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ORIGINAL ARTICLE

Cryptic diversity and biogeographical patterns within the black salamander (*Aneides flavipunctatus*) complex

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ABSTRACT

Aim Phylogeographical structure in the black salamander (*Aneides flavipunctatus*) was inferred using two independent genetic datasets. Concordance between the datasets was sought in order to evaluate earlier suggestions of species-level breaks and evidence of vicariance and long-term isolation within the complex. We hypothesized that major phylogeographical breaks would either correspond to current tectonic plate boundaries or to historical geological processes.

Location North-western California and southern Oregon (USA).

Methods Three mitochondrial DNA (mtDNA) genes were sequenced for 240 black salamanders from 136 localities, and up to 13 nuclear DNA loci were sequenced for 145 black salamanders representing 93 localities. Phylogenetic analysis of our mitochondrial dataset was performed to recover major lineages, while spatial clustering analysis of our nuclear dataset was utilized to identify points of concordance with our mtDNA phylogeny. Levels of gene flow were estimated for all contact zones.

Results Our mitochondrial phylogeny recovered four major lineages. Cluster analysis of our nuclear dataset is consistent with a four-population scheme, with the boundaries matching those of the mtDNA lineages. Gene flow across a contact zone in southern Humboldt between two of the populational units is extremely limited ($2Nm < 1$). In what is identified as the Central Core population, two distinctive subpopulations were delineated based on nuclear data, but mitochondrial data are discordant.

Main conclusions The *Aneides flavipunctatus* complex comprises at least four species-level units. Two of the boundaries between these units are associated with current tectonic plate boundaries. The contact zone between our Northwest and Central Core populations lies adjacent to the Mendocino Triple Junction (MTJ), where the Humboldt and North American plates meet, while the area separating the Santa Cruz and Central Core populations corresponds to the boundary between the Pacific and Humboldt tectonic plates. The phylogeographical break within the Central Core population lies in a region in which uplift occurred that is associated with the historical position of the migrating MTJ.

Keywords
Amphibians, California, Mendocino Triple Junction, mitochondrial DNA, nuclear loci, phylogeography, San Andreas Fault.

INTRODUCTION

The California Floristic Province is a biodiversity hotspot containing numerous endemic species and has been the focus of a large number of biogeographical studies (Myers *et al.*, 2000; Calsbeek *et al.*, 2003; Lapointe & Rissler, 2005; Rissler *et al.*, 2006). Some of the underlying causes of its high biological diversity and the complex biogeographical patterns are related to the geological history of the region and the resulting topographic and environmental heterogeneity.

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North-western California is exceptional because of a wide range of interconnected geological processes. Probably the most famous geological formation of the region is the San Andreas Fault (SAF), a north-west to south-east oriented slip-strike fault that marks the boundary between the North American and Pacific tectonic plates (Atwater, 1989). Another geological feature of north-western California is known as the Mendocino Triple Junction (MTJ), off the coast of southern Humboldt County, where three tectonic plates coincide and the SAF terminates as it joins the Mendocino Fracture Zone (McKenzie & Morgan, 1969; Furlong & Schwartz, 2004). To the north of the MTJ lies the Cascadia Subduction Zone, a region where sea floor spreading is forcing the Gorda and Juan de Fuca plates eastwards, where they subduct beneath the North American Plate (Atwater, 1989). While the SAF and the MTJ are two of the major drivers of the geological evolution of north-western California, few studies have directly linked these processes to lineage divergence or species boundaries of taxa in the region.

Even though the California Floristic Province has been well studied, cryptic species are still being described with the aid of multi-locus datasets and/or fine-scale sampling strategies (Jockusch *et al.*, 2012; Hedin *et al.*, 2013; Papenfuss & Parham, 2013). Organisms that are ecological specialists, or that have low vagility, are particularly good candidates for containing cryptic species because of their tendency to form genetically isolated populations, and they often display substantial phylogeographical structure that can be used to infer evolutionary history. Additionally, low-vagility organisms can elucidate historical geological processes because in some cases they are essentially 'riding' the tectonic plates. In this study we focus our attention on a low-vagility, ecologically specialized salamander that is endemic to the California Floristic Provence.

The black salamander, *Aneides flavipunctatus* (Strauch, 1870), is a terrestrial, direct-developing salamander that inhabits north-western California and extreme southern Oregon (Fig. 1). This species exhibits marked variation in colour pattern and microhabitat preference throughout its geographical range, which encompasses multiple forest types (Lowe, 1950; Lynch, 1981). The geographically isolated population from the Santa Cruz Mountains was originally described as a distinct subspecies (Myers & Maslin, 1948), and the other isolated population from Shasta County was originally described as a distinct species (Cope, 1883), both on the basis of their divergent ecological and morphological traits. In recent years these two isolated populations have been variously treated taxonomically (Collins & Taggart, 2002; Stebbins, 2003; Rissler & Apodaca, 2007). A previous range-wide mitochondrial study recovered four major lineages that were considered to warrant species status (Rissler & Apodaca, 2007). However, sampling was limited and may have been insufficient to recover all of the major clades, or to determine the location of the contact zones between them. Subsequent work identified contact zones between genetically distinct populations at the Willits/Longvale area of Mendocino County, with varying levels of gene flow between the three ecomorphs of the region (Reilly *et al.*, 2012). Additionally, a major genetic break was detected between salamanders in Shasta County and salamanders from the western Klamath Mountains, where gene flow is essentially absent between these populations (Reilly *et al.*, 2013).

Here we use fine-scale geographical sampling and a multilocus dataset to recover species-level genetic lineages within the black salamander species complex. We identify the geographical range of each lineage, localize contact zones between lineages, and estimate gene flow across contact zones. We hypothesize that (1) phylogeographical breaks will be associated with boundaries between tectonic plates, and/or (2) phylogeographical breaks will be associated with historical geological processes associated with the northward migration of the MTJ.

MATERIALS AND METHODS

Genetic sampling

Tissues were obtained from 240 individuals of *Aneides flavipunctatus* from 136 localities throughout north-western California and southern Oregon. DNA sequences used in previous molecular studies of *A. flavipunctatus* (Rissler & Apodaca, 2007; Reilly *et al.*, 2012, 2013) were downloaded from GenBank (accession numbers: AY274627–AY274756, JX544070–JX544733, KF056387–KF056791). Localities for all specimens used in genetic analysis are presented in Appendix S1 (Fig. S1) and Appendix S2 (Table S1) in the Supporting Information. DNA was extracted from tissues by either salt-extraction or using the DNeasy kit (Qiagen, Valencia, CA, USA).

We sequenced three mitochondrial loci, including portions of the *ND4*, cytochrome *b* (*cytb*) and *12S* genes. PCR primer information for all loci can be found in Appendix S2 (Table S2). PCR reactions were carried out using standard procedures before labelling with fluorescent-dye nucleotides by cycle sequencing reactions for both forward and reverse primers. Cycle sequencing products were cleaned by ethanol precipitation and sequenced using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA). Sequence reads were combined in CodonCode Aligner 3.5.2 (CodonCode Corporation, Dedham, MA, USA) and aligned using muscle (Edgar, 2004). Mitochondrial loci were concatenated and treated as a single locus for phylogenetic analyses. For rooting of phylogenetic trees, the *ND4*, *cytb* and *12S* genes were downloaded from GenBank for *Aneides hardii* (AY728226) and sequenced for *Aneides lugubris, Aneides ferreus* and *Aneides vagrans*.

We sequenced up to 13 independent nuclear loci from a total of 145 individuals representing 93 localities. These include 12 anonymous nuclear loci (Reilly *et al.*, 2012) and one intron locus (*POMC*). These markers were amplified and sequenced following the same protocol as described above for mitochondrial DNA (mtDNA) markers. We used

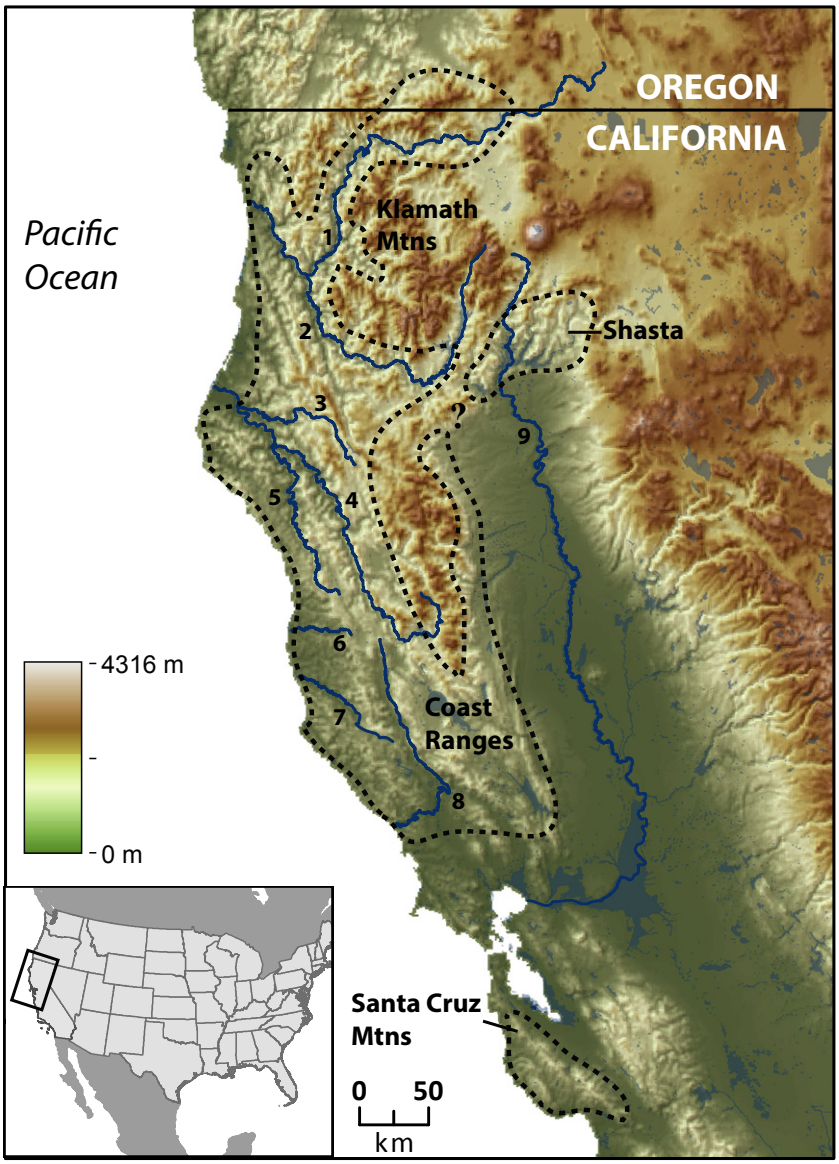

Figure 1 Topographical map of northwestern California with dashed line representing the estimated range limits of *Aneides flavipunctatus*. Numbers correspond to relevant rivers: 1, Klamath; 2, Trinity; 3, Van Duzen; 4, Eel; 5, South Fork Eel; 6, Noyo; 7, Navarro; 8, Russian; 9, Sacramento.

the software program PHASE 2.1 (Stephens *et al.*, 2001; Stephens & Scheet, 2005) to obtain haplotype sequences for each PCR product using the default threshold of 0.9. The software DNASP (Rozas *et al.*, 2003) was used to calculate locus-specific summary statistics such as the number of haplotypes, number of parsimony informative sites, haplotype diversity, nucleotide diversity and Tajima's *D* (Table 1).

MtDNA data analyses

We used both maximum likelihood and Bayesian inference to reconstruct mitochondrial gene trees using the software programs GARLI (Zwickl, 2006) and BEAST 1.8 (Drummond & Rambaut, 2007). Our GARLI run was carried out using the default parameters, and the tree with the highest likelihood served as a guide tree for bootstrapping analyses assessed by 500 bootstrap replicates. The BEAST analysis was run with a GTR+I+G model of sequence evolution as determined by JMODELTEST2 (Darriba *et al.*, 2012). We implemented a strict clock, which is appropriate for shallow phylogenies because of low levels of rate variation between branches (Brown & Yang, 2011). A calibration of 0.8% lineage divergence per million years, estimated for the *cytb* gene in another salamander (Tan & Wake, 1995), was applied to obtain approximate divergence times for nodes. A coalescent tree prior under a constant population size model was implemented because we were examining an intraspecific dataset. Two separate runs of 20 million generations were carried out, sampling every 1000 generations for a total of 20,000 saved trees per run. After removing 10,000 trees from each run the two runs were combined to produce a maximum clade credibility tree. Convergence was assessed by confirming that all effective sample size (ESS) values were greater than 200 using TRACER 1.5 (Rambaut & Drummond, 2009). DNASP (Rozas *et al.*, 2003) was used to calculate population genetic parameters such as haplotype diversity, nucleotide

Table 1 Summary statistics for the 16 loci used in this study of *Aneides flavipunctatus*.

Locus	bp	*n*	total bp	*h*	PS	PIS	hD (+SD)	π	Tajima's *D*
ND4	735	234	171,990	118	195	170	0.985 (0.003)	0.0594	−0.315 ($P > 0.10$)
cytb	385	184	70,840	83	71	61	0.974 (0.005)	0.0496	−0.247 ($P > 0.10$)
12S	627	230	144,210	83	91	63	0.971 (0.005)	0.0324	−0.609 ($P > 0.10$)
POMC	481	294	141,414	36	30	19	0.780 (0.019)	0.0026	−2.051 ($P < 0.05$)
SR1	477	255	121,635	17	18	5	0.577 (0.033)	0.0020	−1.956 ($P < 0.05$)
SR2	357	116	41,412	27	36	26	0.885 (0.022)	0.0216	−0.388 ($P > 0.10$)
SR4	271	159	43,089	23	20	16	0.872 (0.014)	0.0112	−0.917 ($P > 0.10$)
SR7	565	213	120,345	50	55	31	0.891 (0.014)	0.0055	−2.254 ($P < 0.01$)
SR8	640	238	152,320	60	53	27	0.934 (0.008)	0.0067	−1.710 ($0.10 > P > 0.05$)
SR9	185	188	34,780	13	32	30	0.499 (0.043)	0.0402	−0.830 ($P > 0.10$)
SR12	434	222	96,348	36	31	18	0.910 (0.009)	0.0083	−1.425 ($P > 0.10$)
SR15	525	157	82,425	40	30	25	0.928 (0.013)	0.0100	−0.788 ($P > 0.10$)
SR16	273	175	47,775	20	18	12	0.645 (0.038)	0.0057	−1.914 ($P < 0.05$)
SR17	455	205	93,275	60	38	25	0.933 (0.011)	0.0075	−1.673 ($0.10 > P > 0.05$)
SR19	631	270	170,370	46	49	26	0.782 (0.021)	0.0034	−2.302 ($P < 0.01$)
SR20	553	241	133,273	28	25	13	0.731 (0.020)	0.0037	−1.957 ($P < 0.05$)

bp, length of locus in bases; *n*, number of sequences; *h*, number of haplotypes; PS, polymorphic sites; PIS, parsimony informative sites; hD, haplotype diversity; π, nucleotide diversity.

diversity, Tajima's *D* and Fu's F_S within mtDNA lineages (Table 2), and percentage uncorrected sequence divergence and F_{ST} between mtDNA lineages (Table 3).

Population structure

To gain insight into the genetic population structure within *A. flavipunctatus* we examined our nuclear dataset using Geneland (Guillot *et al.*, 2005, 2008; Guedj & Guillot, 2011). This spatial clustering program estimates the number of panmictic groups and locates the geographical boundaries between them by assuming that each putative group is at Hardy–Weinberg equilibrium with linkage equilibrium between loci. Geospatial UTM coordinates were uploaded to be associated with each sample. We set the number of populations to a range from $K = 1$ to $K = 15$ and ran the program for 250,000 iterations, sampling every 100 iterations for a total of 2500 saved iterations per population scheme. This methodology was repeated with 10 replicates. After the first 500 iterations were removed as burn-in from each run, the most probable number of populations was determined and plotted on a map containing posterior probability values for the assignment of each individual to population. We used this method to objectively discover genetically distinct populations, or species, and then determine if these populations were recovered as monophyletic groups in our mtDNA phylogeny. This methodology was repeated on a smaller scale for one recovered population that exhibited substantial genetic variation.

Species tree analysis

The software package *beast (Heled & Drummond, 2010) was used to infer the relationships of our major mtDNA lineages using our nuclear loci. This program models incomplete lineage sorting and intraspecies polymorphism to estimate a phylogeny. For this analysis we treated each major mtDNA lineage as a 'species' and included 2–4 individuals (4–8 haplotypes per gene) for each species. We estimated the phylogeny using both nuclear data only, and nuclear plus mitochondrial data. For each dataset we determined the best model of sequence evolution for each locus using jModel Test2 (Darriba *et al.*, 2012), and made two runs of 100 million generations using different starting seeds, sampling trees every 10,000 generations for a total of 10,000 saved trees per run. A lognormal relaxed clock model with a normal prior distribution and a Yule Process species tree prior was used. After removing the first 1000 trees from each run we combined the remaining 18,000 trees from the two runs to create a maximum clade credibility tree.

Table 2 Population-specific mitochondrial summary statistics for *Aneides flavipunctatus* from north-western California and southern Oregon (USA).

Population	*n*	hD (+ SD)	π	Tajima's *D*	Fu's F_S
Santa Cruz	14	0.803 (0.096)	0.0050	−0.846 ($P > 0.10$)	1.057
Shasta	10	0.893 (0.111)	0.0098	−0.722 ($P > 0.10$)	1.120
Northwest	55	0.891 (0.034)	0.0118	−1.097 ($P > 0.10$)	1.693
Central Core	161	0.988 (0.003)	0.0340	−0.910 ($P > 0.10$)	−31.289

n, number of sequences; hD = haplotype diversity; π = nucleotide diversity.

Table 3 Genetic divergence between mitochondrial lineages of *Aneides flavipunctatus* from north-western California and southern Oregon. The lower diagonal is the percentage uncorrected sequence divergence among lineages, and the upper diagonal is F_{ST} among lineages.

	Santa Cruz	Shasta	Northwest	Central Core
Santa Cruz	–	0.887	0.884	0.623
Shasta	6.8	–	0.729	0.555
Northwest	6.2	4.0	–	0.580
Central Core	5.8	4.7	5.4	–

Contact zone analysis

We estimated gene flow for all contact zones found between parapatric lineages by analysing our 13 nuclear loci under an isolation-with-migration model as implemented in the software IMa2 (Hey, 2010). The software program IMgc (Woerner *et al.*, 2007) was used to identify the largest non-recombining portion of each nuclear locus, which were subsequently used in our IMa2 analyses. After appropriate prior boundaries were determined from trial runs (q = 8, t = 6, m = 15; where q = population size, t = divergence time, and m = migration rate), a final run was performed consisting of a 5 million step burn-in followed by a 10 million step run sampling every 100 steps for a total of 100,000 saved geneologies. Gene flow was assessed using the product of the population size parameter estimate and half of the migration estimate to produce population migration rate ($2Nm$) values (Hey, 2005).

RESULTS

Data characteristics

A total of 1749 bp of mitochondrial DNA was collected from the *ND4*, *cytb* and *12S* genes, which contained a total of 294 parsimony informative sites (Table 1). Our nuclear dataset consisted of 13 independent loci with an average length of 450 bp, consisting of *c.* 2700 haplotypes for a total of over 1.26 million bp of nuclear data (Table 1). Our nuclear dataset contained a total of 273 parsimony informative sites, with an average nucleotide diversity value of 0.01 (Table 1). All new sequences greater than 200 bp in length analysed in this study have been deposited in GenBank (KM197559–KM197987, KM209477–KM209872). Data coverage for each sample is presented in Appendix S2 (Table S3).

MtDNA phylogeny

Both the maximum likelihood and Bayesian methods produced similar gene tree topologies that mostly agree with the results of the previous mtDNA study by Rissler & Apodaca (2007). Detailed tree figures can be found in Appendix S1. The trees, rooted with outgroups, recover a clade from the Santa Cruz Mountains ('Santa Cruz') to be sister to the rest of the species complex; it is estimated to have diverged from other *A. flavipunctatus* approximately 6–7.5 Ma (Fig. 2a). Within the remaining group is a northern clade consisting of two sister clades, corresponding to Rissler & Apodaca's (2007) 'Northwest' and 'Shasta' lineages, and a central clade ('Central Core'). The Northwest and Central Core clades have a sharply defined contact zone that lies in southern Humboldt County (Fig. 2b). The Central Core contains two sister clades (clades 1 and 2) that do not overlap geographically, yet appear to come into contact in multiple regions, including a well-defined contact zone along the Navarro River in central Mendocino County (Fig. 3a). However, these two mtDNA clades within the Central Core do not correspond generally with the two populations defined by Geneland (see below). The Central Core is composed of 11 well-supported, geographically structured clades that are at least 2 million years old (Fig. 3a), and four of the clades within the Central Core's clade 1 (MFER, SFER, CM and IM) were previously identified (Reilly *et al.*, 2012).

The highest values of haplotype diversity and nucleotide diversity belong to the Central Core, while the lowest values are found in Santa Cruz (Table 2). The uncorrected sequence divergence between mtDNA lineages ranges between 4% and 6.8%, with Santa Cruz and Shasta being the most divergent from each other and Northwest and Shasta being the least divergent (Table 3).

Population structure

The Geneland analysis, after burn-in, supported recognition of four populations with strong support; these correspond to our mtDNA lineages (Fig. 2c–f). These include two disjunct populations, one in the Santa Cruz Mountains and another in Shasta County, and two parapatric populations that meet each other in the main portion of the range in southern Humboldt County. While the mtDNA lineages meet at a sharply defined zone, our Geneland results identified a handful of samples along the Humboldt contact zone as hybrids. Hybrid salamanders were detected at the Cape Mendocino region (Af36), the region at the confluence of the Van Duzen and Eel rivers (Af37, Af62, SBR94-5), Ruth Lake (Af115), and Larabee Creek (Af58). From our sampling localities we estimate the width of the contact zone to be roughly 10–20 km wide.

Although our range-wide Geneland results did not detect breaks within the Central Core we chose to analyse this portion of the main range in a separate analysis. The results show strong support for two populations within the Central Core, with the contact zone between them located at the Noyo River area in coastal Mendocino County extending eastward to the Willits/Longvale area in eastern Mendocino County (Fig. 3b–c). This result is not concordant with the mtDNA results, which show a major break located approximately 25–30 km to the south at the Navarro River (Fig. 3a). The distance between the major mtDNA and nuclear DNA

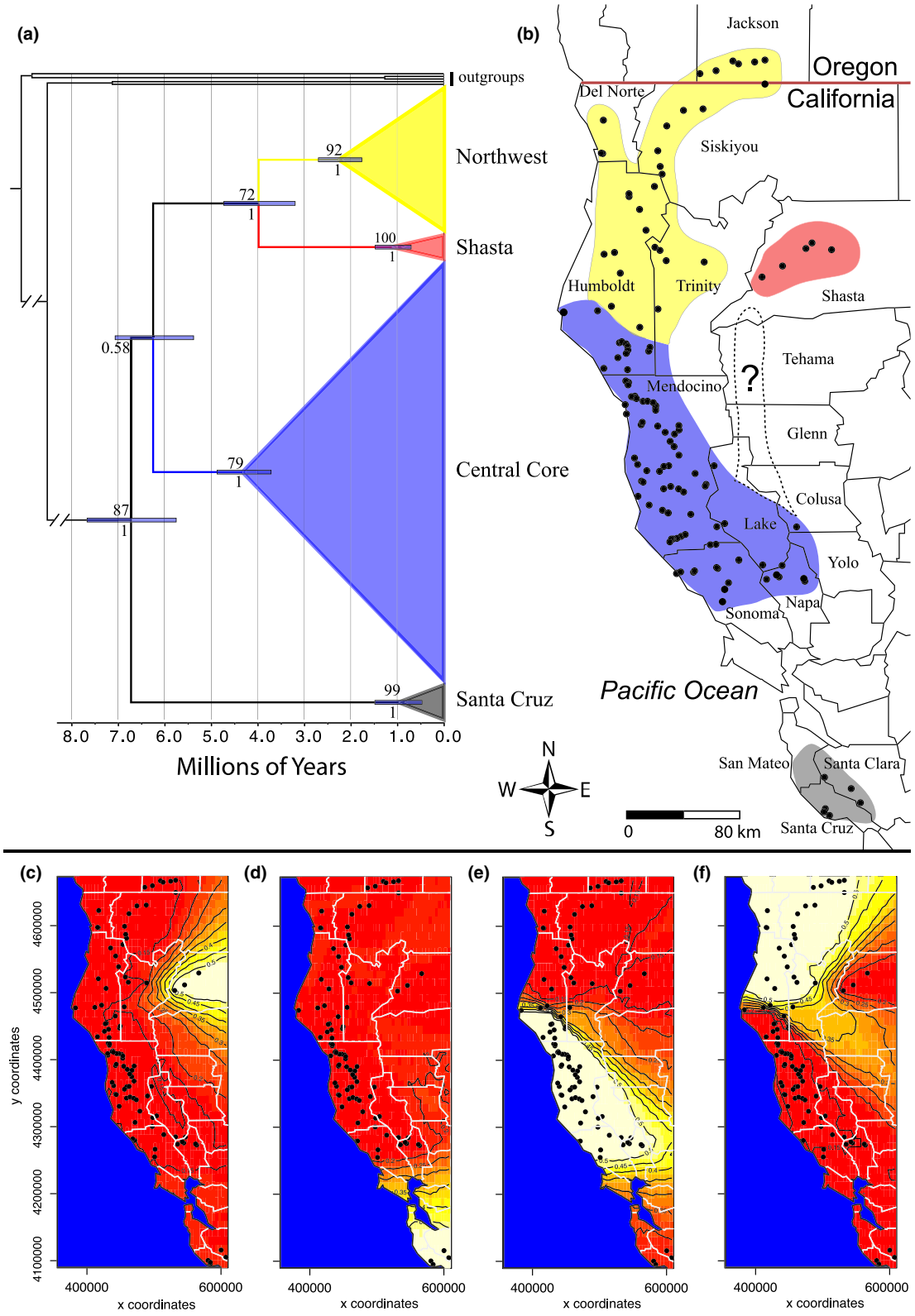

Figure 2 (a) Time-calibrated mtDNA phylogeny for *Aneides flavipunctatus* from north-western California and southern Oregon. Node bars represent 95% confidence intervals. Numbers at nodes represent bootstrap support (above) and posterior probability values (below). (b) Map of localities. Black lines = county lines; black dots = sample localities; dashed line = unsampled area. (c–f) The four populations delimited by Geneland using nuclear data. Black dots = sample localities; white lines = county borders; blue area = Pacific Ocean. Lighter colours correspond to a higher posterior probability value of belonging to a particular population. Maps correspond to the (c) Shasta, (d) Santa Cruz, (e) Central Core, and (f) Northwest populations.

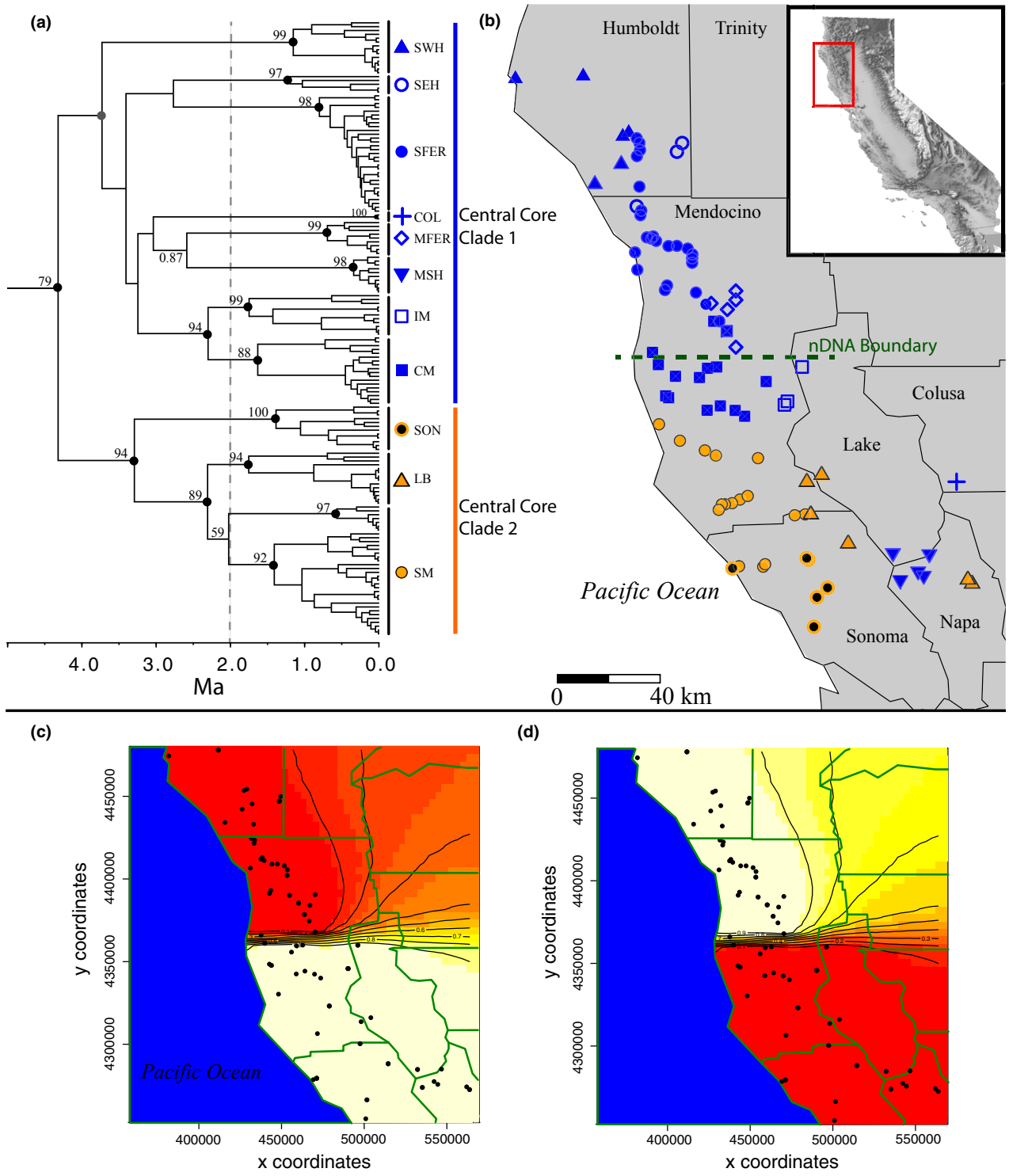

Figure 3 (a) Mitochondrial clades of *Aneides flavipunctatus* within the Central Core. Black circles at nodes represent posterior probability value of 1, and grey circles represent a posterior probability value of > 0.9. Bootstrap support is shown above nodes. SWH, Southwest Humboldt; SHE, Southeast Humboldt; SFER, South Fork Eel River; COL, Colusa; MFER, Main Fork Eel River; MSH, Mount Saint Helena; IM, Inland Mendocino; CM, Central Mendocino; SON, Sonoma; LB, Lake Berryessa; SM, South Mendocino. (b) Map of clade localities. (c–d) GENELAND results for two populations within the Central Core. Lighter colours correspond to a higher posterior probability value of belonging to a particular population. Black dots = sample localities; green lines = county borders; blue area = Pacific Ocean.

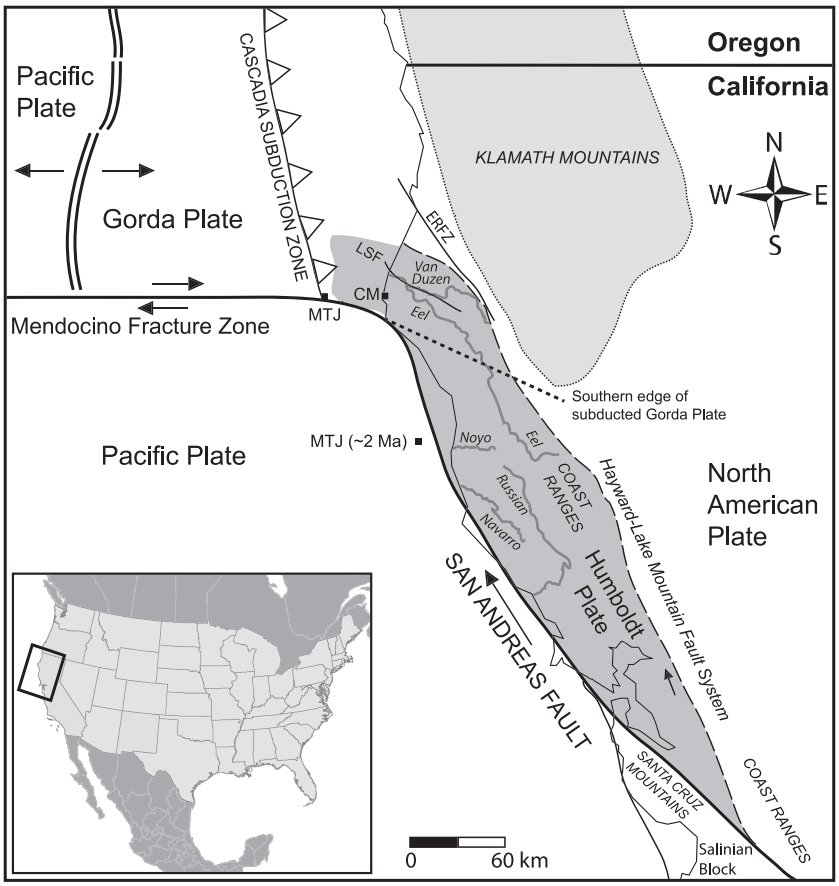

Figure 4 Map of relevant geological and tectonic features of north-western California (Herd, 1978; Kelsey & Carver, 1988; Atwater, 1989; Lock *et al.*, 2006). Light grey lines represent rivers and italic text gives river names. CM, Cape Mendocino; MTJ, Mendocino Triple Junction; LSF, Little Salmon Fault; ERFZ, Eaton Roughs Fault Zone.

(nDNA) contact zones is equal to roughly 10% of the length of the Central Core's range.

Species tree analysis

Our species tree analyses produced similar tree topologies for our nDNA and mtDNA+nDNA datasets (Appendix S1). The main difference is that the mtDNA+nDNA tree finds Santa Cruz to be sister to the rest of the complex while the nDNA tree finds Shasta to be sister to the rest of the complex. In both trees the Northwest and Central Core populations form monophyletic groups that are sister to each other. Neither tree finds a sister relationship between Shasta and Northwest, which is in disagreement with the mtDNA phylogeny. The support values on both trees are low, suggesting that there are conflicting signals among the loci.

Southern Humboldt contact zone

The extent of the contact zone between Northwest and Central Core is narrow, as visualized in the spatial clustering analysis of the nuclear loci (Fig. 2e–f) and the mtDNA lineage boundaries (Fig. 2b). Coalescent analysis of our nuclear loci under an isolation-with-migration model detected low levels of gene flow ($2Nm < 1$) in both directions across the southern Humboldt contact zone. Our estimates show approximately twice as much gene flow from the Central Core into the Northwest [$2Nm = 0.53$; 95% confidence interval (CI) low = 0.10, 95% CI high = 3.05] as in the opposite direction ($2Nm = 0.25$; 95% CI low = 0.03, 95% high = 3.52).

DISCUSSION

The range of *Aneides flavipunctatus* spans multiple tectonic plates (see Fig. 4): (1) the Klamath Mountains, which lie on the North American Plate; (2) the Coast Ranges north of San Francisco Bay, which lie on the Humboldt Plate (also known as the Humboldt Deformation Zone); and (3) the Santa Cruz Mountains, which lie on the Salinian Block of the Pacific Plate (Herd, 1978; Kelsey & Carver, 1988). We have documented four species-level genetic groups within the *A. flavipunctatus* complex: Shasta from Shasta County (North American Plate); Northwest from the western Klamath Mountains (North American Plate); Central Core from the Coast Ranges north of San Francisco Bay (Humboldt Plate); and Santa Cruz from the Santa Cruz Mountains (Pacific Plate). Two of the boundaries between these groups are associated with tectonic plate boundaries.

Shasta and Northwest

The Klamath Mountains are the oldest geological formation within the range of the black salamander and have been relatively stable throughout the Pliocene and Pleistocene

(Harper, 1984). While both the Northwest and Shasta lineages occupy the Klamath Mountains, they are separated by a high elevation mountain ridge that roughly marks the boundary between the older Eastern Klamath Belt (occupied by Shasta) and the rest of the Klamath Mountains to the west, which are younger (Harper, 1984). The geological processes that formed the diverse topography of this region, and in general the geological components, are much older than the estimated divergence time of 4 Ma we have estimated for the Northwest and Shasta populations. A previous study found that *A. flavipunctatus* avoids elevations above 600 m (Lynch, 1981), and the ridge of the Trinity Mountains is over 1000 m in elevation along its entire length. Accordingly, we hypothesize that isolation of these two populations is due to the high elevation Trinity Mountains acting as a barrier to dispersal.

Santa Cruz and Central Core

The Santa Cruz population is isolated from the rest of the range of *A. flavipunctatus* in the Santa Cruz Mountains, which arose from a compressive uplift of the Salinian Block, resulting from a westward bend of the SAF. The mountains formed by the early Pleistocene, at which point they were physically connected with the Coast Ranges to the north in Marin and Sonoma Counties (Dupré *et al.*, 1991). The mountains were not separated from Marin until approximately 600,000 years ago, when the central valley began draining into the San Francisco Bay area (Sarna-Wojcicki *et al.*, 1985; Dupré *et al.*, 1991). While the estimated time of divergence from the rest of the complex of 6–7.5 Ma is much older than the time of physical isolation of the Santa Cruz Mountains (and older than the mountains themselves), we hypothesize that extinction of intermediate populations from Marin and southern Sonoma Counties contributed to the high levels of genetic divergence between the Santa Cruz and Central Core populations. The Santa Cruz Mountains are the northernmost substantial landmass lying on the Pacific Plate so it is not surprising that many other amphibian taxa also exhibit a strong phylogeographical break between populations in the Santa Cruz Mountains and populations north of San Francisco Bay (for a summary see Reilly *et al.*, 2014).

The southern Humboldt contact zone

The Central Core and Northwest populations come into contact in southern Humboldt County between the Van Duzen River and the Eel River. We perceive no existing physical barrier to dispersal that would account for the location of this contact zone; there are no high elevation mountains, and rivers much larger than the Van Duzen (e.g. the Klamath River) are not associated with barriers in the rest of the range. We hypothesize that these lineages were physically isolated for a substantial amount of time, and that they have come into secondary contact. This region of southern Humboldt is known to harbour a major genetic break within the related *Ensatina* complex (Kuchta *et al.*, 2009). Other taxa that exhibit north/south genetic breaks near the Van Duzen River include species of California turret spider (Starrett & Hedin, 2007), and chipmunks of the genus *Tamias* (Sutton & Nadler, 1974). This region also marks the northern range limit of the salamanders *Aneides lugubris*, *Taricha rivularis* and *Taricha torosa*, and the southern range limit of *Plethodon elongatus* (Stebbins, 2003).

The area of contact between Central Core and Northwest populations, known as the 'Humboldt Basin', lies adjacent to the MTJ where the North American, Pacific and Gorda tectonic plates meet (Furlong & Schwartz, 2004; Harden, 2004). The Humboldt Basin is one of the most seismically active regions of California and many of the folds and faults associated with the triple junction extend into southern Humboldt County, where lateral and compressional tectonic processes are active (Furlong & Schwartz, 2004; Harden, 2004). This area is also a transition zone between the younger, more geologically active Coast Ranges, which lie on the Humboldt Plate, and the older more stable Klamath Mountains, which lie on the North American Plate (Fig. 4). The Humboldt Plate is moving northwards with respect to the North American Plate, and in southern Humboldt County it takes a sharp westward turn where it starts to migrate into the Pacific Ocean. The presence of a major genetic break in this region is not surprising, but determining which of the regional processes is responsible is more difficult because there are so many possibilities. The southern edge of the subducted Gorda Plate, the Little Salmon Fault, the Eaton Roughs Fault, and many other faults all lay in this region (Fig. 4). Additionally, the region between the Van Duzen and Eel rivers corresponds to an area of major uplift associated with the MTJ (Lock *et al.*, 2006). But given that southern Humboldt County has been formed recently, this contact zone may be the byproduct of a stable mountain range coming into contact with a young, highly dynamic and continually evolving mountain range.

Diversity within the Central Core

The Central Core occupies the most geologically active and complex region within the range of *A. flavipunctatus*. Slip-strike faults (the Hayward-Lake Mountain Fault system) run parallel to the SAF more than 70 km inland from the fault and the MTJ, and isolate a north-west elongate sliver of the North American continent between it and the SAF. Herd (1978) describes the existence of a microplate termed the 'Humboldt Plate' that moves partly with the North American Plate and partly with the Pacific Plate, and is in the process of being transferred from the North American Plate to the Pacific Plate (Herd, 1978; Kelsey & Carver, 1988). While it is not clear how the complicated genetic patterns within the Central Core arose, examination of the geological history of the Humboldt Plate can help us understand the Central Core's evolutionary history.

Around 6 Ma the palaeo-coastline of north-western California existed along what are now the main stems of the Eel

and Russian rivers, nearly 50 km inland of its present location. The coastline started to expand westwards as the MTJ moved north, causing uplift on its eastern side and forming the Coast Ranges in the process. At 4 Ma the coastline of Sonoma and southern Mendocino counties had extended much further west than the coastline to the north. By 2 Ma the coast of north-western California was nearly at its present-day extent, and a period of extensive uplift of the Coast Ranges occurred between the Eel and Russian rivers (Lock *et al.*, 2006). Before 2 Ma the majority of the coast range drained south to the mouth of the Russian River, but the uplift of central Mendocino caused the drainage direction to reverse, and now the majority of the Coast Ranges drain north into the Eel (Lock *et al.*, 2006). This region of peak uplift between the Eel and Russian rivers is likely to be responsible for the nuclear genetic break observed in the Willits/Longvale region of Mendocino County, which lies on the uplifted coastal plateau separating the Eel and Russian river watersheds (Fig. 3b–c).

Given the number of geographically structured mtDNA clades of a similar age within the Central Core, fragmentation of the range was likely to have been nearly simultaneous (see Larson, 1980). Once these isolates came into secondary contact, gene exchange is likely to have resumed, uniting gene pools in some regions and creating two separate nuclear gene pools that contact each other in the Willits/Longvale area. While patterns of mtDNA and nDNA are discordant within the Central Core, it remains unclear whether genetic differentiation within the Central Core warrants recognition of multiple species and, if so, where those boundaries lie. This uncertainty is apparent when considering the offset mtDNA boundary and the pattern of unidirectional northward gene flow across the Willits/Longvale contact zone (Reilly *et al.*, 2012). A mitochondrial boundary with a divergence time of *c.* 3.5 Ma is associated with the Willits/Longvale area (the focus of Reilly *et al.*, 2012) within the Central Core's Clade 1, but this study uncovered a more substantial mtDNA break further to the south at the Navarro River.

Hypotheses for the discordance of mtDNA and nDNA boundaries include demographic variation between populations (Currat *et al.*, 2008), adaptive introgression of mtDNA across nDNA boundaries (Irwin, 2012), hybrid zone migration (Rohwer *et al.*, 2001), and sex-biased hybridization or dispersal (Rheindt & Edwards, 2011). If the major mtDNA and nDNA breaks within the Central Core are a result of the same historical process, we hypothesize that the discordance is due to the non-adaptive introgression of nDNA genes across stable mtDNA boundaries. Male-biased dispersal has been shown to be common in other plethodontid salamanders (Staub *et al.*, 1995; Liebgold *et al.*, 2010), and female philopatry would be expected to produce stable mitochondrial boundaries. Thus, the mtDNA lineages and boundaries would represent the historical distribution of these salamanders before they came into secondary contact, and nuclear genes would cross contact zones through male-biased dispersal (see García-París *et al.*, 2003). This hypothesis is supported by the following observations: (1) northward gene flow is detected in central Mendocino Co. (Reilly *et al.*, 2012); (2) the nDNA boundary is north of the mtDNA boundary; and (3) the nDNA boundary is concordant with major morphological boundaries (see Reilly *et al.*, 2012).

CONCLUSIONS

Analyses of both mtDNA and nDNA datasets indicate that there are four species-level units within the black salamander species complex: one in the Santa Cruz Mountains, one in Shasta County and two within the main range, which are parapatrically distributed and meet in southern Humboldt County. The southern Humboldt contact zone lies adjacent to the current position of the Mendocino Triple Junction, which is one of the most geologically active regions of California and marks a transition between the newly formed Coast Ranges and the geologically stable Klamath Mountains. Gene flow across the southern Humboldt contact zone is low ($2Nm < 1$) in both directions, suggesting genetic isolation of the two main range species. One of the units identified, the Central Core, contains substantial genetic diversity and phylogeographical structure. Although discordant with mtDNA, nDNA data suggest that the Central Core contains two near-species assemblages of populations that meet in central Mendocino County, an area that experienced extensive uplift approximately 2 Ma when the MTJ was positioned to the west. Formal taxonomic treatment of the entire complex, along with detailed examination of morphological and ecological patterns, will be presented elsewhere.

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REFERENCES

Atwater, T. (1989) Plate tectonic history of the northeast Pacific and western North America. *The geology of North America,* Vol. N, *The eastern Pacific Ocean and Hawaii* (ed. by E.L. Winterer, D.M. Hussong and R.W. Decker), pp. 21–72. Geological Society of America, Boulder, CO.

Brown, R.P. & Yang, Z. (2011) Rate variation and estimation of divergence times using strict and relaxed clocks. *BMC Evolutionary Biology*, **11**, 271.

Calsbeek, R., Thompson, J.N. & Richardson, J.E. (2003) Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. *Molecular Ecology*, **12**, 1021–1029.

Collins, J.T. & Taggart, T.W. (2002) *Standard common and current scientific names for North American amphibians, turtles, reptiles & crocodilians*. Center for North American Herpetology, Lawrence, KS.

Cope, E.D. (1883) Notes on the geographical distribution of Batrachia and Reptilia in western North America. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **35**, 10–35.

Currat, M., Ruedi, M., Petit, R.J. & Excoffier, L. (2008) The hidden side of invasions: massive introgression by local genes. *Evolution*, **62**, 1908–1920.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 722.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Dupré, W.R., Morrison, R.B., Clifton, H.E., Lajoie, K.R., Ponti, D.J., Powell, C.L., II, Mathieson, S.A., Sarna-Wojcicki, A.M., Leithold, E.L., Lettis, W.R., McDowell, P.F., Rockwell, T.K., Unruh, J.R. & Yeats, R.S. (1991) Quaternary geology of the Pacific margin. *The geology of North America,* Vol. K-2, *Quaternary nonglacial geology: conterminous U.S.* (ed. by R.B. Morrison), pp. 141–214. Geological Society of America, Boulder, CO.

Edgar, R.C. (2004) Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797.

Furlong, K.P. & Schwartz, S.Y. (2004) Influence of the Mendocino triple junction on the tectonics of coastal California. *Annual Review of Earth and Planetary Sciences*, **32**, 403–433.

García-París, M., Alcobendas, M., Buckley, D. & Wake, D.B. (2003) Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. *Evolution*, **57**, 129–143.

Guedj, B. & Guillot, G. (2011) Estimating the location and shape of hybrid zones. *Molecular Ecology Resources*, **11**, 1119–1123.

Guillot, G., Estoup, A., Mortier, F. & Cosson, J.F. (2005) A spatial statistical model for landscape genetics. *Genetics*, **170**, 1261–1280.

Guillot, G., Santos, F. & Estoup, A. (2008) Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics*, **24**, 1406–1407.

Harden, D.R. (2004) *California geology*, 2nd edn. Prentice Hall, Upper Saddle River, NJ.

Harper, G.D. (1984) Middle to Late Jurassic tectonic evolution of the Klamath Mountains, California-Oregon. *Tectonics*, **3**, 759–772.

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. *Molecular Ecology*, **22**, 508–526.

Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.

Herd, D.G. (1978) Intercontinental plate boundary east of Cape Mendocino, California. *Geology*, **6**, 721–725.

Hey, J. (2005) On the number of New World founders: a population genetic portrait of the peopling of the Americas. *PLoS Biology*, **3**, 965–975.

Hey, J. (2010) Isolation with migration models for more than two populations. *Molecular Biology and Evolution*, **27**, 905–920.

Irwin, D.E. (2012) Local adaptation along smooth ecological gradients causes phylogeographic breaks and phenotypic clustering. *The American Naturalist*, **180**, 35–49.

Jockusch, E.L., Martínez-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.

Kelsey, H.M. & Carver, G.A. (1988) Late Neogene and Quaternary tectonics associated with northward growth of the San Andreas transform fault, northern California. *Journal of Geophysical Research*, **93**, 4797–4819.

Kuchta, S.R., Parks, D.S., Mueller, R.L. & Wake, D.B. (2009) Closing the ring: historical biogeography of the salamander ring species *Ensatina eschscholtzii*. *Journal of Biogeography*, **36**, 982–995.

Lapointe, F.J. & Rissler, L.J. (2005) Congruence, consensus, and the comparative phylogeography of codistributed species in California. *The American Naturalist*, **166**, 290–299.

Larson, A. (1980) Paedomorphosis in relation to rates of morphological and molecular evolution in the salamander *Aneides flavipunctatus*. *Evolution*, **34**, 1–17.

Liebgold, E.B., Brodie, E.D., III & Cabe, P.R. (2010) Female philopatry and male-biased dispersal in a direct-developing salamander, *Plethodon cinereus*. *Molecular Ecology*, **20**, 249–257.

Lock, J., Kelsey, H., Furlong, K. & Woolace, A. (2006) Late Neogene and Quaternary landscape evolution of the northern California Coast Ranges: evidence for Mendocino triple junction tectonics. *Geographical Society of America Bulletin*, **118**, 1232–1246.

Lowe, C.H. (1950) Speciation and ecology in salamanders of the genus *Aneides*. PhD Thesis, University of California, Los Angeles, CA.

Lynch, J.F. (1981) Patterns of ontogenetic and geographic variation in the black salamander, *Aneides flavipunctatus*. *Smithsonian Contributions to Zoology*, **324**, 1–53.

McKenzie, D.P. & Morgan, W.J. (1969) The evolution of triple junctions. *Nature*, **224**, 125–133.

Myers, G.S. & Maslin, T.P. (1948) The California plethodont salamander, *Aneides flavipunctatus* (Strauch): with description of a new subspecies and notes of other western *Aneides*. *Biological Society of Washington*, **61**, 127–128.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Papenfuss, T.J. & Parham, J.F. (2013) Four new species of California legless lizards (*Aniella*). *Breviora*, **536**, 1–17.

Rambaut, A. & Drummond, A.J. (2009) *Tracer v1.5.0.* Available at: http://beast.bio.ed.ac.uk/ (accessed on 23 May 2013).

Reilly, S.B., Marks, S.B. & Jennings, W.B. (2012) Defining evolutionary boundaries across parapatric ecomorphs of Black Salamanders (*Aneides flavipunctatus*) with conservation implications. *Molecular Ecology*, **21**, 5745–5761.

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.

Reilly, S.B., Portik, D.M., Koo, M.S. & Wake, D.B. (2014) Discovery of a new, disjunct population of a narrowly distributed salamander (*Taricha rivularis*) in California presents conservation challenges. *Journal of Herpetology*, **48**, 371–379.

Rheindt, F.E. & Edwards, S.V. (2011) Genetic introgression: an integral but neglected component of speciation in birds. *The Auk*, **128**, 620–632.

Rissler, L.J. & Apodaca, J.J. (2007) Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the Black Salamander (*Aneides flavipunctatus*). *Systematic Biology*, **56**, 924–942.

Rissler, L.J., Hijmans, R.J., Graham, C.H., Moritz, C. & Wake, D.B. (2006) Phylogeographic lineages and species comparisons in conservation analyses: a case study of California herpetofauna. *The American Naturalist*, **167**, 655–666.

Rohwer, S., Bermingham, E. & Wood, C. (2001) Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution*, **55**, 405–422.

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X. & Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Sarna-Wojcicki, A.M., Meyer, C.E., Bowman, H.R., Hall, N.T., Russell, P.C., Woodward, M.J. & Slate, J.L. (1985) Correlation of the Rockland ash bed, a 400,000-year-old stratigraphic marker in northern California and western Nevada and implications for middle Pleistocene paleogeography of central California. *Quaternary Research*, **23**, 236–257.

Starrett, J. & Hedin, M. (2007) Multilocus geneologies reveal multiple cryptic species and biogeographical complexity in the California turret spider *Antrodiaetus riversi* (Mygalomorphae, Antrodiaetidae). *Molecular Ecology*, **16**, 583–604.

Staub, N.L., Brown, C.W. & Wake, D.B. (1995) Patterns of growth and movements in a population of *Ensatina eschscholtzii platensis* (Caudata: Plethodontidae) in the Sierra Nevada, California. *Journal of Herpetology*, **29**, 593–599.

Stebbins, R.C. (2003) *Western reptiles and amphibians*, 3rd edn. Peterson Field Guide Series, New York, NY.

Stephens, M. & Scheet, N.J. (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data inputation. *American Journal of Human Genetics*, **76**, 449–462.

Stephens, M., Smith, N.J. & Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, **68**, 978–989.

Sutton, D.A. & Nadler, C.F. (1974) Systematic revision of three townsend chipmunks (*Eutamias townsendii*). *The Southwestern Naturalist*, **19**, 199–212.

Tan, A.M. & Wake, D.B. (1995) MtDNA phylogeography of the California newt, *Taricha torosa* (Caudata, Salamandridae). *Molecular Phylogenetics and Evolution*, **4**, 383–394.

Woerner, A.E., Cox, M.P. & Hammer, M.F. (2007) Recombination-filtered genomic datasets by information maximization. *Bioinformatics*, **23**, 1851–1853.

Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Thesis, The University of Texas, Austin, TX.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Map of sample localities (Fig. S1), detailed tree figures with sample labels (Figs S2–S5), and species trees (Fig. S6).

Appendix S2 Museum voucher and sampling locality information (Table S1), primer information (Table S2), and data coverage (Table S3).

BIOSKETCHES

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