607	*N. palmeri*	561	P0017 COII
P0001	Climate	99	AF147608	*N. panamintinus*	561	P0001 COII
P0002	Climate	99	AF147608	*N. panamintinus*	561	P0002 COII
P0003	Climate	99	AF147608	*N. panamintinus*	561	P0002 COII
P0017	Deer Creek	99	AF147607	*N. palmeri*	561	P0017 COII
P0018	Deer Creek	99	AF147607	*N. palmeri*	561	P0017 COII
P0006	Mary Jane	exact	AF147607	*N. palmeri*	561	
P0007	Mary Jane	exact	AF147607	*N. palmeri*	561	
MC1	McWilliams	99	AF147607	*N. palmeri*	561	P0017 COII
MC2	McWilliams	NA	NA	NA	NA	poor sequence
Cyt *b*						
BR1	Bristlecone	99	AF147655	*N. palmeri*	604	BR
BR2	Bristlecone	99	AF147655	*N. palmeri*	604	BR
P0001	Climate	99	JN42474	*N. panamintinus*	604	P0001
P0002	Climate	99	JN42474	*N. panamintinus*	604	P0002
P0003	Climate	99	JN42474	*N. panamintinus*	604	P0002
P0017	Deer Creek	exact	AF147655	*N. palmeri*	604	
P0018	Deer Creek	exact	AF147655	*N. palmeri*	604	
P0006	Mary Jane	exact	AF147655	*N. palmeri*	604	
P0007	Mary Jane	exact	AF147655	*N. palmeri*	604	
MC1	McWilliams	exact	AF147655	*N. palmeri*	604	
MC2	McWilliams	exact	AF147655	*N. palmeri*	604	