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Pleistocene refugia and their effects on the phylogeography and genetic structure of the wolf spider *Pardosa sierra* (Araneae: Lycosidae) on the Baja California Peninsula

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Abstract. The phylogeographic structure of some species distributed across the Baja California Peninsula has been traditionally hypothesized as resulting from vicariant events thought to have occurred between 1–3 Mya. Climatic fluctuations during the Pleistocene have also been shown to influence the distribution patterns of species, and vicariant patterns may have been erased as a consequence of population contractions or expansions into or out of refugia generated during the last glacial maximum ca. 21,000 years ago. Thus, there is still some uncertainty regarding the relative role of vicariance in shaping the modern biota of Baja California. To understand the evolutionary history of the wolf spider *Pardosa sierra* Banks, 1898 on the peninsula, a phylogeny of this species and closely related taxa was generated using a fragment of the mitochondrial gene cytochrome c oxidase subunit I (CO1). Sequences of a fragment of the CO1 gene for 38 individuals from 14 sampling sites along the entire distribution range of *P. sierra* were used to infer phylogeographic patterns, and five nuclear microsatellite loci were also used to genotype 296 individuals from seven of these 14 locations. The current and past potential distributions from two Pleistocene periods were estimated using niche-based distribution modeling, and scenarios of colonization from detected refugia were simulated. We found that Californian populations of *P. sierra* diverged from peninsular populations 4 Mya, this divergence coinciding with the northern-gulf split. However, we did not detect genetic breaks in regions where the mid-peninsular and Isthmus of La Paz canals were presumably formed, either with mitochondrial DNA sequences or microsatellite loci. Two refugia were further detected at the geographic ends of the peninsula, these likely preceding subsequent habitat expansion.

Keywords: Mitochondrial DNA, microsatellite, vicariance, climate change, niche modeling

The Baja California Peninsula (BCP; Fig. 1) has been a model landscape for phylogeographic studies due to its geographic isolation, landscape heterogeneity and high levels of endemism (Jezkova et al. 2009; Wilson & Pitts 2012; Graham et al. 2014; Dolby et al. 2015). In several studies, population divergences or phylogeographic breaks have been detected in a diversity of taxa (Upton & Murphy 1997; Riddle et al. 2000; Murphy & Aguirre-León 2002; Nason et al. 2002; Zink 2002a; Crews & Hedin 2006; Lindell et al. 2006; Graham et al. 2014; Lira-Noriega et al. 2015). To explain the causes of such phylogeographic breaks, hypotheses that include vicariance events have been proposed, with the most important being these: (1) the marine transgression of the Gulf of California in the northern-gulf region (south of California and Arizona), with separation from the rest of the BCP occurring 3 million years ago (Mya) (Riddle et al. 2000; Hafner & Riddle 2005); (2) the mid-peninsular channel, which formed approximately 1 to 1.6 Mya in the nearby Vizcaíno Desert (Upton & Murphy 1997; Hafner & Riddle 2005; Crews & Hedin 2006; Lindell et al. 2006); and (3) the Isthmus of La Paz Channel, which separated the Cape Region of the BCP 3 Mya (Riddle et al. 2000; Hafner & Riddle 2005; Garrick et al. 2013).

Alternative non-vicariant biogeographic hypotheses have also been proposed, most of these highlighting the potential effects of cyclic climatic changes during Pleistocene glacial and interglacial periods (Hewitt 1996, 2004; Hafner & Riddle 2005; Lindell et al. 2006; Riddle & Hafner 2006; Leaché & Mulcahy 2007; Garrick et al. 2009, 2013). In particular, the Last Glacial Maximum (LGM) ca. 21,000 years ago (Dansgaard et al. 1993) may have forced many species from the deserts of the Northern Hemisphere to find refuge south of their previous distributions (Hewitt 1996, 2000, 2004; Van Devender 2002; Graham et al. 2013; Harl et al. 2014). The Cape Region, at the southern tip of the BCP, was likely used as a refugium for species that could not tolerate cold (Murphy & Aguirre-León 2002; Garrick et al. 2013). However, the number and location of refugia is species dependent, as each likely responded differently to climatic events (Harl et al. 2014). Similarly, it is also probable that cycles of range contraction and range expansion generated by climatic oscillations (Grismer 2002) may have erased the genetic fingerprints produced by earlier vicariance events (Crews & Hedin 2006). This raises uncertainty about whether the phylogeographic patterns observed are due to hypothesized vicariance events or climatic changes or both.

Pardosa sierra Banks, 1898 is a species of wolf spider (Lycosidae) that is endemic to the BCP (Correa-Ramírez et al. 2010); it is intolerant of cold temperatures (Van Dyke & Lowrie 1975) and strongly dependent on water bodies (Punzo & Farmer 2006; Correa-Ramírez 2010; Jiménez et al. 2015).

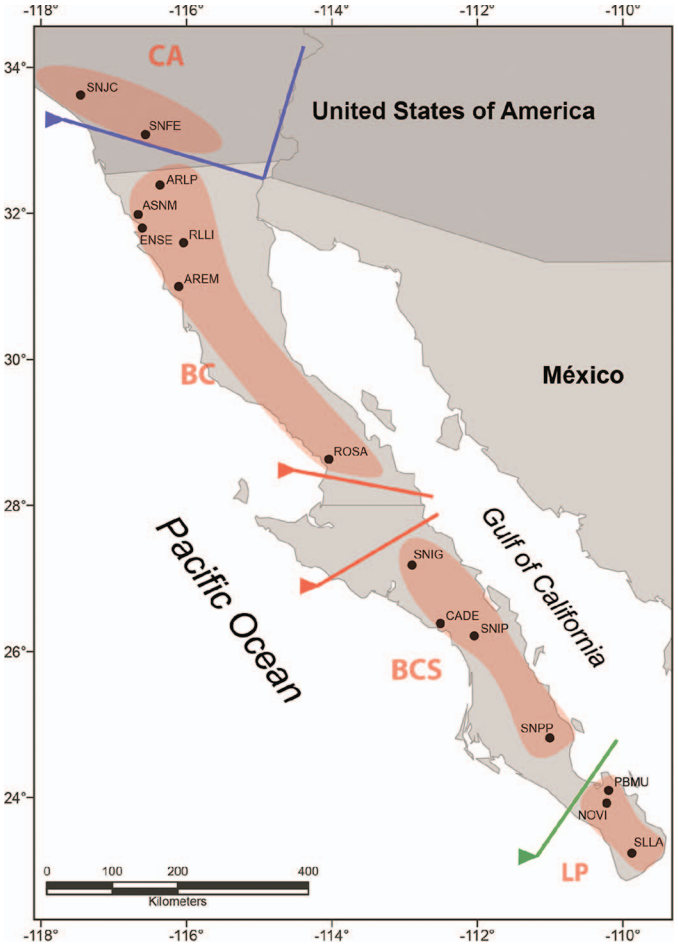

Figure 1.—*Pardosa sierra* collection sites in the Baja California Peninsula. For the acronyms of each collecting site refer to Table 1. Lines indicate the hypothetical vicariance events of the northern-gulf (*blue*), the Vizcaíno mid-peninsular channel (*red*) and the Isthmus of La Paz (*green*). CA = California; BC = Baja California; BCS = South Baja California; LP = La Paz.

Given these biological constraints, it is expected that populations of *P. sierra* would have been affected by changes to their habitat due to climatic variations during the Pleistocene, as has been shown for other desert species of Rodentia (Jezkova et al. 2009), Hymenoptera (Wilson & Pitts 2012), and Scorpiones (Graham et al. 2013), among others (Van Devender 2002).

In this study, mitochondrial DNA sequences and nuclear microsatellites were used to analyze the phylogeographic patterns and genetic structure of *P. sierra* populations across the BCP, with the aim of addressing the following questions: What is the phylogeographic pattern of *P. sierra* along the BCP? How and when did current distribution patterns potentially originate? Does the genetic structure inferred using microsatellite loci coincide with phylogeographic patterns detected using mtDNA sequences? Did *P. sierra* use refugia in more than one climatically suitable area during the LGM? If so, where were these refugia? We expect that any genetic signal resulting from potential vicariance events that occurred before or during the Pleistocene were erased due to refuge-colonization processes caused by climatic changes after the LGM. Finally, if *P. sierra* expanded its distribution from southern refugia after the LGM, we predict a decreasing gradient of microsatellite genetic diversity from the south region to the north of the BCP.

METHODS

Samples and study locations.—Between 2006 and 2011, 296 adult *P. sierra* specimens were hand collected from the margins of rivers, ponds and other suitable habitats along the BCP and southwestern United States (Fig. 1; Table 1). We collected 38 specimens from 14 localities throughout the range of the species for mitochondrial DNA sequence analysis, and 296 specimens were sampled from only seven locations along the BCP for microsatellite genotyping (Table 1). The specimens were preserved in 95% ethanol and deposited in the Colección Aracnológica y Entomológica del Centro de Investigaciones Biológicas del Noroeste (CAECIB).

DNA extraction and genetic characterization using mitochondrial markers and microsatellites.—Total genomic DNA was extracted from the legs of each specimen as reported by Aljanabi & Martinez (1997). DNA quality was verified on 1% agarose gels (buffer TAE 1x, GelRed 10x), and DNA concentration (ng/µl) was quantified with a NanoDrop 8000 (Thermo Fisher Scientific, Wilmington, DE). A mitochondiral fragment of the cytochrome c oxidase subunit I (CO1) gene was amplified by polymerase chain reaction (PCR) and sequenced using the primers CO1P- L and CO1P-R (Correa-Ramírez et al. 2010). PCR reactions were performed with a PTC-200 DNA Engine Thermal Cycler (BioRad Laboratories, Hercules, CA) in a total reaction volume of 15 µl (~50 ng genomic DNA, 0.40 mM each primer, 2.5 mM mM $MgCl_2$, 0.2 mM each dNTP, 1x PCR buffer and 0.5 U Taq polymerase (Invitrogen, Carlsbad, CA)). A total of 35 temperature cycles were performed, each cycle consisted of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 52°C, extension for 30 seconds at 72°C, and included an initial denaturation step for 4 minutes at 94°C, and a final extension step for 10 minutes at 72°C. PCR products were visualized using electrophoresis in 1.5% agarose gels. The sequencing of PCR products was performed using the BigDye Terminator sequencing method in an ABI PRISM 3730XL sequencer (PE Applied Biosystems; Macrogen, Seoul, Korea).

Five microsatellite loci previously described for *P. sierra* in Molecular Ecology Resources Primer Development Consortium et al. (2010) were used. The final PCR reaction volume was 15 µl with ~30 ng genomic DNA, 1x PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 200 µM each dNTP, 0.4 µM each primer, 1.5–2.5 mM MgCl2 and 0.15 U Taq DNA Polymerase (Invitrogen, Carlsbad, CA). The PCR conditions were 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, annealing at 52°C for 30 sec, 72°C for 30 sec, and a final extension of 72°C for 5 min. PCR reactions were performed in an MJ Research PTC-200 thermal cycler. PCR products were visulaized by electrophoresis on 6% polyacrylamide gels with 7.5 M urea. Gels were silver-stained according to Benbouza et al. (2006). The allele size was determined by their relative position on the gel compared to the molecular marker (nucleotide ladders of 10 and 50 bp, Invitrogen).

Phylogeography and population genetic structure.—To assess whether the phylogeographic pattern of *P. sierra* along the

Table 1.—Sampling locations of *Pardosa sierra* in the Baja California Peninsula and frequency of haplotypes of mitochondrial gene CO1. n^a = number of individuals analyzed for each microsatellite locus.

Location	ID	Lat (°N)	Long (°W)	n^a	Haplotypes CO1 (*n*)
USA, CA, Sn. Juan Creek	SNJC	33.638	−117.421	-	*Hap3* (4)
USA, CA, Sn. Felipe	SNFE	33.066	−116.553	-	*Hap*3 (1), *Hap*7 (3)
Mexico, BC, Arroyo Las Palomas	ARLP	32.373	−116.354	-	*Hap*1 (2), *Hap*6 (2)
Mexico, BC, Arroyo Sn. Antonio Minas	ASNM	31.968	−116.658	-	*Hap*1 (4)
Mexico, BC, Ensenada	ENSE	31.783	−116.6	26	*Hap*1 (1), *Hap*5 (3)
Mexico, BC, Rancho Las Liebres	RLLI	31.584	−116.03	-	*Hap*2 (2)
Mexico, BC, Arroyo El Mejin	AREM	30.980	−116.094	-	*Hap*6 (1)
Mexico, BC, El Rosarito	ROSA	28.616	−114.033	10	*Hap*1 (1), *Hap*5 (1)
Mexico, BCS, Sn. Ignacio	SNIG	27.174	−112.869	-	*Hap*1 (1)
Mexico, BCS, Cadejé	CADE	26.366	−112.5	52	*Hap*1 (2), *Hap*4 (2)
Mexico, BCS, Sn. Isidro-La Purísima	SNIP	26.2	−112.033	52	*Hap*1 (1)
Mexico, BCS, Sn. Pedro de la Presa	SNPP	24.833	−110.983	52	-
Mexico, BCS, Presa de la Buena Mujer	PBMU	24.088	−110.191	-	*Hap*1 (2)
Mexico, BCS, El Novillo	NOVI	23.916	−110.216	52	*Hap*1 (3)
Mexico, BCS, Sierra de la Laguna	SLLA	23.233	−109.866	52	*Hap*1 (2)

Baja California peninsula was correlated with geological vicariance events, the 14 locations were clustered *a priori* into four groups, California (CA), Baja California (BC), Baja California Sur (BCS) and La Paz (LP), based on the positions of the hypothetical trans-peninsular channels (Fig. 1). An analysis of molecular variance (AMOVA) was performed using CO1 sequences among these groups. Additionally, genetic differentiation values (F_{ST}) were estimated by applying 10,000 permutations using ARLEQUIN 3.5 (Excoffier & Lischer 2010).

To determine the genetic structure of *P. sierra*, microsatellite loci were analyzed using STRUCTURE 2.3.4 software (Pritchard et al. 2000). First, we performed an analysis without the LOCPRIOR option to obtain an initial *K*, and afterwards we ran it again using this *K* value as LOCPRIOR. In both analyses the admixture model was used, with a burn-in period of 100,000 and $1x10^6$ MCMC repetitions thereafter. The number of populations was estimated using the ΔK value using Evanno's method (Evanno et al. 2005) in STRUCTURE HARVESTER Web v0.6.94 (Earl & VonHoldt, 2012). The results of 15 independent runs were processed and visualized using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004) respectively. Finally, the genetic differentiation values (F_{ST}) were evaluated using the infinite allele model (Weir & Cockerham 1984) in ARLEQUIN, using the Bonferroni correction to adjust the significance level for multiple tests (Rice 1989).

Genetic diversity.—To observe haplotype diversity, haplotype frequencies from the CO1 sequences were calculated using DnaSp 5.10 (Librado & Rozas 2009). To estimate population growth of *P. sierra*, the R2 test was performed (Ramos-Onsins & Rozas 2002), with 10,000 coalescence simulations using DnaSP v5 (Librado & Rozas 2009). To detect potential relationships among haplotypes, a statistical parsimony network was estimated for CO1 with a 95% confidence criterion, using TCS 1.13 software (Clement et al. 2000).

To detect a genetic diversity gradient along the peninsula, we calculated the Pearson correlation coefficient between the latitude of each location with its corresponding heterozygosity (H_E) and allelic diversity (A/L) of microsatellites previously obtained from GENEPOP 4.0.7. (Rousset 2008). To observe if there is a relationship between distance and genetic identity between populations, we tested for isolation-by-distance, using the Mantel test (Slatkin 1993) with 10,000 permutations in IBDWSin v3.23 (Jensen et al. 2005). Recent population bottleneck analysis was performed with a two-tailed Wilcoxon sign-rank test for heterozygosity excess under a two-phased mutation model (TPM; Di Rienzo et al. 1994; Miller et al. 2012), using the program BOTTLENECK 1.2.02 (Piry et al. 1999).

Phylogenetic analysis.—In addition to the newly-generated CO1 sequences from this study, additional sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/; accessed April 2014) for another 28 taxa, these included different species of *Pardosa* C.L. Koch, 1847 and closely related genera (Appendix 1). For Bayesian phylogenetic inference we used MRBAYES 3.2.2 (Ronquist & Huelsenbeck 2003), and JMODELTEST 2.1.6 software (Darriba et al. 2012) was used to identify an optimal GTR+I+G model of molecular evolution for the un-partitioned CO1 matrix under the Akaike Information Criterion (AIC). Bayesian analysis was performed using two independent runs consisting of $10x10^6$ MCMC generations, with sampling every 1,000 generations. A 50% majority-rule consensus tree was generated after 10% burn-in.

Estimation of divergence time.—To test whether phylogeographic patterns detected were temporally concordant with hypothetical vicariance events, interspecific and population divergence times were estimated in BEAST 1.8 (Drummond et al. 2012), using both fossil and rate calibration methods with CO1. The first analysis included individuals of *P. sierra*, plus other *Pardosa* species and related genera. The GTR+I+G substitution model was used based on the results of JMODELTEST 2.1.6, along with a lognormal relaxed clock model and Yule speciation process to model the tree prior. The Yule process evaluates the relative ages of nodes contributing to the prior distribution of nodal ages (Ho & Phillips 2009). A lycosid macrofossil from the Miocene found in Dominican amber (Penney 2001) was used as a root calibration point, with a minimum age constraint of 20 Mya. We implemented a hard minimum bound (Ho & Phillips 2009)

to a well-identified macrofossil with a lognormal distribution (mean 0, SD 1.0, offset 20). The fossil specimen used here is considered the first representative of the family Lycosidae from the fossil record (Penney 2001); previously recorded fossils from the Carboniferous, Tertiary, Miocene, or Pliocene assigned to genera of Lycosidae were likely misidentified species that correspond to other families (Penney 2001).

We also performed a second BEAST analysis including only *P. sierra* specimens, using the Coalescent constant size to model the tree prior. The tree was rate calibrated using the substitution rate of 0.0115 substitutions per site per million years for CO1, proposed for insects by Brower (1994) and also used for spiders in phylogeographic studies (e.g., Chang et al. 2007). For both BEAST analyses, two independent runs of $40x10^6$ generations were performed, sampling every 2,000 generations. The results were analyzed in TRACER 1.5 (Rambaut & Drummond 2007). To ensure parameter convergence and the effective sampling size (ESS), all parameters and trees for both independent runs were combined in LOGCOMBINER 1.8 (Rambaut & Drummond 2007) with a burn-in of 25% for the first trees in TREEANNOTATOR 1.8 (Rambaut & Drummond 2007). Finally, the results were summed up in a single tree (maximum clade credibility tree) and visualized in FIGTREE 1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Niche-based distribution model.—To identify geographic areas that might have served as refugia for *P. sierra* during and after the LGM, we generated a niche-based distribution model (NBDM; Segurado et al. 2006) using records of *P. sierra* collected for this study at 31 localities in the States of Baja California and Baja California Sur in Mexico, and California in the United States of America (Appendix 2). To infer the NBDMs the records were loaded into the 'maximum entropy machine-learning algorithm' MAXENT 3.2.2 (Phillips et al. 2006). Current bioclimatic variables used (BIO 1–19) were downloaded from WorldClim (Hijmans et al. 2005; www.worldclim.org) with a resolution of 1 km^2 using 75% of the records for training and the remaining 25% for validation of the model. To run MAXENT, default parameters were used with 1,000 iterations. The models were evaluated with the area under the curve (AUC) method. The algorithm compensates for co-linearity between variables using a method for regularization that deals with feature selection. There is thus less of a need to remove correlated variables (Elith et al. 2011), as the algorithm ranks the contribution of each during the analysis. The results were projected in QUANTUM GIS 2.2 Valmiera software. To explore the potential occurrence of a species in the past, the models generated under current climatic conditions were projected onto the LGM (21,000 years ago) and interglacial (140,000–120,000 years ago) scenarios. The climatic layers from the past were downloaded from WorldClim for LGM scenarios developed by the Paleoclimate Modelling Intercomparison Project Phase II (Braconnot et al. 2007), the Community Climate System Model (CCSM; Collins et al. 2004), the Model for Interdisciplinary Research on Climate (MIROC; Hasumi & Emori 2004) and, for the interglacial period, we used the layers developed by Otto-Bliesner et al. (2006). The CCSM and MIROC models simulate climatic conditions that were calculated as prevailing during the LGM, however a stronger decrease in temperature is assumed for the CCSM compared to the MIROC model (Otto-Bliesner et al. 2006). For assessing variable importance in each model a jackknife test was performed in MAXENT.

RESULTS

Phylogeography and population genetic structure.—The mitochondrial CO1 gene studied had 630 base pairs, and in the sample of 38 specimens, 7 unique haplotypes were detected (Fig. 2a, b; Table 1; GenBank accession numbers in Appendix 1). No population expansion was detected with the CO1 gene ($R2 = 0.099$; $P = 0.239$). The haplotype network shows that the haplotypes tend to be grouped according to their geographical regions. Haplotype 1 (*Hap1*) was the most abundant in the southern region of the BCP, whereas *Hap2* and *Hap6* were only detected in the northern region of the BCP. Additionally, *Hap3* and *Hap7* were only observed in California (USA), seven mutational steps apart with respect to the remaining haplotypes (Fig. 2a, b; Table 1); this coincides with the geographical separation of clades detected in the phylogenetic analysis (Fig. 3). The AMOVA detected that the Californian locations are different from all the BCP locations ($F_{CT} = 0.7$, $P = 0.001$), whereas inside the BCP using another test, no differences were observed between the BC, BCS and LP groups ($F_{CT} = 0.1$, $P = 0.07$) (results not shown). STRUCTURE clustered the seven locations of the BCP into five populations, although genetic mixing exists between them (Fig. 2 c, d), which in north-south latitudinal order are I (Ensenada), II (El Rosarito and Cadejé), III (San Isidro La Purísima and El Novillo), IV (San Pedro de La Presa) and V (Sierra de La Laguna). Pairwise F_{ST} values showed genetic differences between BCP populations (F_{ST} varied from 0.01 to 0.04, $P < 0.001$, Table 2).

Using microsatellite data, the genetic diversity detected was 9.4–17.6 for A/L and between 0.81–0.84 for H_E (Table 2). The Mantel test for the microsatellites ($r = 0.36$, $P = 0.9$), and correlation analyses between latitude and the population heterozygosity ($r = –0.64$, $P = 0.2$), and between latitude and A/L ($r = –0.82$, $P = 0.08$) were not significant. The bottleneck analysis was significant for the Wilcoxon sign-rank test only for northern populations I ($P = 0.03$) and II ($P = 0.03$).

Phylogenetic relationships.—The Bayesian 50% majority-rule consensus tree (Fig. 3) recovered the samples of *P. sierra* as monophyletic ($PP = 1.0$), and sister to *P. atromedia* Banks, 1904 (but with low nodal support; $PP = 0.69$). The sister-groups to *P. sierra* + *P. atromedia* were a polytomic assemblage of taxa including *P. valens* Barnes, 1959, *P. steva* Lowrie & Gertsch, 1955, *P. sura* Chamberlin & Ivie, 1941, and *P. vadosa* Barnes, 1959. Within the *P. sierra* lineage, only two subclades were detected: the California clade (CA) from the USA, and the Baja California Peninsula (BCP) clade from Mexico, each reciprocally monophyletic. This analysis did not detect divergence among populations of *P. sierra* from the BCP (Fig. 3).

Estimation of divergence times.—BEAST analysis using a fossil calibration inferred a divergence date for the separation of the CA clade from the BCP clade of ca. 4 Mya (95% HPD 8.52–1.65 Mya; Fig. 4). The CA clade started diversifying around 1.6 Mya (95% HDP 5.9–0.76 Mya) and the BCP clade around 2.4 Mya (95% HDP 4.3–0.3 Ma). Alternatively, for the

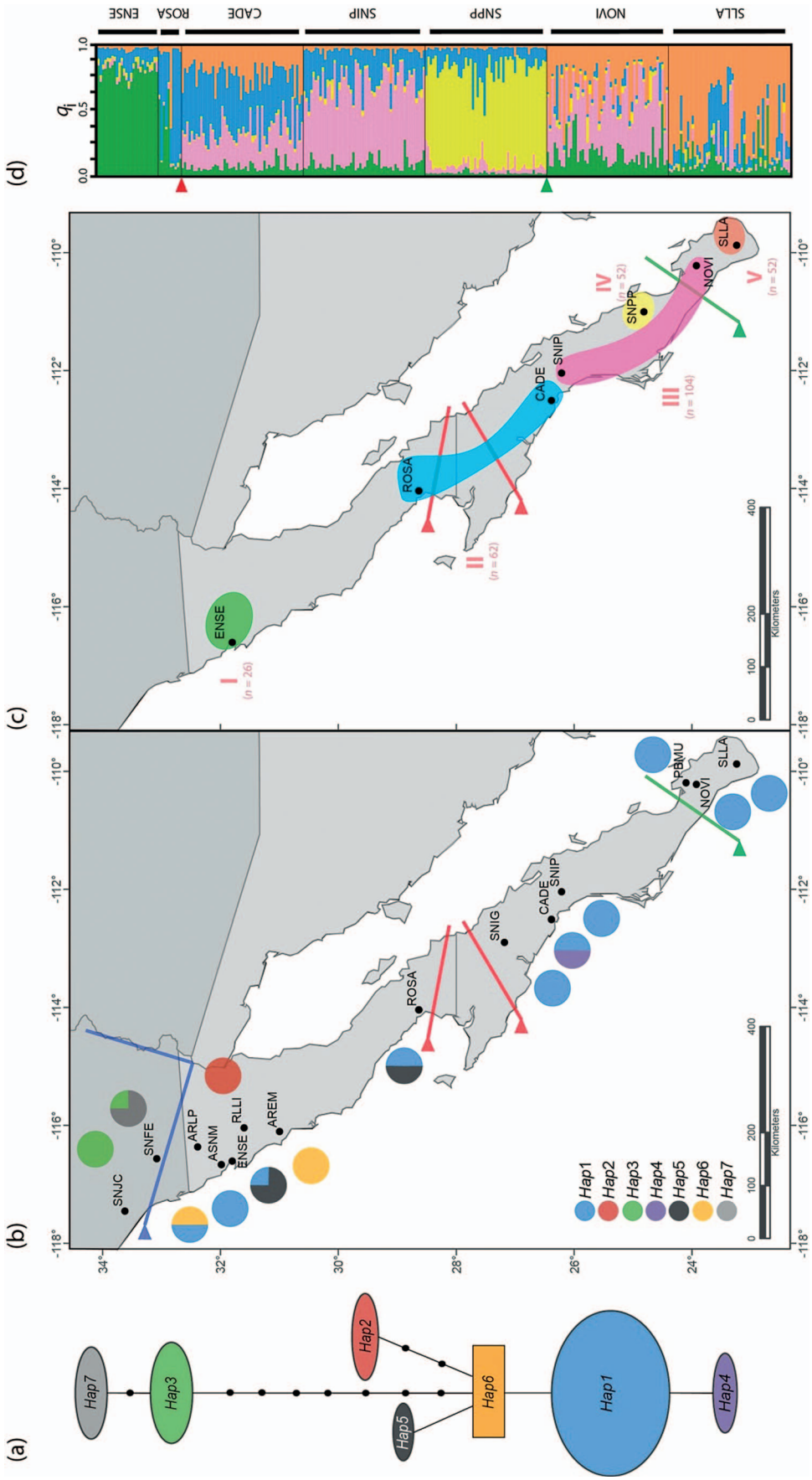

Figure 2.—Phylogeographic pattern and genetic structure of *Pardosa sierra* in the Baja California Peninsula. (a) Statistical parsimony network based on 38 CO1 sequences. The areas of the box and circles are proportional to the haplotype frequency, and each nucleotide substitution between haplotypes is represented by a dot. (b) Latitudinal distribution of seven haplotypes of the mtDNA gene CO1. The acronyms of each collecting site are described in Table 1. (c) Number of genetically homogeneous populations (I–V) detected, using five microsatellite loci in STRUCTURE. Abbreviation (*n*) indicates sample size per population. (d) In the diagram, each horizontal bar represents an individual and each color represents the membership proportion (q_i) corresponding to each population defined by STRUCTURE. Lines and arrows on the maps indicate the putative channel transgressions of the northern-gulf (*blue* in b), the mid-peninsular Vizcaíno channel (*red* in b, c, and d) and the Isthmus of La Paz (*green* in b, c, and d).

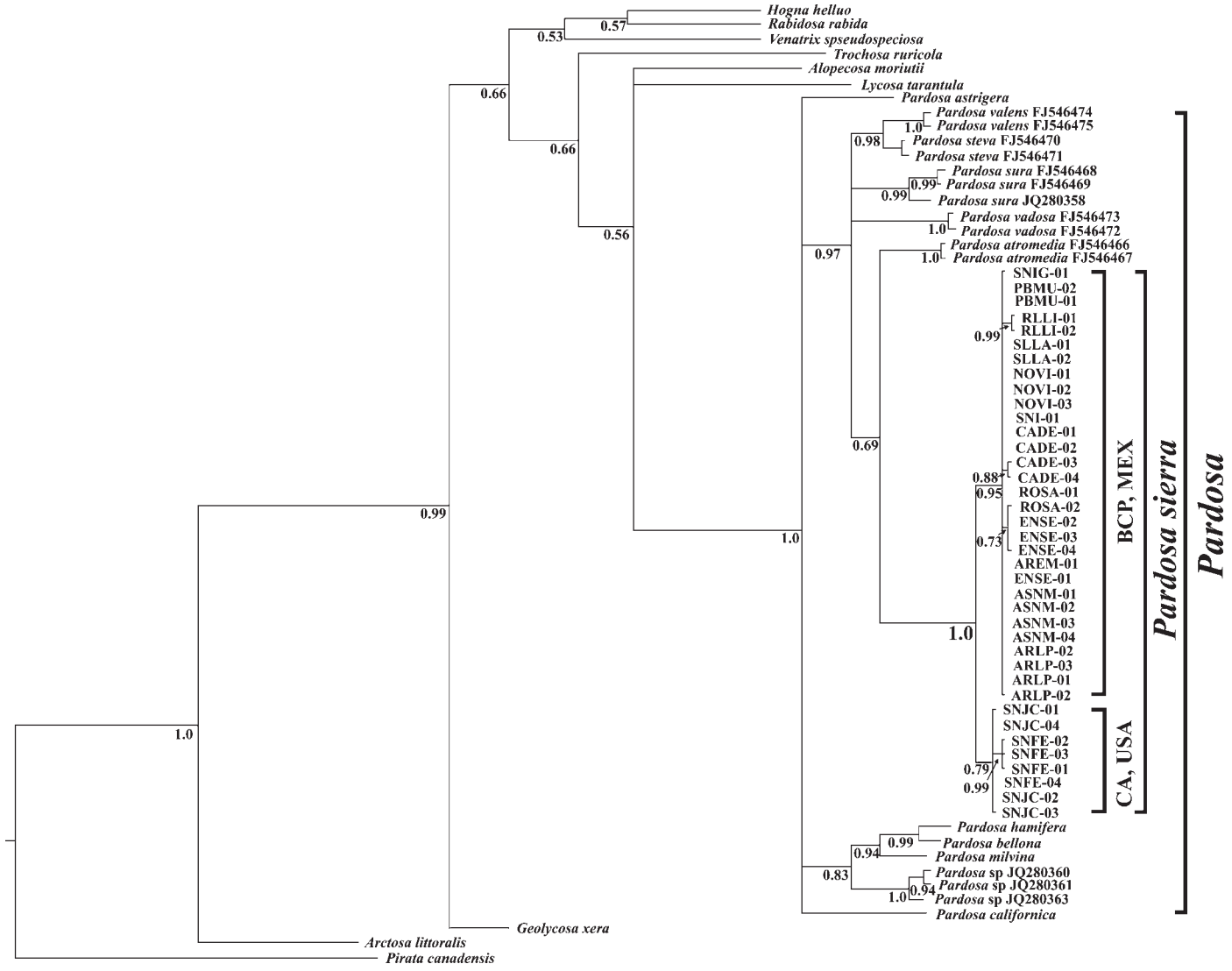

Figure 3.—Bayesian 50% majority-rule consensus tree of mitochondrial CO1 sequences, for populations of *Pardosa sierra* and related Lycosidae. Numbers below the nodes indicate the posterior probability values. BCP = Baja California Peninsula, MEX = Mexico, CA = California, USA. For acronyms of the location of each specimen see Table 1.

Table 2.—Values of genetic differentiation (F_{ST}) estimated by pairs of populations, allelic diversity (A/L), expected (H_E) and observed (H_O) heterozygosity, based on the allelic frequencies of five microsatellite loci of *Pardosa sierra* from the Baja California Peninsula. Values below the diagonal show the pairwise F_{ST} values and above the diagonal are shown the corresponding probability (values *** $P < 0.0001$). I (Ensenada), II (Rosarito-Cadejé), III (San Isidro La Purisima and El Novillo), IV (San Pedro de la Presa), V (Sierra de La Laguna).

	Locations							
	I	II	III	IV	V	*A/L*	H_O	H_E
I	-	***	***	***	***	9.4	0.55	*0.81*
II	0.033	-	***	***	***	13.6	0.58	*0.84*
III	0.044	0.012	-	***	***	17.6	0.62	*0.83*
IV	0.035	0.025	0.037	-	***	14	0.46	*0.82*
V	0.038	0.012	0.020	0.039	-	14.8	0.57	*0.84*

rate-calibrated BEAST analysis, the split between both clades (CA and BCP) was inferred as being much more recent (0.6 Mya; 95% HPD 1.04–0.22 Mya; tree not shown), with diversification from 0.1 Mya (95% HDP 0.23–0.007 Ma) and 0.2 Mya (95% HDP 0.37–0.06 Ma) for the CA and BCP clades, respectively.

Niche-based distribution modeling.—The model projected for the interglacial period (140,000–120,000 years ago) indicated expanded areas of potential habitat in the northern and southern BCP (Fig. 5a). The models CCSM and MIROC projected for the LGM (21,000 years ago) revealed that the potential habitat of *P. sierra* was concentrated in the latitudinal geographic ends of the BCP and that an important wide band of inadequate habitat was projected in the center of the BCP (AUC = 0.75–0.99 for both models; Fig. 5b, c). Currently, the distribution predicted by MAXENT agrees with the known distribution of *P. sierra* (AUC = 0.945; Fig. 5d). The most important climatic variables identified by the

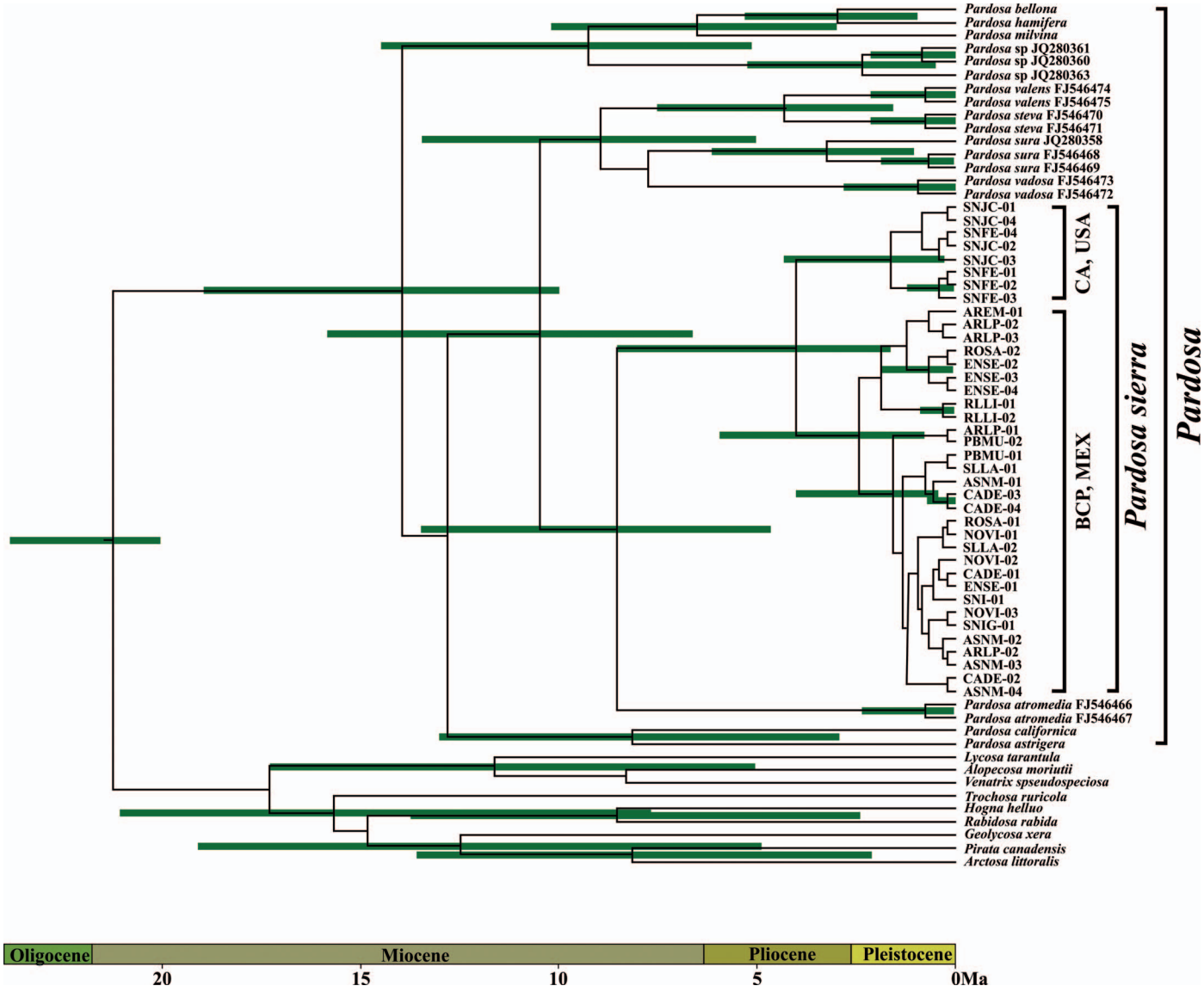

Figure 4.—Fossil calibrated BEAST chronogram of mitochondrial CO1 sequences, for populations of *Pardosa sierra* and related Lycosidae. Green bars indicate the 95% highest posterior density estimates of divergence time for each node. BCP = Baja California Peninsula, MEX = Mexico, CA = California, USA. For acronyms of the location of each specimen see Table 1.

jackknife test (by percentage contribution to the generation of the model) were annual temperature range ($Pc = 58$), precipitation in the driest month ($Pc = 12.8$) and mean diurnal range ($Pc = 8.2$).

DISCUSSION

Phylogeography and population genetic structure.—The observed mitochondrial genetic differentiation of *Pardosa sierra* populations from California versus the rest of the Baja California Peninsula coincides with the occurrence of the alleged vicariance event known as the northern-gulf (Riddle et al. 2000). Similar phylogenetic patterns have also been detected in this region in several vertebrate species, e.g., the toad *Bufo punctatus* (Riddle et al. 2000), the rodent *Thomomys bottae* (Alvarez-Castañeda & Patton 2004), the lizard *Xantusia* sp. (Sinclair et al. 2004) and the boa *Lichanura trivirgata* (Wood et al. 2008). The concordance in phylogeographic signal among these different taxa suggests that they may have responded in parallel to the same underlying process/es (Arbogast & Kenagy 2001; Zink 2002a). Therefore, it is probable that in this region, the northern-gulf vicariance event has influenced the genetic architecture of *P. sierra*. Both clades are reciprocally monophyletic (Avise 2000), and this is most likely due to the occurrence of an ancient barrier (Zink 2002a). However, low migration rate and reinforcement could be alternative explanations for this phylogeographic pattern (Munguía-Vega 2011; Dolby et al. 2015). Another explanation for reciprocal monophyly between clades would be that clade CA constitutes a cryptic species. The morphological characteristics of Californian specimens from this study (color pattern, size and male pedipalp) do not differ from those reported for *P. sierra* (Correa-Ramírez et al. 2010), however the female epigynum shows some variation in its internal

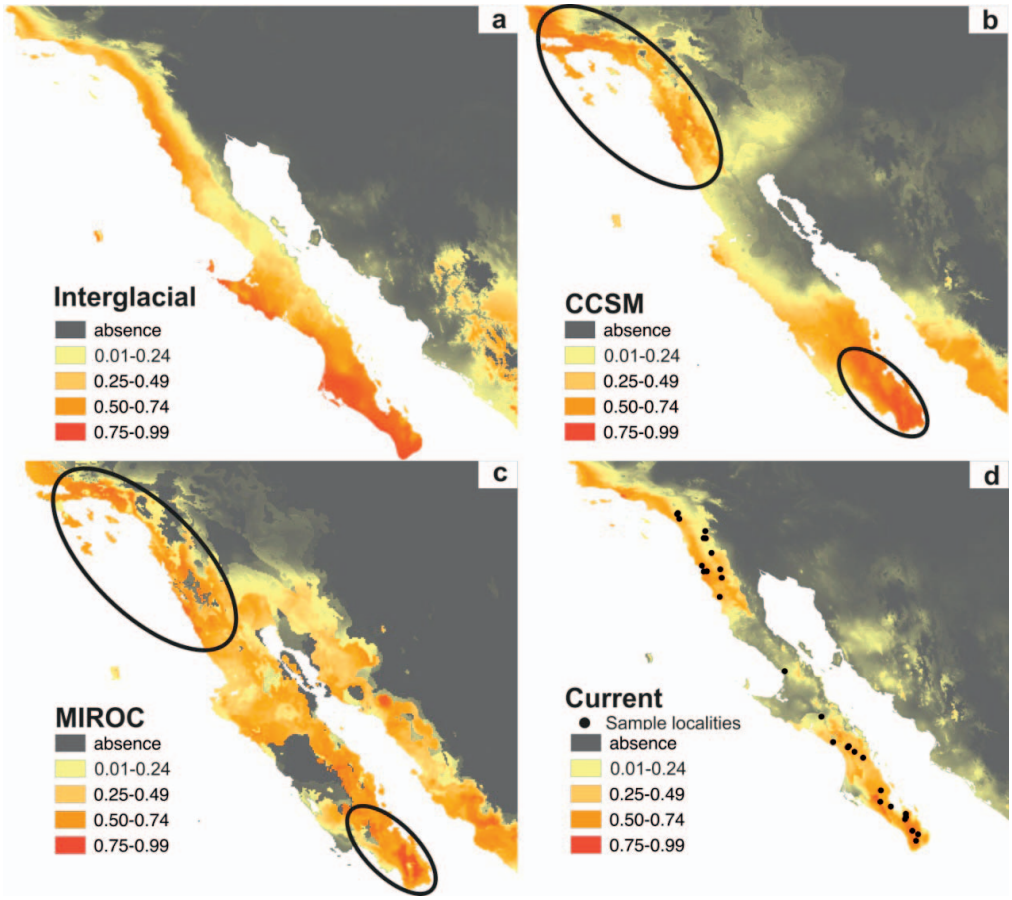

Figure 5.—Niche-based distribution models of *Pardosa sierra*. (a) Model for the last interglacial period (140,000–120,000 years ago). (b) Community Climate System Model corresponding to the last glacial maximum (LGM) 21,000 years ago. (c) Model for Interdisciplinary Research on Climate during the LGM. (d) Actual time model. Colored bars indicate the probability of a favorable habitat for *P. sierra*, with red indicating the highest value. Points in actual model (d) indicate the locations used to generate ENMs, and circles (b and c) indicate potential refugia during the LGM according to AUC = 0.75–0.99 range.

anatomy (i.e., structure of the spermathecae and copulatory ducts); the latter provides some support for a possible cryptic complex, with diversification during the Late Miocene/Early Pliocene (Starrett & Hedin 2006) in *P. sierra*. Indeed, cryptic speciation in spiders has been detected in the same region (Ramirez & Chi 2004), and is potentially explained by high rates of diversification in spiders more generally (Starrett & Hedin 2006). To better delimit species we need to include more individuals and additional molecular markers (i.e., unlinked nuclear genes), perform detailed morphological analyses, and reconcile multiple gene trees (Starret & Hedin 2006) using new statistical methods based as coalescent-theory (Yang & Rannala 2010; Ence & Carstens 2011; Fujita et al. 2012).

Although a phylogeographic break was observed around the mid-peninsular channel and Isthmus of La Paz (Crews & Hedin 2006) in spiders of the genus *Homalonychus* Marx, 1891 (Homalonychidae), in our study phylogeographic breaks were not detected among *P. sierra* populations along the BCP (Fig. 3). These results and a paucity of geological evidence refute the hypothesis of the occurrence of a mid-peninsular channel (Grismer 2002; Murphy & Aguirre-León 2002; Crews & Hedin 2006; Garrick et al. 2009), or if it did exist, it did not have a lasting impact on the genetic structure of *P. sierra* in this region. Indeed, although the signal from the CO1 data did not reveal population expansion, the wide distribution of a single haplotype (*Hap1*), the few haplotypes with restricted distribution (Fig. 2b) and the divergence time inferred (Fig. 4) all suggest that *P. sierra* populations were already present along the BCP at least 2.4 Mya.

Fossil and rate calibrations for divergence date estimation using BEAST resulted in unsurprisingly different estimates of divergence times. We used a relaxed molecular clock model for both analyses, with the fossil calibrated analysis estimating much deeper ages for all nodes. Each method has its limitations, and using substitution rates alone has been criticized due to relaxation of the strict molecular clock in many taxa (Ho & Larson 2006; Ho 2007), and the problems posed by applying substitution rates from other species (Zink 2002b). Meanwhile, the use of fossils to calibrate trees can be erroneous given the uncertainty of geological dating (Magallón 2004) and the incomplete nature of the geological record. In this study, we focus on the results of our fossil calibrated BEAST analysis, which are concordant with phylogeographic splits inferred for other vertebrate taxa (i.e., *Peromyscus eremicus*, *Chaetodipus baileyi*, *Bufo punctatus*) in the northern-gulf around 3 Mya (Riddle et al. 2000).

The genetic structure of *P. sierra*, as detected by microsatellite markers (Fig. 2c, d; Table 2), was not concordant with geographic breaks expected under a model of vicariance along the BCP. Despite the difference in mutation rates between microsatellite molecular markers and CO1 (Hedrick 1999), in this study both markers failed to detect phylogeographic signals related to the mid-peninsular and Isthmus of La Paz channels. This evidence could support the hypothesis that climatic phenomena configured the current genetic architecture of *P. sierra* populations, or that these phenomena erased any mitochondrial genetic signal that might have originated from these vicariance events.

Pleistocene refugia.—The location of Pleistocene refugia depends on how each species responded to the cyclic climate oscillations of this period (Harl et al. 2014). This is evident in the few studies that have focused on identifying potential refugia during the Pleistocene in the Baja California Peninsula. For some plant species, such as totem pole cactus (*Lophocereus schottii*; Nason et al. 2002) and cardon (*Pachycereus pringlei*; Gutiérrez 2015), and some animal species, such as the gnatcatcher *Polioptila californica* (Zink et al. 2000), refugia have been observed towards the south of the peninsula. In contrast, for some arthropod species, the peninsula's central region acted as a refugium (Wilson & Pitts 2012; Graham et al. 2014). In the case of *P. sierra*, two geographic areas located in the north and south end of the peninsula most likely acted as separate refugia (Fig. 5b, c), and these areas coincide with the refugia suggested by Hafner & Riddle (1997). According to the niche-based distribution modeling analysis, *P. sierra* probably occupied a greater geographic range along the BCP during the last interglacial period (140,000–120,000 years ago; Fig. 5a). However, according to the simulation results, when the temperature decreased during the LGM (21,000 years ago), its potential habitat became fragmented. The lack of a relationship between latitude and microsatellite heterozygosity and between latitude and allelic diversity supports the presence of two refugia, because if only one had existed in the south, more genetic diversity would be expected in this region and there would be a gradient of decreasing genetic diversity towards the north (as has been suggested for other species in the BCP; Riddle & Hafner 2006; Valdivia 2014; Gutiérrez 2015). Moreover, the existence of two populations of origin in opposing regions of the BCP could be the reason that a distance isolation structure was not detected.

The presence of greater haplotype diversity in the populations of the north and absence of exclusive haplotypes in the south (Fig. 2b), suggests that southern populations originated from those in the north before the refugia fragmented them. This is consistent with northern haplotypes of CA (*Hap7* and *Hap3*), which are more divergent relative to the other haplotypes (Fig. 2a). The wide distribution of only a single haplotype (*Hap1*) across the peninsula suggests that this could be considered an ancestral haplotype (Avise 2000; Cuenca et al. 2003), derived from northern populations. The fact that *Hap1* is present on opposite sides of the BCP (Fig. 2b) supports the hypothesis that *P. sierra* took refuge in these locations on the peninsula. Likewise, the bottleneck detected using microsatellite loci in populations I and II (Fig. 2c) could explain the low CO1 diversity in the southern populations, given that this region coincides geographically with the area where CO1 transitions to a single haplotype (*Hap1*) from the north toward the south of the peninsula. However the window of time detected by BOTTLENECK software using microsatellite loci is only about ~25–250 generations in accordance with Cornuet & Luikart (1996) and Luikart et al. (1998), therefore it is necessary to analyze genetic markers with low mutation rates to test if a bottleneck could be related to the middle peninsular vicariant event. Finally, the intolerance of *P. sierra* to cold and its sensitivity to changes in rainfall or humidity (Van Dyke & Lowrie 1975) are constraits that limit these spiders to preferentially inhabit areas surrounding water bodies and oases (Punzo & Farmer 2006; Correa-Ramírez 2010; Jiménez et al. 2015). Interestingly, variation in sea levels could be related to changes in the variables that most influenced the potential niche of the species during the Pleistocene (e.g., temperature and humidity), and may therefore be implicated in refugia occurring in more than one area of the BCP. Certainly, if cold intolerance forced *P. sierra* to occupy those disparate areas (Fig. 5b, c), as is likely, then it is plausible that postglacial recolonization processes were accountable for erasing the genetic signal that may have been produced by other vicariance events in the central and southern regions of the Baja California Peninsula.

Based on these data, the northern-gulf channel appears to be the only vicariance event that left a lasting phylogeographic signal in *P. sierra*. This conclusion is supported by strong genetic differentiation between California (USA) populations and those from the Baja California Peninsula. The genetic differentiation patterns revealed by mitochondrial sequences and microsatellites failed to detect genetic breaks in regions where the mid-peninsular and Isthmus of La Paz canals were presumably formed. Two refugia were detected at both ends of the Baja California peninsula during the Pleistocene, specifically between the last interglacial period (140,000–120,000 years ago) and the last glacial maximum period (21,000 years ago).

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Appendix 1.—GenBank accesion numbers of mitochondrial (COI) marker. The species listed were used in phylogenetic analysis for Lycosidae.

Species	GenBank Accesion number
Alopecosa moriutii	AB564729
Arctosa littoralis	JQ280374
Geolycosa xera	DQ151816
Hogna helluo	JQ280373
Lycosa tarantula	KC550668
Pardosa astrigera	AY836055
Pardosa atromedia	FJ546466
Pardosa atromedia	FJ546467
Pardosa bellona	JQ280362
Pardosa californica	JQ280357
Pardosa hamifera	JQ280364
Pardosa milvina	JQ280356
Pardosa sierra Hap1	FJ546464
Pardosa sierra Hap2	JQ280371
Pardosa sierra Hap3	JQ280366
Pardosa sierra Hap4	Fj546465
Pardosa sierra Hap5	KT364484
Pardosa sierra Hap6	JQ280372
Pardosa sierra Hap7	JQ280367
Pardosa steva	FJ546470
Pardosa steva	FJ546471
Pardosa sura	FJ546468
Pardosa sura	FJ546469
Pardosa sura	JQ280358
Pardosa vadosa	FJ546472
Pardosa vadosa	FJ546473
Pardosa valens	FJ546474
Pardosa valens	FJ546475
Pardosa sp	JQ280361
Pardosa sp	JQ280360
Pardosa sp	JQ280363
Pirata canadensis	KF368671
Rabidosa rabida	DQ029232
Trochosa ruricola	AB564731
Venatrix pseudospeciosa	JQ240195

Appendix 2.—Records of *Pardosa sierra* used to generate potential refuges for the Interglacial and during and after the Last Glacial Maximum periods. All data were collected for this study in the states of Baja California and Baja California Sur in Mexico and California in the United States of America.

Location	Lat	Long
USA, CA, Sn. Juan Creek	33.639	−117.422
USA, CA, Sn. Juan Creek I	33.607	−117.444
USA, CA, Sn. Juan Creek, LakeLand Ville	33.607	−117.444
USA, CA, Margarita Truck Trail	33.454	−117.377
USA, CA, Sn. Felipe Creek	33.066	−116.553
USA, CA, Descanso Town	32.843	−116.605
USA, CA, Pine Creek	32.838	−116.538
México, BC, Arroyo Las Palomas	32.374	−116.355
México, BC, Arroyo Sn. Antonio Minas	31.969	−116.659
México, BC, Arroyo Sn. Salvado	31.853	−116.078
México, BC, Arroyo Sn. Carlos I	31.793	−116.501
México, BC, Arroyo Sn. Carlos II	31.786	−116.505
México, BC, Ensenada	31.783	−116.600
México, BC, Rancho Las Liebres	31.584	−116.033
México, BC, Arroyo El Mejín	30.980	−116.095
México, BC, El Rosarito	28.617	−114.033
México, BCS, Sn Ignacio	27.175	−112.869
México, BCS, Cadejé	26.367	−112.500
México, BCS, Carambuche-Sn. Isidro	26.237	−112.002
México, BCS, Sn. Isidro-La Purísima	26.200	−112.033
México, BCS, Arroyo Sn. José	26.059	−111.820
México, BCS, Arroyo Sn. Javier	25.871	−111.546
México, BCS, Sn. Pedro de la Presa	24.833	−110.983
México, BCS, El Pilar-Las Pocitas	24.472	−111.001
México, BCS, Rancho Camarón	24.320	−110.669
México, BCS, Presa de la Buena Mujer	24.088	−110.191
México, BCS, Playitas	23.986	−110.187
México, BCS, El Novillo	23.917	−110.217
México, BCS, Camino a Sierra de La Laguna	23.550	−109.984
México, BCS, El Chorro, Santiago	23.439	−109.804
México, BCS, Sierra de la Laguna	23.233	−109.867