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Conservation Genetics of Kangaroo Mice, Genus *Microdipodops*

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Abstract The two currently recognized species of kangaroo mice, *Microdipodops megacephalus* and *M. pallidus*, inhabit sandy soils of the Great Basin Desert in western North America. Given their habitat specificity and the fluctuating climate throughout the Pleistocene, kangaroo mice likely endured a turbulent biogeographic history that resulted in disjunct distributions and isolation of genetic lineages. Recent phylogenetic investigations using mitochondrial data have revealed several mitochondrial clades within this genus that may represent cryptic species. These mitochondrial clades are genetically unique, occupy relatively small distributions, and, as such, may be at an increased risk of extinction due to climate change and extensive recent habitat alteration. Herein, we apply haplotype network, population genetic, and historical demographic analyses to mitochondrial data of each *Micropdipodops* species and mitochondrial clade to assess conservation genetics within kangaroo mice. Results indicate that each mitochondrial clade is a distinct lineage with little to no gene flow occurring among clades. Additionally, historical demographic analyses support past population expansions and identify locations of past refugium for each distinct lineage. Although mitochondrial data indicate that the clades appear to be in approximate genetic equilibrium and have not suffered any extreme bottlenecks over time, there is still concern for the survival of smaller and more vulnerable *Microdipodops* subpopulations due to impending habitat threats in the Great Basin Desert.

Keywords Biogeography · Conservation genetics · Great Basin Desert · Kangaroo mice · *Microdipodops* · Mitochondrial DNA

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Introduction

The Great Basin Desert of North America is a high latitude and high elevation arid desert, encompassing most of Nevada as well as portions of California, Oregon, Idaho, and Utah. This cold desert is characterized by a series of basins and more than 160 north-south oriented mountain ranges established by an active tectonic history over the last 17 million years (Ma; Davis 2005 and references therein). Alternating islands of basins and ranges as well as the rise

and fall of pluvial lake levels (Benson 1981; Benson et al. 1990; Grayson 1993), shifting climatic patterns (Antevs 1952; Grayson 2006; Beever et al. 2011), and floristic transitions (Reveal 1979) have resulted in multiple potential barriers to dispersal. Isolation is especially common for taxa distributed on mountaintop islands, possessing limited dispersal abilities, or having specific habitat requirements (e.g., some species of butterflies, beetles, plants, fish, and mammals; Smith 1978; Austin and Murphy 1987; Yandell 1992; Britten et al. 1994, 1995; Porter and Rust 1996; Epps et al. 1998; Fleishman et al. 2001; Beever et al. 2003; Floyd et al. 2005; Kramer et al. 2011). Many species, however, may not be affected by the heterogeneous topography of the Great Basin and have widespread distributions (e.g., some species of birds, mammals, plants, dragonflies, and damselflies; Johnson 1975, 1978; Wells 1983; Wilcox et al. 1986; Rogers 1991b, a; Johnson and Marten 1992; Hamrick and Godt 1996; Lawlor 1998; Simpkin et al. 2000). Although the Great Basin Desert is rich in animal, plant, and geologic diversity (Fiero 1986; Davis 2005), the sustainability of flora and fauna in this desert is threatened by climate change (McDonald and Brown 1992; Beever et al. 2003; Grayson 2005; Fleishman and Dobkin 2009), habitat alteration and subsequent loss of native sagebrush and grass communities from the introduction of nonnative plant species (e.g., cheatgrass; Knapp 1996; Pellant et al. 2004), altered frequencies of wild fires (Whisenant 1990; Miller and Rose 1999), and the cultivation and irrigation of sandy basins (Hafner and Hafner 1998a, 1998b). These threats have resulted in the reduction of available habitat in the Great Basin, which can negatively affect those populations and species isolated within basins or on mountaintops, possibly leading to their extinction (Visser 2008; Blois and Hadly 2009).

Kangaroo mice (Heteromyidae: *Microdipodops*) may be especially at risk. These rodents are endemic to western North America and are restricted to the Great Basin Desert (Figs. 1 and 2; Hall 1941; Hafner 1981; Hafner et al. 1996). Only two species of *Microdipodops* are currently recognized: *M. megacephalus* Merriam (the dark kangaroo mouse) and *M. pallidus* Merriam (the pallid kangaroo mouse; Hafner 1981; Hafner and Hafner 1983; Patton 2005). Both kangaroo mouse species are rare and among the least abundant nocturnal rodents of the Great Basin (Hall 1941; Hafner et al. 2006, 2008; Hafner and Upham 2011). Low species diversity and rarity within *Microdipodops* may be due to ecological specializations; kangaroo mice are restricted to open habitats with xeric, sandy soils and have tight associations with specific flora. *Microdipodops megacephalus* is found primarily in association with sagebrush (*Artemisia* Linneaus) and/or rabbit brush (*Chrysothamnus* Nuttall), having a broad tolerance for various amounts of gravel overlay in sandy soils, and occupying generally higher elevations and a larger geographic range compared to *M. pallidus* (Hafner and Upham 2011). *Microdipodops pallidus* is considered highly specialized, occupying a rather small geographic range and preferring deeper and finer soils (no gravel overlay) in association with greasewood (*Sarcobatus* Nees von Esenbeck) and saltbush (Atriplex Linnaeus; Hall 1941; Hafner 1981; Hafner et al. 2008). These specific habitat requirements, the tectonic history of the Great Basin, and recent anthropogenic changes have resulted in a highly fragmented distribution for both *Microdipodops* species (Figs. 1 and 2).

Due to these impending threats, kangaroo mice have been the topic of several recent phylogeography studies (Hafner et al. 2006, 2008; Hafner and Upham 2011). These studies support the rarity of both *M. megacephalus* and *M. pallidus* (2.67% and 2.88% trapping success, respectively) and indicate that the abundance of both species is decreasing (Hafner and Upham 2011). Although their common names imply otherwise, kangaroo mice are quite similar morphologically. Genetically, however, kangaroo mice represent rather ancient lineages that are genetically isolated (Hafner et al. 1979, 2007). Recent studies using molecular sequence data also have found morphologically cryptic lineages within *M. megacephalus* and *M. pallidus*. There are likely four cryptic lineages within *M. megacephalus* (the allopatric eastern, western, central, and Idaho clades in Fig. 1; both the eastern and central clades can be further subdivided into two geographical subunits; Hafner and Upham 2011) and two cryptic lineages within *M. pallidus* (the parapatric western and eastern clades in Fig. 2; the eastern clade is further subdivided into three geographical subunits; Hafner et al. 2008). These studies also found that kangaroo mice diverged much earlier than expected (Hafner et al. 2007, 2008; Hafner and Upham 2011), and may have invaded the Great Basin with considerable genetic diversity coincident with the formation of sandy habitats in the Pleistocene (Smith 1982; Mehringer 1986). After this initial invasion, it is probable that populations of both *M. megacephalus* and *M. pallidus* were restricted to favorable sandy habitat patches and underwent further genetic divergence. Analyses of haplotype sharing further revealed that populations of kangaroo mice have adjusted their distributions and that particular areas (such as southern Nevada) may have served as refugia in response to past climate changes (Hafner et al. 2008; Hafner and Upham 2011).

Because of geophysical and genetic isolation of small populations in the face of habitat alteration, kangaroo mice are ideal model systems for studying the effects of population structuring in a changing environment; these taxa are in dire need of an assessment of genetic diversity for conservation purposes. It is the goal of this study to identify the major genetic units (based on mitochondrial DNA) within

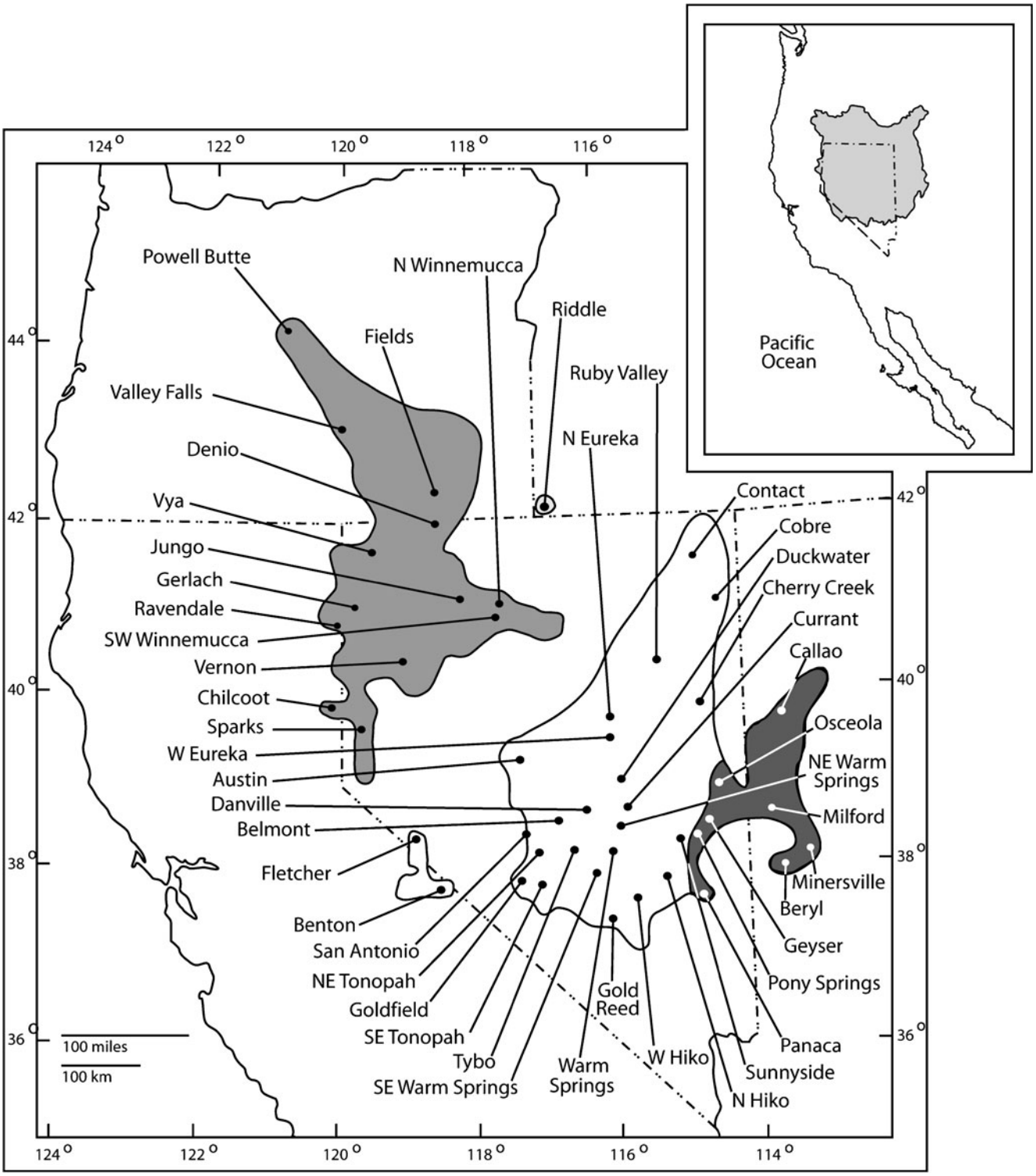

Fig. 1 Geographic distribution of dark kangaroo mouse, *Microdipodops megacephalus*, redrawn from Hafner and Upham (2011). Western North America and the Great Basin Desert (shaded area; Cronquist et al. 1972) are shown in the inset map. The outline of the state of Nevada is shown in both maps for orientation. Filled circles indicate the 47 sampling localities and the shaded regions correspond to the four principal clades identified in Hafner and Upham (2011). The medium gray, light gray, white, and dark gray regions correspond to the western, Idaho, central, and eastern clades, respectively

the dark and the pallid kangaroo mouse, and within each genetic group, to examine historical demographic patterns and identify possible conservation units for management purposes.

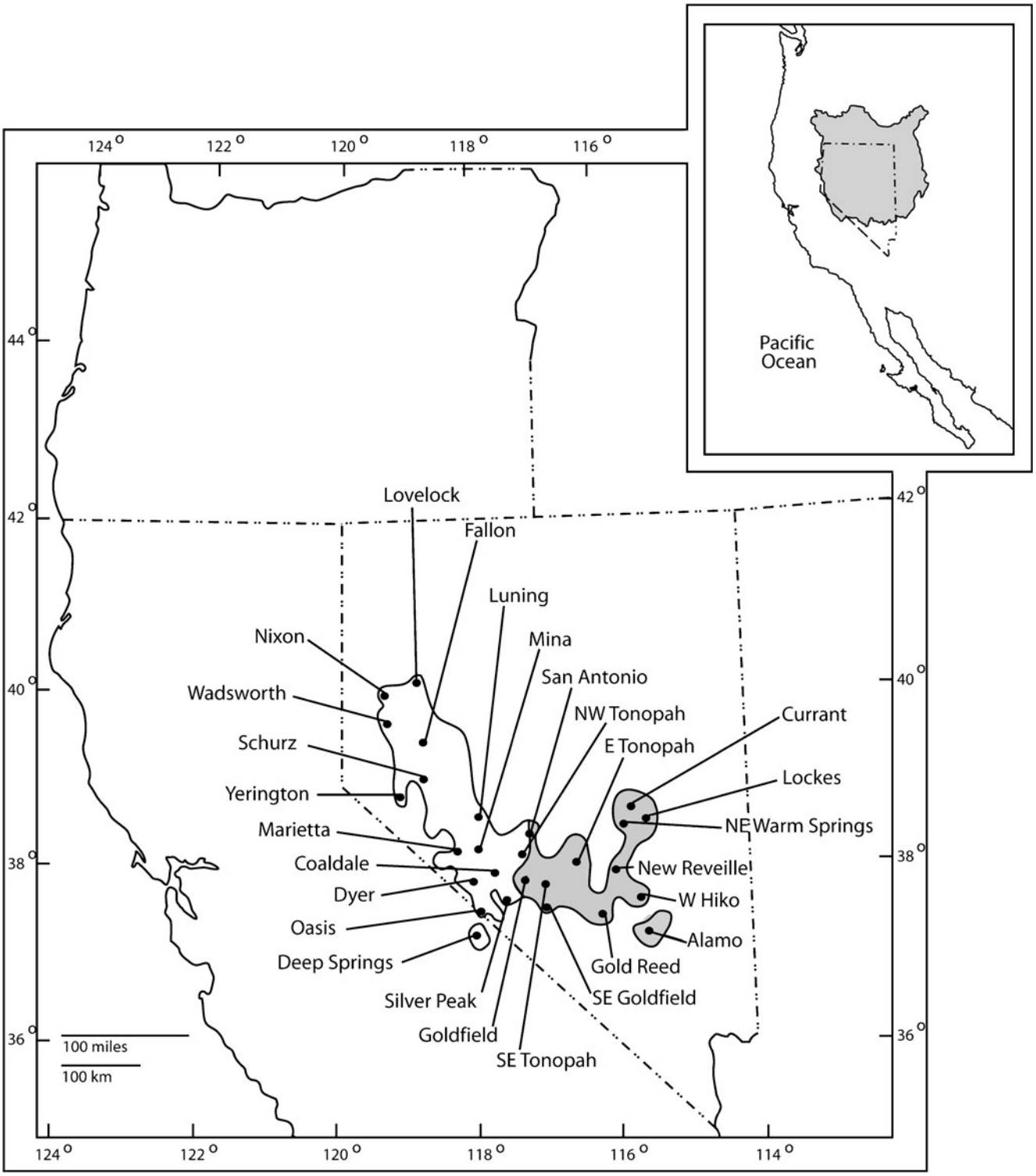

Fig. 2 Geographic distribution of the pallid kangaroo mouse, *Microdipodops pallidus*, redrawn from Hafner et al. (2008). Western North America and the Great Basin Desert (shaded area; Cronquist et al. 1972) are shown in the inset map. The outline of the state of Nevada is shown in both maps for orientation. Filled circles indicate the 27 sampling localities and the shaded regions correspond to the two principal clades identified Hafner et al. (2008). The white and dark gray regions correspond to the western and eastern clades, respectively

Materials and Methods

Specimens Examined

One hundred eighty-six specimens of *M. megacephalus* from 47 localities, and 98 specimens of *M. pallidus* from 27 localities were examined to document genetic diversity within the genus *Microdipodops* (Table 1; Figs. 1 and 2). Three mitochondrial gene fragments were analyzed: 16S ribosomal RNA (16S; 542 base pairs [bp]), cytochrome *b* (*Cytb*; 403 bp), and transfer RNA for glutamic acid and five noncoding bases ($tRNA^{Glu}$; 45 bp). All kangaroo mouse mitochondrial data were previously collected in studies conducted by Hafner et al. (2006) and Hafner and Upham (2011; *M. megacephalus*), and Hafner et al. (2008; *M.* pallidus) and are freely available on GenBank. The analyses performed here differ from previous studies in their focus on population genetics compared to phylogenetic analyses. Construction of haplotype networks and population genetic analyses are more appropriate to tease apart the evolutionary history of recent divergences such as the genetic groups within each *Microdipodops* species (Clement et al. 2000; Posada and Crandall 2001; Excoffier and Heckel 2006). For all analyses listed below, 16S (542 bp) and *Cytb* (403 bp) were analyzed separately, as well as in a combined framework with $tRNA^{Glu}$ and the five noncoding bases for a total of 990 bp.

Network, Population Genetic, and Historical Demographic Analyses

In 2011, Hafner and Upham calculated the predicted number of haplotypes from each distributional area for both *M. megacephalus* and *M. pallidus* and determined that haplotype sampling was generally complete and thorough. Thorough haplotype sampling is a necessary prerequisite for subsequent network and population genetic analyses. Haplotype networks were constructed separately for each species of kangaroo mouse. A statistical parsimony analysis (Templeton et al. 1992) using TCS 1.21 software (Clement et al. 2000) was performed to assemble the most parsimonious haplotype tree (with linkages between taxa representing mutational events) and estimate a 95% plausible set for all haplotype connections.

The computer programs Arlequin v3.5 (Excoffier et al. 2005) and DnaSP (v. 5.1; Rozas et al. 2003) were used to calculate a variety of population genetic statistics including haplotype diversity (h), nucleotide diversity (π), uncorrected genetic distance, a measure of genetic divergence (FST; Weir and Cockerham 1984), and Tajima's D test of selective neutrality (Tajima 1989). Population structure and population pairwise ϕ_{ST} values were assessed with an analysis of molecular variance (AMOVA; Excoffier et al. 1992). In these analyses, populations were defined a priori as one panmictic population of *M. megacephalus*, one panmictic population of *M. pallidus*, and by *M. megacephalus* and *M. pallidus* mitochondrial clades (treated as populations) as determined by Hafner and Upham (2011; Table 1) and Hafner et al. (2008; Table 1), respectively. Significance was assessed by 10,000 randomization replicates.

We used the program SAMOVA 1.0 (Dupanloup et al. 2002) to conduct a spatial analysis of molecular variance to attempt to detect genetic barriers between inferred populations (general localities; Table 1). Due to program requirements, only unique haplotypes were analyzed (88 for *M. megacephalus* and 42 for *M. pallidus*). These unique haplotypes were the same analyzed in Hafner and Upham (2011) and Hafner et al. (2008). SAMOVA aims to identify groups of populations (K) that are maximally differentiated from each other (F_{CT} index) without defining populations a priori. Analyses were run for 10,000 iterations and 100 initial conditions for K=1, 2, 3, …, 10 groups. To assess possible correlation between population pairwise genetic distance and geographical distance, Mantel tests were performed using the program Alleles In Space (AIS; Miller 2005). Geographic distances were calculated by hand from geographic coordinates prior to the SAMOVA and Mantel tests.

To examine the demographic history of both *M. megacephalus* and *M. pallidus* and each mitochondrial clade (treated as a population), the smoothness of the observed haplotype frequency distribution was quantified using Harpending's raggedness index (Harpending 1994) and Fu's F_S test of selective neutrality (Fu 1997) in Arlequin; these measures distinguish between populations recently expanded and stationary populations. The shape of the mismatch distribution also was examined as it indicates a recent population expansion (unimodal distribution; Rogers and Harpending 1992) or a relatively stable demographic history (bimodal or multimodal distribution; Ray et al. 2003). The adequacy of an observed mismatch distribution to fit the expected model of demographic expansion was evaluated statistically by use of the mismatch sum of squared deviations (SSD) and the raggedness index.

The demographic history of each *Microdipodops* clade (except the Idaho clade) as determined by Hafner et al. (2008; Table 1) and Hafner and Upham (2011; Table 1) was examined using Bayesian skyline plot (BSP) reconstructions run in BEAST v1.6.1 (Drummond and Rambaut 2007). The coalescent-based BSP analyses use a population-level set of DNA sequences to estimate (i) changes in effective population size through time, and (ii) time of coalescence for all included haplotypes (Drummond et al. 2005). The resulting graphical depiction of past population dynamics includes credibility intervals (95% HPD) of

Table 1 Localities (listed alphabetically by general locality), number of samples (*n*), museum vouchers, and mitochondrial clades (determined by Hafner and Upham (2011) and Hafner et al. (2008)) of *Microdipodops megacephalus* and *M. pallidus* specimens examined in this study. Museum abbreviations are as follows: Moore Laboratory of Zoology (MLZ, Occidental College), Museum of Southwestern Biology (MSB, University of New Mexico), Monte L. Bean Life Science Museum (BYU, Brigham Young University), San Diego Natural History Museum (SDNHM), Idaho Museum of Natural History (IMNH, Idaho State University), and the Museum of Vertebrate Zoology (MVZ, University of California, Berkeley)

Locality	*n*	Museum Vouchers	Clade
Microdipodops megacephalus			
Austin: 6.2 mi S, 19.6 mi W Austin, 6150 ft, Lander Co., Nevada	4	MLZ 1748–1751	Central
Belmont: 3.2 mi N, 4.2 mi E Belmont, 7000 ft, Nye Co., Nevada	4	MLZ 2027–2030	Central
Benton: 5 mi N Benton, 5600 ft, Mono Co., California	6	MLZ 1740–1742, MLZ 1915–1917	Central
Beryl: 0.7 mi N, 6.3 mi E Beryl, 5125 ft, Iron Co., Utah	8	MLZ 2145–2152	Eastern
Callao: 7.7 mi S, 2.7 mi E Callao, 4500 ft, Juab Co., Utah	2	MSB 35599, 35600	Eastern
Callao: 5.5 mi S, 7.8 mi E Callao, 4400 ft, Juab Co., Utah	1	MSB 35602	Eastern
Cherry Creek: 7.2 mi N, 8.8 mi E Cherry Creek, 5850 ft, White Pine Co., Nevada	1	MLZ 1965	Central
Chilcoot: 1.7 mi N Chilcoot, 5100 ft, Plumas Co., California	1	MLZ 1756	Western
Chilcoot: 1.5 mi N Chilcoot, 5100 ft, Plumas Co., California	1	MVZ 158930	Western
Cobre: 0.9 mi S, 0.4 mi W Cobre, 5900 ft, Elko Co., Nevada	2	MLZ 2067, 2068	Central
Contact: 10.9 mi S, 2.5 mi W Contact, 5700 ft, Elko Co., Nevada	2	MLZ 2069, 2070	Central
Currant: 4.9 mi S, 28.2 mi W Currant, 6000 ft, Nye Co., Nevada	2	MLZ 2005, 2006	Central
Danville: 6.1 mi S, 2.4 mi E Danville, 6800 ft, Nye Co., Nevada	3	MLZ 2021–2023	Central
Denio: 0.6 mi S Denio, 4200 ft, Humboldt Co., Nevada	2	MSB 35530, 35531	Western
Duckwater: 8.4 mi N, 17.5 mi W Duckwater, 6350 ft, Nye Co., Nevada	3	MLZ 1997–1999	Central
N Eureka: 22.8 mi N, 3.6 mi W Eureka, 5850 ft, Eureka Co., Nevada	4	MLZ 1956, 1957, MSB 35526, 35527	Central
W Eureka: 6.2 mi N, 9.5 mi W Eureka, 6000 ft, Eureka Co., Nevada	2	MLZ 2031, 2032	Central
Fields: 2.4 mi N, 3.4 mi E Fields, 4050 ft, Harney Co., Oregon	9	MLZ 2007–2015	Western
Fletcher: 1/4 mile N Fletcher, 6100 ft, Mineral Co., Nevada	2	MLZ 1744, 1745	Central
Gerlach: 28.5 mi N, 27.8 mi W Gerlach, 4700 ft, Washoe Co., Nevada	5	MLZ 2089–2093	Western
Gerlach: 28.2 mi N, 27.6 mi W Gerlach, 4700 ft, Washoe Co., Nevada	5	MLZ 2094–2098	Western
Gerlach: 24.5 mi N, 25.0 mi W Gerlach, 4800 ft, Washoe Co., Nevada	1	MLZ 2099	Western
Gerlach: 24.0 mi N, 24.8 mi W Gerlach, 4800 ft, Washoe Co., Nevada	5	MLZ 2100–2104	Western
Gerlach: 22.4 mi N, 23.6 mi W Gerlach, 4800 ft, Washoe Co., Nevada	5	MLZ 2105–2109	Western
Geyser: 5.3 mi S, 1.6 mi E Geyser, 5900 ft, Lincoln Co., Nevada	2	MLZ 1974, 1975	Eastern
Geyser: 5.2 mi S, 1.9 mi E Geyser, 5900 ft, Lincoln Co., Nevada	4	MLZ 1976–1979	Eastern
Geyser: 5.1 mi S, 2.3 mi E Geyser, 5900 ft, Lincoln Co., Nevada	4	MLZ 1980–1983	Eastern
Goldfield: 12.0 mi N, 2.5 mi W Goldfield, 4860 ft, Esmeralda Co., Nevada	1	MLZ 1747	Central
Gold Reed: 2.9 mi S, 3.1 mi E Gold Reed, 5350 ft, Nye Co., Nevada	1	MLZ 2053	Central
Gold Reed: 2.9 mi S, 4.0 mi E Gold Reed, 5330 ft, Nye Co., Nevada	5	MLZ 2054–2058	Central
N Hiko: 31 mi N, 1 mile W Hiko, 5100 ft, Lincoln Co., Nevada	1	MLZ 1960	Central
W Hiko: 6 mi N, 31 mi W Hiko, 4800 ft, Lincoln Co., Nevada	2	MLZ 1815, 1816	Central
Jungo: 13.8 mi N, 11.2 mi E Jungo, 4200 ft, Humboldt Co., Nevada	5	MLZ 2124–2128	Western
Milford: 16.1 mi S, 19.6 mi E Garrison, 5400 ft, Millard Co., Utah	3	MLZ 2079–2081	Eastern
Milford: 19.3 mi S, 18.4 mi E Garrison, 5100 ft, Millard Co., Utah	6	MLZ 2082–2087	Eastern
Milford: 11.2 mi N, 39.6 mi W Milford, 5200 ft, Beaver Co., Utah	1	MLZ 2088	Eastern
Minersville: 4.2 mi S, 15.8 mi W Minersville, 5050 ft, Beaver Co., Utah	8	MLZ 2071–2078	Eastern
Minersville: Escalante Desert, 38°09.118′ N, 113°12.946′ W, 1540 m, Beaver Co., Utah	2	BYU 30100, 30101	Eastern
Osceola: 6.0 mi S, 4.2 mi W Osceola, 5800 ft, White Pine Co., Nevada	3	MLZ 1942–1944	Eastern
Panaca: 24 mi W Panaca, 4600 ft, Lincoln Co., Nevada	4	MLZ 1752–1755	Eastern
Pony Springs: 9.0 mi N, 10.8 mi W Pony Springs, 6020 ft, Lincoln Co., Nevada	2	MLZ 2059, 2060	Eastern
Powell Butte: Becker Ranch, Powell Butte, Crook Co., Oregon	1	SDNHM 16431	Western
Ravendale: 4.4 mi N, 13.6 mi E Ravendale, 5650 ft, Lassen Co., California	3	MLZ 2110–2112	Western
Ravendale: 4.7 mi N, 10.8 mi E Ravendale, 5350 ft, Lassen Co., California	2	MLZ 2113–2114	Western

Table 1 (continued)

Locality	*n*	Museum Vouchers	Clade
Riddle: Starr Valley, NW ¼ Section 19, T16S, R5W, B.M., Owyhee Co., Idaho	1	IMNH 259	Idaho
Riddle: 1/2 mi N Nevada, 2 1/2 mi E Oregon, Owyhee Co., Idaho	1	IMNH 693	Idaho
Ruby Valley: 13.2 mi S, 0.6 mi E Ruby Valley, 6000 ft, Elko Co., Nevada	1	MLZ 2033	Central
San Antonio: 3.7 mi N, 3.2 mi E San Antonio, 5600 ft, Nye Co., Nevada	2	MLZ 1761, 1762	Central
Sparks: 6 mi N, 4 mi E Sparks, 4600 ft, Washoe Co., Nevada	3	MLZ 1757–1759	Western
Sunnyside: 1.3 mi S, 4.9 mi W Sunnyside, 5200 ft, Nye Co., Nevada	1	MLZ 1966	Central
NE Tonopah: 13.8 mi N, 7.9 mi E Tonopah, 5800 ft, Nye Co., Nevada	4	MLZ 1961–1964	Central
SE Tonopah: 9.8 mi S, 9.9 mi E Tonopah, 5200 ft, Nye Co., Nevada	1	MLZ 1831	Central
Tybo: 1.0 mi N, 8.5 mi W Tybo, 6200 ft, Nye Co., Nevada	2	MLZ 1799, 1800	Central
Valley Falls: 36 mi N, 14 mi E Valley Falls, 4300 ft, Lake Co., Oregon	10	MLZ 1987–1996	Western
Vernon: 0.5 mi S, 11.5 mi W Vernon, 4450 ft, Pershing Co., Nevada	1	MLZ 1760	Western
Vya: 3.2 mi N, 11.5 mi E Vya, 5600 ft, Washoe Co., Nevada	3	MLZ 1984–1986	Western
Warm Springs: 5.9 mi N, 10.2 mi E Warm Springs, 5200 ft, Nye Co., Nevada	1	MLZ 2024	Central
Warm Springs: 6.4 mi N, 10.1 mi E Warm Springs, 5200 ft, Nye Co., Nevada	1	MLZ 2025	Central
Warm Springs: 7.7 mi N, 9.5 mi E Warm Springs, 5200 ft, Nye Co., Nevada	1	MLZ 2026	Central
NE Warm Springs: 19.2 mi N, 13.4 mi E Warm Springs, 6000 ft, Nye Co., Nevada	5	MLZ 1905, MLZ 1948–1951	Central
SE Warm Springs: 12.7 mi S, 0.4 mi E Warm Springs, 6000 ft, Nye Co., Nevada	5	MLZ 1968–1972	Central
N Winnemucca: 7 mi N Winnemucca, 4600 ft, Humboldt Co., Nevada	2	MSB 35533, 35534	Western
SW Winnemucca: 5.5 mi S, 9.2 mi W Winnemucca, 4300 ft, Humboldt Co., Nevada	1	MSB 35535	Western
Microdipodops pallidus			
Alamo: 4.5 mi S, 32.5 mi W Alamo, 4600 ft, Lincoln Co., Nevada	3	MSB 35536–35538	Eastern
Coaldale: 1.8 mi S, 5.3 mi E Coaldale, 4797 ft, Esmeralda Co., Nevada	1	MLZ 1817	Western
Currant: 4.9 mi S, 28.2 mi W Currant, 6000 ft, Nye Co., Nevada	5	MLZ 2000–2004	Eastern
Deep Springs: 7.2 mi S, 4.0 mi W Deep Springs, 4920 ft, Inyo Co., California	2	MLZ 1767, 1768	Western
Deep Springs: 4.6 mi S, 3.9 mi W Deep Springs, 5000 ft, Inyo Co., California	2	MLZ 1769, 1770	Western
Deep Springs: 2.4 mi S, 2.3 mi W Deep Springs, 5050 ft, Inyo Co., California	6	MLZ 1771–1776	Western
Dyer: 7.0 mi N, 0.5 mi W Dyer, 4900 ft, Esmeralda Co., Nevada	5	MLZ 1785–1789	Western
Fallon: 4.3 mi N Fallon, 3900 ft, Churchill Co., Nevada	1	MLZ 1947	Western
Goldfield: 12.0 mi N, 2.5 mi W Goldfield, 4860 ft, Esmeralda Co., Nevada	2	MLZ 1743, 1746	Eastern
SE Goldfield: 4.6 mi S, 19.8 mi E Goldfield, 4950 ft, Nye Co., Nevada	2	MLZ 2051, 2052	Eastern
Gold Reed: 3.0 mi S, 4.3 mi E Gold Reed, 5330 ft, Nye, Co., Nevada	2	MLZ 1958, 1959	Eastern
W Hiko: 6 mi N, 31 mi W Hiko, 4800 ft, Lincoln Co., Nevada	4	MLZ 1811–1814	Eastern
Lockes: 9.6 mi S, 3.8 mi W Lockes, 4800 ft, Nye Co., Nevada	4	MLZ 2017–2020	Eastern
Lovelock: 2.5 mi N, 22.5 mi W Lovelock, 3950 ft, Pershing Co., Nevada	1	MLZ 1967	Western
Luning: 9.8 mi N, 10.8 mi E Luning, 5350 ft, Mineral Co., Nevada	5	MLZ 1805–1809	Western
Luning: 12.7 mi N, 9.2 mi E Luning, 5050 ft, Mineral Co., Nevada	1	MLZ 1810	Western
Marietta: 0.4 mi S, 0.5 mi E Marietta, 4950 ft, Mineral Co., Nevada	3	MLZ 1777–1779	Western
Mina: 8.9 mi S, 1.2 mi E Mina, 4400 ft, Mineral Co., Nevada	5	MLZ 1780–1784	Western
New Reveille: 0.9 mi N, 10.3 mi E New Reveille, 4900 ft, Nye Co., Nevada	2	MLZ 1940–1941	Eastern
Nixon: 6.4 mi N, 1.0 mi W Nixon, 4200 ft, Washoe Co., Nevada	1	MLZ 1794	Western
Oasis: 0.2 mi S, 1.5 mi E Oasis, 5050 ft, Mono Co., California	2	MLZ 1790, 1791	Western
Oasis: 1.0 mi S, 4.0 mi E Oasis, 5100 ft, Mono Co., California	2	MLZ 1792, 1793	Western
San Antonio: 0.5 mi S San Antonio, 5400 ft, Nye Co., Nevada	3	MLZ 1796–1798	Eastern, Western
Schurz: 7.3 mi N, 2.6 mi W Schurz, 4287 ft, Mineral Co., Nevada	3	MLZ 1818–1820	Western
Silver Peak: 5.1 S, 1.1 mi E Silver Peak, 4300 ft, Esmeralda Co., Nevada	2	MLZ 1945, 1946	Western
E Tonopah: 0.5 mi N, 32.0 mi E Tonopah, 5600 ft, Nye Co., Nevada	4	MLZ 1801–1804	Eastern
NW Tonopah: 9.2 mi N, 8.1 mi W Tonopah, 4850 ft, Nye Co., Nevada	1	MLZ 1973	Western

Table 1 (continued)

Locality	*n*	Museum Vouchers	Clade
SE Tonopah: 11.0 mi S, 10.0 mi E Tonopah, 5200 ft, Nye Co., Nevada	5	MLZ 1821–1825	Eastern
SE Tonopah: 10.6 mi S, 10.0 mi E Tonopah, 5200 ft, Nye, Co., Nevada	5	MLZ 1826–1830	Eastern
Wadsworth: 1.0 mi N, 1.0 mi W Wadsworth, 4200 ft, Washoe Co., Nevada	1	MLZ 1795	Western
NE Warm Springs: 19.2 mi N, 13.4 mi E Warm Springs, 6000 ft, Nye Co., Nevada	5	MLZ 1906, 1952–1955	Eastern
Yerington: 11.7 mi S, 3.5 mi E Yerington, 4690 ft, Lyon Co., Nevada	3	MLZ 1832–1834	Western
Yerington: 11.1 mi S, 2.8 mi E Yerington, 4640 ft, Lyon Co., Nevada	5	MLZ 1835–1839	Western

combined phylogenetic and coalescent uncertainty. Analyses were run using a piecewise-constant Bayesian skyline coalescent tree prior with the number of groups set as the number of taxa divided by five to avoid model over-parameterization (Ho and Shapiro 2011). Previous studies have supported clock-like evolution for the combined dataset (16S and *Cytb*) for both *Microdipodops* taxa (Hafner et al. 2008; Hafner and Upham 2011). Therefore, we used a rate estimate of 0.0903 substitutions/site (Hafner and Upham 2011) and two mean age estimates to calculate a *Microdipodops*-specific substitution rate. These age estimates were 7.05 million years ago (Ma; Hafner and Upham 2011) and 8.06 Ma (Hafner et al. 2007) for the divergence of *M. pallidus* and *M. megacephalus*. Dividing the rate by twice the age estimate gave the per-lineage substitution rate of 6.4×10^9 subs/site/year and 5.6×10^9 subs/site/year, respectively. Fixed-rate analyses were run using a strict molecular clock and the GTR+I+G model of nucleotide substitution as deemed most appropriate using MrModeltest 2.3 (Nylander 2004). Trees resulting from runs of 1×10^7 and 3×10^7 generations were combined after 10-20% burnin to reach stable parameter estimates (i.e., all runs were checked for convergence, effective sample sizes>100, and sufficient mixing), and used to generate BSP reconstructions in Tracer v 1.5 (Rambaut and Drummond 2007). All data were re-plotted to common timescales using the R computer programming language (R Development Core Team 2011).

Results

Only results from the combined 990 bp dataset are presented below. Results from analyses of individual genes are similar to those of the combined dataset and are available upon request. As in previous findings, 16S was less informative than the other mitochondrial gene fragment (Hafner et al. 2006, 2008; Hafner and Upham 2011).

Network Analyses

Network analyses of the combined dataset for *M. megacephalus* result in 88 haplotypes when gaps were treated as a fifth character (87 haplotypes when gaps were treated as missing data) and four unconnected subnetworks (separated by 13 or more steps; Fig. 3a–d). These four unconnected subnetworks correspond to the eastern, central, western, and Idaho clades in Hafner and Upham (2011). No haplotypes are shared among the four units, and only one haplotype is found in the Idaho clade. Similar to what Hafner and Upham (2011) found in their phylogenetic study, the eastern and central clades are each further subdivided into two subclades. Of the two subdivisions in the eastern *M. megacephalus* clade (25 haplotypes; Fig. 3a), one is primarily a western subclade including Geyser, Milford, Minersville, Osceola, Panaca, and Pony Springs, and the other is an eastern subclade consisting of the localities of Beryl, Callao, Geyser, Milford, and Minersville (Fig. 1). Haplotypes from Geyser, Minersville, and Milford are found in both subclades (shown in gray in Fig. 3a). The most common (i.e., ancestral) haplotype in the eastern clade consists of nine individuals from the localities of Beryl and Minersville. Three of the haplotypes (12%) are present at two or more of the sampled localities and all localities are represented by more than one haplotype. The central *M. megacephalus* clade (39 haplotypes; Fig. 3b) is divided into two parts: a central subclade and a western subunit. Note that haplotypes from the Belmont, N Eureka, and NE Tonopah localities are found in both subunits (Fig. 1; shown in gray in Fig. 3b). The western subunit is the smaller of the two subunits in the central clade and consists primarily of private haplotypes from the western end of the distribution (including the localities Fletcher and Benton which represent a disjunct population in the Mono Basin of California and Nevada; Fig. 1). Five of the haplotypes (13%) within the central clade are present at two or more localities, and 72% of the sampled localities are represented by more than one haplotype. The ancestral haplotype in the central clade consists of

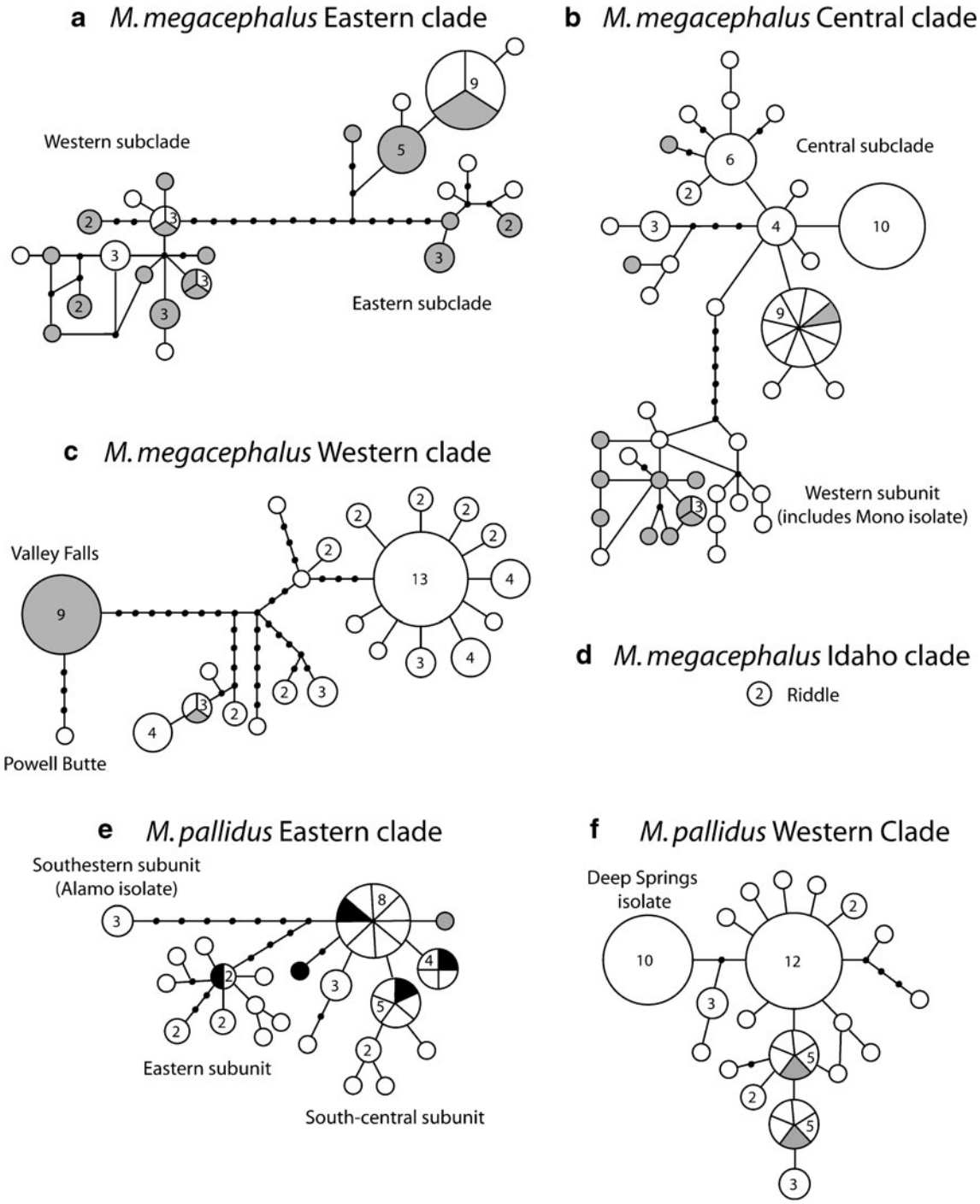

Fig. 3 Statistical parsimony haplotype network for dark (*Microdipodops megacephalus*; **a-d**) and pallid (*M. pallidus*; **e-f**) kangaroo mice based on the combined 990 bp mitochondrial dataset. Network analyses for *M. megacephalus* support four unconnected subnetworks which correspond to the eastern (**a**), central (**b**), western (**c**), and Idaho (**d**) clades in Hafner and Upham (2011). No haplotypes are shared among the four *M. megacephalus* clades, although haplotypes are shared among subclades within the eastern (**a**) and central (**b**) clades (*shown in gray*). Gray shading in the western *M. megacephalus* clade (**c**) corresponds to the 2 Valley Falls haplotypes. Network analyses of *M. pallidus* support two unconnected subnetworks which correspond to the eastern (**e**) and western (**f**) clades in Hafner et al. (2008). No haplotypes are shared between the two *M. pallidus* clades, but specimens from the same locality (San Antonio) are found in both clades (*shown in gray*). In the eastern clade (**e**), specimens from NE Warm Springs are found in both the eastern and south-central subunits (*shown in black*). Each connection represents a single mutational step with inferred haplotypes represented by small black circles. Observed haplotypes are shown as large circles with haplotype frequency indicated within each circle

ten individuals from multiple localities: Gold Reed, SE Tonopah, Sunnyside, Warm Springs, NE Warm Springs, and SE Warm Springs (Figs. 1 and 3b). Another common haplotype consisting of nine individuals from the central subclade is also distributed across multiple localities (Currant, Goldfield, Gold Reed, W Hiko, NE Tonopah, and Warm Springs). In the western *M. megacephalus* clade (23 haplotypes; Fig. 3c), the northernmost localities (Powell Butte and Valley Falls) make up a separate subunit of two haplotypes. Valley Falls is represented by a total of two haplotypes and the second haplotype is shared with specimens from Denio and Jungo (shown in gray in Fig. 3c). The other 11 localities are distributed throughout the remainder of the network. The most common haplotype in the western clade consists of 13 individuals from multiple localities (Fields, Gerlach, Ravendale, and Vya), and 4 of the haplotypes (17%) are present at two or more sampling localities (Figs. 1 and 3c). The majority of the sampling localities (62%) consist of more than one haplotype.

Network analyses of the combined dataset for *M. pallidus* result in 42 haplotypes (when gaps were treated as either missing data or a fifth character) and two unconnected subnetworks (separated by 13 or more steps; Fig. 3e–f). These two unconnected subnetworks correspond to the eastern and western clades in Hafner et al. (2008). Although no haplotypes are shared between the two units, specimens from the same locality (San Antonio) are found in both subnetworks (shown in gray in Fig. 3e–f). In the eastern *M. pallidus* clade (22 haplotypes; Fig. 3e), six of the haplotypes (27%) are present at two or more localities, a majority of the sampled localities (75%) are represented by more than one haplotype, and the most common haplotype consists of eight individuals from multiple localities (Goldfield, SE Goldfield, E Tonopah, SE Tonopah, and NE Warm Springs). There are three subunits within the eastern clade: 1) a southeastern subunit with a divergent haplotype from the isolated Alamo locality, 2) an eastern subunit consisting of ten haplotypes from Gold Reed, W Hiko, Lockes, New Reveille, and NE Warm Springs and, 3) a south-central subunit with 11 haplotypes from Currant, Goldfield, SE Goldfield, SE Tonopah, San Antonio, and NE Warm Springs (Figs. 2 and 3e). The distribution of these three subunits, however, does not correspond perfectly to geography and specimens from the NE Warm Springs are found in both the eastern and south-central subunits (Fig. 2; shown in black in Fig. 3e). In contrast to the eastern clade, there is little structure within the western *M. pallidus* clade (Fig. 3f). Three of the haplotypes (15% of the 20 haplotypes) are present at two or more of the sampled localities, and 56% of the sampled localities are represented by more than one haplotype. The ancestral haplotype in the western clade consists of 12 individuals from Coaldale, Dyer, Luning, Marietta, Mina, Schurz, and Silver Peak. There is also another common haplotype consisting of ten individuals all from the isolated Deep Springs locality (Figs. 2 and 3f).

Population Genetic and Historical Demographic Analyses

Analysis of molecular variation (AMOVA) of *M. megacephalus* reveals significant population structuring with 83.87% of the variation distributed among the groups (pairwise ϕ_{ST}=0.839; Table 2). When populations are defined as mitochondrial clades, all ϕ-statistics are significant with the majority of the variation (82.71%) allocated among groups (Table 2). Pairwise estimates of mitochondrial DNA F_{ST} for mitochondrial clades calculated in DnaSP range from 0.651 (eastern and central clades) to 0.869 (central and Idaho clades).

The program SAMOVA determines that the best partitioning scheme of genetic diversity for *M. megacephalus* is obtained with K=6–8. These results are in agreement with phylogenetic and other population analyses (see above) corresponding to the four mitochondrial clades and additional divisions within each clade. At K=6, 7, and 8, F_{CT} values were 0.829, 0.843, and 0.857, respectively; all values are statistically significant (P<0.05). Subdivisions greater than K=8 did not produce additional informative clusters, mostly identifying geographically isolated populations. Mantel tests detect significant correlation between geographical distance and population pairwise genetic distance when all samples from each mitochondrial clade are analyzed (r=0.792, P<0.001). When clades are analyzed individually, isolation by distance also is detected (r values ranged from 0.307 to 0.50; P<0.05). These results support increased genetic divergence over geographic distance and confirm the presence of genetically isolated clades and sharing of genetic information within clades.

Similarly, AMOVA of *M. pallidus* finds significant population structuring with 92.34% of the variation distributed among the groups (pairwise ϕ_{ST}=0.9234; Table 2). When populations are defined as mitochondrial clades, the majority of the variation (91.85%) is allocated among groups and all ϕ-statistics are significant (Table 2). Pairwise estimate of mitochondrial DNA F_{ST} calculated in DnaSP is 0.792 between the eastern and western clades.

In agreement with previous phylogenetic and population genetic analyses (see above), the program SAMOVA determines that the best partitioning scheme of genetic diversity is obtained with K=2 corresponding to the two *M. pallidus* mitochondrial clades (F_{CT}=0.851; P<0.005). At K=3, San Antonio is recognized as a distinct partition and subdivisions greater than K=4 did not produce additional informative clusters (isolated populations were identified as K

Table 2 Analysis of molecular variance (AMOVA) indicating degree and significance of population structuring of the combined 990 bp dataset for all populations and the four mitochondrial clades (central, eastern, western, and Idaho Clades) of the dark kangaroo mouse (*Microdipodops megacephalus*), and for all populations and the two mitochondrial clades (eastern and western) of the pallid kangaroo mouse (*M. pallidus*). Significance of variance component (*P*) was tested by permutation according to Excoffier et al. (1992)

Source of Variation	Variance components	Percentage of variance	Fixation indices	P
Microdipodops megacephalus				
All populations				
Among populations	3	83.87	ϕ_{ST}=0.8387	*P*<0.0001
Within populations	182	16.13		
Mitochondrial clades				
Among clades	3	82.71	ϕ_{CT}=0.8271	*P*<0.0001
Among populations within clades	43	10.19	ϕ_{SC}=0.5898	*P*<0.0001
Within populations	139	7.09	ϕ_{ST}=0.9291	*P*<0.0001
Microdipodops pallidus				
All populations				
Among populations	1	92.34	ϕ_{ST}=0.9234	*P*<0.0001
Within populations	96	7.66		
Mitochondrial clades				
Among clades	1	91.85	ϕ_{CT}=0.9185	*P*<0.0001
Among populations within clades	26	5.47	ϕ_{SC}=0.6715	*P*<0.0001
Within populations	70	2.68	ϕ_{ST}=0.9732	*P*<0.0001

increased). Isolation by distance is detected by mantel tests when all samples from each mitochondrial clade are analyzed together (r=0.686, *P*<0.001) and when mitochondrial clades are analyzed separately (r values ranged from 0.332 to 0.426; *P*<0.001). These results confirm the presence of two genetically isolated clades as well as sharing of genetic information within each clade of *M. pallidus*.

Mismatch distribution analysis of all *M. megacephalus* samples analyzed as one panmictic population reveals a multimodal shape (Table 3) that suggests a relatively stable demographic history. Specifically, intermediate values of haplotype and nucleotide diversity as well as nonsignificant Fu's F_s and Tajima's D indicate that the overall demographic history of *M. megacephalus* is relatively stable. However, nonsignficant values for mismatch SSD and the raggedness index support the null hypotheses of population expansion for all samples of *M. megacephalus* (Table 3). When the eastern and central mitochondrial clades are examined separately as distinct populations, mismatch analyses result in bimodal distributions and nonsignificant SSD and

Table 3 Population genetic and historical demographic statistics for the combined 990 bp dataset of the dark kangaroo mouse (*Microdipodops megacephalus*) and the pallid kangaroo mouse (*M. pallidus*). Statistics are for all samples (one panmictic population) and the mitochondrial clades determined in previous phylogenetic analyses (central, eastern, western, and Idaho clades for *M. megacephalus*; eastern and western clades for *M pallidus*). Statistics include sample size (*n*), haplotype diversity (*h*), nucleotide diversity (π), Tajimas's D, Fu's F_s, mismatch distribution parameters for the sudden expansion model (sum of squared deviation, SSD; θ_0, population size before expansion; θ_1, population size after expansion; τ, expansion parameter; Harpending's raggedness index, Ragged; shape of the mismatch distribution; Mismatch). Asterisks indicate statistically significant values (*P*<0.05). Fu's F and Tajima's D tests of neutrality exclude sites with gaps

	n	*h*	π	Tajima's D	Fu's F_s	SSD	θ_0	θ_1	τ	Ragged	Mismatch
Microdipodops megacephalus											
All Samples	186	87	0.03787	1.6925	−7.2386	0.011	0	81.687	57.867	0.0037	Multimodal
Central	69	39	0.0065	−0.7344	−22.8458*	0.028	0	10.97	11.967	0.0310	Bimodal
Eastern	50	25	0.0101	0.2873	−3.7346	0.007	0.002	19.073	15.475	0.0095	Bimodal
Western	65	23	0.0099	−0.2826	−1.0689	0.036*	0.002	22.883	15.947	0.0223	Bimodal
Idaho	2	1	0	–	–	–	–	–	–	–	
Microdipodips pallidus											
All Samples	98	42	0.0276	2.1254	0.2668	0.501*	0	99999	0.375	0.01	Bimodal
Eastern	44	22	0.0058	−0.8537	−6.7362*	0.0392	0.002	11.363	10.60	0.0351	Bimodal
Western	54	20	0.0025	−1.5954*	−11.7276*	0.0046	0	99999	2.61	0.042	Unimodal

raggedness indices; thus supporting population expansions in these clades (Table 3). The central clade is notable for high haplotype diversity, low nucleotide diversity, and a significantly negative Fu's F_s, all of which are strong indicators of past population expansion (Table 3). The western clade seems to show conflicting results: a significant mismatch SSD and bimodal distribution (Table 3) indicates rejection of the null hypothesis of population expansion, but a nonsignificant raggedness index argues for acceptance of the expansion model in this lineage.

Mismatch distribution analysis of all *M. pallidus* samples analyzed as one panmictic population reveals a significant mismatch SSD, which supports rejection of the null hypothesis of significant population expansion (Table 3). Although intermediate haplotype and nucleotide diversity values as well as nonsignificant Fu's F_s and Tajima's D also suggest the lack of population expansion (Table 3), the raggedness index of the mismatch distribution of *M. pallidus* is not significant, therefore supporting the model of population expansion (Table 3). When the two mitochondrial clades are examined separately as distinct populations, a nonsignificant SSD values and raggedness indices, unimodal distributions, and significantly negative Fu's F_s values all indicate a history of demographic expansion for both the eastern and western clades of *M. pallidus* (Table 3).

Bayesian skyline plots examining the demographic history of each *Microdipodops* clade (except Idaho) yielded similar results regardless of the mean age estimate (7.05 Ma or 8.06 Ma) used to calculate the per-lineage substitution rate. BSP reconstructions differed only in the estimated time of coalescence for all extant haplotypes, with the slower rate finding consistently older times of coalescence (data available upon request). Additionally, when relaxed clock methods (uncorrelated lognormal) were used, they returned similar results to the fixed rate (strict clock) analyses with slightly broader confidence intervals (data available upon request). Results using the fixed rate of 6.4×10^9 subs/site/year are reported here.

Time of coalescence for all extant haplotypes within each *M. megacephalus* clade ranged from 0.5 to 0.75 Ma (Fig. 4a–c). Although a slight population expansion is indicated for the eastern clade and a small expansion and subsequent decline is indicated for the western clade, these population changes are not significant. However, there does appear to be a significant population expansion for the central clade. Time of coalescence for all extant haplotypes within the *M. pallidus* eastern and western clades ranged from 0.1 to 0.43 Ma (Fig. 4d–e). Although population expansions are indicated for both of the eastern and western clades of *M. pallidus*, these expansions are not significant.

Discussion

Recent molecular studies of Hafner et al. (2008) and Hafner and Upham (2011) indicate the existence of morphologically cryptic species within the two currently recognized species of *Microdipodops*. It appears that *M. megacephalus* and *M. pallidus* represent species groups containing four and two cryptic species, respectively. The results of our network, SAMOVA, and isolation by distance analyses support previous phylogenetic findings of cryptic species within *Microdipodops* (Hafner et al. 2008; Hafner and Upham 2011), corresponding to the eastern, central, western, and Idaho clades of *M. megacephalus* (Figs. 1, 3a and d) and the eastern and western clades of *M. pallidus* (Figs. 2, 3e and f). This study extends our understanding of the patterns of genetic diversity in *Microdipodops* by providing a population-genetic characterization of the major clades and an assessment of their demographic histories. Such baseline data are necessary for future conservation efforts.

Network Analyses, Population Genetics and Historical Demography

Within *M. megacephalus*, network and population genetic analyses of the eastern clade find that the most common haplotypes are from the eastern subclade (i.e., localities of Beryl and Minersville; Figs. 1 and 3a). This indicates that kangaroo mice from southwestern Utah may represent the ancestral population for the eastern clade, a possible source for recent radiations and expansions to the north and west (Table 3; Fig. 4a). Indeed, the location of this ancestral population (immediately south of the Great Salt Lake) suggests that the demographic history of the eastern clade may be linked intimately with the lacustral intervals of the ancient Pleistocene Lake Bonneville.

Within the central clade of *M. megacephalus*, kangaroo mice from multiple localities in the central subclade appear to represent the ancestral population (localities Gold Reed, SE Tonopah, Sunnyside, Warm Springs, NE Warm Springs, and SE Warm Springs; Figs. 1 and 3b) and population genetic analyses support a strong signal of population expansion (Table 3; Fig. 4b). Evidence of population expansion in the central clade also was supported in directional analyses of phylogeographic patterns (DAPP; Hafner et al. 2008) performed in Hafner and Upham (2011). These DAPP analyses uncovered a web orientation pattern of haplotype sharing for the central clade of *M. megacephalus*, indicating the presence of a southern refugium during cooler climatic periods followed by subsequent expansions to the north during warmer times (possibly during the last 100,000 years; Fig. 4b). Interestingly, kangaroo mice isolated in the

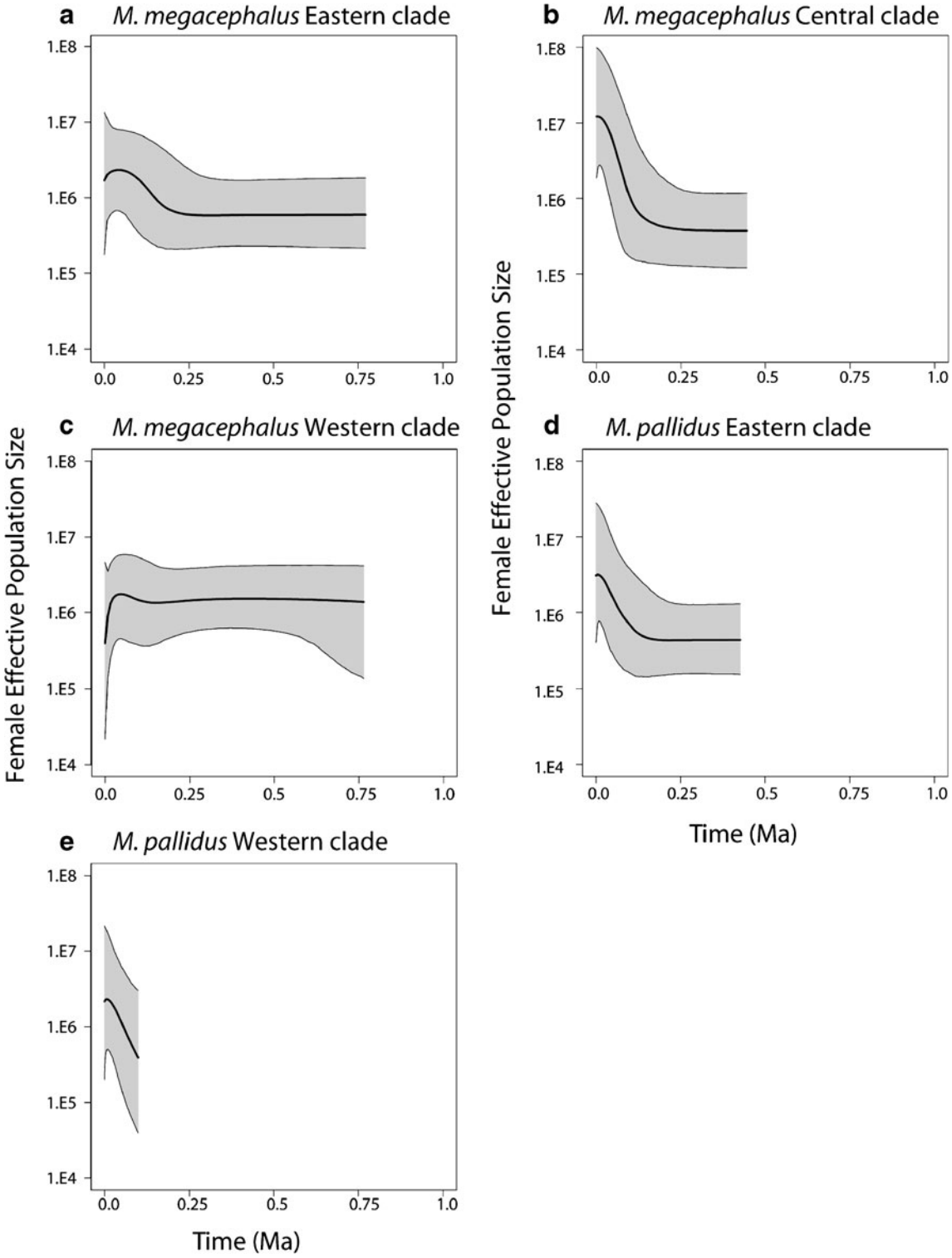

Fig. 4 Bayesian skyline plots for dark (*Microdipodops megacephalus* **a-c**) and pallid (*M. pallidus*; **d-e**) kangaroo mice based on the combined 990 bp mitochondrial dataset. For all plots, *x*-axis values are millions of years before present (Ma) and *y*-axis values are estimates of female effective population size (N_{ef}). The thick black line is the median estimate and the shaded areas correspond to the 95% HPD estimate

Mono Basin of the central clade's western subunit (localities Benton and Fletcher; Figs. 1 and 3b) consist of unique haplotypes that are not shared with other *M. megacephalus*.

In the *M. megacephalus* western clade, the ancestral population seems to be represented in northwestern Nevada (localities of Fields, Gerlach, Ravendale, and Vya; Figs. 1

and 3c), with individuals radiating both to the north and the south in recent times. DAPP analyses in Hafner and Upham (2011) also found a north-south directional trend of haplotype sharing for the western clade, indicating that populations possibly adjusted their distributions in response to climatic changes. The web orientation and north-south patterns resulting from the Hafner and Upham (2011) DAPP analyses, the findings reported here, and decreased habitat availability in the northern extremes of the central and western clades (Hafner and Upham 2011) support source-sink dynamics in these *M. megacephalus* clades. In fact, the northern populations may be at a greater risk for extinction as seen by lack of abundance in these areas (Hafner and Upham 2011) as well as lack of population expansions (Table 3) and apparent bottlenecks in the BSP reconstructions (Fig. 4c).

Our analyses of *M. pallidus* provide corroboration and enhanced understanding of the eastern and western clades that were recognized by Hafner et al. (2008). In the eastern clade of *M. pallidus*, the presumptive ancestral haplotype is found in populations occupying two disjunct regions of the south-central subunit: the locality of NE Warm Springs to the northeast and the localities of Goldfield, SE Goldfield, E Tonopah and SE Tonopah to the southwest (Fig. 2). Previous DAPP analyses found a distinct northeast-southwest directional pattern in this clade (Hafner et al. 2008), implying that kangaroo mice in this region may have adjusted their distributions in response to past climatic changes. Population genetic analyses and BSP reconstructions also indicate expansions within the eastern clade may have recently occurred (Table 3; Fig. 4d). Although only three samples were available from the peripheral isolate of Alamo (Fig. 2), these samples are genetically unique, quite diverged from the rest of the eastern clade, and show no genetic variation (Fig. 3e).

The presumed ancestral haplotype of the western clade of *M. pallidus* is distributed over a large geographic area in southwestern Nevada (localities Coaldale, Dyer, Luning, Marietta, Mina, Schurz, and Silver Peak; Fig. 2). Phylogenetic analyses (Hafner et al. 2008) indicate very little structure in the western clade, supporting possible recent population expansion in this clade (Table 3; Fig. 4e). There is slightly more phylogenetic structure in the southern portions of this clade (Fig. 3f; Hafner et al., 2008), indicating that this region may have served as a refugium during colder historical times. The distinct northwest-southeast pattern of gene exchange in the western clade reported from the DAPP analyses of Hafner et al. (2008) is consistent with this interpretation. Kangaroo mice from the peripheral location of Deep Springs are genetically distinct (minimally two mutational steps from other known haplotypes) and geographically isolated from other populations in the western clade by a rather dramatic ridge of mountains at the southern terminus of the White Mountains. It is likely that the population from Deep Springs underwent a substantial genetic bottleneck at some point in the past as evidenced by the lack of mitochondrial DNA variation based on the ten individuals sampled. DAPP analyses from Hafner et al. (2008) coupled with the results presented here imply that populations of *M. pallidus* may have adjusted their distributions in response to past climatic changes and that the southern Great Basin may have been a refugium at the height of pluvial periods (Hafner et al. 2008). These refugia and source populations probably persisted throughout the turbulent Pleistocene and were sufficiently large to preserve and accumulate nucleotide substitutions over time.

For all haplotypes examined within each mitochondrial clade, BSP reconstructions revealed only very recent demographic shifts in the last 1 Ma indicating the impact of recent pluvial history in the formation of sandy habitats and facilitating the adaptive divergence of sand-adapted organisms. However, the initial divergence of *M. megacephalus* and *M. pallidus* occurred in the Miocene (Hafner et al. 2007), well before the formation of the sandy habitats within the Great Basin, and major lineage divergences within *Microdipodops* pre-dated the tumultuous climatic events of the Pleistocene (Hafner and Upham 2011). Fossils found outside the Great Basin from the late Blancan (1.9–2.9 Ma) also support that kangaroo mice did not evolve *in situ* in the Great Basin and they instead invaded this region relatively recently (perhaps in the early Pleistocene; Smith 1982; Mehringer 1986; Hafner and Upham 2011). Thus, the recent coalescence times as well as the lack of more ancient demographic shifts reported in the BSP reconstructions may represent the true history of each clade. Interestingly, Hafner and Upham (2011) found that mitochondrial clades of *Microdipodops* are in approximate genetic equilibrium based on haplotype-area curves either as a result of population sizes not fluctuating wildly during the pluvial history of the Pleistocene or that genetic equilibrium formed since the end of the Pleistocene. Our BSP reconstructions appear to primarily support the former explanation (Fig. 4); that is, BSP analyses in most cases show extended periods of stable population sizes throughout the latter portion of the Pleistocene. However, equilibrium may also have formed quite recently after population expansions evident in several of the clades. Although coalescent error increases towards the root of a genealogy (Ho and Shapiro 2011), it is also possible that the mitochondrial data used here are not informative enough to reveal more ancient demographic signals (mitochondrial data corresponds to the amount of time since all haplotypes in a clade coalesced). Use of additional, slower-evolving, markers such as nuclear DNA may be necessary to tease apart historical signals within each *Microdipodops* clade.

BSP reconstructions also suggest slight differences in times of coalescence and demographic events for each *Microdipodops* clade (Fig. 4). In combination with the other assessments of historical demography, there are strong indications of past population expansions for the *M. megacephalus* eastern and central clades and both of the *M. pallidus* clades. Population expansion is rejected in the *M. megacephalus* western clade based on the mismatch SSD, but a nonsignificant raggedness index and a negative (albeit nonsignificant) Fu's F_S indicates expansion (Table 3). This discrepancy suggests that members of this clade underwent a complex demographic history (i.e., recent bottleneck, migration, or population subdivision). Given unique geographical distributions, as well as the likelihood that each clade represents a cryptic species, differences are not unexpected. Variation among estimates of genetic diversity may explain observed differences in times of coalescence; clades within *M. megacephalus* show generally higher values of nucleotide diversity and longer times to coalescence than the *M. pallidus* clades (Table 3; Fig. 4). Despite distinct differences in geographic distributions and genetic diversity, population sizes do not appear to vary drastically among the clades (Fig. 4), with differences in demographic histories occurring largely within the past 200,000 years.

It is important to note that all estimates in this study were made using a few mitochondrial markers, 16S, *Cytb*, and $tRNA^{Glu}$. Using additional and non-maternally inherited loci may provide more accurate estimates because historical population dynamics estimated using a single locus (or linked loci that are all maternally inherited) may be inaccurate due to stochastic processes associated with that locus (Heled and Drummond 2008). Thus, additional analyses using other population-specific genetic markers (i.e., microsatellites) should provide a more full understanding of the population dynamics and estimates of effective population sizes within each *Microdipodops* clade. The recent description of microsatellite markers for *Microdipodops* (Lance et al. 2010) will facilitate that work which is already ongoing (Andersen, Hafner, and Light, pers. comm.) and will likely shed new light on the demographic history and conservation genetics of *Microdipodops*.

Implications for Conservation and Management

Although the Great Basin Desert is rich in animal, plant, and geologic diversity (Fiero 1986; Davis 2005), the sustainability of flora and fauna in this desert is threatened by a variety of factors, both historical and recent. An active tectonic history in addition to shifting climatic patterns, floristic transitions, and fluctuating pluvial lake levels may have served to isolate populations of many species in the area, potentially resulting in the establishment of new species. More recently, however, climate change, wild fires, invasive plants, livestock grazing, and agriculture have reduced the amount of available habitat for many species in the Great Basin. While these recent events may result in further divergence of isolated populations in altered selective regimes, it is feared that these processes may negatively affect many species and lead to their extinction. Indeed, Chaplin et al. (2000) ranks the Great Basin as second in imperiled species numbers among ecoregions of the United States.

Presently, species of kangaroo mice are not recognized as being imperiled by any government agency or conservation group. The present conservation status (IUCN Red List Category) of *M. megacephalus* and *M. pallidus* is "Least Concern" (Linzey and Hammerson 2008; Linzey et al. 2008). This conservation status of kangaroo mice, however, lags behind current scientific knowledge and belies the actual situation. Newly available information on kangaroo mice (i.e., Hafner et al. 2008; Hafner and Upham 2011; this study) documents unanticipated cryptic lineages, rarity and declining abundance, populations identified as being at risk or extinct, temporal demographic trends, and detailed patterns of genetic diversity over geography. It seems clear that a thorough reassessment of the conservation status of kangaroo mice is required.

Recent studies of kangaroo mice questioned the fate of certain populations and provided evidence of local extinction of other populations (Hafner et al. 2008; Hafner and Upham 2011). Additionally, Hafner and Upham (2011) showed that the abundance of both species is decreasing. Taken together, these reports signal concern for the survival of *Microdipodops* populations, especially those geographically isolated populations that harbor unique genetic characteristics. All major clades and subclades of *Microdipodops* recognized in this study represent evolutionary significant units (*sensu* Moritz 1994) and warrant the attention of conservationists and wildlife managers. Of all kangaroo mouse populations, the Idaho clade of *M. megacephalus* deserves the most serious attention; these kangaroo mice represent a major cryptic lineage and are known from only a few specimens from a tiny isolated area on the northern periphery of the distribution of kangaroo mice. As detailed by Hafner and Upham (2011), virtually all of the more northern populations of *M. megacephalus* seem to be in peril; nearly all of these populations show low abundance and are isolated to tiny areas or appear locally extinct (including the type locality). Importantly, concern for the welfare of the northern populations affects all four clades of *M. megacephalus* and includes some of the most genetically distinct members of the genus (e.g., Callao from the eastern clade, Valley Falls and Powell Butte of the western clade, and Riddle of the Idaho clade; Figs. 1 and 3). Populations of kangaroo mice from the Mono isolate also deserve conservation recognition because of its high genetic diversity (as

evidenced by its many private haplotypes; Fig. 3b) and its unique biogeographical history (Hafner et al. 2006; Hafner and Upham 2011). The peripheral isolates associated with *M. pallidus*, Alamo and Deep Springs localities (Figs. 2 and 3), also warrant special conservation recognition and further study because of their genetic uniqueness, tiny geographical range, uncertain status, and habitat alteration (see Hafner et al. 2008); these isolates are likely in peril.

Microdipodops are sand-obligate mammals endemic to the Great Basin. Likely due to their extreme ecological specializations, kangaroo mice have a highly fragmented distribution (Figs. 1 and 2) and are one of the least abundant nocturnal rodents of the Great Basin (Hall 1941; Hafner et al. 2006, 2008; Hafner and Upham 2011). Because of their stenotopic ecologies, however, kangaroo mice may serve as valuable indicators of healthy desert ecosystems. Documented reductions in their abundance or absence in areas from which they were known previously should provide advance notice of the ecological effects associated with environmental changes. This study provides baseline demographic and population-genetic data pertaining to kangaroo mice that should assist conservation efforts and management decisions. Future conservation efforts for *Microdipodops* should focus on ensuring the welfare of the smaller and more vulnerable subpopulations while simultaneously working to maintain the genetic diversity represented across each species.

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