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From the mountains to the coast and back again: Ancient biogeography in a radiation of short-range endemic harvestmen from California ☆

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ABSTRACT

The harvestmen genus *Calicina* is represented by 25 short-range endemic species occurring in the western Sierra Nevada, Transverse and Coast Ranges of California. Our principal aim was to reconstruct the temporal and spatial biogeographic history of this arachnid lineage. We inferred a time-calibrated species tree for 21 of 25 described *Calicina* species using multiple genes and multilocus coalescent-based methods. This species tree was used as a framework for algorithmic biogeographic and divergence time analyses, and a phylogenetic canonical correlation analysis (CCA) was used to examine the relationship between morphological evolution and environmental variables. Species tree and biogeographic analyses indicate that high-elevation Sierran taxa are early-diverging in *Calicina*, with subsequent biogeographic "criss-crossing" of lineages from the Sierra Nevada to the Coast Ranges, back to the Sierra Nevada, then back to Coast Ranges. In both the Sierra Nevada and Coast Ranges, distantly-related parapatric lineages essentially never occur in sympatry. CCA reveals that in both the Coast Ranges and the Sierra Nevada, distant phylogenetic relatives evolve convergent morphologies. Our evidence shows that *Calicina* is clearly dispersal-limited, with an ancient biogeographic history that provides unique insight into the complex geologic evolution of California since the mid-Paleogene.

1. Introduction

The California Floristic Province is a well-known biodiversity hotspot, containing more endemic species of plants and animals than any North American region of comparable size (Myers et al., 2000; Lancaster and Kay, 2013). This rich diversity is at least in part a reflection of the complex geologic history of the region, characterized by tectonic activity, volcanism, marine inundations, and fluctuating climatic regimes (reviewed in Hall, 2007; Schierenbeck, 2014). The study of dispersal-limited animal taxa is particularly informative for biogeographic inference, as these taxa often retain biogeographic signal that allows for the elucidation of both historical and contemporary patterns of diversification. Studies of such taxa in California have revealed fine-scale resolution of spatial patterns of divergence, and have provided evidence for both short and long-term barriers to dispersal. For example, phylogeographic studies of salamanders and mygalomorph spiders have demonstrated extreme population subdivision at microgeographic scales and evidence for cryptic speciation (e.g., Martínez-Solano et al., 2007; Bond and Stockman, 2008; Kuchta et al., 2009b; Martínez-Solano and Lawson, 2009; Reilly et al., 2013; Leavitt et al., 2015; Reilly and Wake, 2015). Other studies have revealed unexpected long-distance dispersal events, providing novel insight into biogeographic processes (e.g., Lapointe and Rissler, 2005; Satler et al., 2011; Hedin et al., 2013). Despite this wealth of biogeographic studies, relatively few studies have examined the spatial and temporal biogeographic history of entire species radiations in California endemic animal taxa.

Over 6500 species of harvestmen, contained in four suborders, are currently known (Kury, 2013). Of these, the suborder Laniatores is the most diverse with over 4100 described species. Laniatores of the family Phalangodidae are small, slow-moving harvestmen typically found in mesic microhabitats. Phalangodids comprise the largest Nearctic family of Opiliones, including over 100 described species. California is particularly rich in short range endemic phalangodids (66 species from nine genera, Ubick and Briggs, 2008), but these have yet to be studied from a molecular phylogenetic perspective.

Previous studies hypothesized the genus *Calicina* to be the most early-diverging Nearctic phalangodid lineage (Ubick and Briggs, 1989, 2008). The 25 described species of *Calicina* occur in uplands of the western Sierra Nevada, Transverse, and Coast Ranges of Cal-

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ifornia; no species have ever been collected in the Central Valley. *Calicina* are small-bodied (0.77–1.67 mm) microhabitat specialists found under medium to large-sized rocks or beneath decomposing logs that are in undisturbed contact with the soil. Most species have a limited period of annual activity during which adults can be found on the surface and upper layers of the soil matrix (Ubick and Briggs, 1989). These characteristics, in combination with the fact that almost all *Calicina* species have very small geographic distributions, suggest that *Calicina* are highly dispersal-limited. *Calicina* taxa have been placed into four species groups (*mariposa*, *digita*, *palapraeputia*, and *serpentinea*) and nine subgroups based on variation in penis glans morphology (Fig. 1). The only published phylogeny for *Calicina* is based on eight genitalic characters – at the base of the tree is a trichotomy between the *mariposa*, *digita*, and *serpentinea* species groups, with the monotypic *palapraeputia* species group hypothesized to be sister to the *serpentinea* group (Fig. 1).

Calicina is hypothesized to be a dispersal-limited lineage whose biogeographic history may reflect various geologic processes that have shaped California. The presence of representatives of all *Calicina* species groups in the southwestern Sierra Nevada suggests this region as a center of origin for the genus (Ubick and Briggs, 1989). Ubick and Briggs (1989, fig. 20) specifically hypothesized that this initial diversification was associated with lineage isolation on ancient exotic terranes. Sierran exotic terranes are extremely old, i.e., Mesozoic in age or older (Saleeby, 2011; Ingersoll, 2012; Millar et al., 2012; Clemens-Knott et al., 2013), and an alternative hypothesis is that initial Sierran divergence instead reflects Oligocene (~35–25 Ma) uplift (Harden, 1998; Hall, 2007; Schierenbeck, 2014). Ubick and Briggs similarly invoked western exotic terranes as key in the diversification of Coastal taxa, which were inferred to have dispersed westwards across the Central Valley multiple times independently (1989, figs. 20 and 21). An alternative here is that Sierran versus Coastal taxa have a more complex history, perhaps

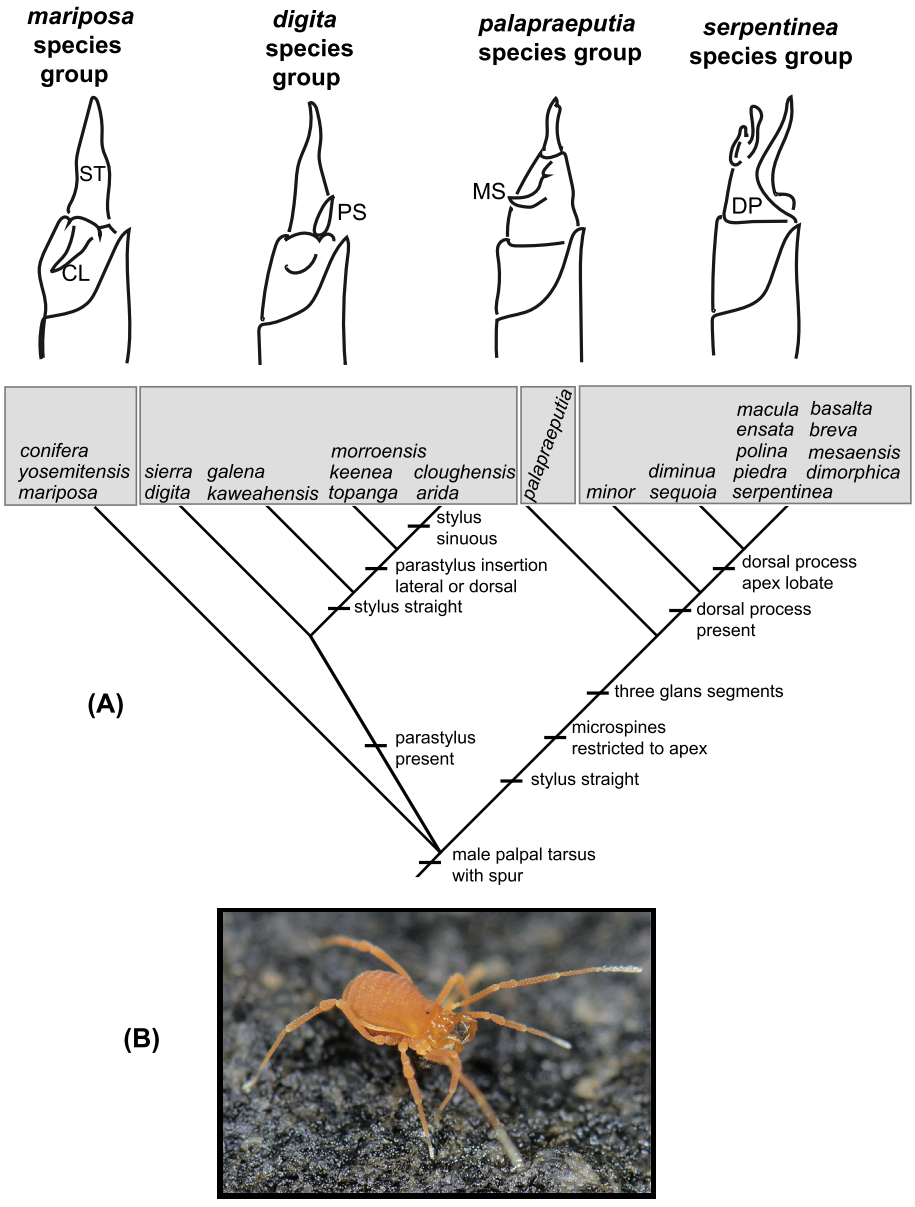

Fig. 1. (A) *Calicina* species group and subgroup composition and relationships following Ubick and Briggs (1989), including apomorphic character states. Representative penis sketches for each group are shown, lateral view. ST = stylus, CL = collar lobe, PS = parastylus, MS = middle segment, DP = dorsal process. (B) *Calicina ensata* from Trimmer Springs Road, Fresno County.

with biogeographic connections in both directions (e.g., as observed in mygalomorph spiders, Satler et al., 2011; Hedin et al., 2013; Leavitt et al., 2015).

Although individual *Calicina* species are distinguished mostly by variation in male genitalic morphology, there are also interesting large-scale trends in somatic morphology. Many *Calicina* species exhibit varying degrees of reduction in body size, pigmentation, and number of tarsal segments. A study of ontogenetic transformations indicated that these character states were likely the result of the secondary retention of juvenile characteristics, or paedomorphosis. Ubick and Briggs (1989) hypothesized that paedomorphism in *Calicina* arose via progenesis (acceleration of sexual maturity), as almost all highly paedomorphic *Calicina* species are found exclusively in xeric habitats, where small size and shorter life cycles would be favored. Additionally, many *Calicina* species have partial or complete eye loss. Based on examinations of early instars of species with blind and well-developed eyes, Ubick and Briggs (1989) concluded that eye loss in *Calicina* was not paedomorphic, but the result of the evolution of troglomorphism (cave-associated morphology) in particular surface habitats. They also hypothesized that the reduced pigmentation and body size of paedomorphic taxa might also reflect troglomorphic adaptations.

We conducted a systematic study of *Calicina* with aims to: (1) infer a species tree for *Calicina* using multilocus DNA sequence data and multispecies coalescent methods, (2) test alternative biogeographic hypotheses outlined above by estimating divergence times and reconstructing ancestral ranges, and (3) examine patterns of morphological evolution and identify potential climatic correlates.

2. Materials and methods

2.1. Taxon sampling

Fieldwork focused on published localities (Ubick and Briggs, 1989), with collections generally conducted during the winter and spring months following seasonal rains. Specimens intended for molecular or morphological data collection were preserved in 100% EtOH (at −80 °C) or 80% EtOH, respectively. Specimens were collected from across the range of the genus, including 21 of the 25 described *Calicina* species (Fig. 2). All four *Calicina* species groups and nine subgroups were sampled. A potential undescribed species from Kaweah Cave in Sequoia National Park was also sampled. Despite our efforts, the Sierran species *C. basalta*, *C. conifera*, *C. macula*, and *C. keenea* were not sampled (Fig. 2). The final specimen sample included 47 ingroup specimens from 43 localities (Fig. 2, Appendix A). The Californian phalangodids *Sitalcina lobata* and *Texella bifurcata* were used as outgroup taxa in most phylogenetic analyses (see below for exceptions).

2.2. Molecular phylogenetics

To aid in marker development, a transcriptome for *C. topanga* was sequenced using Illumina HiSeq technology with 50-bp paired-end reads and assembled using Trinity software (Grabherr et al., 2011). Illumina raw reads have been submitted to the NCBI Sequence Read Archive (see Appendix A). Custom primers for polymerase chain reaction (PCR) amplification were designed to target several candidate nuclear loci (Hedin et al., 2012) by comparing the transcriptomes of *C. topanga*, *S. lobata* (from Hedin et al., 2012) and *T. bifurcata*. PCR experiments targeted five nuclear gene regions consisting of four protein-coding regions and one 3′ untranslated region (UTR), as well as fragments of mitochondrial COI and nuclear ribosomal 28S (Table 1). Genomic DNA was extracted from leg tissue (3–4 legs) using the Qiagen DNEasy kit (Qiagen, Valencia, CA). DNA fragments were amplified using PCR, purified, and directly Sanger sequenced in both forward and reverse directions. Sequences were trimmed and assembled into contigs using Geneious Pro R7 (http://www.geneious.com/), and haplotypes from heterozygous sequences were reconstructed using PHASE v2.1 (Stephens et al., 2001). Alignments were performed using MUSCLE (Edgar, 2004) implemented in Geneious.

PartitionFinder v1.1.1 (Lanfear et al., 2012) was used to select models of sequence evolution for protein coding genes. Data blocks were specified for codon positions, and the Bayesian Information Criterion (BIC) was used to select the best-fit partitioning scheme and models of sequence evolution. For noncoding regions (28S and the 2ARegulatory UTR), the BIC was used to select models of sequence evolution using jModelTest v2.1.4 (Darriba et al., 2012).

Species trees were inferred using ∗BEAST implemented in BEAST v1.8 (Heled and Drummond, 2010; Drummond et al., 2012). Each gene fragment was initially analyzed using an uncorrelated lognormal (UCLN) relaxed clock model (Drummond et al., 2006). Parameter estimates associated with the clock rate for each gene were then examined using Tracer v1.6 (Rambaut et al., 2014). If the 95% highest posterior density (HPD) of the coefficient of variation for any individual gene included zero, a strict molecular clock was specified for that locus. A strict clock could not be rejected for five of the seven fragments (Table 1). To account for clock rate heterogeneity, a UCLN relaxed clock was specified for COI and 28S.

∗BEAST analyses were run for 250 million generations and sampled every 25,000 generations with substitution models, clock models, and trees unlinked among loci, with the exception of the 2Aregulatory exon and UTR. Because these regions are presumed to belong to a single recombinational unit, these data partitions were run with both clock models and trees linked. Two different rooting strategies were used in ∗BEAST species tree inference. For one set of analyses, transcripts from *S. lobata* and *T. bifurcata* were included as outgroup sequences (hereafter referred to as outgroup-rooted analysis). Another set of species tree analyses were run using only ingroup taxa, and rooted using the molecular clock (hereafter referred to as ingroup-only analysis). Three independent ∗BEAST analyses were run for each rooting strategy, with convergence and stationarity of parameter estimates assessed using Tracer v1.6 (Rambaut et al., 2014). Individual tree files from repeated runs were combined using LogCombiner (Rambaut and Drummond, 2014). The first 2000 samples (20% of logged generations) of each run were discarded as burn-in, and the program TreeAnnotator was used to construct a maximum clade credibility (MCC) tree (Rambaut and Drummond, 2013).

Because Laniatores (and Phalangodidae) lack a detailed Mesozoic/early Cenozoic fossil record, and because of general uncertainly in the age of the most recent common ancestor (tMRCA) of Laniatores (Hedin et al., 2012; Sharma and Giribet, 2014), three different procedures were used to estimate absolute divergence times for *Calicina*. First, a well-calibrated arthropod COI clock rate of 2.69% (ucld mean = 0.0169) per million years (Ma) was specified for the COI data partition (Papadopoulou et al., 2010) and used in both ingroup-only and outgroup-rooted ∗BEAST analyses (conducted as above). Second, a COI clock rate (ucld mean = 0.01115) estimated for the Laniatores genus *Sclerobunus* (Derkarabetian et al., 2010) was applied to ingroup-only and outgroup-rooted ∗BEAST analyses. Finally, a concatenated BEAST analysis was run using 28S and COI data (from GenBank) for an increased panel of Laniatorean taxa. This taxon sample directly mirrored the non-synthetonychiid Laniatores sample of Sharma and Giribet (2014), but also included a large set of Nearctic phalangodids, including our original 28S and COI *Calicina* data. 28S alignments were performed using MUSCLE. A gamma-distributed prior was specified for the root node with an alpha of 3, a beta of 30, and an offset of 199. The prior distribution was truncated to an interval of 206–485, resulting in a 95% confidence interval of 218–410 Ma

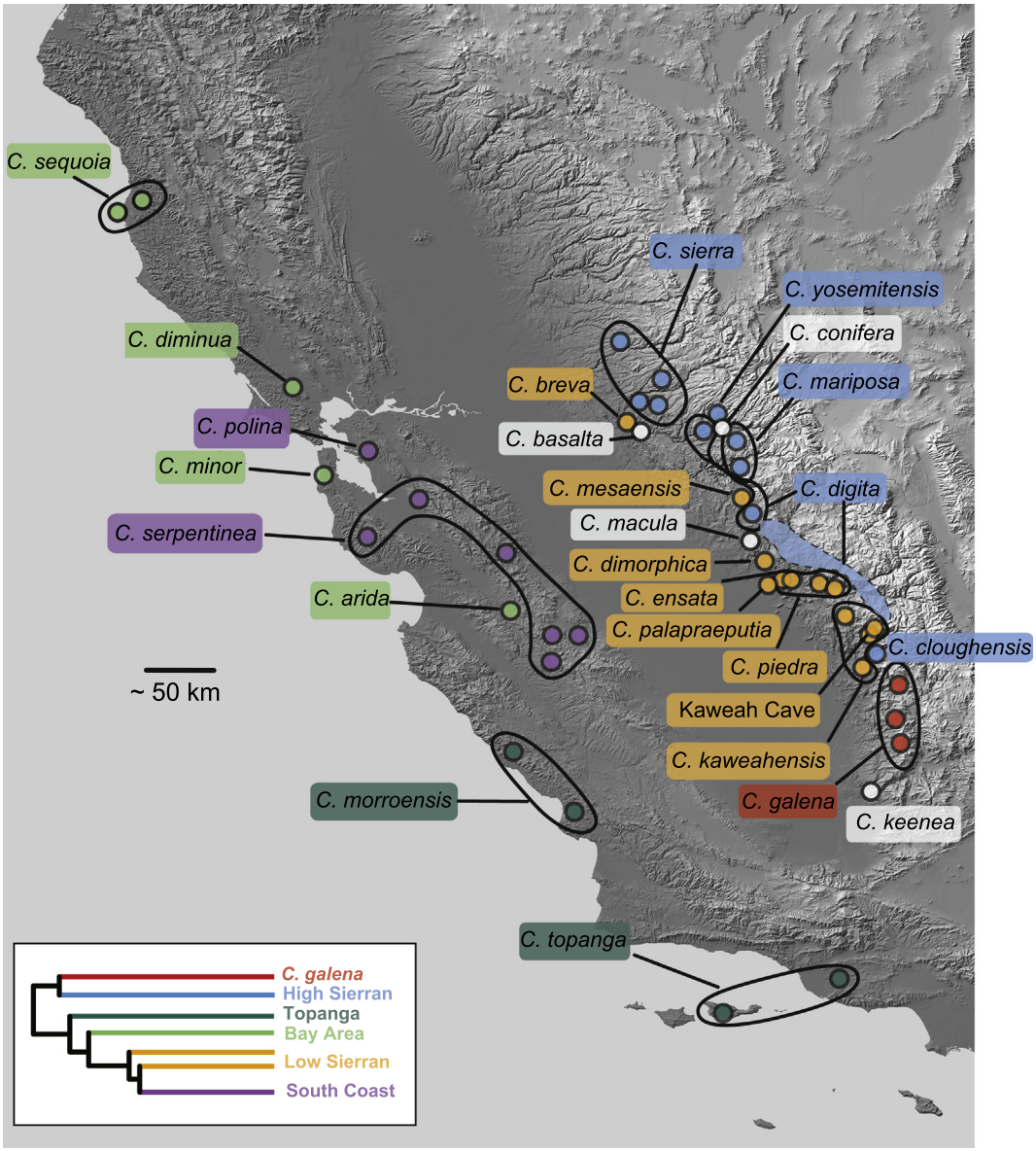

Fig. 2. Map of *Calicina* species localities included in this study. Species names and localities are color coded according to the major clades recovered in ∗BEAST species tree analyses. Unsampled species are shown in white; unsampled southern populations of *C. digita* designated by blue outlined area surrounded by dashed line. Inset: simplified ∗BEAST topology. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Characteristics of gene regions included in phylogenetic analyses.

Gene name	Orthologous to *Ixodes* protein	Matrix completeness (%)	Aligned length (bp)	Parsimony informative sites	Clock model
COI	–	87	808	372	UCLN
28S	–	70	1123	64	UCLN
2Aregulatory exon	ISCW003443	96	617	91	Strict
2Aregulatory UTR	ISCW003443	100	642	107	Strict
RNA polymerase II transcription factor	ISCW004924	94	484	122	Strict
Uroporphyrinogen decarboxylase	ISCW020804	94	686	218	Strict
Ski-interacting protein	ISCW021146	98	614	148	Strict

for the tMRCA of non-synthetonychiid Laniatores. This prior distribution spans the range of *median* divergence times estimated by Sharma and Giribet (2014, fig. 5), while the use of a gamma-distributed prior favors the youngest of these median age estimates. Three independent concatenated BEAST analyses were run for 100 million generations and sampled every 10,000, with substitution and clock models unlinked among loci, and trees linked. As in ∗BEAST analyses, UCLN clock models were specified for the 28S and COI partitions.

2.3. Biogeographic analysis

The R-package BioGeoBEARS was used to infer biogeographic history (Matzke, 2013). This maximum likelihood-based method

implements several biogeographic models including the LAGRANGE DEC model (Ree et al., 2005; Ree and Smith, 2008), and maximum likelihood implementations of the models used in DIVA (Dispersal-Vicariance Analysis; Ronquist, 1997) and BayArea (Landis et al., 2013). BioGeoBEARS also allows for the estimation of the likelihood of founder effect speciation events, where one of the daughter lineages occupies a new area outside of the ancestral range (ABCD > ABCD, E). This is estimated as an additional free parameter *j* that can be added to any of the models implemented in the program.

BioGeoBEARS was run using the MCC trees resulting from both the ingroup-only and outgroup-rooted *BEAST analyses using the Papadopoulou et al. (2010) rate. Three areas were represented: the Sierra Nevadas (SN), the Coast Ranges (CR), and the Transverse Ranges (TR). These areas represent naturally defined geomorphic provinces based on geology, faults, topographic relief, and climate (California Geological Survey, www.conservation.ca.gov). Each species was coded as being present or absent in each of these three areas. Default starting values were used for all parameters. The likelihood scores of nested models (e.g., DEC and DEC+ *j*, etc.) were compared using likelihood ratio tests, and AIC scores were calculated for all six models.

2.4. Character evolution

Morphological data were collected for all *Calicina* species represented in the final taxon sample. Scute length, leg II length, and eye development were scored for 212 SDSU and California Academy of Sciences (CAS) specimens (raw data were submitted to **the Dryad Digital Repository: doi:10.5061/dryad.70h1h**). Scute length (measured dorsally at midline) and leg II length (combined leg segments, left leg) were treated as continuous characters, and eye development was scored as a binary character (i.e., well-developed vs. loss of retinal pigmentation). Measurements were taken using an Olympus SZX12 dissecting microscope with an ocular micrometer. Because the canonical correlation analysis (see below) requires a single trait value per species, the average scute length and leg II length were calculated for each species. Measurements were taken for multiple specimens of each sex per species if available. A paired *t*-test on average scute length and leg II length for each species found no significant differences in these measurements across sexes.

Altitudinal and climatic layers for nineteen quarterly and annual measurements of temperature and precipitation (Bioclimatic layers 1–19) were obtained from the WorldClim dataset v.1.4 at 30 arc-second (1 km) resolution (Hijmans et al., 2005). Climatic data for all known published (Ubick and Briggs, 1989) and Hedin lab collection localities for *Calicina* specimens were extracted using the program DIVA-GIS (Hijmans et al., 2004). Raw climatic data were deposited into the Dryad Digital Repository: doi:10.5061/dryad.70h1h. Climatic data were extracted for a total of 194 localities, and values were averaged across each species. Highly correlated variables were removed following Jezkova et al. (2011). This resulted in a reduced set of nine climatic layers used for downstream statistical analyses (see Appendix C). In order to further reduce the dimensionality of the climatic data, a principal component analysis (PCA) was performed, and the average scores for each species from the first two principal components were retained and used in subsequent comparative analyses.

A phylogenetic canonical correlation analysis (CCA) was used to examine the relationship between climatic variation and morphology. This multivariate method allows for the calculation and analysis of correlation between character sets while accounting for the non-independence of species due to phylogeny (Revell and Harrison, 2008). The per species average PC1 and PC2 scores from the PCA of climatic variables were used as one character matrix, and average scute length, leg II length, and eye development were compiled into a second matrix. Pagel's λ was estimated for the phylogenetic transformation, and *p*-values were generated to test the null hypothesis that the *i*th and all subsequent canonical correlations are 0. The phylogenetic CCA was performed using the 'phytools' package in R (Revell, 2012), and canonical loadings were calculated using the "comput" function in the 'CCA' package (González et al., 2008).

3. Results

3.1. Phylogeny

Unphased DNA sequences for all gene regions have been submitted to GenBank (Appendix A); phased matrices have been submitted to **the Dryad Digital Repository: doi:10.5061/dryad.70h1h**. *BEAST topologies using the ingroup-only and outgroup rooting strategies were identical, except for the placement of *C. galena*. The ingroup-only analysis placed *C. galena* as sister to a weakly supported clade (PP = 0.81) containing members of an early-diverging Sierran clade including *C. yosemitensis*, *C. cloughensis*, *C. digita*, *C. mariposa*, and *C. sierra* (**High Sierran clade**, Fig. 3), while the outgroup-rooted analysis placed *C. galena* as sister to all remaining *Calicina* species with strong support. Because the outgroups *Sitalcina* and *Texella* are quite divergent from *Calicina*, perhaps attracting the long branch leading to *C. galena* toward the base of the phylogeny, downstream biogeographic and character evolution analyses were conducted using the MCC trees and posterior distributions resulting from both analyses. For the remainder of this paper, ingroup-only results are illustrated and discussed, with qualitatively similar out-group rooted results mentioned where justified.

In addition to the strongly supported early-diverging **High Sierran clade**, several other lineages are strongly supported and informally named here (Figs. 2 and 3). A **Topanga clade** corresponds to the sampled topanga subgroup members (*C. topanga* and *C. morroensis*), and is distributed in the western Transverse Ranges (including Santa Cruz Island) north to Morro Bay. A strongly-supported **Bay Area** clade includes *C. diminua*, *C. sequoia*, *C. arida*, and *C. minor*. These taxa occur in the inner South Coast Ranges north through the San Francisco Bay Area to Humboldt County. The last major lineage recovered is comprised mostly of taxa from the foothills of the southern and central Sierra Nevada (**Low Sierran group**). However, nested within this group are two Coastal taxa (**South Coast clade**, *C. serpentinea* and *C. polina*), which occur from north of the Bay Area to the inner South Coast Ranges, although our samples are only from south (*C. serpentinea*) and east (*C. polina*) of the Bay Area (Fig. 2).

The *mariposa*, *digita*, and *serpentinea* species groups were not recovered as monophyletic in the *BEAST species trees (compare Figs. 1 and 3). Additionally, the monotypic *palapraeputia* group (Ubick and Briggs, 1989; Fig. 1) was recovered as nested within a strongly supported clade containing some members of the *serpentinea* group (Fig. 3). Of the nine subgroups hypothesized in Ubick and Briggs (1989), only the topanga subgroup was recovered as monophyletic with strong support.

3.2. Divergence time estimation

Median and 95% HPD estimates from alternative *BEAST and BEAST analyses are summarized in Table 2. A BEAST chronogram for the non-synthetonychiid Laniatores rooting (with GenBank numbers) is included as Appendix B. In general, we prefer the analyses suggesting the youngest of ages for *Calicina*. Other analyses suggest more ancient divergences within *Calicina* that appear geologically unrealistic (e.g., divergences of Coast Range taxa before

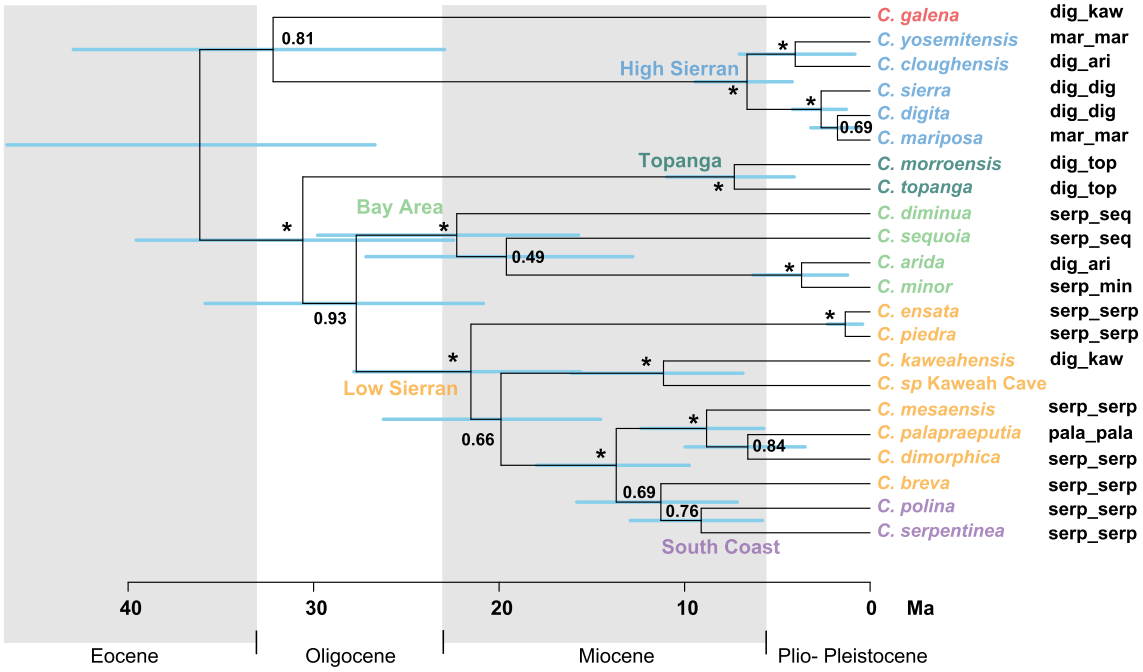

Fig. 3. Ingroup-only MCC ∗BEAST species tree with median divergence time estimates (in Ma) and 95% highest posterior densities of divergence time estimates, based on Papadopoulou et al. (2010) COI rate. Support values shown as posterior probabilities; nodes with posterior probabilities greater than 0.95 denoted with an asterisk. Species are color-coded according to major clades recovered. Species group and subgroup membership as hypothesized in Ubick and Briggs (1989) indicated to the right of each species name. Abbreviations: ari = arida, dig = digita, kaw = kaweahensis, mar = mariposa, min = minor, pala = palapraeputia, serp = serpentinea, seq = sequoia, top = topanga. Intervals for geologic epochs from the U.S. Geological Survey Geologic Names Committee, 2007, Divisions of geologic time—Major chronostratigraphic and geochronologic units: U.S. Geological Survey Fact Sheet 2007-3015, 2 p. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Divergence time estimates resulting from alternative clock analyses.

Model	Papadopoulou rate – IG	Papadopoulou rate – OUT	Sclero rate – IG	Sclero rate – OUT	Deep laniatores rooting
tMRCA of genus *Calicina*	36.14 (26.68–46.53)	41.40 (30.33–54.66)	86.92 (64.17–113.51)	92.04 (67.15–121.37)	54.51 (36.39–77.83)
tMRCA of Bay Area and Low Sierran + South Coast	27.70 (20.84–35.85)	28.47 (22.92–41.64)	66.74 (49.58–88.14)	63.43 (51.89–93.38)	39.08 (25.66–55.86)
tMRCA of Topanga	7.32 (4.09–10.98)	7.65 (4.16–11.61)	17.96 (10.36–27.64)	16.74 (9.11–25.13)	20.87 (9.10–36.04)

Note: median values, 95% HPD values in parentheses.

Table 3
BioGeoBEARS results based on ingroup-only ∗BEAST topology. Bold values indicate models of range evolution with probabilities significant at $\alpha = 0.05$ and preferred by the Akaike Information Criterion (AIC).

Model	LnL	Degrees of Freedom	D statistic (LRT)	*p*-value (LRT)	AIC
DEC+ *j*	−12.87	3	8.37	**0.0038**	**31.73**
DEC	−17.05	2	–	–	38.1
DIVALIKE+ *j*	−12.66	3	7.54	**0.0060**	**31.31**
DIVALIKE	−16.43	2	–	–	36.86
BAYAREALIKE+ *j*	−14.21	3	25.5	**4.4e−07**	**34.41**
BAYAREALIKE	−26.96	2	–	–	57.91

Coast Range habitats existed, etc. – see Section 4.1). However, even these "young age" analyses suggest that *Calicina* is an ancient genus. Estimates using the Papadopoulou et al. (2010) rate on the ingroup-only topology indicate a median age for the genus at 36.1 Ma (95% HPD of 26.7–46.5 Ma; Table 2, Fig. 3). All other analyses suggest even more ancient divergences.

3.3. Historical biogeography

BioGeoBEARS reconstructions gave qualitatively similar results from both the ingroup-only and outgroup-rooted analyses. Likelihood ratio tests of the fit of nested models overwhelmingly favor models that include founder effect speciation (*p*-values for each comparison <0.01, Table 3). Because the DEC+ *j* and DIVALIKE+ *j* models were statistically indistinguishable and favored over the BAYAREALIKE+ *j* model in the ingroup-only analysis (Table 3), the results of these two models are reported here (Fig. 4). These models are unable to distinguish between a Sierra Nevada versus Coast Range ancestral range for *Calicina*. The analysis inferred a Sierran distribution for the High Sierran clade and *C. galena*, and a Coastal distribution for the clade containing remaining *Calicina*, with subsequent dispersal back to the Sierra Nevada around 22 million years ago. Within this Sierra Nevada group, another dispersal event was inferred back to the Coast Ranges around 9 million years ago.

3.4. Character evolution

The first two principal components of the reduced set of nine climatic layers collectively explained 83.5% of the variation in species occurrence (51.1% and 32.4%, respectively; see Appendix C). Altitude and mean temperature of the wettest quarter load strongly and oppositely on PC1, suggesting a strong influence of these climatic variables on *Calicina* distribution. The other seven

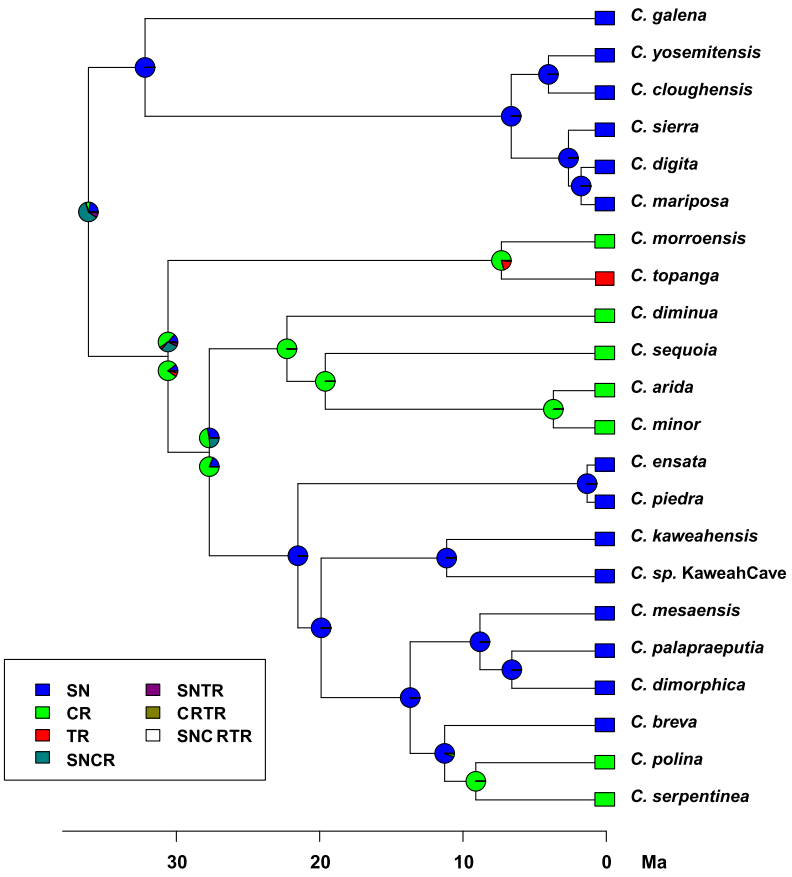

Fig. 4. BioGeoBEARS ancestral range reconstructions on the ingroup-only species tree under the preferred models (DEC+ *j* and DIVALIKE+ *j*). Both reconstructions are shown for nodes that greatly differed among models, with the reconstruction for DEC+ *j* shown above and DIVALIKE+ *j* shown below. Abbreviations: SN = Sierra Nevada, CR = Coast Ranges, TR = Transverse Ranges.

climatic variables load roughly equally on PC1 and PC2. When localities are labeled by region (i.e., Coastal, Sierran, and Transverse), there is apparent separation between the Coastal and Sierran groups on a biplot of the first two principal components, suggesting that *Calicina* habitats are defined by different sets of climatic variables on either side of the Central Valley (Appendix C). Most species localities in the Sierra Nevada fall along a narrow and well-defined axis of temperature and precipitation, such that localities with the highest annual temperatures tend to experience the lowest precipitation during the hottest and driest quarter of the year. In contrast, most Coastal localities tend to experience more even temperatures with low temperature seasonality and smaller diurnal ranges and increased isothermality, or "temperature evenness" throughout the year. Interestingly, these localities also experience greater seasonality in precipitation, indicating that, in general, there are greater extremes of precipitation levels throughout the year in Coastal localities. These localities mostly correspond to species that are found in arid serpentine grassland and oak woodland habitats (e.g., *C. morroensis*, *C. polina*, *C. serpentinea*, and *C. arida*).

The results of the phylogenetic CCA based on the ingroup-only ∗BEAST topology indicate a significant relationship between position in climatic and morphological shape space (Fig. 5). The first canonical component in this comparison was significant ($p = 0.015$). This component indicates a relationship between climatic variation in locality (represented by mean PC1 and PC2 scores for each species) and mean scute and leg II length, and eye development. Both PC1 and PC2 scores were shown to be correlated with position in morphological space (canonical loadings = 0.61 and 0.52, respectively). Scute length and leg II length were shown to be positively correlated with canonical component 1 (canonical loadings = 0.55 and 0.45, respectively), while eye development (coded as 0 = well-developed, 1 = blind) was strongly negatively correlated with CC1 (canonical loading = −0.73).

Although members of the Low Sierran group are more closely related to Topanga and Bay Area clades (Fig. 3), they are more similar to species of the High Sierran clade in morphological space (Fig. 5). Similarly, the two species of the South Coast clade (*C. serpentinea* and *C. polina*) are phylogenetically nested within the Low Sierran group, but are morphologically more similar to the other Coastal taxa of the Bay Area and Topanga clades. These results suggest that selective pressures associated with the different climates on either side of the Central Valley may be driving morphological evolution in *Calicina*. An exception involves *C. sequoia* – this species is very small but has well-developed eyes (unlike other small-bodied *Calicina*), and it occurs in the Coastal Redwoods of northern California in areas that have lower annual mean temperatures but high precipitation (unlike other Coastal species).

4. Discussion

4.1. Calicina historical biogeography

Our species tree analyses indicate that high-elevation Sierran taxa are early-diverging in *Calicina*, with subsequent biogeographic "criss-crossing" of lineages from the Sierra Nevada to the Coast Ranges, back to the Sierra Nevada, then back to Coast Ranges (Fig. 2). This pattern suggests that speciation of the stem lineages of *Calicina* was largely driven by allopatry associated with vicariance, followed by secondary colonization of both Sierran and

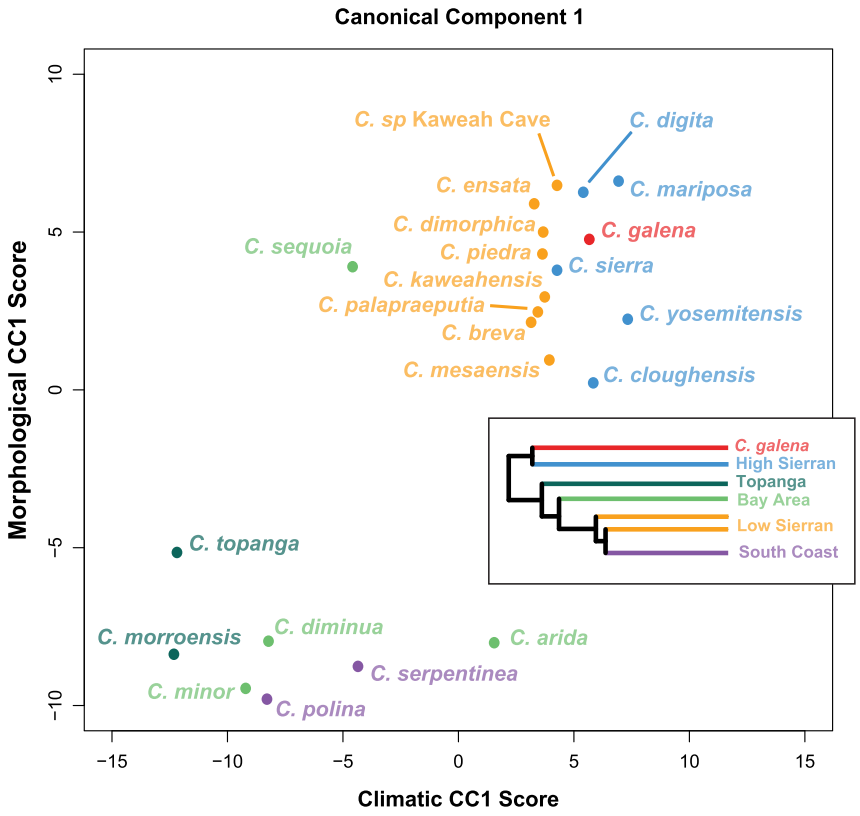

Fig. 5. Scatterplot of climatic and morphological CC1 scores resulting from the CCA. Species names are color-coded according to major clades recovered in the species tree analyses. Inset: simplified *BEAST topology. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Coastal habitats. This criss-crossing results in distantly-related phylogenetic lineages occurring in geographic proximity. Throughout the central and southern Sierra Nevada, a pattern of elevational tiering is seen with *C. galena* + High Sierran clade species found at higher elevations, and species of the Low Sierran group in adjacent foothills to the west. Here we presume that *C. digita*, including unsampled southern populations (Fig. 2), is monophyletic. A similar parapatric pattern occurs in the Coast Ranges, with Bay Area clade species generally occupying more western habitats, and the two South Coast species found in more eastern habitats (Fig. 2). Intriguingly, species in the phylogenetically independent radiations both in the Coast Ranges and the Sierra Nevada retain strict allopatry/parapatry, even though these species are apparently anciently diverged (Figs. 2 and 3). A similar pattern has been documented in a radiation of crickets in New Caledonia, whose distributional history has been largely driven by allopatric speciation followed by secondary sympatry due to recent range expansion (Nattier et al., 2012).

Although the results of the molecular clock and biogeographic analyses allow for the interpretation of patterns of diversification in a geologic context, there is clear uncertainty in the absolute timing of *Calicina* diversification. We conducted multiple calibration analyses and prefer the youngest of dates implied by these analyses, but these dates still indicate ancient divergences in *Calicina*. Older calibrations place divergences of Coastal taxa in the Paleocene or Eocene (~65–40 Ma), when the western edge of the early Sierra Nevada formed the California coastline (reviewed in Schierenbeck, 2014).

The deepest divergences in *Calicina* separate high-elevation Sierran species from a clade containing the remaining species in the genus. The median age of this split in "young calibrations" coincides with the Oligocene (~35–25 Ma), which was characterized by the gradual uplift of the Sierra Nevada from the basin and range landscape of the present-day Great Basin (Harden, 1998; Schierenbeck, 2014). Importantly, geological evidence suggests that terrestrial habitats were available in the southern Sierra Nevada region during this general time frame. West of the Sierra Nevada, a low inland sea occupied most of western California, and the Coast Ranges were forming (Harden, 1998).

During the late Oligocene and early Miocene, various non-marine rock formations were being deposited to the southern Coast as the East Pacific Rise subducted under the North American Plate. This growth of the southern Coast likely provided a colonization opportunity for *Calicina*, as the next cladogenic event in the genus corresponds to the separation of the lineage that eventually gave rise to the Topanga clade in the Transverse and southern Coast Ranges (Fig. 3). The gradual development of the San Andreas Fault from 24 to 12 Ma, and associated northwest movement of the North American Plate (Hall, 2007; Gottscho, 2014; Schierenbeck, 2014) may have had a role in the separation and radiation of the Bay Area clade. The estimated time frame (roughly 22 Ma) for diversification of this clade is consistent with these geologic events. These species are associated with the Franciscan assemblage, a complex mélange of terranes that was accreted to the North American Plate during the subduction of the Farallon Plate (Furlong and Schwartz, 2004). Finally, the median age of the Low Sierran group is estimated to be around 22 Ma, which coincides with a period of uplift from 25 to 10 Ma during which the Sierra Nevada increased from roughly 1000–2500 m in elevation (Schierenbeck, 2014; Fig. 3).

Nested deeply within the Low Sierran group are two Coastal taxa, *C. serpentinea* and *C. polina* (Fig. 3). The results of the species tree inference and the ancestral range reconstruction suggest that this trans-valley pattern is the result of an ancient (i.e., mid-late Miocene) east to west dispersal event from the central Sierra Nevada foothills to the Coast Ranges (Figs. 3 and 4). Similar

"trans-valley" spatial patterns have been found in various salamanders, including *Batrachoseps attenuatus* (Jockusch and Wake, 2002; Wake, 2006; Martínez-Solano et al., 2007), *Ensatina escholtzii xanthoptica* (Kuchta et al., 2009a, 2009b), and *Aneides lugubris* (Lapointe and Rissler, 2005), which have disjunct distributions at latitudes near the San Francisco Bay. These studies recover a relatively recent (i.e., mid-late Pleistocene) west to east "trans-valley" leak (i.e., from the Coast Ranges to the Sierra Nevada). Two trapdoor spider species *Aliatypus californicus* and *A. erebus* also exhibit similar intraspecific patterns across the Central Valley in this region, but these patterns were inferred to have east to west directionality (Satler et al., 2011). Based on gene tree topology and levels of mitochondrial divergence, the phylogeographic patterns in these trapdoor spiders are inferred to be older than those recovered in salamanders. A recent study of the mygalomorph spider genus *Calisoga* also found that clades in the Bay Area are related to clades in the Sierra Nevada (Leavitt et al., 2015). This relationship appears to have east–west directionality as well, with south Coast Range populations nested within a larger Sierran clade. Although divergence dates have not been estimated for the mygalomorph phylogeographic breaks, these findings suggest that the landscape of the Central Valley has undergone intermittent periods of suitability conducive to dispersal (see also Hedin et al., 2013).

4.2. Comparative biogeography

Although many studies focus on phylogeographic and/or comparative phylogeographic patterns in California taxa (Calsbeek et al., 2003; Lapointe and Rissler, 2005; Feldman and Spicer, 2006; Chatzimanolis and Caterino, 2007; Schierenbeck, 2014), fewer studies have focused on species-level historical biogeographic patterns within California endemic species radiations. Exceptions include several studies of trapdoor spiders and salamanders (e.g., Kuchta et al., 2009a; Hedin et al., 2013; Leavitt et al., 2015). *Batrachoseps* salamanders are particularly similar to *Calicina* in many regards. *Batrachoseps* are morphologically specialized for subterranean life, are extremely sedentary, and are able to persist in small patches of suitable habitat (Yanev, 1980). The distribution of *Batrachoseps* in California is also similar to that of *Calicina*. The subgenus *Batrachoseps* contains 19 species and ranges almost continuously up the Pacific Coast and throughout most of the Sierra Nevada, with some isolated populations in the Central Valley. This subgenus includes four species groups: the *attenuatus*, *nigriventris*, *diabolicus*, and *pacificus* groups. Diversification in these groups is thought to be largely driven by the extremely low vagility of these organisms and their ability to persist in small suitable patches of habitat in geologically dynamic landscapes (Jockusch et al., 2001, 2012).

Batrachoseps is hypothesized to be ancient; early studies hypothesized that the genus originated in the early Eocene (Wake et al., 1978) and fossil evidence indicates that the genus was present in the Sierra Nevada in essentially modern form during the Miocene (Peabody, 1959; Yanev, 1980). The first comprehensive molecular study of the genus inferred divergence times from allozyme genetic distances (Yanev, 1978, 1980). The genus was inferred to have originated during the mid-Eocene (~40 Ma), with divergences of 30–25 Ma among widely distributed species (according to the existing taxonomy at the time) and about 8–10 Ma among allopatric populations within species. This biogeographic scenario supports a vicariance-dominated history for *Batrachoseps*, largely influenced by the evolution of the San Andreas Fault, and the uplift of the Sierra Nevada, Coast Ranges, and Transverse Ranges throughout the mid-Miocene. Although taxonomic designations in the genus have changed over the last few decades, subsequent studies generally agree with this biogeographic context for the genus (Jackman et al., 1997; Jockusch and Wake, 2002; Wake, 2006). Additionally, recent studies of divergence dates estimate that crown group began to diversify during the mid to late Miocene (Jockusch et al., 2001; Martínez-Solano et al., 2007, 2012).

Overall, the phylogenetic and biogeographic patterns recovered in this study suggest that *Calicina*, like *Batrachoseps*, has inhabited California since the Eocene. Based on molecular clock estimates, *Calicina* is presumed to be a relatively ancient lineage whose evolutionary and biogeographic history has been shaped by the dynamic geological processes of California over the last 30 million years.

4.3. Character evolution

CCA recovered broad separation of *Calicina* species in climatic and morphological space (Fig. 5). The Sierran species were recovered in a tight cluster, suggesting a strong association of climatic variation in species localities with morphology. Coastal species exhibited more variation in climate and morphology, but the highly paedomorphic species (*C. morroensis*, *C. diminua*, *C. minor*, and *C. polina*) were recovered in a cluster, with the exception of *C. sequoia* and *C. arida*, whose climatic associations appear to be unique for Coastal species (Fig. 5). The Low Sierran group, inferred to have Coastal ancestry, is more similar to the High Sierran clade in morphological space than it is to close relatives of the Bay Area clade (Fig. 5). Similarly, species of the South Coast clade, with Sierran ancestry, have converged morphologically with the Bay Area and Topanga clades. These repeated patterns of convergent evolution provide support for a strong association between climate variation and morphological evolution in *Calicina*.

4.4. Calicina systematics

Ubick and Briggs (1989) hypothesized that *Calicina* species belong to one of nine subgroups in four larger species groups, based primarily on male genitalic characters. Molecular phylogenetic analyses do not support the monophyly of any species groups containing more than one species, and only one subgroup, the topanga subgroup, was recovered in this study (Fig. 3). However, reconciliation of this apparent discord only requires the morphological reconsideration of a few key taxa, as noted below.

First, both species of the arida subgroup (*C. cloughensis, C. arida*) appear misplaced on the morphological tree (compare Figs. 1 and 3). Ubick and Briggs (1989) noted "the two species of the arida subgroup have an unusual glans morphology for their group". Males of the cave-dwelling *C. cloughensis* actually lack parastyli and have a collar lobe like the *mariposa* species group; this is consistent with the molecular placement of this taxon. Males of *C. arida* have a penis somewhat similar to *C. diminua* and *C. sequoia*, again consistent with the molecular placement of this taxon. Whether or not *C. arida* and *C. minor* possess synapomorphic genitalic similarities should be re-examined. Second, both species of the kaweahensis subgroup (*C. galena, C. kaweahensis*) appear similarly misplaced on the morphological tree. *Calicina galena* has a very distinctive, autapomorphic male glans morphology (Ubick and Briggs, 1989; fig. 8). The distinctiveness of this morphology matches the divergent molecular placement of this taxon. The molecular phylogenetic placement of *C. kaweahensis* is perhaps the most difficult to reconcile with genitalic morphology – the glans morphology of this taxon differs obviously from *C. galena*, but whether or not this species has a dorsal process needs to be re-evaluated. Finally, the ovipositor structure of *C. palapraeputia* (*palapraeputia* species group) suggests a close relationship with members of the serpentinea subgroup (e.g., distribution of microspines restricted to the apical surface). This also suggests that the C. *palapraeputia* middle

glans segment is homologous to the serpentinea subgroup dorsal process (see Fig. 1), as hypothesized by Ubick and Briggs (1989).

Although four Sierran species were not sampled for this study (Fig. 2), their distribution and genitalic morphology (following Ubick and Briggs, 1989) make it possible to hypothesize their phylogenetic placement. We hypothesize that *C. conifera* is a member of the High Sierran clade, that *C. basalta* and *C. macula* are members of the Low Sierran group, and that *C. keenea* is a member of the Topanga clade. The inclusion of *C. keenea* in a future study would provide a key biogeographic link between Sierran taxa and Transverse Range/Coastal lineages.

5. Conclusions

This research highlights the need for further studies of species level systematics in phalangodids, particularly in California, where most of the diversity of Nearctic phalangodids is found. Within *Calicina*, the inclusion of all described species would improve the understanding of interspecies relationships and the historical biogeography of the genus. In particular, inclusion of southern populations of *C. digita* would increase the resolution of biogeographic patterns in the High Sierran clade and provide more evidence for the pattern of elevational tiering discovered here. Because most *Calicina* species are microhabitat specialists with extremely restricted ranges, these taxa could be at a high risk of extinction due to climate change and habitat disturbance. The study of such taxa is important, as the insights gained from the study of short-range endemic taxa can help enhance both conservation and research outcomes (Harvey et al., 2011).

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Appendices A–C. Supplemental material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.02.002.

References

Bond, J., Stockman, A., 2008. An integrative method for delimiting cohesion species: finding the population-species interface in a group of Californian trapdoor spiders with extreme genetic divergence and geographic structuring. Syst. Biol. 57, 628–646. http://dx.doi.org/10.1080/10635150802302443.

Calsbeek, R., Thompson, J.N., Richardson, J.E., 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. Mol. Ecol. 12, 1021–1029. http://dx.doi.org/10.1046/j.1365-294X.2003.01794.x.

Chatzimanolis, S., Caterino, M.S., 2007. Toward a better understanding of the "transverse range break": lineage diversification in southern California. Evolution 61, 2127–2141.

Clemens-Knott, D., van der Kolk, D.A., Sturmer, D.M., Saleeby, J.B., 2013. The Goldstein Peak Formation, central California: record of a nonmarine intra-arc basin within the Early Cretaceous Sierra Nevada arc. Geosphere 9, 718–735. http://dx.doi.org/10.1130/GES00886.1.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Derkarabetian, S., Steinmann, D.B., Hedin, M., 2010. Repeated and time-correlated morphological convergence in cave-dwelling harvestmen (Opiliones, Laniatores) from montane western North America. PLoS ONE 5, e10388. http://dx.doi.org/10.1371/journal.pone.0010388.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88. http://dx.doi.org/10.1371/journal.pbio.0040088.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Feldman, C.R., Spicer, G.S., 2006. Comparative phylogeography of woodland reptiles in California: repeated patterns of cladogenesis and population expansion. Mol. Ecol. 15, 2201–2222.

Furlong, K.P., Schwartz, S.Y., 2004. Influence of the Mendocino triple junction on the tectonics of coastal California. Annu. Rev. Earth Planet. Sci. 32, 403–433.

González, I., Déjean, S., Martin, P.G., Baccini, A.others, 2008. CCA: an R package to extend canonical correlation analysis. J. Stat. Softw. 23, 1–14.

Gottscho, A.D., 2014. Zoogeography of the San Andreas Fault system: great Pacific Fracture Zones correspond with spatially concordant phylogeographic boundaries in western North America: zoogeography of the San Andreas Fault system. Biol. Rev. http://dx.doi.org/10.1111/brv.12167.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., et al., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652.

Hall, C.A., 2007. Introduction to the Geology of Southern California and its Native Plants. University of California Press.

Harden, D.R., 1998. California Geology. Prentice Hall.

Harvey, M.S., Rix, M.G., Framenau, V.W., Hamilton, Z.R., Johnson, M.S., Teale, R.J., Humphreys, G., Humphreys, W.F., 2011. Protecting the innocent: studying short-range endemic taxa enhances conservation outcomes. Invertebrate Systematics 25, 1–10. http://dx.doi.org/10.1071/IS11011.

Hedin, M., Starrett, J., Akhter, S., Schönhofer, A.L., Shultz, J.W., 2012. Phylogenomic resolution of paleozoic divergences in harvestmen (Arachnida, Opiliones) via analysis of next-generation transcriptome data. PLoS ONE 7, e42888. http://dx.doi.org/10.1371/journal.pone.0042888.

Hedin, M., Starrett, J., Hayashi, C., 2013. Crossing the uncrossable: novel trans-valley biogeographic patterns revealed in the genetic history of low-dispersal mygalomorph spiders (Antrodiaetidae, *Antrodiaetus*) from California. Mol. Ecol. 22, 508–526. http://dx.doi.org/10.1111/mec.12130.

Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580. http://dx.doi.org/10.1093/molbev/msp274.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., et al., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978.

Hijmans, R.J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I., Rojas, E., 2004. DIVA-GIS. Vsn. 5.0. A geographic information system for the analysis of species distribution data.

Ingersoll, R.V., 2012. Composition of modern sand and Cretaceous sandstone derived from the Sierra Nevada, California, USA, with implications for Cenozoic and Mesozoic uplift and dissection. Sed. Geol. 280, 195–207. http://dx.doi.org/10.1016/j.sedgeo.2012.03.022.

Jackman, T.R., Applebaum, G., Wake, D.B., 1997. Phylogenetic relationships of bolitoglossine salamanders: a demonstration of the effects of combining morphological and molecular data sets. Mol. Biol. Evol. 14, 883–891.

Jezkova, T., Olah-Hemmings, V., Riddle, B.R., 2011. Niche shifting in response to warming climate after the last glacial maximum: inference from genetic data and niche assessments in the chisel-toothed kangaroo rat (*Dipodomys microps*). Glob. Change Biol. 17, 3486–3502. http://dx.doi.org/10.1111/j.1365-2486.2011.02508.x.

Jockusch, E.L., Martinez-Solano, I., Hansen, R.W., Wake, D.B., 2012. Morphological and molecular diversification of slender salamanders (Caudata: Plethodontidae: *Batrachoseps*) in the southern Sierra Nevada of California with descriptions of two new species. Zootaxa 3190, 1–30.

Jockusch, E.L., Wake, D.B., 2002. Falling apart and merging: diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West. Biol. J. Linn. Soc. 76, 361–391.

Jockusch, E.L., Yanev, K.P., Wake, D.B., 2001. Molecular phylogenetic analysis of slender salamanders, genus *Batrachoseps* (Amphibia: Plethodontidae), from central coastal California with descriptions of four new species. Herpetol. Monogr. 15, 54–99. http://dx.doi.org/10.2307/1467038.

Kuchta, S.R., Parks, D.S., Mueller, R.L., Wake, D.B., 2009a. Closing the ring: historical biogeography of the salamander ring species *Ensatina eschscholtzii*. J. Biogeogr. 36, 982–995. http://dx.doi.org/10.1111/j.1365-2699.2008.02052.x.

Kuchta, S.R., Parks, D.S., Wake, D.B., 2009b. Pronounced phylogeographic structure on a small spatial scale: geomorphological evolution and lineage history in the salamander ring species *Ensatina eschscholtzii* in central coastal California. Mol. Phylogenet. Evol. 50, 240–255. http://dx.doi.org/10.1016/j.ympev.2008.10.019.

Kury, A.B., 2013. Order opiliones sundevall, 1833. In: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa, vol. 3703, pp. 27. http://dx.doi.org/10.11646/zootaxa.3703.1.7.

Lancaster, L.T., Kay, K.M., 2013. Origin and diversification of the California flora: re-examining classic hypotheses with molecular phylogenies. Evolution 67, 1041–1054. http://dx.doi.org/10.1111/evo.12016.

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. Syst. Biol. 62, 789–804. http://dx.doi.org/10.1093/sysbio/syt040.

Lanfear, R., Calcott, B., Ho, S.Y., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.

Lapointe, F., Rissler, L.J., 2005. Congruence, consensus, and the comparative phylogeography of codistributed species in California. Am. Nat. 166, 290–299. http://dx.doi.org/10.1086/431283.

Leavitt, D.H., Starrett, J., Westphal, M.F., Hedin, M., 2015. Multilocus sequence data reveal dozens of putative cryptic species in a radiation of endemic Californian mygalomorph spiders (Araneae, Mygalomorphae, Nemesiidae). Mol. Phylogenet. Evol. 91, 56–67. http://dx.doi.org/10.1016/j.ympev.2015.05.016.

Martínez-Solano, I., Jockusch, E.L., Wake, D.B., 2007. Extreme population subdivision throughout a continuous range: phylogeography of *Batrachoseps attenuatus* (Caudata: Plethodontidae) in western North America. Mol. Ecol. 16, 4335–4355. http://dx.doi.org/10.1111/j.1365-294X.2007.03527.x.

Martínez-Solano, Í., Peralta-García, A., Jockusch, E.L., Wake, D.B., Vázquez-Domínguez, E., Parra-Olea, G., 2012. Molecular systematics of *Batrachoseps* (Caudata, Plethodontidae) in southern California and Baja California: mitochondrial-nuclear DNA discordance and the evolutionary history of *B. major*. Mol. Phylogenet. Evol. 63, 131–149. http://dx.doi.org/10.1016/j.ympev.2011.12.026.

Martínez-Solano, I., Lawson, R., 2009. Escape to Alcatraz: evolutionary history of slender salamanders (*Batrachoseps*) on the islands of San Francisco Bay. BMC Evol. Biol. 9, 38.

Matzke, N.J., 2013. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5, 242–248.

Millar, C.I. et al., 2012. Geologic, climatic, and vegetation history of California. In: The Jepson Manual: Vascular Plants of California, second ed. University of California Press, Berkeley, pp. 49–68.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nattier, R., Grandcolas, P., Elias, M., Desutter-Grandcolas, L., Jourdan, H., Couloux, A., Robillard, T., 2012. Secondary sympatry caused by range expansion informs on the dynamics of microendemism in a biodiversity hotspot. PLoS ONE 7, e48047. http://dx.doi.org/10.1371/journal.pone.0048047.

Papadopoulou, A., Anastasiou, I., Vogler, A.P., 2010. Revisiting the insect mitochondrial molecular clock: the Mid-Aegean Trench calibration. Mol. Biol. Evol. 27, 1659–1672. http://dx.doi.org/10.1093/molbev/msq051.

Peabody, F.E., 1959. Trackways of Living and Fossil Salamanders. University of California Press.

Rambaut, A., Drummond, A.J., 2014. LogCombiner v2. 1.1.

Rambaut, A., Drummond, A.J., 2013. TreeAnnotator v1. 7.0. Institute of Evolutionary Biology, University of Edinburgh.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1. 6. Computer Program and Documentation Distributed by the Author. Website http://beast.bio.ed.ac.uk/Tracer (accessed 27 July 2014).

Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311.

Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14. http://dx.doi.org/10.1080/10635150701883881.

Reilly, S.B., Mulks, M.F., Reilly, J.M., Jennings, W.B., Wake, D.B., 2013. Genetic diversity of black salamanders (*Aneides flavipunctatus*) across watersheds in the Klamath Mountains. Diversity 5, 657–679. http://dx.doi.org/10.3390/d5030657.

Reilly, S.B., Wake, D.B., 2015. Cryptic diversity and biogeographical patterns within the black salamander (*Aneides flavipunctatus*) complex. J. Biogeogr. 42, 280–291. http://dx.doi.org/10.1111/jbi.12413.

Revell, L.J., 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3, 217–223.

Revell, L.J., Harrison, A.S., 2008. PCCA: a program for phylogenetic canonical correlation analysis. Bioinformatics 24, 1018–1020. http://dx.doi.org/10.1093/bioinformatics/btn065.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Saleeby, J., 2011. Geochemical mapping of the Kings-Kaweah ophiolite belt, California—Evidence for progressive mélange formation in a large offset transform-subduction initiation environment. In: Geological Society of America Special Papers. Geological Society of America, pp. 31–73.

Satler, J.D., Starrett, J., Hayashi, C.Y., Hedin, M., 2011. Inferring species trees from gene trees in a radiation of California trapdoor spiders (Araneae, Antrodiaetidae, *Aliatypus*). PLoS ONE 6, e25355. http://dx.doi.org/10.1371/journal.pone.0025355.

Schierenbeck, K.A., 2014. Phylogeography of California: An Introduction. University of California Press.

Sharma, P.P., Giribet, G., 2014. A revised dated phylogeny of the arachnid order Opiliones. Front. Genet. 5. http://dx.doi.org/10.3389/fgene.2014.00255.

Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68, 978–989.

Ubick, D., Briggs, T.S., 1989. The harvestmen family Phalangodidae. 1. The new genus *Calicina*, with notes on *Sitalcina* (Opiliones: Laniatores). Proc. Calif. Acad. Sci. 46, 95–136.

Ubick, D., Briggs, T.S., 2008. The harvestman family Phalangodidae. 6: Revision of the *Sitalcina* complex (Opiliones: Laniatores). Proc. Calif. Acad. Sci. 59, 1–108.

Wake, D.B., 2006. Problems with species: patterns and processes of species formation in salamanders. Ann. Mo. Bot. Gard. 93, 8–23.

Wake, D.B., Maxson, L.R., Wurst, G.Z., 1978. Genetic differentiation, albumin evolution, and their biogeographic implications in plethodontid salamanders of California and southern Europe. Evolution, 529–539.

Yanev, K.P., 1980. Biogeography and distribution of three parapatric salamander species in coastal and borderland California. The California Islands: Proceedings of a Multidisciplinary Symposium. Santa Barbara Museum of Natural History, Santa Barbara, California, pp. 531–550.

Yanev, K.P., 1978. Evolutionary Studies of the Plethodontid Salamander Genus *Batrachoseps*. University of California, Berkeley.