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Phylogeography of Beck's Desert Scorpion, *Paruroctonus becki*, reveals Pliocene diversification in the Eastern California Shear Zone and postglacial expansion in the Great Basin Desert

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ABSTRACT

The distribution of Beck's Desert Scorpion, *Paruroctonus becki* (Gertsch and Allred, 1965), spans the 'warm' Mojave Desert and the western portion of the 'cold' Great Basin Desert. We used genetic analyses and species distribution modeling to test whether *P. becki* persisted in the Great Basin Desert during the Last Glacial Maximum (LGM), or colonized the area as glacial conditions retreated and the climate warmed. Phylogenetic and network analyses of mitochondrial cytochrome *c* oxidase 1 (*cox1*), 16S rDNA, and nuclear internal transcribed spacer (ITS-2) DNA sequences uncovered five geographically-structured groups in *P. becki* with varying degrees of statistical support. Molecular clock estimates and the geographical arrangement of three of the groups suggested that Pliocene geological events in the tectonically dynamic Eastern California Shear Zone may have driven diversification by vicariance. Diversification was estimated to have continued through the Pleistocene, during which a group endemic to the western Great Basin diverged from a related group in the eastern Mojave Desert and western Colorado Plateau. Demographic and network analyses suggested that *P. becki* underwent a recent expansion in the Great Basin. According to a landscape interpolation of genetic distances, this expansion appears to have occurred from the northwest, implying that *P. becki* may have persisted in part of the Great Basin during the LGM. This prediction is supported by species distribution models which suggest that climate was unsuitable throughout most of the Great Basin during the LGM, but that small patches of suitable climate may have remained in areas of the Lahontan Trough.

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1. Introduction

The Great Basin Desert is perhaps the most biologically unique of the currently recognized North American deserts (Hafner and Riddle, 2011). The region is positioned between the Sierra Nevada to the west and the Rocky Mountains to the east, and occurs further north and at a higher elevation than the other North American deserts (Cronquist et al., 1972; Olson et al., 2001). These factors together make the summer climate less severe, and the winters longer and colder. Accordingly, the Great Basin is sometimes referred to as the only 'cold' or 'temperate' desert in North America (Grayson, 1993).

During the late Pleistocene, the Great Basin climate was at times even colder, with temperatures fluctuating between cool glacial periods and warm interglacials (Spaulding, 1990; Thompson, 1990). Such climatic fluctuations probably made conditions even more extreme for desert organisms. Based on macrofossil data from packrat middens, many Great Basin plants are thought to have undergone severe range shifts, especially since the Last Glacial Maximum (LGM approximately 21 Kya), in response to Pleistocene climate fluctuations (Thompson, 1990; Thompson and Anderson, 2000). Although arid shrub-steppe vegetation was able to persist in some northern areas during the most recent Pleistocene glacial-interglacial cycles (Madsen et al., 2001; Wilson and Pitts, 2010), phylogeographic data suggest that several arid-adapted taxa may have colonized the Great Basin only recently, following the LGM (Nowak et al., 1994; Hornsby and Matocq, 2011). Other arid-adapted species, however, appear to have remained in the Great Basin during the Pleistocene in spite of severe habitat changes. These species potentially endured fluctuations in climate by shifting realized niches (within more stable fundamental niches), a phenomenon referred to as 'niche drifting' (Jezkova et al., 2011). Furthermore, genetic data and species distribution models (SDMs) suggest that some montane species in the Great Basin responded to Pleistocene climates by shifting ranges along elevational gradients (Walteri and Guralnick, 2008; Galbreath et al., 2009, 2010), colonizing lower elevations during glacial periods and higher elevations during interglacials. Information from

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fossils, phylogenetics, and SDMs all portray an extensive resorting of plant and animal communities within the Great Basin as the climate changed between the LGM and the current interglacial.

At the southern end of the Great Basin, phylogeographic datasets are beginning to reveal the biogeographical history of the Mojave Desert (Bell et al., 2010). Occurring at lower latitudes and lower mean elevations, the Mojave experiences more extreme summers than the Great Basin, but the winters are less severe (Redmond, 2009). The Mojave contains flora and fauna from many of the surrounding regions with few endemics, making it a transitional desert between the cooler Great Basin to the north and the warmer Peninsular and Sonoran deserts to the south (Bell et al., 2010; Hafner and Riddle, 2011). During glacial extremes, the geographical distributions of many arid-adapted organisms in the Mojave are thought to have contracted into areas associated with desert basins and drainages, where some were isolated long enough to form distinct lineages (see review in Bell et al., 2010). In the northern Mojave, there is also evidence that some regions harbored arid-adapted taxa that are now primarily distributed throughout the Great Basin (Jezkova et al., 2011).

The Beck's Desert Scorpion *Paruroctonus becki* (Gertsch and Allred, 1965) occurs in both the Mojave and Great Basin. We investigated the phylogeography of this common, arid-adapted scorpion to better understand how organisms with distributions spanning these deserts responded to climatic warming since the LGM. Our primary aim was to test whether *P. becki* persisted in the Great Basin during the LGM, or colonized the area relatively recently as glacial conditions retreated and climates warmed. Our secondary aim was to assess whether phylogeographic patterns among *P. becki* populations might reveal aspects about lineage diversification in the Mojave.

To accomplish these objectives, we sequenced a portion of the mitochondrial gene cytochrome *c* oxidase 1 (*cox1*), from samples collected throughout the range of *P. becki.* In an attempt to further resolve phylogenetic patterns derived from the *cox1* data, we also sequenced a portion of 16S ribosomal RNA (16S) from a subset of samples that characterized the majority of the *cox1* variation (exemplars from the major *cox1* groups), producing a larger dataset when concatenated with the *cox1* data. We then assessed the matrilineal genealogy within a temporal context using a relaxed molecular clock, and used demographic analyses to compare the genetic data to expectations under models of population expansion. Relying solely on mitochondrial DNA (mtDNA) for evolutionary reconstructions is controversial (e.g., Zink and Barrowclough, 2008; Edwards and Bensch, 2009). We therefore sequenced a variable nuclear gene, the internal transcribed spacer region (ITS-2), from a subset of individuals. In addition, we constructed climate-based species distribution models to examine the potential distribution of suitable climate for *P. becki* during the LGM for comparison with our interpretations derived from genetic data.

Phylogeographic studies often expose previously overlooked patterns in morphological variation among populations, sometimes with important taxonomic implications (e.g. Fouquet et al., 2007; Wood et al., 2008; Köhler and Glaubrecht, 2010). We discuss our results in light of current taxonomic hypotheses for *P. becki* and two morphologically similar species from California, *Paruroctonus variabilis* Hjelle and *Paruroctonus silvestrii* Borelli.

2. Materials and methods

2.1. Taxon sampling

Using ultraviolet light detection (Stahnke, 1972), we collected 89 samples of *P. becki* from 51 locations throughout the Mojave and Great Basin (Fig. 1; Table 1). Four specimens representing three congeners were included as outgroups: *Paruroctonus boreus* (Girard), *P. silvestrii*, and *P. variabilis*. Legs were removed from each specimen for DNA isolation and stored in 95% ethanol at −80°, with the vouchers retained in 70% ethanol. All tissues and vouchers were accessioned at the American Museum of Natural History.

2.2. Molecular techniques

We isolated genomic DNA from leg tissues by using either a standard phenol-chloroform extraction or a DNeasy Extraction Kit (Qiagen Inc., Valencia, CA, USA). We sequenced a fragment of the *cox1* gene using the primers LE1r (Esposito, 2011) and COImodF (Bryson et al., 2013). This gene was chosen because it has proven useful in intraspecific studies of scorpions (Prendini et al., 2003; Graham et al., 2013) and other arachnids (Prendini et al., 2005; Thomas and Hedin, 2008; Wang et al., 2008). Based on a preliminary assessment of the *cox1* dataset (see Results), a subset of samples representing most of the genetic structure observed in this gene was selected for additional sequencing. Fragments of 16S were sequenced using primers from Gantenbein et al. (1999) and nuclear ITS-2 using primers from Ji et al. (2003).AmpliTaq Gold (Applied Biosystems, Inc., Foster City, CA, USA) was used for polymerase chain reactions, with *cox1* fragments amplified at 50–54 °C for 34 cycles, 16S at 50 °C for 30 cycles, and ITS-2 at 50 °C for 35 cycles. Fluorescence-based cycle sequencing was conducted using the PCR primers (Appendix B) and a BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Qiagen Inc., Valencia, CA, USA), with electrophoresis on an ABI 3130 automated sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequences were edited using SEQUENCHER v. 4.9 (Gene Codes Corp., Inc., Ann Arbor, MI, USA) and DnaSP v. 5 (Librado and Rozas, 2009). All sequences were deposited in GenBank (accession numbers KF548316–KF548479).

2.3. Phylogenetics and divergence dating

We assessed phylogenetic patterns using Bayesian inference (BI) implemented in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003) on the Cyberinfrastructure for Phylogenetic Research cluster (CIPRES Gateway v 3.1) at the San Diego Supercomputer Center. Best-fit models of nucleotide substitution were determined for the *cox1* haplotype data under several codon partitions (each codon position separately, positions 1 + 2 and 3 separate, and unpartitioned) using jModeltest v. 0.1.1 with the Akaike information criterion (Posada, 2008). Substitution models calculated for different gene partitions were also explored for a concatenated mtDNA dataset (*cox1* + 16S). A best-fit partitioning scheme for each dataset was then determined by assessing Bayes factors on the harmonic mean marginal likelihood values (Nylander et al., 2004). Our biogeographical interpretations were based on final runs of 10 million generations using the appropriate partitioning scheme and substitution models. Trees were sampled every 1000 generations with the first 2.5 million generations discarded as burn-in after confirming chain stationarity using TRACER v. 1.5 (Rambaut and Drummond, 2007) and AWTY (Nylander et al., 2008).

We used the parsimony option (Polzin and Daneshmand, 2003) in NETWORK v. 4.5.1.6 (Fluxus Technology Ltd. 2004) to construct separate median-joining networks of mtDNA and nuclear haplotypes (Bandelt et al., 1999). The mtDNA network was limited to the *cox1* sequences of *P. becki*. Samples of *P. variabilis* were included in the network of nuclear haplotypes. Three individuals contained a heterozygous site in the ITS-2 sequence data, and haplotypes for these individuals were therefore reconstructed using PHASE implemented in DnaSP (Stephens and Donnelly, 2003).

We used a relaxed molecular clock in BEAST v. 1.5.4 (Drummond and Rambaut, 2007) to estimate diversification times within the *P. becki* mtDNA phylogeography. Best-fit substitution models

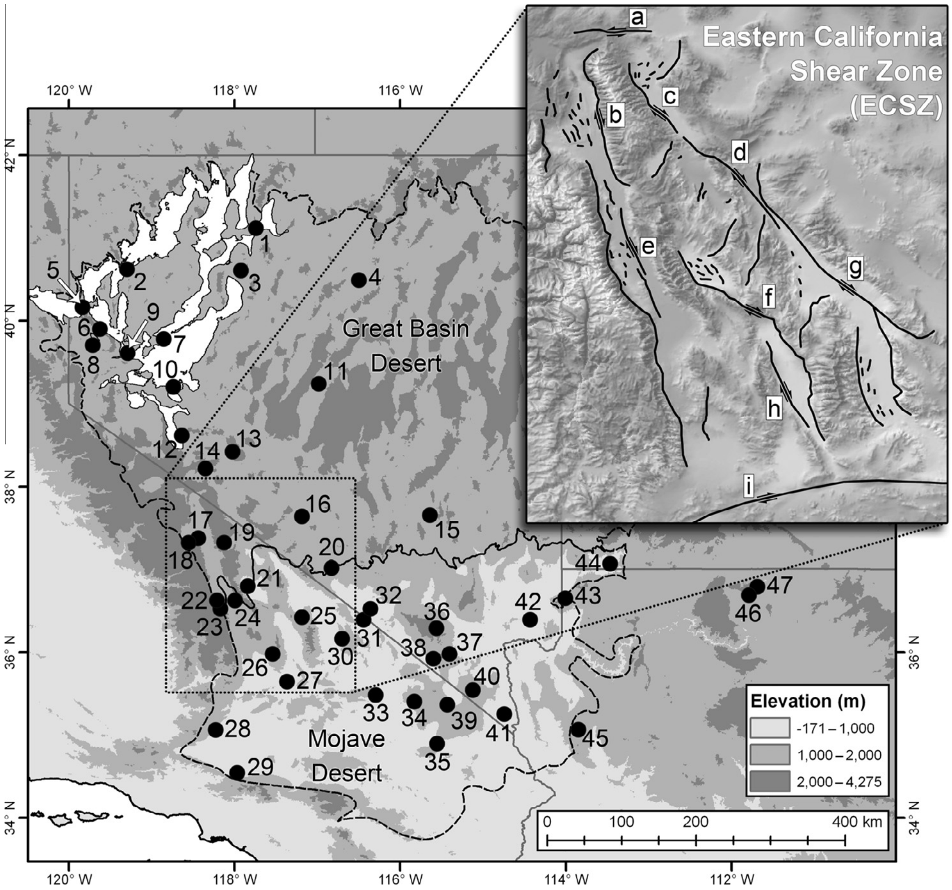

Fig. 1. Map of the study area showing sample locations for *Paruroctonus becki* (Gertsch and Allred) (black circles), with numbers corresponding to localities listed in Table 1. Dashed lines represent the approximate borders of the Mojave and Great Basin Deserts. Inset indicates major faults within the Eastern California Shear Zone. The distribution of Pleistocene Lake Lahontan at its pluvial maximum is indicated by the white area in northwest Nevada. Strike-slip faults are indicated by dark lines with arrows and letters as follows: a = Coaldale Fault, b = White Mountains Fault zone, c = Fish Lake Valley Fault zone, d = Furnace Creek Fault, e = Owens Valley Fault, f = Hunter Mountain Fault, g = Death Valley Fault, h = Panamint Valley Fault, i = Garlock Fault. Dark lines without arrows indicate normal faults.

were chosen for each gene (no codon partitions) using jModeltest, and divergence dates were estimated using an uncorrelated lognormal clock model. A mean mutation rate of 0.007 substitutions/site/million was selected for the *cox1* partition based on data from a distantly related group of scorpions (Gantenbein et al., 2005), with a mean standard deviation of 0.003 (Gantenbein and Largiadér, 2002). We ran BEAST for 40 million generations with a Yule tree prior, and samples were retained every 1000 generations. TRACER was used to confirm stationarity of the Markov Chain Monte Carlo, as well as to determine the adequacy of the effective sample sizes (ESS > 200 for each estimated parameter).

2.4. Demographic analyses and landscape interpolation

We conducted mismatch distribution tests of demographic expansion in four of the geographically-structured groups identified in the phylogenetic and network analyses, using Arlequin v. 3.11 (Excoffier et al., 2005). These distributions were run with 10,000 bootstrap replicates, with the sum of square deviations (SSD) between the observed and simulated data used to assess statistical significance. Arlequin was also used to calculate Fu's F (Fu, 1997) for these groups, on the assumption that groups which underwent recent demographic expansion would have significantly negative F values, indicating deviations from mutation-drift equilibrium.

We developed a landscape interpolation of genetic distances across the Great Basin to assess genetic diversification, limiting the assessment to genetic samples identified from this region (see Section 3). Alleles In Space v. 3.11 (Miller, 2005) was used to calculate pairwise genetic distances among sites from the *cox1* sequence data as determined by a Delaunay triangulation-based connectivity network. Distance values were calculated as residuals from a regression of genetic and geographic distances to correct for spatial autocorrelation among samples (Miller et al., 2006). The values were then interpolated onto a 50 × 50 grid representing a minimum convex polygon around the targeted samples, with distance weighting set to 0.25.

2.5. Species distribution modeling

We used 47 unique locality records associated with the genetic samples and 18 additional records from the literature (Gertsch and Soleglad, 1966; Haradon, 1985) to construct SDMs. Google Earth (http://earth.google.com) was used to estimate latitude and longitude for records lacking coordinates. Records that could not be pinpointed to an area less than five kilometers in diameter were discarded to match the resolution of the modeling rasters (2.5 arc-min).

We constructed SDMs using Maxent v. 3.3.2 (Phillips et al., 2006), with environmental data represented by bioclimatic layers of current climatic trends, seasonality, and extremes of temperature and precipitation (Hijmans et al., 2005). Layers were masked (clipped) to the ecoregions (Olson et al., 2001) that contained occurrence records (Central Basin and Range, Northern Basin and Range, Mojave Basin and Range, Sonoran Desert) to improve model accuracy and reduce problems with extrapolation (Pearson et al., 2002; Thuiller et al., 2004; Randin et al., 2006). Correlations among

Table 1
Sampling localities of *Paruroctonus becki* (Gertsch and Allred) portrayed in Fig. 1.

Locality #	Taxon	State	Locality name	Latitude	Longitude	Vouchers
1	*P. becki*	NV	Winnemucca	41.12564	−117.76308	MG397, 398
2	*P. becki*	NV	Gerlach	40.64307	−119.31268	MG350, 351
3	*P. becki*	NV	Flat Tire	40.601367	−117.91349	LP10560
4	*P. becki*	NV	Beowawe	40.52007	−116.51368	MG15, 18
5	*P. becki*	NV	Honey Lake Valley	40.12901	−119.82145	MG516, 517
6	*P. becki*	NV	Sparks North	39.855278	−119.655889	LP6289
7	*P. becki*	NV	Hot Springs Mt	39.76	−118.87	MG164
8	*P. becki*	NV	Sparks South	39.735998	−119.686388	LP6290
9	*P. becki*	NV	Wadsworth	39.633801	−119.285448	MG514
10	*P. becki*	NV	Blow Sand Mts	39.19902	−118.72205	MG528, 529
11	*P. becki*	NV	Big Smokey Valley	39.239123	−117.000493	MG879, 880
12	*P. becki*	NV	Hawthorne	38.586683	−118.604542	LP6291
13	*P. becki*	NV	Mina	38.429296	−118.065709	MG837, 838
14	*P. becki*	NV	Murietta	38.252972	−118.350441	MG504–506
15	*P. becki*	NV	Tempiute	37.654021	−115.640362	MG918–920
16	*P. becki*	NV	Goldfield	37.61572	−117.226262	MG1080, 1081
17	*P. becki*	CA	Millpond	37.37903	−118.48391	MG385–387
18	*P. becki*	CA	Tungsten	37.3424	−118.52415	LP5007
19	*P. becki*	CA	Deep Springs Valley	37.31298	−118.10313	MG375–378
20	*P. becki*	NV	Sarcobatus Flat	37.040003	−116.815702	MG949–951
21	*P. becki*	CA	Saline Valley	36.753133	−117.86325	LP4984, 4992
22	*P. becki*	CA	Tuttle Creek	36.596765	−118.188562	MG541, 542
23	*P. becki*	CA	Alabama Hills	36.59985	−118.183417	LP4991
24	*P. becki*	CA	Long John Cyn	36.63608	−118.00033	MG361–363
25	*P. becki*	CA	Panamint Range	36.40923	−117.17596	MG475, 476, 477
26	*P. becki*	CA	China Lake, Mt Springs Cyn	35.944983	−117.543652	LP4373
27	*P. becki*	CA	Trona Pinnacles	35.617533	−117.370104	LP4369
28	*P. becki*	CA	Mojave	35.069529	−118.220224	MG713
29	*P. becki*	CA	Palmdale	34.508208	−117.978967	MG1112, 1113, 1155
30	*P. becki*	CA	Dante's View	36.21697	−116.72399	MG451, 542
31	*P. becki*	CA	Amargosa Dunes	36.432483	−116.421417	LP4995
32	*P. becki*	NV	Ash Meadows	36.466013	−116.377554	LP10450
33	*P. becki*	CA	Avawatz Mts	35.510409	−116.309343	LP4384
34	*P. becki*	CA	Halloran Summit	35.412699	−115.802629	MG1042–1044
35	*P. becki*	CA	Vulcan Mine	34.914776	−115.545667	MG1131, 1132, 1153, 1154
36	*P. becki*	NV	Spring Mts North	36.2458	−115.54299	MG12
37	*P. becki*	NV	Spring Mts South	35.95397	−115.43804	MG136
38	*P. becki*	NV	Mt Potosi	35.9149	−115.55276	MG60
39	*P. becki*	CA	Morning Star Mine	35.362694	−115.422928	LP8502
40	*P. becki*	NV	Nipton Road	35.5197	−115.14	MG224–226
41	*P. becki*	NV	Christmas Tree Pass	35.261226	−114.743965	MG1060–1062
42	*P. becki*	NV	Blue Point	36.399938	−114.446469	MG127–129
43	*P. becki*	AZ	Virgin Mts	36.639712	−114.014165	MG157–159
44	*P. becki*	UT	Warner Valley	37.042092	−113.453533	LP7250
45	*P. becki*	AZ	Hualapai Mts	35.076556	−113.882472	MG1151, 1152
46	*P. becki*	AZ	Soap Creek Cyn	36.72607	−111.75808	MG183, 184
47	*P. becki*	AZ	Cliff Dwellers Lodge	36.74677	−111.751569	LP7698
	P. boreus	NV	Humboldt River	41.3708	−115.78369	MG323
	P. silvestrii	CA	Zuma Ridge	34.034676	−118.82002	LP3199
	P. variabilis	CA	Panoche	36.578931	−120.73528	LP6287, MG710

the layers were assessed from values of grid cells containing locality records to avoid over-fitting the models and improve model transferability (Peterson et al., 2007). When the Pearson's correlation coefficient was greater than 0.75 (Rissler et al., 2006; Rissler and Apodaca, 2007) among layers, one of the layers was selected for retention in Maxent runs. Nine layers were selected for modeling, representing quarterly climates rather than monthly climates, precipitation during the driest quarter over precipitation during the warmest quarter, and annual temperature range instead of mean diurnal range: Bio3, isothermality; Bio5, maximum temperature of the warmest month; Bio6, minimum temperature of the coldest month; Bio7, temperature annual range; Bio8, mean temperature of the wettest quarter; Bio9, mean temperature of the driest quarter; Bio15, precipitation seasonality; Bio16, precipitation of the wettest quarter; Bio17, precipitation of the driest quarter.

We ran Maxent using logistic output and default settings except for random seeding. Cross-validation was used to assess model robustness by dividing presence points into five groups and running five iterations with different groups selected for each run. Thus, 20% of the presence points were used as test points and 80% for model training (Nogués-Bravo, 2009). Model performance was assessed by the default evaluation of the area under the receiver operating characteristic curve (AUC). To extrapolate models to LGM climates, distribution models were projected onto simulated climates for the LGM (c. 21 ka) derived from the Community ClimateSystem Model (CCSM; Otto-Bliesner et al., 2006) and the Model for Interdisciplinary Research on Climate (MIROC; Hasumi and Emori, 2004). Climatic suitability was depicted in ArcGIS v. 9.2 (ESRI, Redlands, CA, USA) by converting continuous Maxent outputs into binary grids using the maximum training sensitivity plus specificity threshold (Liu et al., 2005; Jiménez-Valverde and Lobo, 2007).

3. Results

3.1. Phylogenetics and divergence dating

We sequenced 747 bp of *cox1* for 92 samples, 484 bp of 16S for 47 samples, and 241 bp of ITS-2 for 33 samples. The concatenated dataset of *cox1* and 16S sequences included 45 samples. The *cox1* phylogeny yielded several geographically-structured groups, although not all were strongly supported (Fig. 2). *Paruroctonus variabilis* and *P. becki* were rendered paraphyletic by the grouping of one *P. variabilis* sample with all *P. becki* samples, except those from the Providence Mountains (Fig. 2). Other geographically-structured groups were comprised of individuals from the Great Basin, the western Mojave, and the eastern Mojave, with a few samples in the latter group also extending onto the Colorado Plateau in northern Arizona. Of the geographically-structured groups, only those in the Great Basin and White-Inyo Range were strongly supported. In general, individuals collected from the same or nearby localities grouped together with strong BI support, although three areas (localities 21, 31, and 40) contained haplotypes from different groups.

The topology of the tree obtained by analyses of the concatenated mtDNA dataset (Fig. 3) was similar to that obtained by analysis of *cox1* alone (Fig. 2), but with a few important differences. The *P. becki* samples from the Providence Mountains that were divergent in the *cox1* analysis instead formed the most basal divergence within a weakly supported group containing all other *P. becki* samples, rendering the species monophyletic. Although Bayesian support was weak, *P. variabilis* samples from the San Joaquin Valley in California were monophyletic in the concatenated tree, and sister to a clade containing *P. becki*. As in the *cox1* tree, samples from the White-Inyo Range were strongly supported as a monophyletic group (referred to hereafter as the 'White-Inyo Clade'). Samples from the western Mojave grouped with low Bayesian support in the *cox1* tree, but strong support in the concatenated tree (referred to hereafter as the 'Western Mojave Clade'). Samples from throughout the eastern Mojave formed a weakly supported group with those from the Great Basin in both the *cox1* and concatenated analysis (referred to hereafter as the 'Eastern Clade', although we recognize the limited phylogenetic support for this group). Within the Eastern Clade, most of the samples distributed across the Great Basin formed a strongly supported group, consisting of very similar haplotypes (referred to hereafter as the 'Great Basin Clade'; Figs. 2–4).

The haplotype network constructed from the *cox1* data (Fig. 5a) portrayed a pattern similar to that of the concatenated mtDNA tree (Fig. 3). Samples from the Great Basin Clade formed a star-shaped pattern that was at least 20 mutation steps from the other samples within the Eastern Clade, which also formed a distinct, though diverse subnetwork. Two of the four samples from the Providence Mountains nested within the Eastern Clade, and the other two formed a divergent group positioned between the samples from the Western Mojave Clade (37 mutation steps removed) and the White-Inyo Clade (41 steps removed).

In the nuclear haplotype network, all *P. becki* haplotypes were two mutation steps from the most common haplotype consisting of samples from the Eastern Clade, and including both mitochondrial groups from the Providence Mountains (Fig. 5b). The only sample successfully sequenced for ITS-2 from the White-Inyo Clade shared a haplotype with samples from the Western Mojave Clade, only one mutation step removed from the most common haplotype. The two *P. variabilis* samples comprised the most divergent haplotype in the nuclear network, but this haplotype was only two mutational steps from the most similar *P. becki* haplotype.

Results from the BEAST analysis of the concatenated mtDNA data (Fig. 6) suggested that divergence between *P. becki* and the most closely related outgroup, *P. variabilis*, took place between the Late Miocene and early Pliocene. Subsequently, initial diversification within *P. becki* appears to have begun with the divergence of a lineage represented in the Providence Mountains sometime in the Late Miocene to mid-Pliocene. The Eastern Clade, the White-Inyo Clade, and the Western Mojave Clade were estimated to have diverged during the Pliocene, and the remaining diversification, including that between the Great Basin Clade and the other samples in the Eastern Clade, was estimated to have occurred during the Pleistocene.

3.2. Demographic analyses and landscape interpolation

Mismatch analyses resulted in sum of squared deviation (SSD) values that were nonsignificant for the Western Mojave and the White-Inyo clades, implying that these data do not deviate from models of demographic expansion (Table 2). The SSD value for the Eastern Clade was significant, but the significance was lost when the Great Basin Clade was analyzed independently. Furthermore, the Great Basin Clade exhibited a unimodal distribution (Fig. 7), and was the only grouping that yielded a significantly negative Fu's *F* value, both of which would be expected for a population that has undergone expansion.

The interpolation of residual genetic distances for the *cox1* data from the Great Basin Clade (Fig. 4b) displayed a pattern of decreasing genetic diversity from the northwest to the southeast. The highest diversity occurred in the extreme northwest of the current distribution of *P. becki*, an area within the Lahontan Basin.

3.3. Species distribution models

The SDMs performed significantly better than random, as AUC scores were high (>0.95) for both training and testing data (Fig. 8). Under current climatic conditions, the SDM for *P. becki* depicted suitable climate over most of the low and mid-elevation areas within the Mojave Desert, Great Basin, and the southern Colorado Plateau. Additional areas were also depicted to the north along the Snake River Plain of southern Idaho, in arid parts of southeastern Oregon, and in the northwest part of the Sonoran Desert in southern California (in an area also referred to as the 'Colorado Desert').

Both LGM models (CCSM and MIROC) depicted suitable climate throughout the Mojave and western Sonoran deserts, as well as throughout the length of western California and northern Baja California (Fig. 8). In contrast, the MIROC model depicted areas of suitable climate during the LGM that extended further south along the coast of Sonora, Mexico, but not as far north in California. The MIROC model also indicated that climate was generally unsuitable throughout the higher elevations of southern California, whereas the CCSM model indicated suitable climate in all but the highest elevations of southern California. Both models depicted unsuitable climate in the Great Basin during the LGM, except for very small patches in the western Great Basin (within the Lahontan Basin). Neither model depicted suitable climate on the Colorado Plateau during the LGM.

4. Discussion

4.1. Postglacial colonization of the Great Basin

Our primary objective was to determine whether *P. becki* persisted throughout its current distribution in the Great Basin Desert during the LGM, or whether the species colonized the area recently, as glacial conditions retreated and climate warmed. Phylogenetic and demographic analyses of mtDNA provide convincing evidence that *P. becki* recently colonized a large part of the western Great Basin following the LGM. The pattern was not as expected, however, because the genetic data, along with predictions from the SDMS, suggest that *P. becki* may have persisted in some part of the Great Basin during the LGM and then expanded to its current

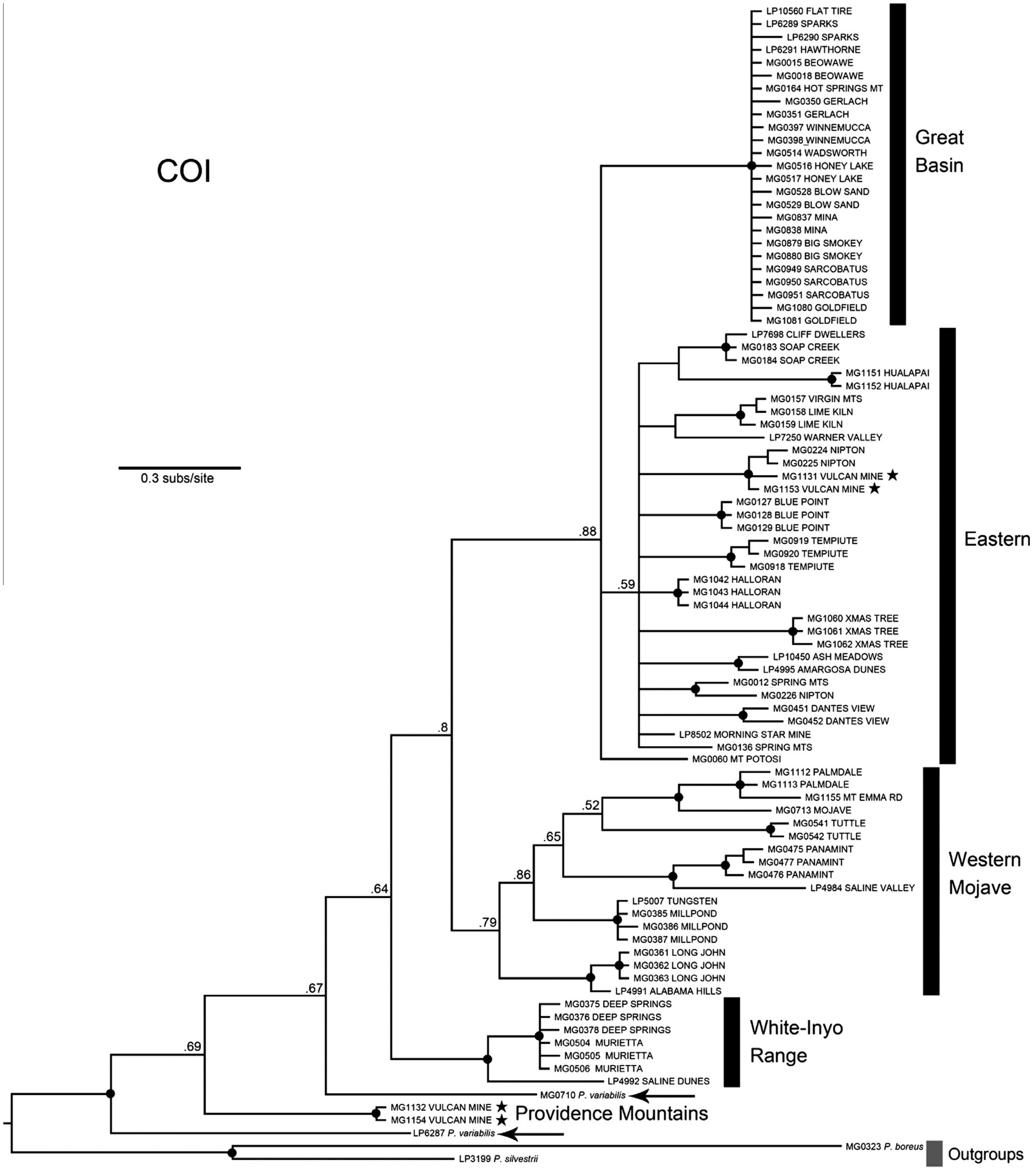

Fig. 2. Majority rule (50%) consensus tree depicting results of Bayesian phylogenetic analysis of *cox1* mtDNA sequences for *Paruroctonus becki* (Gertsch and Allred). Vertically arranged bars indicate geographically-structured groups of haplotypes. Arrows point to two *Paruroctonus variabilis* Hjelle samples. Stars indicate individuals from the Providence Mountains. Nodes with Bayesian posterior probability values indicating strong support (PP > 0.9) are represented by black circles. Support values are indicated above nodes.

widespread distribution in the western Great Basin when the climate warmed.

The SDMs projected onto LGM conditions depicted suitable climate across much of the Mojave and, importantly, in small low-elevation sub-basins in the western Great Basin (Fig. 8). According to the CCSM model, the predicted area encompasses Walker Lake (Fig. 8b), whereas the MIROC model predicted sub-basins further north that include Pyramid Lake and the Smoke Creek Desert (Fig. 8c). These sub-basins occur within the Lahontan Trough, which filled with water and coalesced to form Lake Lahontan

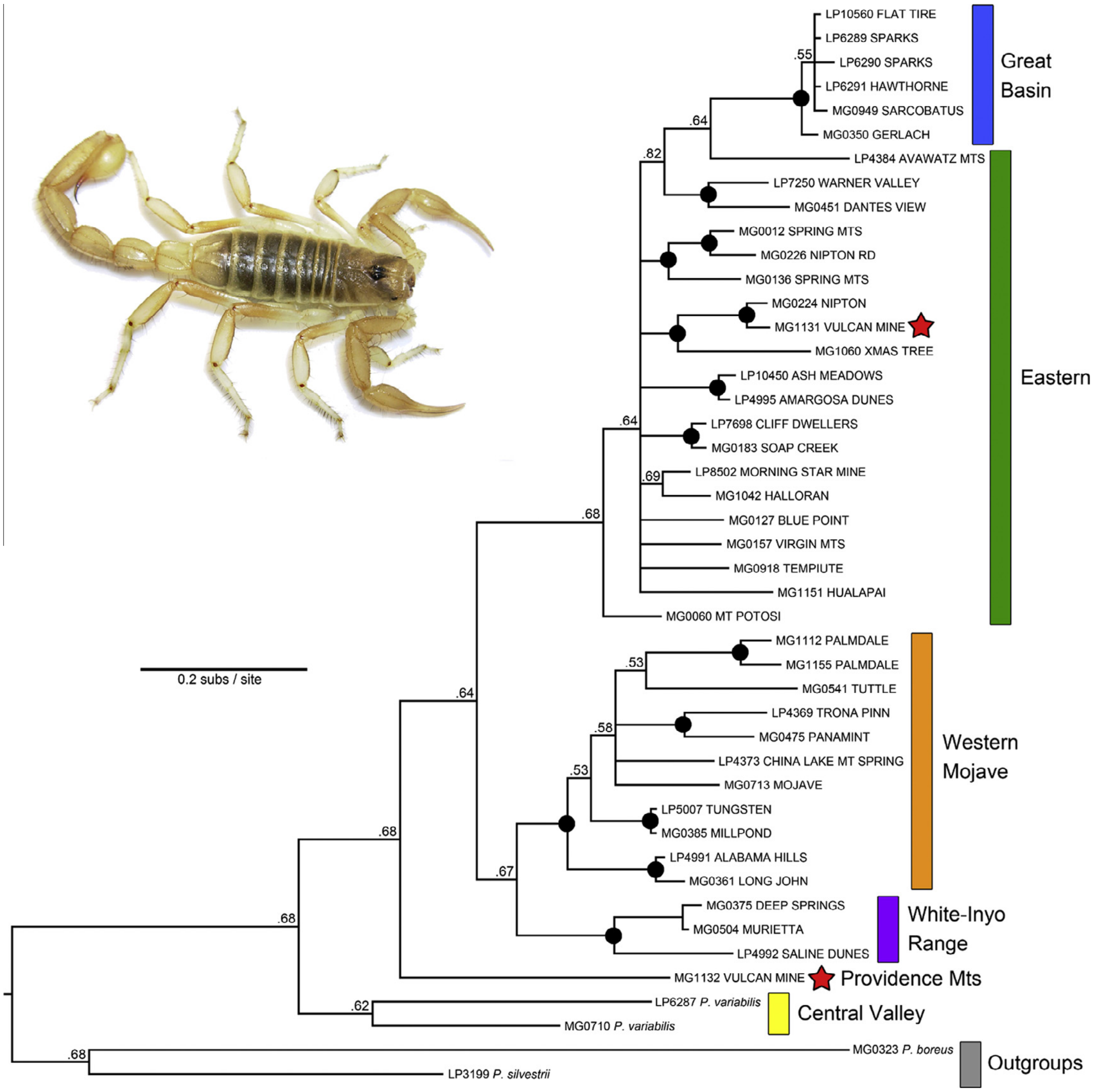

Fig. 3. Majority rule (50%) consensus tree depicting results of Bayesian phylogenetic analysis of concatenated *cox1* and 16S mtDNA sequences for *Paruroctonus becki* (Gertsch and Allred). Vertically arranged bars indicate geographically-structured groups of haplotypes. Nodes with Bayesian posterior probability values indicating strong support (PP > 0.9) are represented by black circles. Support values are indicated above nodes.

during pluvial maxima (Figs. 1 and 4; Benson, 1991). Therefore, at times most, if not all, of the areas identified as suitable during the LGM were underwater. If *P. becki* persisted in this region, perhaps it was only along slopes adjacent to the lake. This may have caused a population bottleneck at that time, explaining the low genetic diversity within the Great Basin Clade.

In the mtDNA haplotype network, most of the samples from the Great Basin (the Great Basin Clade) formed a star-shaped pattern (Fig. 5a), which is usually indicative of recent range expansion (Avise, 2000). Demographic analyses also support a model of recent expansion for the Great Basin Clade (Table 2). According to the foremost models of colonization, the gradual expansion model (reviewed in Koizumi et al., 2012) and the leading edge model (reviewed in Jezkova et al., 2011), genetic differentiation should be greater among older populations than among populations from recently colonized regions. Based on this prediction, the landscape interpolation of genetic distances within the Great Basin Clade (Fig. 4b) lends additional support to the interpretation that *P. becki* expanded its distribution from one or more smaller areas in the western Great Basin.

This geographical expansion appears to have occurred in eastward and southeastward directions. Westward expansion was probably inhibited by the Sierra Nevada, but no obvious biogeographical barriers occur to the north. Despite searches in apparently suitable habitat, we were unable to find *P. becki* in more northern areas, perhaps reflecting physiological or ecological limitations associated with higher latitudes that were inaccurately portrayed by the SDMs. Individuals from the Great Basin Clade were found within close proximity to individuals from several other groups in the south, but never in sympatry (Fig. 4), suggesting there might be some degree of high density blocking (Waters, 2011), or potential competition, among the different *P. becki* groups.

4.2. Pre-pleistocene diversification

Our estimate of Late Miocene divergence between the putative sister species, *P. becki* and *P. variabilis*, corresponds with the marine embayment of the San Joaquin Valley (Hall, 2002). *Paruroctonus variabilis* is endemic to the eastern slopes of the California coastal ranges that border the San Joaquin Valley (Hjelle, 1982), so divergence between these species could have occurred when marine waters isolated the Coastal Ranges from arid regions further inland.

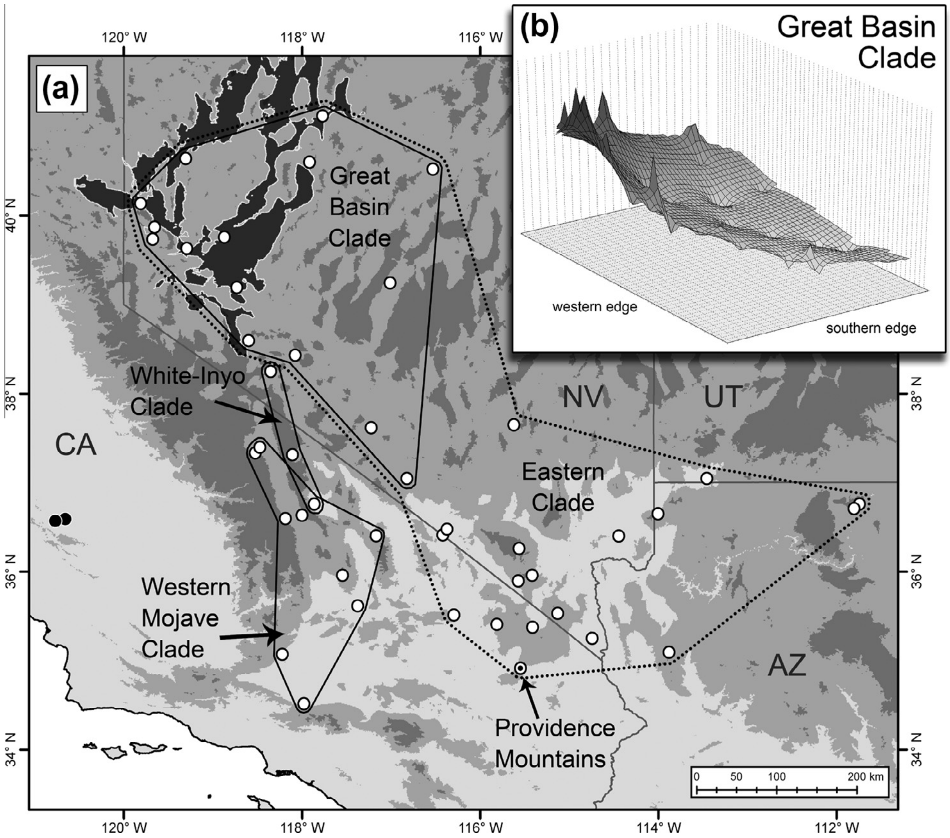

Fig. 4. Map depicting the distribution of major groups of mtDNA haplotypes within the phylogeography of *Paruroctonus becki* (Gertsch and Allred) discussed in the text (a), and a landscape interpolation of genetic distances for samples in the Great Basin (b). Sample localities used for genetic analyses are indicated by white dots. The dark shading with the white outline portrays Lake Lahontan at its pluvial maximum. Solid lines indicate strongly supported clades, whereas the dotted line outlines the weakly supported Eastern Clade.

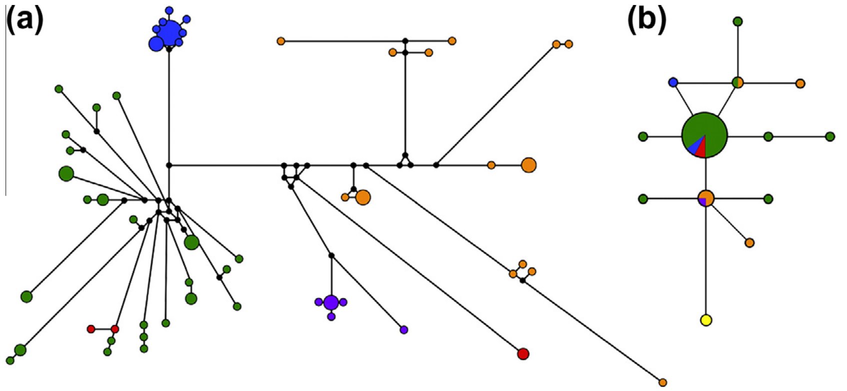

Fig. 5. Haplotype networks for *Paruroctonus becki* (Gertsch & Allred) based on mitochondrial *cox1* (a) and nuclear ITS-2 (b) sequence data. Circle sizes are proportional to haplotype frequencies. Color indicates geographical groups identified in Fig. 4. The yellow circle in the nuclear network (b) represents *Paruroctonus variabilis* Hjelle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Interestingly, nodes representing divergence among the Western Mojave Clade, the Eastern Clade, and the White-Inyo Clade have mean estimates within the Pliocene. These clades appear to meet in the eastern California Shear Zone, a geologically active area of predominantly right-lateral strike-slip faults (Fig. 1) thought to accommodate motion between the Pacific and North America plates (Frankel et al., 2010). This region experienced intense extensional tectonics during the Pliocene (Phillips, 2008). Extensional faulting is thought to have first begun along the east side of the White-Inyo Range at the Fish Lake Valley fault zone (6.9–4 Ma), forming deep desert valleys (Reheis and Sawyer, 1997). Subsequent faulting on the west side of the White-Inyo Range took place during the late Pliocene, causing a rapid deepening of a preexisting valley and forming the modern low-lying Owens Valley along the White Mountains and Owens faults (Stockli et al., 2003; Phillips, 2008). The location (Fig. 1) and timing of these events appear to match the placement (Fig. 4) and estimated divergence times (Fig. 6) among the three clades. Populations of *P. becki* may have become divided on each side of the expanding low-elevation valleys east of the White-Inyo Range.

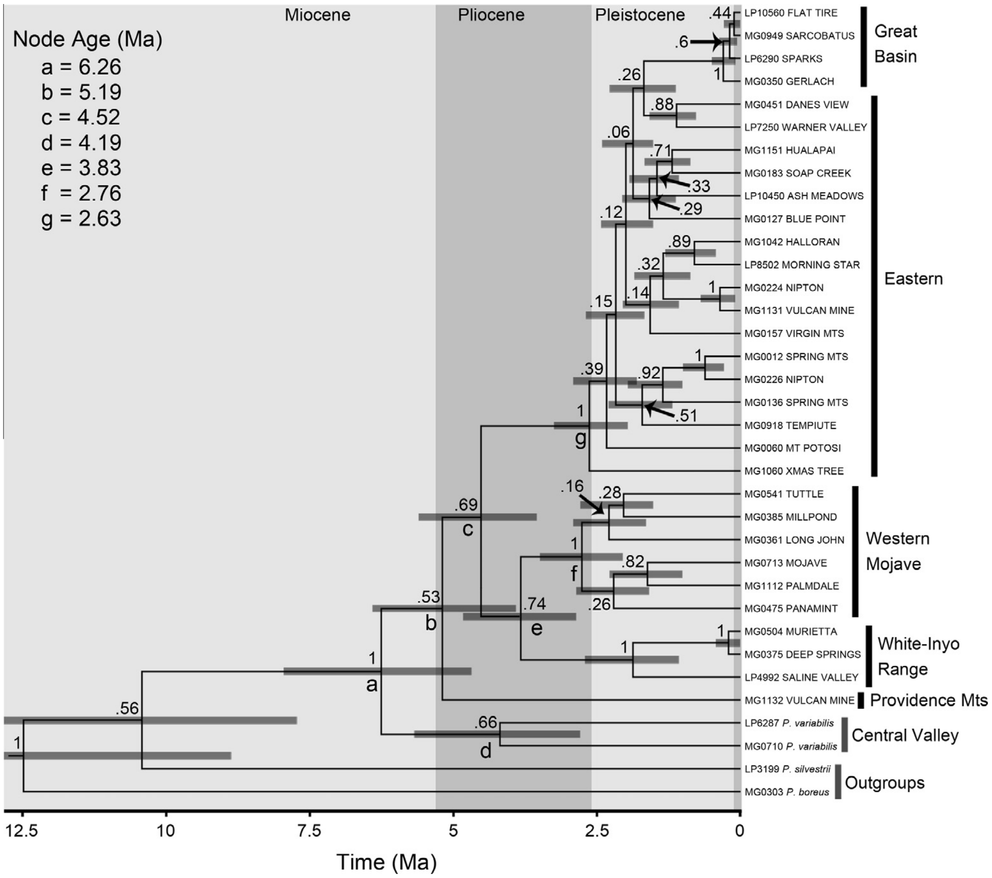

Fig. 6. Rate-calibrated chronogram for *Paruroctonus becki* (Gertsch and Allred). Posterior probabilities are indicated for nodes. Letters below nodes identify mean divergence date estimates within the Miocene or Pliocene. Bars represent highest posterior densities (95%) around mean date estimates.

Table 2
Results of goodness-of-fit tests of population expansion based on mismatch distributions and Fu's *F* for geographically-structured groups of haplotypes in *Paruroctonus becki* (Gertsch and Allred) identified by phylogenetic (Figs. 2 and 3) and network (Fig. 4) analyses. Asterisks indicate non-significant SSD values (>0.05) and significant *F* values (<0.02) which imply that the data do not differ from expectations under models of expansion.

	Western Mojave clade	White-Inyo clade	Eastern group	Great Basin clade
Goodness-of-fit test				
Distribution curve	Multimodal	Multimodal	Multimodal	Unimodal
SSD	0.016072	0.081827	0.0173	0.000059
P	0.134*	0.134*	0.012	1.0*
Fu's *F*	−0.27583	0.1211	−5.41313	−5.47584
P	0.432	0.488	0.087	<0.001*

Formation of the Owens Valley in the Late Pliocene may then have further sundered *P. becki* populations within the region, facilitating the formation of the Western Mojave and White-Inyo clades.

The divergent mtDNA haplotypes that we recovered from the Providence Mountains are curious (Figs. 2, 3 and 5a). Two of four samples from this locality (Figs. 1 and 4) grouped with other eastern Mojave samples, whereas the other two were placed basal to the other *P. becki* samples (Figs. 2 and 3). The nuclear haplotypes of these latter samples were identical to the haplotypes recovered in samples from the Eastern Clade, and no obvious morphological differences were observed between them. Assessment of the sequence data revealed no evidence of pseudogenes (numts), such as double peaks, indels, frameshifts, or premature stop codons (Bertheau et al., 2011). Other potential explanations include incomplete lineage sorting (retention of ancestral polymorphism), disequilibrium of mtDNA caused by microbial symbionts (Hurst and Jiggins, 2005), or mitochondrial introgression from a divergent population or related species not sampled. We favor the latter explanation. The southernmost record for *P. becki* is from the Coachella Valley area of the Colorado Desert (Gertsch and Soleglad, 1966), a subdivision of the Sonoran Desert. If *P. becki* is discovered in the area between the Coachella Valley and the Providence Mountains, these populations may resolve the patterns observed in samples from the Providence Mountains.

4.3. Taxonomic considerations

Although a morphological assessment was beyond the scope of this project, we observed several morphological differences among the specimens from which DNA sequences were obtained. First, a conspicuous morphological similarity was observed between *P. becki* and its putative sister species, *P. variabilis*. This is surprising because *P. variabilis* was at first considered to be a "light race" of another species, *P. silvestrii*, distributed throughout southern California and adjacent areas in northern Baja California (Gertsch and Soleglad, 1966; Hjelle, 1982). The original description of *P.*

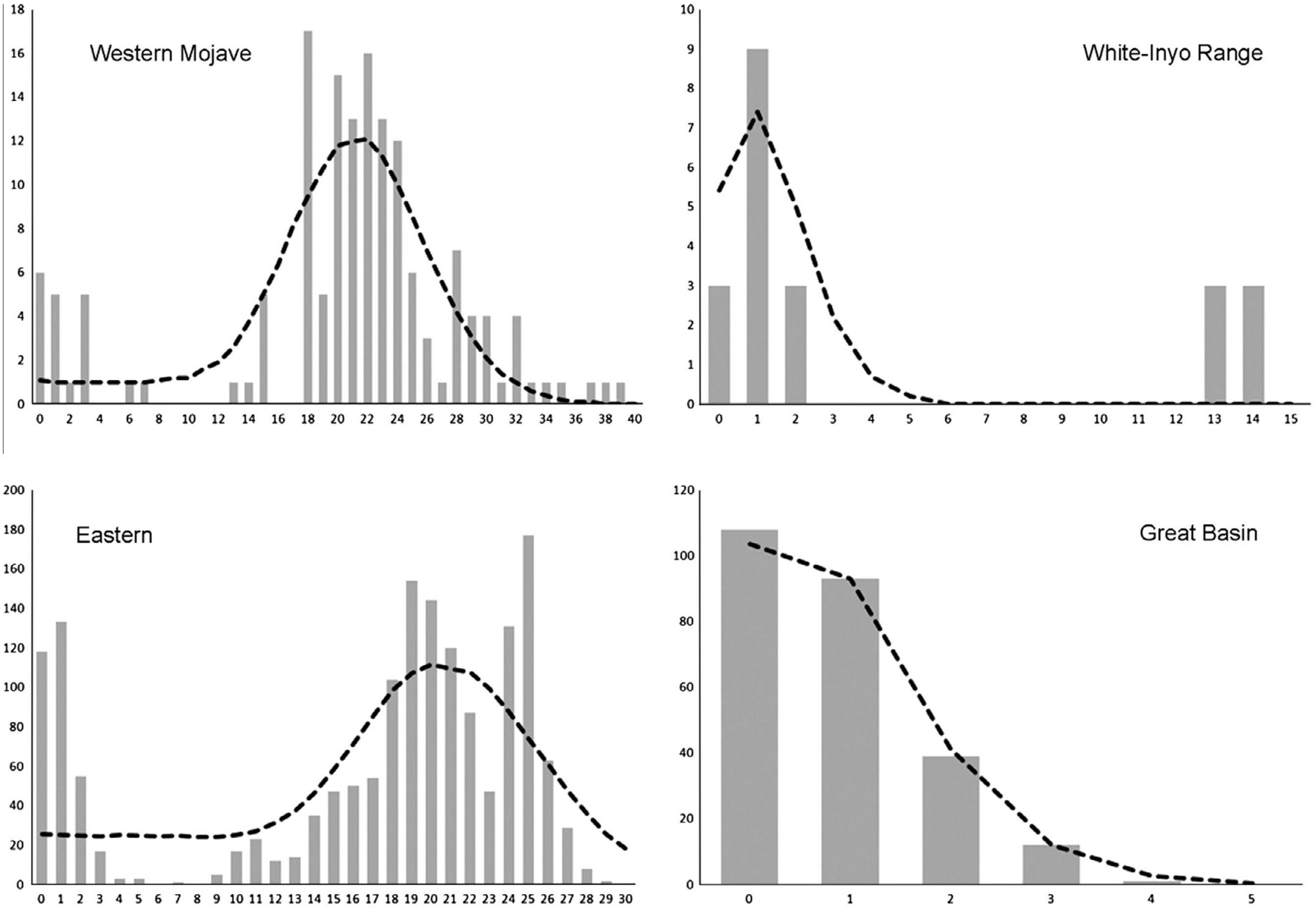

Fig. 7. Mismatch distributions based on *cox1* data for four mtDNA groups of *Paruroctonus becki* (Gertsch and Allred) recovered in phylogenetic analyses. Dashed lines represent the expected distribution if populations underwent demographic expansion, whereas bars indicate the observed frequency (*y*-axis) of pairwise differences (*x*-axis).

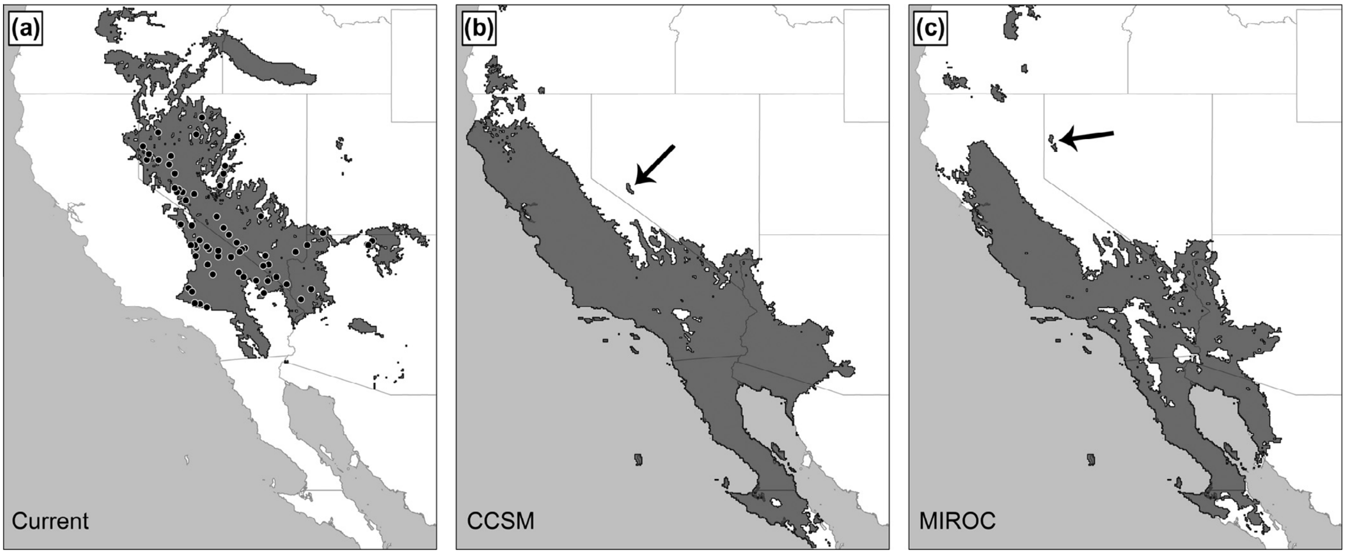

Fig. 8. Graphical results from species distribution models of *Paruroctonus becki* (Gertsch and Allred) generated using Maxent and displayed using the maximum training sensitivity plus specificity threshold. Models represent climate predicted as suitable (dark shading) during current conditions (a) and LGM conditions estimated from CCSM (b) and MIROC (c) climatic simulations. Black circles represent occurrence records used to generate the models. Arrows indicate potential northern refugia within the Great Basin Desert.

variabilis compares it to*P. silvestrii* and *P. becki*, however, distinguishing *P. variabilis* from the latter by the following combination of characters: distinct denticles opposite biscuspid tooth on the ventral margin of the cheliceral fixed finger; more dorsal marbling; and different electrophoretic patterns of venom proteins. Venom is of little practical utility because it is not typically used to diagnose scorpion species, and the degree to which venom proteomes vary within scorpion species has yet to be satisfactorily assessed. Dorsal marbling was also found to be an unreliable diagnostic character. Although most *P. becki* are superficially similar in color and degree of marbling of the dorsum, some populations (e.g. Locality 32: Ash Meadows) contained phenotypes with darker marbling than that of *P. variabilis.* We examined several *P. becki* specimens, representing each major group, but none possessed cheliceral denticles. Consequently, the presence of cheliceral denticles appears to be diagnostic for *P. variabilis*. Based on this morphological difference and the differences in mitochondrial and nuclear DNA sequence data presented herein, we could not refute the current taxonomic status of these two species. Nevertheless, both species would benefit from more rigorous morphological evaluation and redescription.

4.4. Concluding remarks

Floristic responses to late Pleistocene climate fluctuations are relatively well characterized for the Great Basin due to the wealth of data that have been extracted from packrat middens throughout southwestern North America (Betancourt et al., 1990). The geographical ranges of Great Basin plant species are thought to have responded in two ways: taxa were either ‘orthoselective’ and relatively insensitive to climate change, experiencing little change to their geographical ranges (e.g., shifted along elevational gradients but did not undergo large geographic changes); or taxa shifted to new areas, following a ‘migration’ model (Nowak et al., 1994). Using this logic, a model of late Pleistocene distributional response may be developed for the Great Basin terrestrial fauna.

Rodent species that occupy basins within the region appear to fall into both categories. Recent phylogeographic assessments of the chisel-toothed kangaroo rat, *Dipodomys microps* Merriam (Jezkova et al., 2011), and the pallid kangaroo mouse, *Microdipodops pallidus* Merriam (Hafner et al., 2008), suggest that both species remained in the Great Basin during the LGM (rendering them orthoselective). Phylogeography of the bushy-tailed woodrat, *Neotoma cinerea* (Ord), suggests that it followed the migration model, as the LGM distribution of this species appears to have shifted to the south (Hornsby and Matocq, 2011). Other recent studies of arid-adapted reptile species, the desert horned lizard, *Phrynosoma platyrhinos* Girard (Jezkova, 2010) and the western North American nightsnake, *Hypsiglena torquata* (Günther) (Mulcahy, 2008), suggest that both species probably followed the migration model. Data from *P. becki* also fit a migration model, but with the caveat that this scorpion may have persisted within small parts of the western Great Basin, and recently expanded to occupy a larger part of the region.

The phylogeography of *P. becki* also uncovered an unexpected degree of phylogeographic structure across the Mojave Desert. Given that mitochondrial groupings were not strongly supported by nuclear data, and the fact that no obvious morphological divergences were discerned from samples collected throughout the species’ range, we conclude that *P. becki* probably represents a single species. Such patterns underscore the need for integrative approaches to delimit species and assess relationships among little-known terrestrial invertebrates like scorpions.

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