



## Cryptic genetic diversity and complex phylogeography of the boreal North American scorpion, *Paruroctonus boreus* (Vaejovidae)



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### ABSTRACT

Diverse studies in western North America have revealed the role of topography for dynamically shaping genetic diversity within species through vicariance, dispersal and range expansion. We examined patterns of phylogeographical diversity in the widespread but poorly studied North American vaejovid scorpion, *Paruroctonus boreus* Girard 1854. We used mitochondrial sequence data and parsimony, likelihood, and Bayesian inference to reconstruct phylogenetic relationships across the distributional range of *P. boreus*, focusing on intermontane western North America. Additionally, we developed a species distribution model to predict its present and historical distributions during the Last Glacial Maximum and the Last Interglacial Maximum. Our results documented complex phylogeographic relationships within *P. boreus*, with multiple, well-supported crown clades that are either geographically-circumscribed or widespread and separated by short, poorly supported internodes. We also observed subtle variation in predicted habitat suitability, especially at the northern, eastern and southern edges of the predicted distributional range under past climatic conditions. The complex phylogenetic relationships of *P. boreus* suggests that historical isolation and expansion of populations may have occurred. Variation in the predicted distributional range over time may implicate past climatic fluctuations in generating the patterns of genetic diversity observed in *P. boreus*. These findings highlight both the potential for cryptic biodiversity in widespread North American scorpion species and the importance of phylogeographical studies for understanding the factors responsible for generating the biodiversity of western North America.

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### 1. Introduction

The landscape of intermontane western North America, from the Rocky Mountains in the east to the Sierra Nevada and Cascade ranges in the west, developed over 40 million years of continuous tectonic activity (Flesch et al., 2000; Lipman, 1980; Mousumi et al., 2009). The geography and climate of this region varies from low-lying desert valleys, intermediate elevation deciduous and boreal forests to tundra-like mountain peaks, often over relatively short distances. The flora and fauna of this region have experienced dramatic shifts in climate and topography during their evolutionary history (Wells, 1983). Studies of geographical patterns of genetic diversity across this landscape offer insights into the roles of geography and climate in structuring genetic variation in the biota of western North America.

The complex geography and ecology of intermontane western North America is associated with multifarious patterns of genetic and biological diversity. Species boundaries and genetic diversity in the intermontane west are linked either to geographical barriers or transitions between ecoregions. The first pattern is exemplified by species with distributions limited to a single ecoregion (*Hadrurus arizonensis*, Graham et al., 2013b; e.g., *Perognathus flavus*, Neiswenter and Riddle, 2010) or clades delimited by these ecoregions (e.g., *Chionactis occipitalis*, Wood et al., 2008; arachnids, reptiles, amphibians and mammals, Wood et al., 2013). The second pattern is exemplified by species with clades defined by geographical barriers (e.g., *Gastrophryne olivaceous*, Streicher et al., 2012). A third pattern is exemplified by species with little geographical structure across intermontane western North America, (e.g., *Bufo punctatus*, Jaeger et al., 2005). Of course, geographical barriers are often associated with shifts in ecological conditions, and the phylogeographical relationships within a single species may exhibit elements of all three patterns.

Geography often changes abruptly in intermontane western North America, hence even relatively minor shifts in climate may

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alter the presence, extent and connection of different ecosystems. Bioclimatic modeling and paleoevidence suggest that habitat shifts occurred rapidly over the past several hundred thousand years (Grayson, 1987, 2006; Waltari and Guralnick, 2009). Historical fluctuations in the ecology of this region may have varied the available habitat and amount of gene flow among populations of many species, potentially generating complex patterns of phylogenetic diversity (Grayson, 1987, 2006; Waltari and Guralnick, 2009).

We investigated the phylogeography of the boreal scorpion, *Paruroctonus boreus* Girard, 1854 (Fig. 1), of the endemic North American scorpion family Vaejovidae Thorell, 1876, to examine how genetic diversity is structured across the Great Basin, how historical climate fluctuations can impact predicted species distributions, and how historical species distributions may be linked to current geographical patterns of genetic diversity in widespread species. *Paruroctonus boreus* has one of the most widespread distributions of any North American scorpion (Fig. 2), extending from northern Arizona in the USA to British Columbia in Canada, and from western Nebraska to central Oregon, USA (Gertsch and Sologlad, 1966; Haradon, 1985; Sissom and Franke, 1981). *Paruroctonus boreus* tends to be restricted to sagebrush and similar habitats in the north of its distribution (Allred, 1975; Johnson, 2004; Zack and Looney, 2012), but occurs in a more varied array of habitats in the south (Allred and Gertsch, 1976). It is well known that scorpions have low vagility and limited capability for long-distance dispersal (Prendini, 2001; Yamashita and Polis, 1995) and this was recently corroborated by phylogeographical studies (Bryson et al., 2013a; Yamashita and Polis, 1995).

We analyzed two mitochondrial markers to examine how genetic diversity is distributed across the geographical range of *P. boreus*, focusing on the Great Basin. We also modeled the predicted species distribution of *P. boreus* during the present and during past periods of climatic fluctuation. Our phylogeographical

results document complex genetic structure among populations of *P. boreus*, whereas the species distribution models suggest historical variation in its predicted distribution. We interpret these data in the context of previous phylogeographical and paleoclimatic modeling research to gain insights into the patterns and processes that structured the genetic diversity of species in the Great Basin and western North America.

## 2. Methods

### 2.1. Field collecting and geographic sampling

We collected scorpions using long-wave ultraviolet lights at night and by turning rocks during the summers of 2002–2010. Sixty-eight samples of *P. boreus* were sequenced from 30 localities, representing populations that cross potential biogeographical barriers, and span most of the geographical range of the species (Table 1; Fig. 2). The related species, *Paruroctonus utahensis* (Williams, 1968), was sequenced as an outgroup. Vouchers and tissue samples were stored in 95% ethanol and deposited in the collections of the Denver Museum of Nature and Science (DMNS), Colorado, and the American Museum of Natural History (AMNH), New York (Table 1).

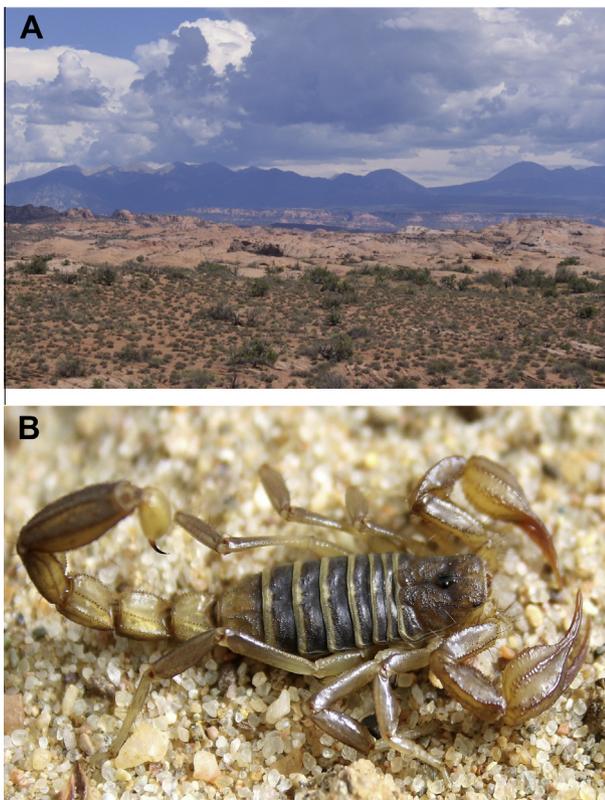
We also downloaded sequence data from GenBank (Table 1) for three other congeners, *Paruroctonus becki* Gertsch & Allred, 1965, *Paruroctonus silvestrii* Borelli, 1909 and *Paruroctonus variabilis* Hjelle, 1982, and two other, more distantly related scorpion taxa, *Uroctonus mordax* Thorell, 1876 and *Centruroides gracilis* Latreille, 1804, as outgroups.

### 2.2. Molecular methods

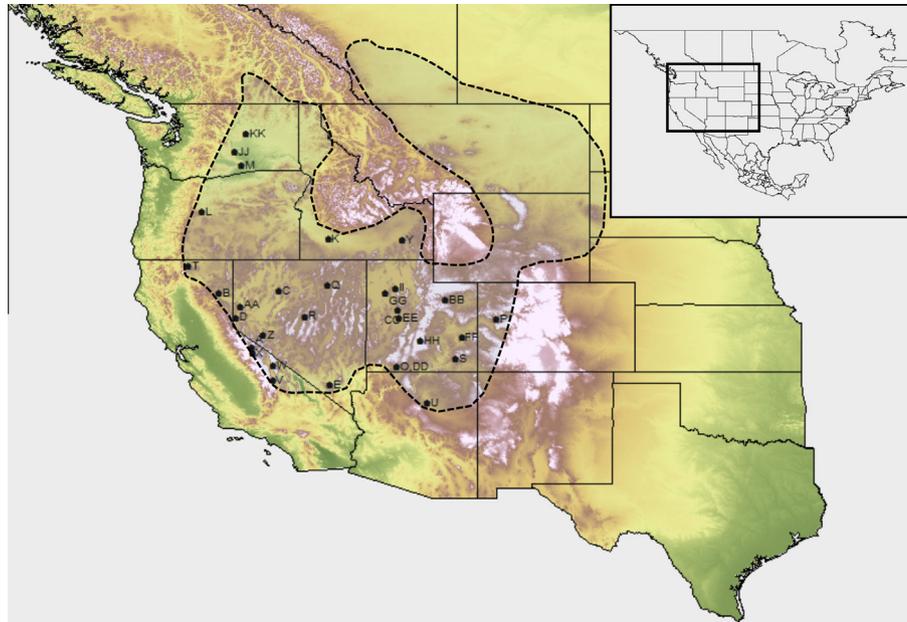
DNA was extracted from scorpion tissues using the DNEasy Blood and Tissue Kit (Qiagen). We sequenced two mitochondrial markers, the ribosomal 16S subunit (16S) and Cytochrome c Oxidase I (COI), which have been shown to reveal phylogenetic structure at the species and subspecies level in scorpions (Bryson et al., 2013a; Parmakelis et al., 2006). We amplified a fragment of the 16S gene using primers and conditions described by Gantenbein et al. (1999) and Prendini et al. (2003, 2005) and a fragment of the COI gene using primers and conditions described by Prendini et al. (2003, 2005) and Vink et al. (2005). We used the ExoSAP-IT kit (United States Biochemical) to prepare PCR products for sequencing. Prepared PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.). We precipitated and rehydrated our samples using ethanol/sodium acetate and HPLC-purified formamide (HIDI). Samples were analyzed on an ABI PRISM 3100xl automated sequencer at the Genomics Core Facility of the University of Texas-Arlington and an ABI PRISM 3730xl automated sequencer at the Sackler Institute for Comparative Genomics of the AMNH.

### 2.3. Analytical methods

Sequences were assembled and edited using Sequencher (GeneCodes Co., Ann Arbor), aligned in MEGA v. 5.2.2 (Tamura et al., 2011) using the ClustalW algorithm (Larkin et al., 2007), and adjusted manually if necessary. Each COI sequence was translated to ensure that it was in an open reading frame. The aligned 16S and COI fragments were concatenated for analysis. We determined the most appropriate model of molecular evolution by ranking models in MEGA using the Bayesian Information Criterion (Kass and Raftery, 1995), and selected similar top-ranking models for both genes. We calculated the number of haplotypes, haplotype diversity, number of polymorphic sites, and nucleotide diversity for both gene regions using DNaSP v. 5 (Librado and Rozas,



**Fig. 1.** (A) San Juan County, southeastern Utah, USA, typical habitat of the boreal North American vaejovid scorpion, *Paruroctonus boreus*. (B) Habitus of *P. boreus* (male) from Mt. Charleston, Nevada, USA.



**Fig. 2.** Hypothetical distribution of the boreal North American vaejovid scorpion, *Paruroctonus boreus*, indicated with dashed line, showing localities from which samples were obtained for DNA analysis, indicated with black dots (codes for localities in Table 1; inset showing the study area in the US).

2009). We also tested for selection on *COI* using the codon-based Z-test in MEGA, which infers selection based on variation in the ratio of synonymous to nonsynonymous mutations.

Aligned sequence data were analyzed with three phylogenetic methods: parsimony, maximum likelihood (ML), and Bayesian inference. We conducted parsimony analysis in MEGA, using a close-neighbor-interchange parsimony search on random trees (a branch-and-bound heuristic parsimony search yielded similar topologies). ML analyses were conducted on the concatenated dataset, partitioned by gene and codon position for *COI*, with RAxML (Stamatakis, 2006), using RAxML-HPC Blackbox v. 7.4.2 on the CIPRES Science Gateway Server (Miller et al., 2010). Data were analyzed under the GTR+G model of molecular evolution with the gamma distribution specified for five categories. Nodal support for both parsimony and ML methods was determined by bootstrapping with 1000 pseudoreplicates. We also conducted Bayesian inference on the concatenated dataset, partitioned by gene and codon position for *COI*, using MrBayes v. 3.1 (Huelsenbeck and Ronquist, 2001). We implemented the GTR+I+G model of molecular evolution with five discrete gamma categories, and used default parameters of MrBayes, except for a variable rate prior. Markov-chain Monte Carlo (MCMC) searches were conducted for 4 million generations (sampling frequency of once every 100 generations) using four chains (three heated and one cold). We determined whether the MCMC searches had converged by (1) assessing when the standard deviation of split frequencies had declined to below 0.01; (2) examining log-likelihood versus generation plots; and (3) using the program AWTY (Wilgenbusch et al., 2004) to examine other output parameters. We discarded the first 25% of trees as burnin, and then visualized and manipulated phylogenies in Figtree v. 1.3.1 (Rambaut, 2007).

#### 2.4. Species distribution modeling

We added 30 localities associated with our genetic samples to 59 localities obtained from records in museum databases (<http://symbiota1.acis.ufl.edu/scan/portal/>) and the literature (Gertsch and Soleglad, 1966; Johnson and Allred, 1972) to build our species distribution models (SDM). Records without geographical

coordinates were georeferenced using Google Earth (<http://earth.google.com>) to obtain a relative longitude and latitude for modeling. Only records that could be resolved with a reasonable level of geographical accuracy were included. We used MAXENT 3.3.2 (Phillips et al., 2006) to construct SDMs of *P. boreus* due to its robust performance in relation to other models (Elith et al., 2006) and its statistical validation (Elith et al., 2011). We used all nineteen bioclimatic data layers along with altitude at 0.5' (0.9 × 0.9 km) and 2.5' (4 × 4 km) spatial resolution from the WorldClim dataset (<http://www.worldclim.org>; (Hijmans et al., 2005), for three different time periods: present day (PD), Last Glacial Maximum (LGM, ca. 21,000 ybp), derived from the Community Climate System Model (CCSM; (Otto-Bliesner et al., 2006) and Last Interglacial Maximum (LIG, ca. 140,000–120,000 ybp) derived from the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2; (Braconnot et al., 2007). Analyses were performed using version 06-1-2013 of the database (<http://pmip2.lscce.ipsl.fr>).

Environmental data were imported into ArcMap (version 10.1) and extracted (clipped) with a 'mask' of the western USA represented by the following states: Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington and Wyoming. We restricted the geographical area of the model to states potentially inhabited by *P. boreus* during historical or present times. Raster data, along with the 89 presence localities, were converted to ASCII files for analysis in MAXENT, and modeling performed as described (Phillips et al., 2006).

### 3. Results

#### 3.1. Mitochondrial sequence diversity in *P. boreus*

We sequenced 942 base-pairs (bp) of *COI* from 68 specimens and 289 bp of *16S* from 49 specimens of *P. boreus*, collected at 30 unique localities. Both *COI* and *16S* were variable across the geographical range of *P. boreus*. *COI* was more variable, with more haplotypes, greater haplotype diversity, and greater nucleotide diversity, than *16S* (Table 2). As for many protein-encoding genes (Hughes et al., 2003), the ratio of synonymous to nonsynonymous

Table 1

Locality ID (on Fig. 2), location, county, state, elevation, and Genbank accession numbers for both 16S and COI.

ID	Taxa	Locale	GPS N	GPS W	County	State	Elevation (m)	16S	COI
A10	<i>Paruroctonus boreus</i>	A	38.05445	119.1253	Mono	CA	2083		
A8	<i>P. boreus</i>	A	38.05445	119.1253	Mono	CA	2083		
A19	<i>P. boreus</i>	A	38.05445	119.1253	Mono	CA	2083		
A28	<i>P. boreus</i>	A	38.05445	119.1253	Mono	CA	2083		
B1	<i>P. boreus</i>	B	40.46	120.57	Lassen	CA	1672		
B16	<i>P. boreus</i>	B	40.46	120.57	Lassen	CA	1672		
B2	<i>P. boreus</i>	B	40.46	120.57	Lassen	CA	1672		
B36	<i>P. boreus</i>	B	40.46	120.57	Lassen	CA	1672		
B4	<i>P. boreus</i>	B	40.46	120.57	Lassen	CA	1672		
B9	<i>P. boreus</i>	B	40.46	120.57	Lassen	CA	1672		
C30	<i>P. boreus</i>	C	40.60137	117.9135	Pershing	NV	1630		
D20	<i>P. boreus</i>	D	39.39393	119.8388	Washoe	NV	1828		
D22	<i>P. boreus</i>	D	39.39393	119.8388	Washoe	NV	1828		
D3	<i>P. boreus</i>	D	39.39393	119.8388	Washoe	NV	1828		
D30	<i>P. boreus</i>	D	39.39393	119.8388	Washoe	NV	1828		
E1	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
E15	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
E2	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
E33	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
H11	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
H15	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
H4	<i>P. boreus</i>	E	36.3688	115.6287	Clark	NV	2123–2255		
K16	<i>P. boreus</i>	K	42.90284	115.6927	Owyhee	ID	760		
K18	<i>P. boreus</i>	K	42.90284	115.6927	Owyhee	ID	760		
K301	<i>P. boreus</i>	K	42.90284	115.6927	Owyhee	ID	760		
K36	<i>P. boreus</i>	K	42.90284	115.6927	Owyhee	ID	760		
L2	<i>P. boreus</i>	L	44.13128	121.3324	Deschutes	OR	983		
L3	<i>P. boreus</i>	L	44.13128	121.3324	Deschutes	OR	983		
L301	<i>P. boreus</i>	L	44.13128	121.3324	Deschutes	OR	983		
L5	<i>P. boreus</i>	L	44.13128	121.3324	Deschutes	OR	983		
L6	<i>P. boreus</i>	L	44.13128	121.3324	Deschutes	OR	983		
MJF1	<i>P. boreus</i>	M	46.2	119.6	Benton	WA	457		
M4	<i>P. boreus</i>	M	46.2	119.6	Benton	WA	457		
O11	<i>P. boreus</i>	O	37.19	112.66	Kane	UT	1983		
O33	<i>P. boreus</i>	O	37.19	112.66	Kane	UT	1983		
O9	<i>P. boreus</i>	O	37.19	112.66	Kane	UT	1983		
P1	<i>P. boreus</i>	P	39.33517	108.203	Mesa	CO	1734		
P13	<i>P. boreus</i>	P	39.33517	108.203	Mesa	CO	1734		
P16	<i>P. boreus</i>	P	39.33517	108.203	Mesa	CO	1734		
P201	<i>P. boreus</i>	P	39.33517	108.203	Mesa	CO	1734		
P21	<i>P. boreus</i>	P	39.33517	108.203	Mesa	CO	1734		
Q11	<i>P. boreus</i>	Q	40.82	115.73	Elko	NV	1626		
Q6	<i>P. boreus</i>	Q	40.82	115.73	Elko	NV	1626		
Q9	<i>P. boreus</i>	Q	40.82	115.73	Elko	NV	2011		
R10	<i>P. boreus</i>	R	39.4455	116.7561	Lander	NV	2011		
R2	<i>P. boreus</i>	R	39.4455	116.7561	Lander	NV	2011		
R3	<i>P. boreus</i>	R	39.4455	116.7561	Lander	NV	2011		
R4	<i>P. boreus</i>	R	39.4455	116.7561	Lander	NV	2011		
T201	<i>P. boreus</i>	T	41.67267	121.9462	Siskiyou	CA	1463		
S1	<i>P. boreus</i>	S	37.5611	110.0087	San Juan	UT	1476		
S501	<i>P. boreus</i>	S	37.5611	110.0087	San Juan	UT	1476		
LP 6288	<i>P. boreus</i>	AA	39.85531	–119.656	Washoe	NV	1412		
LP 6401	<i>P. boreus</i>	BB	40.18441	–110.463	Duchesne	UT	1745		
LP 7260	<i>P. boreus</i>	CC	39.70557	–112.583	Millard	UT	1442		
LP 8492	<i>P. boreus</i>	DD	37.17601	–112.627	Kane	UT	1871		
LP 6399	<i>P. boreus</i>	EE	39.38104	–112.563	Millard	UT	1434		
LP 7795	<i>P. boreus</i>	FF	38.48722	–109.74	Grand	UT	1816		
LP 6855	<i>P. boreus</i>	GG	40.49886	–113.153	Tooele	UT	1286		
LP 6905	<i>P. boreus</i>	HH	38.34883	–111.571	Wayne	UT	2120		
LP 6398	<i>P. boreus</i>	II	40.70906	–112.687	Tooele	UT	1463		
LP 8864	<i>P. boreus</i>	JJ	46.81694	–119.88	Grant	WA	360		
LP10488	<i>P. boreus</i>	KK	47.59052	–119.379	Grant	WA	367		
LP 7794	<i>P. boreus</i>	U	35.57117	–111.286	Coconino	AZ	1309		
LP 5052	<i>P. boreus</i>	V	36.59972	–118.183	Inyo	CA	1787		
LP 3193	<i>P. boreus</i>	W	37.25461	–118.156	Inyo	CA	2110		
LP 4990	<i>P. boreus</i>	X	37.79982	–119.079	Inyo	CA	2376		
LP 1975	<i>P. boreus</i>	Y	42.85538	–112.396	Bannock	ID	1521		
LP 7258	<i>P. boreus</i>	Z	38.59453	–118.599	Mineral	NV	1262		
LP6287	<i>Paruroctonus variabilis</i>							KF548407.1	KF548323.1
MG0012	<i>Paruroctonus becki</i>							KF548424.1	KF548330.1
LP3199	<i>Paruroctonus silvestrii</i>							KF548406.1	KF548318.1
LP10556	<i>Paruroctonus utahensis</i>								
	<i>Centruroides gracilis</i>							DQ990822	AY995830.1
	<i>Uroctonus mordax</i>							NC010782.1	NC010782.1

**Table 2**  
Summary of genetic variation in *P. boreus* for both Cox-1 and 16S.

Statistic	Cox-1	16S
Sequence length	946	291
<i>h</i> (number of haplotypes)	36	20
<i>H<sub>d</sub></i> (haplotype diversity)	0.981	0.929
<i>S</i> (polymorphic sites)	216	28
$\pi$ (nucleotide diversity)	0.049	0.031

mutations (*dN/dS* ratio) suggested that *COI* has experienced purifying selection.

### 3.2. Phylogenetic relationships among *P. boreus* populations

Parsimony, ML, and Bayesian inference of *16S* and *COI*, analyzed separately and concatenated, recovered similar topologies. We therefore present only the ML tree, with nodal support from all three phylogenetic reconstruction methods on a concatenated dataset (Fig. 3). We recovered the monophyly of *Paruroctonus* and *P. boreus*. *Paruroctonus becki* grouped sister to *P. variabilis*, to the exclusion of the other *Paruroctonus* species included in the analysis, whereas *P. silvestrii* grouped sister to a clade comprising *P. boreus* and *P. utahensis* (see Fig. 3).

Major clades within *P. boreus* were mostly well-supported but geographically complex (Fig. 3). The ten major clades recovered could be categorized into three main types: (1) clades that were entirely geographically circumscribed (i.e., comprising individuals from the same or geographically proximate populations); (2) clades mostly comprising individuals from a single population, but including a few individuals from geographically disparate localities; and (3) geographically widespread clades. Geographically-circumscribed clades (2, 5, 6) were largely restricted to the northern part of the distributional range of *P. boreus*, although this category includes some clades (7 and 10) from further south. Both types of geographically-widespread clades were found further south in the distributional range of *P. boreus*. Clades 3, 4, and 8 were comprised mostly of individuals from the same or geographically proximate populations, whereas clades 1 and 9 comprised individuals from geographically widespread populations. One sample from locality V (Inyo County, CA) did not form part of any major clade, but was placed sister to clades 9 and 10 (with low nodal support). Although most individuals from the same populations grouped together into clades (e.g., localities K in southwestern ID, L from central OR, or M from southern WA), some were scattered among phylogenetically divergent clades (e.g., localities Q from northern NV and O from western UT). Strong nodal support was obtained for the crown clades within *P. boreus*, compared with the deeper relationships among clades, which were instead characterized by short internodes and weak nodal support.

### 3.3. Species distribution modeling

The three species distribution models suggested subtle historical variation in the predicted distribution of *P. boreus*. The PD model accurately predicted the current distribution of *P. boreus*, especially the core of its distributional range, with high confidence (ca. 60–75%). The two models of past climate generally predicted high habitat suitability in the center of the range of *P. boreus*, but differed in the extent of marginal habitat predicted at the edges of its range. The LGM model was largely similar to the PD model, but predicted a greater extent of marginal habitat at the southern and eastern edges of the range, and less habitat in the northern part thereof. The LIG model differed from both the PD and LGM models in predicting a greater extent of marginal habitat at the northeastern edge of the range, but a decrease in suitable habitat in the southern part thereof. The PD and LIG models predicted an

isolated patch of highly suitable habitat (>95%), surrounded by unsuitable habitat (<10%) at locality E in southern Nevada (Fig. 4). In contrast, the LGM model predicted reasonably suitable habitat (approximately 50%) connecting this locality to other predicted suitable habitats. Interestingly, *P. boreus* occurs at high elevations at this locality (Table 1), surrounded by lower valleys. Higher elevations are occupied in the southern part of the distribution, and lower elevations are occupied in the north (Table 1).

## 4. Discussion

The phylogeographical reconstruction of *P. boreus* recovered by our analyses comprises multiple, well supported crown clades that are both geographically-circumscribed and widespread. These clades, in turn, are separated by short, poorly supported internodes. In addition, we observed subtle variation in the predicted distribution of *P. boreus* across its current distribution through time. Below, we compare these results to previous research on the phylogeography and distribution of western North American taxa to make inferences about the processes governing the geographical distribution of genetic diversity in *P. boreus*.

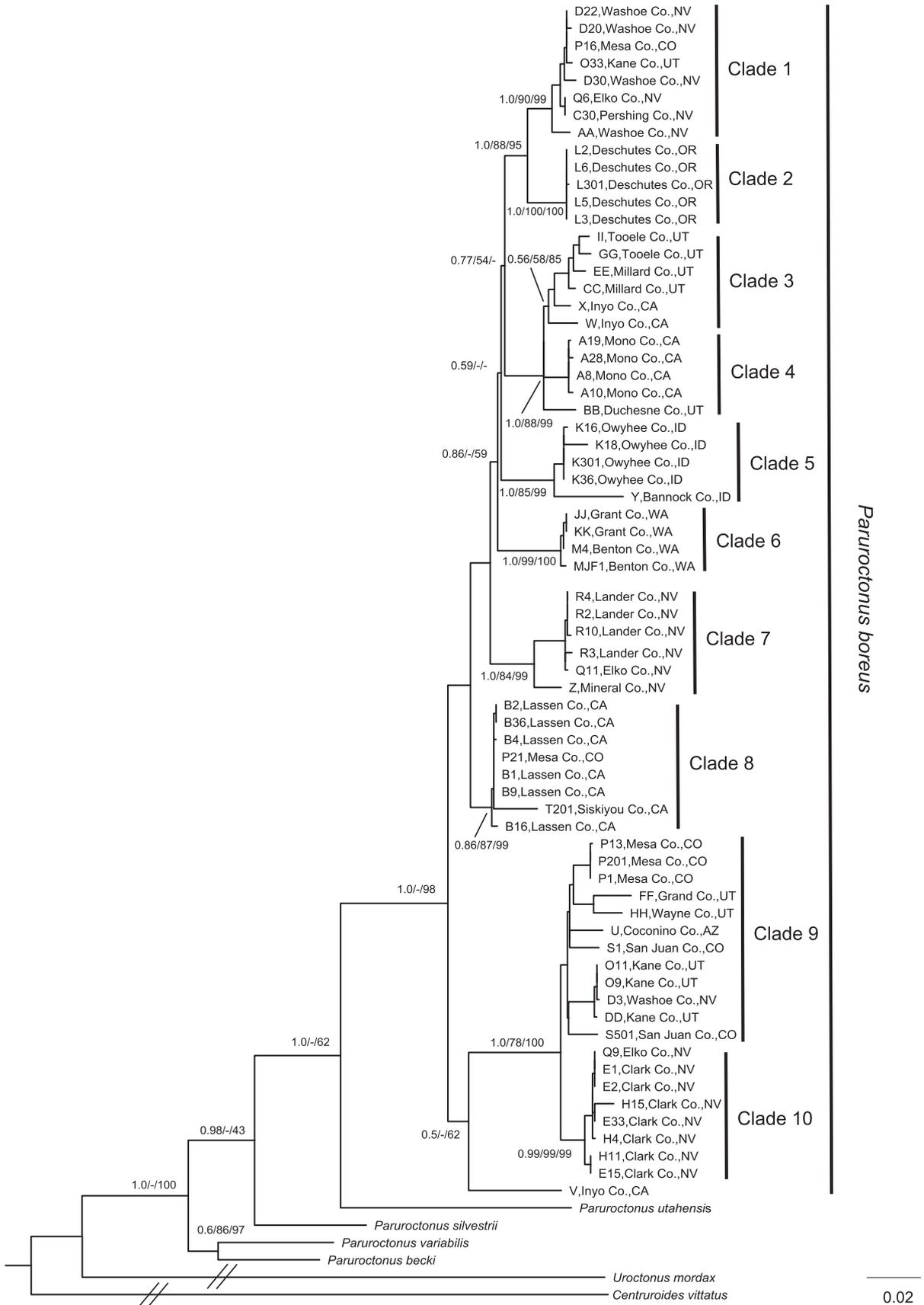
### 4.1. Phylogenetic relationships in *Paruroctonus*

Estep et al. (2005) and Bryson et al. (2013a) provided preliminary evidence for phylogenetic relationships among a limited sample of species in the genus *Paruroctonus*. The phylogenetic relationships among *Paruroctonus* recovered in our study are identical to those recovered in the phylogeographical analysis of *P. becki* (Graham et al., 2013a). Although the consistency of these results is encouraging, only four (Graham et al., 2013a) and five (our study) of the 29 described species of *Paruroctonus* (REVSYS: Systematics of the Scorpion Family Vaejovidae; <http://www.vaejovidae.com>) were included in analyses, to date. Extending this research with additional taxonomic sampling will help to clarify systematic relationships, biogeography and biodiversity within *Paruroctonus*.

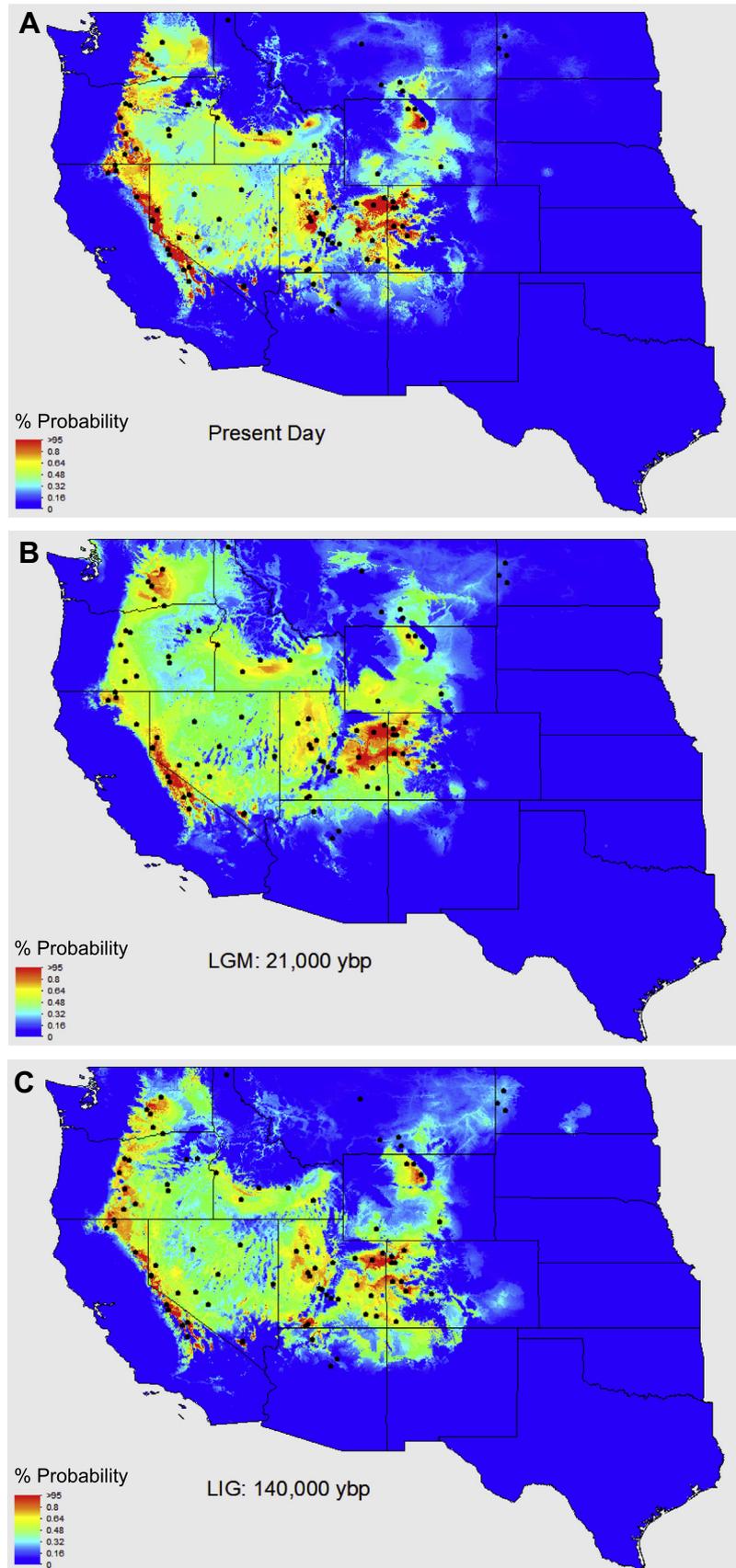
### 4.2. Phylogeography of *P. boreus*

As in many invertebrate taxa, molecular phylogenetic and phylogeographical studies of scorpions are uncommon, relative to the biodiversity of the group. Much of the previous research on scorpion phylogeography focused on Palearctic species (Gantenbein and Lariader, 2003; Graham et al., 2012; Othmen et al., 2009; Parmakelis et al., 2006; Salomone et al., 2007; Shi et al., 2013; Sousa et al., 2010), although recent research addressed Nearctic species (Bryson et al., 2013, 2013b; Estep et al., 2005; Gantenbein et al., 2001; Graham et al., 2013a,b; Yamashita and Rhoads, 2013). The limited number of scorpion taxa thus far studied in western North America usually have geographically-delimited clades that can be defined both by habitat types and geographical barriers (Bryson et al., 2013a; Estep et al., 2005; Gantenbein et al., 2001; Graham et al., 2013a,b; Graham et al., 2012; Yamashita and Polis, 1995). Other animal taxa in the Great Basin display either minimal phylogeographical structure (Jaeger et al., 2005), structure consistent with geographical barriers and habitat types (Wood et al., 2008, 2013), or some combination thereof (Hafner and Upham, 2011; Hafner et al., 2008).

Unlike previously documented phylogeographical patterns in the Great Plains, relationships among *P. boreus* populations recovered in our analysis were characterized by well-defined crown clades separated by short, poorly supported internodes (Fig 3). Whereas some clades, mostly in the northern parts of the distributional range of *P. boreus*, were geographically circumscribed, many



**Fig. 3.** Maximum likelihood reconstruction of relationships within the boreal North American vaejovid scorpion, *Paruroctonus boreus*, based on concatenated fragments of mitochondrial DNA from the Cytochrome Oxidase c I and 16S rDNA genes. Other analytical methods of the separate and concatenated datasets yielded similar topologies. Numbers listed above or below nodes are posterior probabilities from Bayesian inference (left), and nodal support from 1000 bootstrap pseudoreplicates in maximum likelihood analysis (middle) or parsimony analysis (right).



**Fig. 4.** Species distribution model predictions for the boreal North American vaejovid scorpion, *Paruroctonus boreus*, during conditions at the present, and during the Last Glacial Maximum (LGM) and the Last Interglacial Maximum (LIG). Black dots represent locality records. Highly suitable habitats (85–100% probability) are indicated in red or orange, moderately suitable habitats (65–75% probability) in yellow, and unsuitable habitats (<15% probability) in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clades comprised individuals from multiple, geographically disparate populations. Although many biotic and abiotic processes may be implicated in generating complex phylogeographical relationships, there are a few mechanisms, acting alone or in concert, that are likely candidates for generating this pattern of phylogenetic diversity. The short internodes between crown clades could be created by relatively rapid isolation of populations across their geographical range (Lenk et al., 1999; Pfenninger and Posada, 2002). Alternatively, the geographically widespread clades could be the result of rapid expansion or fragmentation of a genetically diverse founder population (Fontenot et al., 2011; Makowsky et al., 2009). Our results do not bear the standard signature of rapid range expansion (Excoffier et al., 2009; Ibrahim et al., 1996) which consists of minimally divergent, geographically widespread haplotypes (it could also indicate a selective sweep). Finally, our results could suggest recent gene flow or introgression among genetically and geographically divergent populations (Lenk et al., 1999; Pfenninger and Posada, 2002). We cannot discriminate among these processes because our sampling was unbalanced (i.e., many samples from single localities and single samples from many localities).

#### 4.3. Geographical distribution of *P. boreus*

Our SDMs identified some variation in the predicted historical distribution of *P. boreus*, compared to their current distribution. Interestingly, the PD model predicts with moderate probability the presence of *P. boreus* in western Nebraska, although this locality was not in our model. *Paruroctonus boreus* has been reported informally from this locality, but we could not find any records of this species from this area in scientific publications or museum databases.

There was notable variation among different models in the extent of marginal habitat predicted in the northern, eastern, and southern extent of the predicted distribution of *P. boreus*. This variation in suitable habitat could plausibly imply that there has been historical variation in the geographical isolation of populations of *P. boreus*, especially at the margins of their distributional range. The Great Basin has unusually clear records of how historical climatic fluctuations have shifted the distributions of some taxa both in elevation and latitude, although comparative data for invertebrates are scarce (Grayson, 1987, 2006; Waltari and Guralnick, 2009). Indeed, range expansion and introgression related to historical climatic shifts have been implicated in phylogeographical patterns in other taxa from the Great Basin (Spellman et al., 2007; Waltari and Guralnick, 2009). We acknowledge the potential pitfalls associated with interpretation of SDMs, which include the troublesome assumptions of predicting distributions for widespread taxa without accounting for physiological or evolutionary adaptation (Cooper et al., 2011; Martinez-Cabrera et al., 2012) and the potential inaccuracy of predicting past and present species distributions from a simple set of temperature and precipitation measures, while ignoring biotic interactions (reviewed in Pearson and Dawson, 2003). We therefore interpret these results cautiously as suggesting a role for past geographical range variation in the complex phylogeography of *P. boreus*.

Based on our analyses, the current elevational distribution of *P. boreus* conforms to the biogeographical patterns of other Great Basin taxa associated with sagebrush communities, i.e., (mammals, Grayson, 2006; Hafner and Upham, 2011; Lawlor, 1998; sage-grouse, Miller and Eddleman, 2000; night snake, Mulcahy, 2008; sage-grouse, Schroeder et al., 2004). There is a clinal elevation decrease from south to north exhibited by *P. boreus* (Table 1). The sagebrush communities have changed dramatically over time by shifting its distribution from the southwest, now dominated by creosote bush, to its current distribution primarily in the Great

Basin (Miller and Eddleman, 2000). This shift, which corresponds to historical climatic fluctuations in western North America (Tausch et al., 1993), fits our model of the predicted historical distribution of *P. boreus*.

## 5. Conclusions

Although there are relatively few biogeographical and phylogeographical studies of terrestrial invertebrates in western North America, recent research has begun to incorporate them in studies of phylogenetic patterns in this region (Graham et al., 2013a; Wilson and Pitts, 2010). Our study provides further evidence for cryptic genetic diversity in North American scorpions, highlighting the importance of phylogeographical studies on poorly studied taxa from this region.

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