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RESEARCH ARTICLE

Rangewide phylogeography and landscape genetics of the Western U.S. endemic frog *Rana boylii* (Ranidae): implications for the conservation of frogs and rivers

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Abstract Genetic data are increasingly being used in conservation planning for declining species. We sampled both the ecological and distributional limits of the foothill yellow-legged frog, *Rana boylii* to characterize mitochondrial DNA (mtDNA) variation in this declining, riverine amphibian. We evaluated 1525 base pairs (bp) of cytochrome *b* and ND2 fragments for 77 individuals from 34 localities using phylogenetic and population genetic analyses. We constructed gene trees using maximum likelihood and Bayesian inference, and quantified genetic variance (using AMOVA and partial Mantel tests) within and among hydrologic regions and river basins. Several moderately supported, geographically-cohesive mtDNA clades were recovered for *R. boylii*. While genetic variation was low among populations in the largest, most inclusive clade, samples from localities at the edges of the geographic range demonstrated substantial genetic divergence from each other and from more central populations. Hydrologic regions and river basins, which represent likely dispersal corridors for *R. boylii,* accounted for significant levels of genetic variation. These results suggest that both rivers and larger hydrologic and geographic regions should be used in conservation planning for *R. boylii*.

Keywords Declining amphibian · California · River basins · Foothill yellow-legged frog · Conservation genetics

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Introduction

Successful conservation and restoration of any declining species depends on the availability of adequate biological information for that species, the preservation of suitable habitat to guarantee long-term viability in the face of climate and land use changes, and the political and cultural will to enable necessary habitat protection and environmental regulation. Species status designations (under the US Endangered Species Act, the IUCN Red List, CITES and related conservation entities) increasingly rely on genetic information, especially for defining appropriate intraspecific management units. Several management-oriented categories have been created to help identify, and therefore protect, components of biological diversity below the species level, including: Evolutionary Significant Units (ESUs), Distinct Population Segments (DPSs), and Management Units (MUs). Although the difference between ESUs, DPSs, and MUs may be somewhat arbitrary (de Guia and Saitoh 2007), the goal of using genetic lineage-based strategies to identify important conservation targets within species continues to have broad appeal (Moritz 2002; Avise 2004).

Recent comparative phylogeographic research in the complex, mountainous regions of the western U.S. has

focused on California as a critical landscape for lineage-based conservation efforts. The California Floristic Province in particular has been identified as one of the world's top 25 biodiversity hotspots (Myers et al. 2000), as well as being one of the major human population centers on earth. Based on broad sampling across a wide variety of taxa, several recent studies have reviewed general phylogeographic trends across California. Additional, detailed sampling of many western U.S taxa, especially aquatic and semiaquatic amphibian and reptile endemics from across their ranges, have further identified within-species patterns of genetic differentiation that may be quite general, or may represent idiosyncratic patterns of single species (Shaffer et al. 2000, 2004a, b; Recuero et al. 2006; Phillipsen and Metcalf 2009; Spinks et al. 2010). However, as recently emphasized by several authors, the broad patterns that occasionally emerge from comparative phylogeography (for example, the Apalachicola river discontinuity in the southeastern US [Walker and Avise 1998; Soltis et al. 2006; Pauly et al. 2007], or the virtually ubiquitous split across the transverse range that separates southern from central California [Calsbeek et al. 2003; Chatzimanolis and Caterino 2007]) depend on detailed, single species analyses (Phillipsen and Metcalf 2009; Polihronakis and Caterino 2010). The generality of these landscape-level patterns, and thus their overall utility in conservation planning, should be tested with additional, rangewide work on codistributed taxa. Here, we add a genetic analysis of the foothill yellow-legged frog, *Rana boylii*, an ecologically unique, riverine anuran. Because *R. boylii* is declining across its range, we used basic ecological associations and the historical spatial distribution of the species to guide our genetic sampling, and focus our discussion on the conservation implications of the resulting analyses.

Rana boylii historically occurred in foothill and mountain streams from northern Baja California to southern Oregon west of the Sierra-Cascade crest, from sea level to approximately 1830 m (6000 ft) elevation (Fig. 1a, Stebbins 2003). Although first described as a full species by Baird (1854), a century of taxonomic uncertainty followed, including several name changes (Zweifel 1968). Since 1955, *R. boylii* has generally been recognized as a distinct species in the family Ranidae (Zweifel 1955), although its relationship to other North American ranids remains contentious. Recent molecular analyses suggest that *R. boylii* falls within a clade of western US species (Zweifel 1955; Case 1978a, b; Macey et al. 2001; Shaffer et al. 2004a; Hillis and Wilcox 2005), and may be the sister group of the *Rana pretiosa/luteiventris* clade (Macey et al. 2001; Hillis and Wilcox 2005). Although the phylogenetic placement of the species remains problematic, all analyses indicate that *R. boylii* is a relatively ancient and phylogenetically distinct taxon (Hillis and Wilcox 2005). What is equally clear is that *R. boylii* has experienced significant population declines, especially in the southern part of its range in the southern Sierra Nevada mountains and south coastal California (Jennings and Hayes 1994; Jennings 1996; Davidson et al. 2002; Stebbins 2003; Lind 2005). Because of this, it is currently listed as a California State Species of Special Concern (California Department of Fish and Game 2009), which provides broad, range-wide protection of the species in California, with the goal of recovery before more formal state or federal listing becomes necessary.

Rana boylii is restricted to stream environments, from small creeks to large rivers. They breed in the spring (typically March through June), and females attach one large, globular egg mass to rocky substrates in relatively shallow water (<1 m). Larvae graze on algae throughout the summer and metamorphose by late summer (Jones et al. 2005; Lind 2005). The ecology of juvenile frogs is not well known, although anecdotal accounts indicate that they may use smaller aquatic environments such as springs and tributary streams (pers. obs., S. Kupferberg and W. Palen pers. comm.). *Rana boylii* reaches sexually maturity at 2–3 years of age. Adults are found in and near streams throughout the year, including the non-breeding season. All life stages are believed to use streams as migratory corridors seasonally and for long distance dispersal, and the limited available evidence suggests that overland movement is uncommon (Zweifel 1955; Jones et al. 2005; Lind 2005).

Because of *R. boylii's* association with lotic environments, we were particularly interested in the extent to which patterns of genetic variation were defined by hydrologic features (e.g., small watersheds, river basins) or whether large geologic events and processes representing longer time scales might overshadow the structuring effects of drainages. For example, recent analyses of a broad range of species in California indicated that congruent genetic breaks seen in many species correspond with major mountain-building events and climatic regimes (Calsbeek et al. 2003; Lapointe and Rissler 2005; Soltis et al. 2006). However, for aquatic taxa, more localized watershed differentiation is an additional key component of phylogeographic structure (Spinks and Shaffer 2005; Phillipsen and Metcalf 2009). Thus our specific objectives were to describe intraspecific genetic variation of *R. boylii* throughout its geographic range and determine whether the observed patterns were consistent with: (1) gene flow barriers due to river basin divides/boundaries, (2) more extensive temporal and spatial geologic events, such as glaciations and mountain-building, or (3) some combination of processes acting at both spatial and temporal scales. We present our mtDNA gene tree results as a comprehensive launching point for future genetic investigations. These data, in combination with recent genetic work on codistributed taxa, can then be used to make future

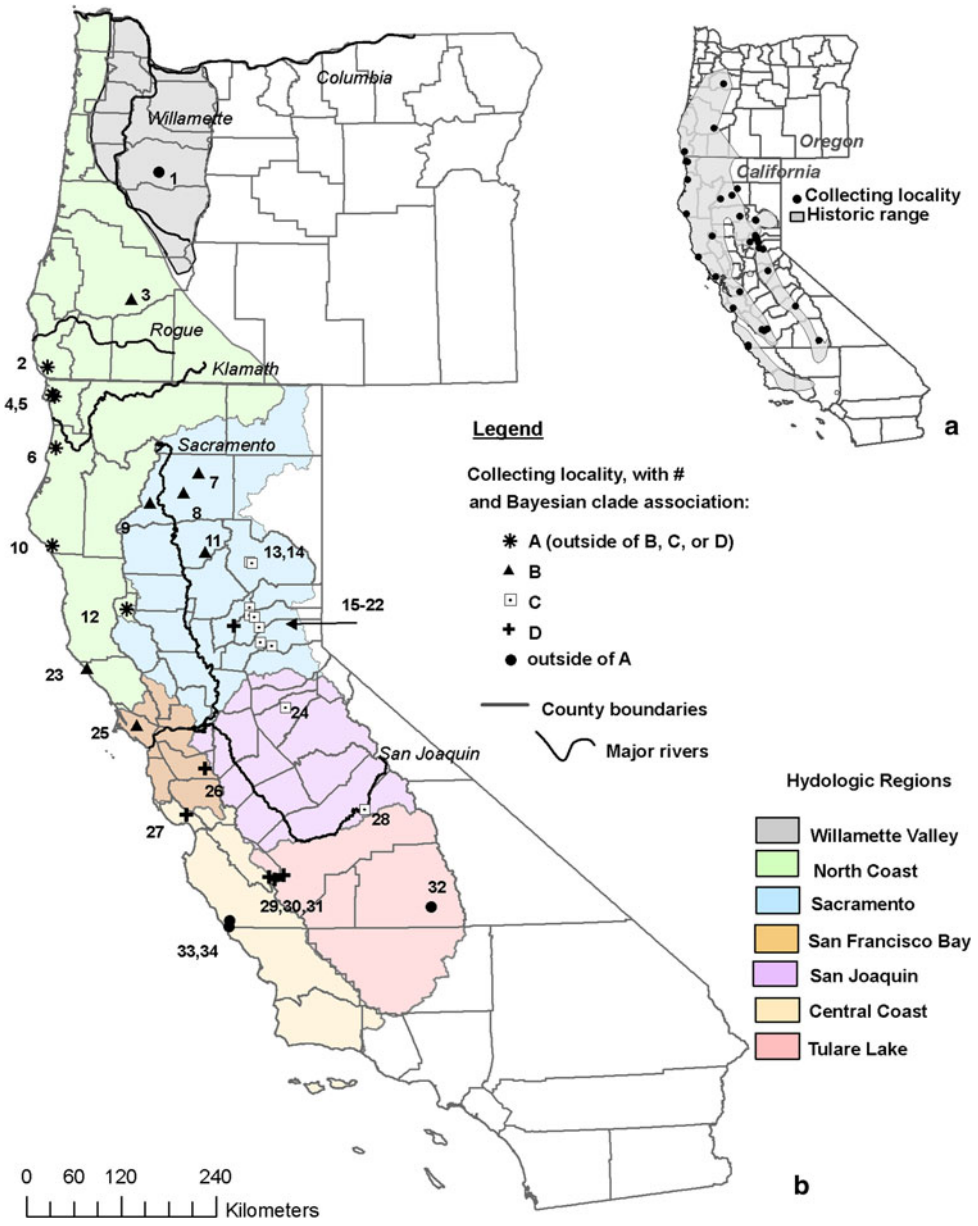

Fig. 1 *Rana boylii* collecting localities relative to the species' historic range (**a**) and Bayesian clade distribution of localities relative to hydrologic regions (**b**). *Rana boylii*'s historic range for California was derived from Zeiner et al. (1988) and the Oregon range was drawn by the senior author from a database of historic localities (Lind 2005). Localities are described by number in Appendix Table 5 and the phylogenetic analysis from which clades are derived is shown in Fig. 2. In this depiction, localities associated with clade A are those that were within that inclusive clade but outside of clades B, C, or D

recommendations on the conservation of this declining species. In addition to mitochondrial DNA (mtDNA), we assessed variation at a single nuclear locus, but recovered little variation.

Materials and methods

Genetic sampling

We sampled genetic material from throughout the current geographic range of *Rana boylii* in California and Oregon (Fig. 1a). The California Department of Water Resources has defined 10 hydrologic regions in the state that integrate current and past geology, tectonics, and climate (Mount 1995). *Rana boylii* historically occurred in seven of these regions and currently occurs in six: North Coast, San Francisco Bay, Central Coast, Sacramento, San Joaquin, and Tulare Lake (Fig. 1b). These six regions include over 30 major river drainages. The relatively large geographic range of *R. boylii* precluded sampling every population, so our goal for tissue collection was to cover the full extent of the historic range such that our first priority was to collect from several populations in each of the six hydrologic regions, and secondarily to collect from as many of the major river drainages as possible within each of the regions (Fig. 1b; e.g., Appendix Fig. 3). The decline of this species in the southern portions of its range (both in the Sierra

Nevada and the coastal ranges) and the extreme northern portion of its range (Stebbins 2003; Jones et al. 2005; Lind 2005) resulted in fewer sampling localities from these areas. Populations of *R. boylii* are not known from the floor of the Great Central Valley of California (Fig. 1a; Appendix Fig. 3), thus we did not attempt to collect in this region. Our sampling outside of California included sites from three of six major river drainages in southwestern Oregon representing two hydrologic regions—one that is contiguous with northern California (North Coast) and a second, the Willamette Valley hydrologic region, which ultimately flows into the Columbia River (U.S. Geological Survey 2004) (Fig. 1b). From one to 30 individuals (whole larvae or sub-adults, adult toe tips) were collected at each locality (Appendix Table 5). Efforts were made to collect larvae from multiple parental groups at each sampling locality by collecting samples over several kilometers at each stream location. Outgroups were selected based on phylogentic relationships inferred from allozymes (Case 1978a) and mtDNA (Green 1986; Macey et al. 2001).

Mapping

We mapped all sample localities using ArcGIS 9.2 Geographical Information System (GIS) software (ESRI 2006). We overlaid the CALWATER database/GIS coverage (CALWATER 2.2—California Interagency Watershed Mapping Committee 1999) on our map of sampling localities to determine the river basin and hydrologic region association for each sampling locality in California. We used descriptions of Oregon watersheds available from the U.S. Geological Survey (2004) to identify river basins and hydrologic regions for the three Oregon localities (Fig. 1b).

Marker selection

Following many recent California amphibian studies, we relied on mtDNA sequence data to examine genetic differentiation over both local and larger geographic scales (Shaffer et al. 2000; Bos and Sites 2001; Macey et al. 2001; Jockusch and Wake 2002; Shaffer et al. 2004a, b; Recuero et al. 2006). We focused on two fragments—nearly complete cytochrome *b* (cyt*b*, 1026 base pairs [bp]) and a portion of mitochondrially encoded NADH subunit 2 (ND2, 499 bp). Both of these fragments have been used for inter and intra-specific analyses in western ranid frogs (e.g. Bos and Sites 2001; Macey et al. 2001; Shaffer et al. 2004a), allowing for comparisons with related species. We also evaluated variation at a single nuclear locus (intron 5 of the tropomyosin gene) for which PCR primers likely to work for anurans were readily available (Friesen et al. 1999).

Laboratory methods

Total genomic DNA was extracted for 77 individual *R. boylii* from 34 localities (1 to 4 individuals per locality; Appendix Table 5) using a standard salt extraction (Sambrook and Russell 2001). Several primer sets were used for DNA amplification and sequencing, including some developed for this study. For cyt*b*, we used the following primer set: *Glu_F* (5′ to 3′ gaaaagctatcgctgttattcaac; T. Engstrom, pers. comm.) and *CytbAR-H_R* (Goebel et al. 1999). PCR conditions were 30 cycles of denaturation at 94°C, annealing at 50°C and extension at 72°C. Sequences were generated using the original PCR primers and two internal primers, *CB755_R* (5′ to 3′ ctggtgtaaaattgtctgggtctc; T. Engstrom, pers. comm.) and *Cytb18rL_F* (Goebel et al. 1999). For ND2, we used GenBank *R. boylii* mtDNA sequences (Macey et al. 2001) to develop primers *ND2_2_F* (5 ′to 3′ tattggccaaacccagcttc), *tRNAtrp_R* (5′ to 3′ ttaaagggcctgagttgcatt), and an internal 3′ end primer *tRNAtrp2_R* (5′ to 3′ ctttgaaggcctttggtctgtatta). PCR conditions for ND2 were 40 cycles of denaturation at 94°C, annealing at 60°C and extension at 72°C. The ND2 sequences were generated using primers *ND2_2_F* and *tRNAtrp2_R*.

For nuclear data, we sequenced intron 5 of the tropomyosin gene for 10 individuals representing 10 localities spanning the geographic range of *R. boylii* (Appendix Table 5, localities 1, 6, 9, 20, 21, 25, 28, 29, 32, 34). For PCR (and sequencing) we used the primers *TROPex5* and *TROPex6* (Friesen et al. 1999); PCR reaction conditions were 30 cycles of denaturation at 94°C, annealing at 65°C and extension at 72°C. All PCR products were sequenced at the University of California Division of Biological Sciences DNA Sequencing Facility (http://dnaseq.ucdavis.edu/) using ABI 3100 or ABI 3730 automated DNA sequencers. Alignments are available from TreeBase (www.treebase.org—study # S10726) and all sequences have been deposited in GenBank (accession numbers HM804086-HM804251).

Phylogenetic and landscape genetic analyses

Sequences were confirmed by aligning and viewing forward and reverse primed fragments in SeqEdit v. 1.0.3 (G. Olsen, Applied Biosystems) and checked for reading frame in Gene Jockey (Taylor 1990). Multiple alignments were conducted using Clustal X v. 1.8 (Thompson et al. 1997) with final minor edits completed manually. We concatenated the cyt*b* and ND2 sequences for phylogenetic analyses and used sequence data from one individual *Rana sierrae* (formerly *R. muscosa*) as an outgroup (Macey et al. 2001; Shaffer et al. 2004a). Two primary methods were used for analysis of mtDNA sequence data. To examine range-wide genetic and biogeographic history and structure, we generated gene trees using maximum likelihood

(ML) and Bayesian inference (BI). To examine genetic differentiation among hydrologic regions and river basins, we used Analysis of Molecular Variance (AMOVA) and partial Mantel tests to assess isolation by distance effects.

Maximum likelihood analyses were performed using PAUP* 4.0b10 (Swofford 2002) with ten random stepwise heuristic searches and tree bisection-reconnection (TBR) branch swapping. Models of molecular evolution for parameter estimation were selected using DTModSel (Minin et al. 2003) with parameters values estimated using PAUP* 4.0b10. We also performed an ML bootstrap analysis with 100 pseudoreplicates (Felsenstein 1985). Bayesian analyses were performed using MrBayes V3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). To determine the optimal partitioning strategy, we performed preliminary Bayesian analyses under three different partitioning schemes: as a single partition (unpartitioned), as two partitions (cyt*b* vs ND2), and as six partitions (by codon position and gene), and then selected the optimal partitioning strategy by comparing Bayes factors (harmonic means) from the three partitioning strategies using the guidelines set out by Kass and Raftery (1995) (Table 1). Preliminary Bayesian analyses were performed with two replicates and four chains for 1×10^6 generations, and the chains were sampled every 1000 generations. We then ran a final Bayesian analysis with the preferred partitioning strategy using two replicates and four chains for 50×10^6 generations and sampled every 5000 generations. Stationarity was determined as the point when the potential scale reduction factor (PSRF) equaled 1, and when the −log likelihood ($-\ln L$) scores plotted against generation time reached a stationary value. Based on these criteria, the first 25% of samples were discarded as burn in. For the partitioned model analyses, we invoked the *prset ratepr = variable* option in MrBayes V3.1.1 in order to accommodate possible among-partition rate variation.

We used AMOVA for sequence data (Excoffier et al. 1992) to explore genetic variation relative to current and historic hydrologic features within the range of *R. boylii*. Hydrologic regions and river basins represent potential dispersal corridors for this highly aquatic frog, which appears to exhibit limited overland movement, so we examined both broad-scale hydrologic regions and local-scale genetic variation among adjacent rivers within our dataset. We partitioned genetic variation within and among seven hydrologic regions (North Coast, San Francisco Bay, Central Coast, Sacramento, San Joaquin, Tulare Lake, and Willamette Valley; Fig. 1b) to test for structuring at this broad scale. We were also interested in whether variation among interconnected river basins within these hydrologic regions contributed to further genetic substructure. The North Coast and Central Coast hydrologic regions are widely recognized by hydrologists and fisheries biologists, especially for anadromous fishes. However, the rivers within these regions drain directly to the Pacific Ocean, and from the perspective of *R. boylii*, the North Coast and Central Coast regions are not hydrologically interconnected with each other or the five remaining regions. The Willamette Valley is similarly isolated as a part of the Columbia River drainage (Fig. 1b). All streams in the Sacramento and San Joaquin hydrologic regions ultimately flow into the lower Sacramento river, and we used this system to test for the isolating effects of watersheds within this single large river basin. We pooled our samples into five quasi-independent drainages: Upper Sacramento, Bear-Feather, Yuba, American, and San Joaquin (see Appendix

Table 1 Models of molecular evolution for parameter estimation, partitioning strategy, and Bayes factor comparisons for the mitochondrial cyt*b* and ND2 data sets

Partitioning strategy	No. partitions	Models	Run #1	Run #2	Combined	Bayes factor comparisons
Unpartitioned	1	GTR + G	−3635.71	−3635.01	−3635.42	528.26
Cyt*b* & ND2	2		−3643.76	−3643.75	−3643.75	544.92
	Cyt*b*	GTR + I				
	ND2	GTR + G				
By codon	6		−3371.98	−3366.15	−3371.29	Preferred
	Cyt*b* 1st	K80				
	Cyt*b* 2nd	F81				
	Cyt*b* 3rd	GTR + G				
	ND2 1st	GTR				
	ND2 2nd	GTR				
	ND2 3rd	GTR				

The preferred partitioning strategy was composed of six positions; cyt*b* 1–3 and ND2 1–3

Table 5, Fig. 3 for details on samples included in each drainage). These drainages are currently hydrologically interconnected via the Sacramento and San Joaquin Rivers, and share a common geologic history. Although the Tulare Lake region is geographically at the southern end of the Great Central Valley, we excluded this region from this analysis because the drainages within it are not hydrologically connected to the San Joaquin River (Mount 1995). We used Arlequin v.2.0 software (Schneider et al. 2000) to conduct two AMOVA analyses; one on sets of populations defined by their occurrence within one of seven hydrologic regions and one on sets of populations defined by their occurrence in one of these five river basins flowing into the Sacramento/San Joaquin Rivers of the Great Central Valley. In addition, we calculated pairwise F_{st} values among hydrologic regions and among river basins to quantify overall levels of mitochondrial divergences.

In order to determine possible effects of isolation by distance (IBD) on genetic variation relative to hydrologic regions, we performed pairwise IBD analyses on the mtDNA for each hydrologic region comparison that was significant in the AMOVA analysis. We used the Alleles in Space (AIS) software (Miller 2005) to generate a matrix of pairwise genetic distances (uncorrected "*p*"), and a matrix of pairwise geographic distances between sampling localities. We then used the Isolation by Distance v3.16 web service (http://ibdws.sdsu.edu/~ibdws/, Jensen et al. 2005) to conduct partial Mantel tests on pairwise hydrologic region comparisons using these genetic and geographic distance matrices and an indicator matrix (0,1) that identified the hydrologic region of each individual. IBD was evaluated as the matrix correlation between genetic and geographical distance correcting for indicator hydrologic region (GG/I) using a partial Mantel test. Differentiation among hydrologic regions corrected for IBD was evaluated as the correlation between genetic distance and hydrologic region correcting for geography (GI/G), also using a partial Mantel test.

Results

Sequence variation

We collected mtDNA sequences for 77 *R. boylii* and one *R. sierrae* (Fig. 1a; Appendix Table 5). The concatenated cyt*b* and ND2 mtDNA fragments provided up to 1525 bp of aligned sequence data for analyses. The nucleotide alignment contained no insertions or deletions across all ingroup (*R. boylii*) samples. However, the *R. sierrae* ND2 fragment contained a 3 bp deletion with respect to *R. boylii*. In the central portion of the geographic range (southern Oregon to central California) of *R. boylii*, genetic variation was relatively low within and among localities (uncorrected *p* range: 0.00–0.009). However, some populations at the northern and southern extremes of the range (Fig. 1b; Appendix Table 5 localities 1, 32, 33, and 34) were much more divergent from the central populations (uncorrected *p* range*:* 0.006–0.022) and from each other (uncorrected *p* range*:* 0.008–0.020). By comparison, all *R. boylii* sequences differed from the *R. sierrae* outgroup by more than 14% (uncorrected *p* range: 0.142–0.148). We also collected up to 517 bp of sequence from intron 5 of the tropomyosin locus for 10 individuals, and these sequences revealed extremely low levels of variation, with only two of the 517 positions variable in three individuals. Uncorrected *p* distances of our nuclear sequences ranged from 0.002 to 0.004 confirming that overall variation is low in *R. boylii* across its range in both nuclear and mitochondrial DNA. The nuclear sequence data was essentially phylogeographically uninformative, and we did not pursue this marker further.

Phylogenetic analyses

Maximum likelihood analyses of the concatenated mtDNA data recovered two optimal trees which differed only by a minor internal branch ($-\ln L$ score = 3645.38204) and were nearly identical to the BI tree. Each analysis revealed moderate bootstrap and Bayesian support values for several geographically cohesive clades. We present the 50% majority rule consensus tree derived from the Bayesian posterior distribution of trees in Fig. 2 with both ML bootstrap and BI posterior probabilities shown for well supported clades (mapped in Fig. 1b). We recovered a large and well-supported clade (clade A, Fig. 2) that included samples from all but four localities: (1) the northernmost in Oregon (south Santium River, locality 1), (2) the southernmost in the Sierra Nevada (Kern River, locality 32), (3) the southernmost coastal locality (Monterey County, draining into San Carpoforo Creek and then into the Pacific Ocean, locality 34), and (4) two of four individuals from the southernmost inland draining site (Los Burros Creek, draining into the Nacimiento River, and then to the Salinas River, locality 33).

Within clade A, there were three moderately well-supported and generally geographically cohesive clades (B, C, and D, Figs. 1b, 2). Clade B contained *R. boylii* from four main streams that flow into the upper Sacramento River, the Umpqua River in southern Oregon, and two coastal streams in Marin and Sonoma counties (localities 3, 7, 8, 9, 11, 23, and 25). Clade C included individuals from drainages flowing west from the Sierra Nevada of California into the Great Central Valley, including the Feather, Yuba, Bear, American, Calaveras, and upper San Joaquin Rivers (localities 13–18, 20–22, 24, and 28). Clade D contained

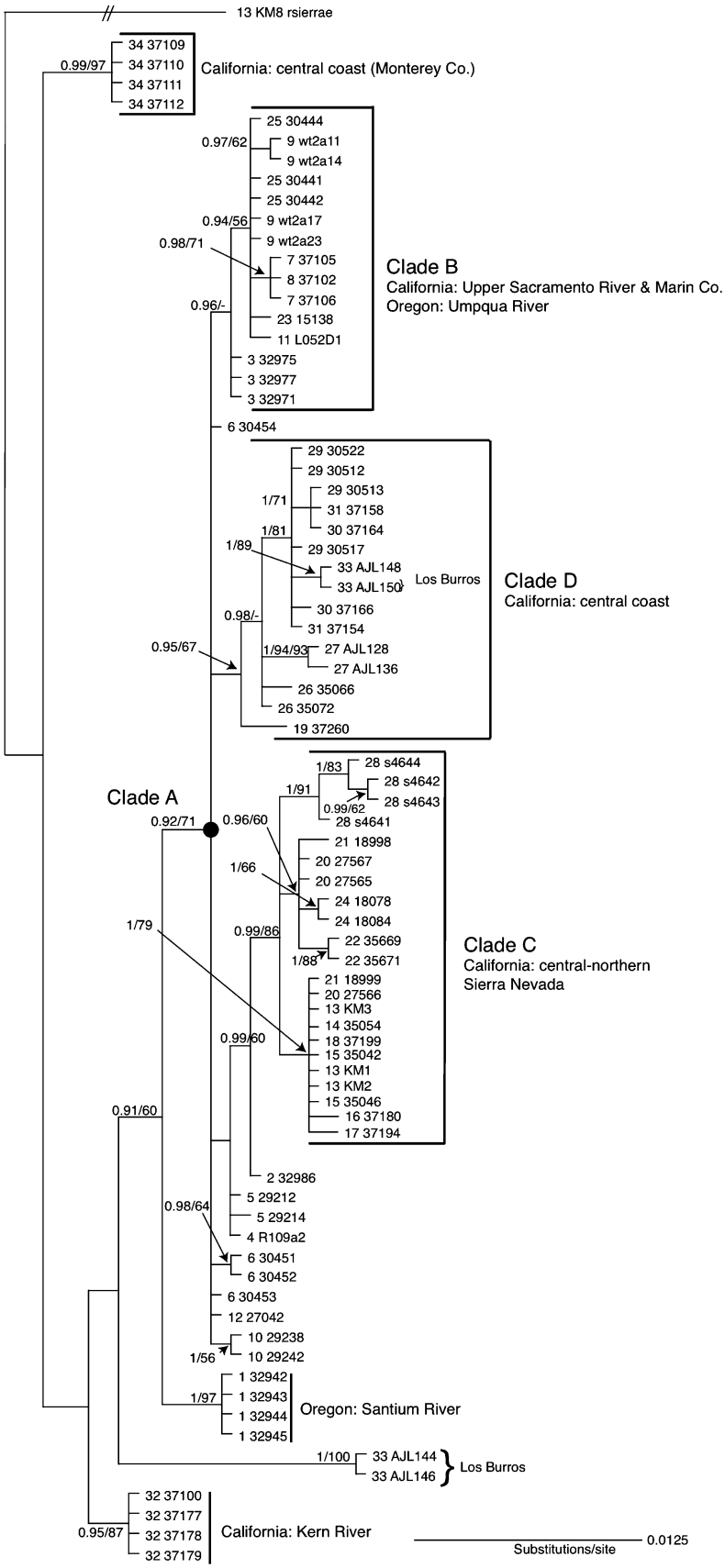

Fig. 2 Majority rule consensus tree for the posterior distribution of trees from the Bayesian analysis of up to 1525 base pairs of concatenated mitochondrial cyt*b* and ND2 data from 77 *Rana boylii* plus one *Rana sierrae* outgroup. Data were partitioned by codon for analysis (Table 1). Branch lengths are the mean branch length from the posterior distribution. Terminals are the locality number followed by sample number (see Appendix Table 5). Numbers above branches are BI posterior probabilities/ML bootstrap support values. Double slash marks indicate a branch not drawn to scale

individuals from coastal (west flowing) and inland (east flowing into the Great Central Valley) streams of the central Coast Range of California (localities 19, 26, 27, 29, 30, 31, and 33). Clade A also contained individuals from several additional localities that did not themselves form a clade but were also excluded from subclades B, C, or D (Fig. 2); these Clade A samples were all from coastal, west-flowing streams of northern California and southern Oregon (localities 2, 4, 5, 6, 10, and 12; Fig. 1b).

Landscape genetics

Within and among the seven occupied hydrologic regions, AMOVA indicated that a significant component of the variation (40%) was among these regions ($F_{st} = 0.3996$, $P = 0.000$, Table 2). At a finer geographic scale, analyses of five hydrologically interconnected populations within the Sacramento–San Joaquin river basin revealed that genetic variation was higher among river basins than it was within river basins (60.56% vs 39.44%, respectively, Table 2). Although these two different scales of analyses are not strictly comparable, they are consistent with the interpretation that both individual drainages and larger hydrologic features are important landscape components that contribute to among-population genetic substructure within *R. boylii*.

Table 2 Genetic variation of populations of *R. boylii* as determined by AMOVA within and among hydrologic regions and Sacramento–San Joaquin river basins

Source	d.f.	Percent of variation, with P for F_{st}
Among hydrologic regions	6	39.96, $P = 0.000$
Within hydrologic regions	70	60.04
Among rivers in the Sacramento–San Joaquin basin	4	60.56, $P = 0.000$
Within rivers in the Sacramento–San Joaquin basin	26	39.44

F_{st} values among hydrologic regions were generally high and significant; 17 of the 21 pairs were significantly different after Bonferroni correction. The relatively consistent F_{st} values among all the pairs indicate that the overall AMOVA is not being driven by any one particular locality (Table 3a). Of the significant pairs, partial Mantel tests indicated that five pairs showed significant differentiation with respect to hydrologic region after accounting for isolation by distance effects; North Coast vs Willamette, North Coast vs Central Coast, North Coast vs San Joaquin, North Coast vs Tulare Lake, and San Joaquin vs Tulare Lake (Table 4, below diagonal). For the hydrologically interconnected Sacramento/San Joaquin river basin populations, all pairwise F_{st} values among three of the

Table 3 Pairwise F_{st} values (below diagonal) and associated P values (above diagonal) for populations of *Rana boylii* from AMOVA's of (a) hydrologic regions and (b) hydrologically connected rivers in the larger Sacramento–San Joaquin river basin

Hydrologic region	Localities included	Nucleotide diversity	Haplotype diversity	Wil	NoC	SFB	CeC	Sac	SaJ	TuL
(a)										
Willamette (Wil)	1	0.000 (0.000)	0.000 (0.000)	–	**0.000**	0.018	**0.000**	**0.009**	**0.000**	**0.000**
North Coast (NoC)	2, 3, 4, 5, 6, 10, 12, 23	0.001 (0.001)	0.914 (0.425)	0.853	–	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
San Francisco Bay (SFB)	25, 26,	0.002 (0.002)	0.700 (0.218)	0.818	0.251	–	0.099	**0.000**	**0.000**	0.045
Central Coast (CeC)	27, 30, 31, 33, 34	0.011 (0.006)	0.901 (0.058)	0.376	0.305	0.163	–	**0.000**	**0.000**	0.090
Sacramento (Sac)	7, 8, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22	0.004 (0.002)	0.857 (0.059)	0.673	0.278	0.282	0.364	–	**0.000**	**0.000**
San Joaquin (SaJ)	24, 28	0.003 (0.002)	0.867 (0.129)	0.841	0.681	0.598	0.346	0.322	–	**0.000**
Tulare Lake (TuL)	29, 32	0.006 (0.003)	0.679 (0.122)	0.582	0.518	0.279	0.076	0.486	0.570	–

River Basin	Localities Included	Nucleotide Diversity	Haplotype Diversity	Upper Sac.	Bear-Feather	Yuba	American	San Joaquin
(b)								
Upper Sacramento	7, 8, 9, 11	0.001 (0.001)	0.821 (0.101)	–	**0.000**	**0.000**	**0.009**	**0.000**
Bear-Feather	13, 14, 20	0.001 (0.001)	0.476 (0.171)	0.845	–	0.135	0.081	**0.000**
Yuba	15, 16, 17, 18, 19	0.003 (0.002)	0.933 (0.122)	0.712	−0.002	–	0.081	**0.000**
American	21, 22	0.003 (0.002)	0.833 (0.222)	0.782	0.272	0.229	–	0.045
San Joaquin	24, 28	0.003 (0.002)	0.867 (0.129)	0.759	0.488	0.409	0.335	–

Boldface P values are significant after a Bonferroni adjustment; $\alpha = 0.007$ for (a) and $\alpha = 0.01$ for (b). Nucleotide and haplotype (gene) diversity estimates include an estimate of variance in parentheses

Table 4 Matrix of r (top cell entry) and P (bottom cell entry) values from isolation by distance partial Mantel tests of statistically significant comparisons in the hydrologic region AMOVA analysis (Table 3)

Hydrologic region	Wil	NoC	SFB	CeC	Sac	SaJ	TuL
Willamette (Wil)	–	**0.6401**	ns	0.1993	ns	0.8326	**0.8750**
		0.0010		0.0190		0.0160	**0.0010**
North Coast (NoC)	**0.8278**	–	**0.2691**	−0.1253	**0.3256**	**0.5155**	0.1811
	0.0010		**0.0030**	0.9600	**0.0010**	**0.0010**	0.0170
San Francisco Bay (SFB)	ns	0.0695	–	ns	**0.4172**	**0.6498**	ns
		0.2940			**0.0010**	**0.0030**	
Central Coast (CeC)	0.1828	**0.2686**	ns	–	0.1616	0.1859	ns
	0.0210	**0.0010**			0.0440	0.0500	
Sacramento (Sac)	ns	−0.0593	−0.1706	0.0925	–	**0.6861**	**0.4183**
		0.9860	0.9880	0.1440		**0.0010**	**0.0010**
San Joaquin (SaJ)	−0.4522	**0.4799**	0.3921	−0.0657	−0.3626	–	**0.7176**
	0.9630	**0.0010**	0.0170	0.7250	0.9990		**0.0010**
Tulare Lake (TuL)	−0.8438	**0.3014**	ns	ns	0.0218	**0.4973**	–
	0.9990	**0.0060**			0.4570	**0.0050**	

Above the diagonal is the correlation of genetic and geographic distance, correcting for indicator hydrologic region variable (GG/I). Cells below the diagonal show the genetic-hydrologic region variable test correcting for geography (GI/G). Values in bold are significant after Bonferroni adjustment ($\alpha = 0.007$) and ns indicates not significant in the hydrologic region AMOVA

populations (Bear-Feather, Yuba, and American) were not significantly different from each other, and the American River also did not differ from the San Joaquin. The remaining river basins were significantly different from each other and different from the Bear-Feather, Yuba, and American (Table 3b). Haplotype diversity was relatively high for all but one river basin (Bear-Feather) with no extreme values in nucleotide diversity (Table 3b).

For Bayesian clades with multiple sampling sites, there was not a strong correlation between the geographic distribution of clades and hydrologic regions; clade B had representatives in 4 of 7 hydrologic regions, clade C in 2 of 7, and clade D in 4 of 7 (Fig. 1b). The six sites that fell within clade A but outside of clades B, C, and D were all in the North Coast hydrologoic region.

Discussion

As an inhabitant of mid-elevation, flowing waters in California and Oregon, *R. boylii* presents a valuable model species for landscape-level genetic analysis. Phylogeographic and landscape genetic analyses provide insights into the history of populations across their geographic range, at different spatial scales. The combined results suggest potential areas for conservation actions, which we discuss below.

Phylogeography of *Rana boylii*

At the phylogeographic level, we detected several features that have important implications for the conservation of *R. boylii*. First, a series of peripheral populations are differentiated by relatively deep splits from those at the "core" of the range (clade A). These populations occur both at the northern limit of the species in central Oregon (locality 1) and to the south, on both the Sierrian (locality 32) and coast range (localities 33, 34) sides of the Great Central Valley (Fig. 1b). The extreme southern populations from southeastern Los Angeles County are extinct and were not included in our sampling, but our analyses of the closest extant populations to the north (localities 33, 34) suggest that these may also have been genetically distinct. This pattern of phylogeographic outliers at the edge of the species range suggests that these populations have a long history of isolation from those in central California, and therefore have relatively high conservation value. In northern California, the general pattern across many taxa is one of relatively low phylogeographic diversity (summarized in Spinks and Shaffer 2005), and *R. boylii* joins a small group of exceptions (e.g. Polihronakis and Caterino 2010) to this general trend. These genetic patterns are somewhat supported by Zweifel's (1955) analyses of range-wide geographic variation in coloration and morphology. Zweifel examined 565 *R. boylii*, and described substantial color pattern variation between southern Sierra Nevada (Kern County) and north coast populations as well as more subtle differences in morphology (head length to width, tibia length to body length ratios) among populations in Marin, Monterey, and other central coast counties. However, based on the overall variation in these features across all specimens and the existence of many intermediate characters, no subspecific designations were proposed (Zweifel 1955).

High clade diversity in the south is consistent with the pattern seen in a number of codistributed taxa. Our southern Sierra Nevada sample (locality 32) fell far outside of the clade containing the rest of the samples from the Sierra Nevada to the north. This break is consistent with mtDNA data for other amphibian and reptile taxa in this bioregion and supports the interpretation of a shared biogeographic history of populations rather than an idiosyncratic gene tree history (Irwin 2002). For example, *Rana muscosa/sierrae* (Macey et al. 2001; Vredenburg et al. 2007), *Emys (Actinemys) marmorata* (Spinks and Shaffer 2005; Spinks et al. 2010), *Ambystoma californiense* (Shaffer et al. 2004b) and *Lampropeltis zonata* (Rodriguez-Robles et al. 1999) all show coincident phylogeographic splits in this area, with populations south of the San Joaquin River in the Tulare Lake basin deeply divergent from those to the north. This region also harbors a number of newly described species of narrowly endemic plethodontid salamanders, further suggesting a long history of isolation (Jockusch and Wake 2002; Wake et al. 2002; see also *Batrachoseps* at http://amphibiaweb.org/). In the southern Coast Ranges, the deep divergence of localities 33 and 34 in southernmost Monterey County indicates that these rare and geographically isolated populations west and south of the Salinas River valley on the central and southern California coast (Fig. 1b) are phylogenetically distinct. Deep genetic discontinuities in Santa Barbara County have recently been discovered in two other aquatic species (Shaffer et al. 2004b, Spinks and Shaffer 2005), further suggesting that this part of the coastal California is a rich source of genetic diversity.

Within the "core" genetic range of *R. boylii* (clade A, Fig. 2), several other patterns of genetic breaks are shared with codistributed taxa. Clade A includes three well-defined subgroups (clades B, C and D, Fig. 2) as well as a series of populations in north-coastal California and southern Oregon (labeled as "A [outside of B, C, or D]" on Fig. 1b). The genetic break between clade B and these clade A populations, somewhere in Mendocino County, is congruent with a similarly placed biogeographic break that was first identified by Good (1989) and summarized for several other taxa in Shaffer et al. (2004a). For example, the stream-dwelling salamanders *Dicamptodon ensatus/tenebrosus* (Good 1989) and stream and pond-breeding frogs *Rana aurora/draytonii* (Shaffer et al. 2004a) each show a phylogenetic split in this same area of coastal northern California. In both cases, these genetic divergences are corroborated by morphological and life history features, justifying species status for each clade. However, unlike these more deeply diverged species pairs, the *R. boylii* populations sampled to the north do not form a distinct clade, suggesting either a more recent separation of the clade B populations from those to the north, or a larger ancestral population size which would require a longer time to monophyly (Hudson and Coyne 2002).

Geologic events have undoubtedly helped shape genetic breaks that are congruent across unrelated taxa (e.g. Calsbeek et al. 2003). However, determining the key events (and when they occurred) is challenging, particularly in the absence of a well-calibrated chronogram. In some cases, however, the pattern of genetic variation and phylogenetic breaks we found in *R. boylii* are consistent with known geologic history, suggesting that geological events have shaped the species genetically. The large genetic divergence of samples from the most southerly California coastal localities (Fig. 2, localities 33 and 34) relative to the rest of *R. boylii* is indicative of a history of isolation. The apparent lack of gene flow across the Great Central Valley (that is, between localities 29–31, 33, 34 on the west side of the Valley and Clade C on the east side; Figs. 1b, 2) is consistent with geologic evidence of marine intrusions in this area and the presence of a large freshwater lake in what is now the San Joaquin Valley/Tulare Lake region (Sarna-Wojcicki et al. 1985; Dupre et al. 1991). Both of these features would have been significant dispersal barriers for this freshwater, stream-associated frog. For the break in the southern Sierra Nevada, Macey et al. (2001) suggested that Pleistocene climatic changes, especially the extent of glacial activity were key factors for the more montane *R. muscosa/sierrae* divergence. Recent studies have shown that the amount and variability of precipitation can affect the distribution of *R. boylii* (Lind 2005) so it is likely that these factors acted in the past as well. The biogeography of a suite of other California species has also been linked to variation in climatic regime (Lapointe and Rissler 2005).

Landscape genetics and among-river variation

Rana boylii is highly aquatic and most of this species' movements and migrations occur within stream networks (Jones et al. 2005; Lind 2005). We found mixed evidence for the historic influence of hydrologic regions and river basins on the biogeography of *R. boylii*. For example, some haplotypes were shared among hydrologic regions and rivers basins, potentially indicating incomplete lineage sorting, ongoing gene flow or some combination of the two. On the other hand, hydrologically interconnected river basins in the larger Sacramento–San Joaquin river basin explained a significant amount of genetic variation in our dataset (Tables 2, 3) indicating that additional important structure exists at the individual river basin level. Within this analysis, three of five river basins were not significantly differentiated (Table 3b; Appendix Fig. 3). Among those five rivers, one pair (the Bear-Feather and Yuba) had a near-zero F_{st} value. These rivers merge before entering the main stem of the Sacramento–San Joaquin system.

Other pairwise F_{st} estimates ranged from 0.845 to 0.229, suggesting that these smaller rivers, although connected by the larger, low-elevation main stems of the Sacramento and San Joaquin Rivers, may not be biologically interconnected for these frogs. A recent study, using a combination of RAPD and mtDNA markers for *R. boylii* sampled from several tributaries in a single river basin, found evidence of genetic differentiation among tributaries with both markers, further suggesting that small watersheds may harbor significant variation in this species (Dever 2007).

The hydrologic region groupings that we used to partition genetic variation represent one scale of potential geographic structuring. Other influential factors include long-term climatic (e.g. glaciations) and geologic (e.g. mountain-building, formation and loss of inland seas, riverine connections to marine environments) events. It has been proposed that the dendritic nature of river systems and the inherent terrestrial barriers between them would result in hierarchical patterns of genetic variation for fish and other aquatic species (Meffe and Vrijenhoek 1988). In practice however, organisms do not necessarily follow this pattern. Rainbow trout (*Oncorhynchus mykiss*), for example show evidence of genetic uniqueness in some river basins but not others (Bagley and Gall 1998). Pleistocene and Holocene climate fluctuations and recent hydrologic mixing among river basins have been invoked to explain congruent as well as incongruent genetic-geographic patterns for some fish species (Froufe et al. 2003; Waters et al. 2007), although the interpretation of these data can be challenging (Peterson and Masel 2009). For *R. boylii*, it is likely that the historical effects of geologic events and associated river basin formation/connectivity and climatic shifts have acted in concert (and probably at different temporal and spatial scales) to influence gene flow among populations of *R. boylii*, while river basin interconnectivity may be the primary organizing factors in more recent times. In support of this interpretation, gene flow inferred from microsatellite data in the Columbia spotted frog (*Rana pretiosa/luteiventris*), ostensibly *R. boylii*'s closest relative, is strongly affected by elevational gradients and is constrained by landscape features such as mountain ridges (Funk et al. 2005).

Frog genetics and river conservation

Rana boylii is currently listed as a Species of Special Concern in California (California Department of Fish and Game 2009) and severe regional declines (Jennings and Hayes 1994; Davidson et al. 2002; Lind 2005) have led to concerns about long-term viability of the species. The hydrologic regions we used to evaluate genetic structuring in *R. boylii* populations integrate current and historical geology and climate (Mount 1995), and we expected these regions to be a primary driver in genetic differentiation for *R. boylii*. We found some support for genetic affinities to hydrologic regions, as well as strong evidence of among-river basin divergence in the subset of rivers we examined. Similar patterns at the watershed level have been found in other aquatic amphibians from western North America (Shaffer et al. 2000), necessitating regional conservation planning at the individual river level.

Several other western U.S. ranid frog taxa exhibit significant intra-specific mtDNA genetic variation, potentially warranting designation as Distinct Population Segments or full species (Green et al. 1997; Macey et al. 2001; Shaffer et al. 2004a). In the case of *R. boylii*, the populations in the southern portion of its range (especially localities 32, 33, and 34) are divergent from the rest of the species, appear to be in decline, and may deserve special conservation attention. A large gap in our sampling exists between these southerly populations and those to the north (Fig. 1a). However, this represents the actual current distribution of the frog, rather than incomplete sampling on our part (Jennings and Hayes 1994; Lind 2005). The population at the northern extreme of the range in Oregon may also warrant some special management efforts, although additional data on extant, intervening populations in Oregon would help to clarify the uniqueness of the northerly populations.

Protection of freshwater ecosystems has lagged behind conservation efforts for terrestrial and marine environments. Integrating information on landscape scale features (e.g., river basins, hydrologic regions) with more localized watershed scale elements (particular river reaches, headwater streams, etc.) and biodiversity assessments, is an emerging approach for freshwater species conservation (Abell et al. 2007). Both our phylogenetic and hydrologic region analyses indicated that genetic variation in *R. boylii* is highly structured along hydrologic boundaries. Future conservation efforts and especially genetically-based management strategies for *R. boylii* should strive to preserve these patterns, as they represent elements of historical lineages that could not be recovered if lost. Unfortunately, some of this deep lineage diversity may have already been lost in the southern part of this species' range where extirpation has been the greatest (Davidson et al. 2002; Lind 2005). The most effective strategy for *R. boylii* conservation in California and Oregon may be to use genetic data from codistributed riverine species to prioritize and protect drainages that harbor unique and historic biological diversity.

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Appendix

See Table 5 and Fig. 3.

Table 5 Locality information for *R. boylii* and outgroup samples

Locality number	Specimen numbers	Locality description	Hydrologic regions, (for AMOVA)	Sacramento–San Joaquin river basin (for AMOVA)
Rana boylii				
1	HBS 32942, 32943, 32944, 32945	South Santium River, approx. 6 km upstream of Foster Reservoir, above Sweet Home, Linn Co., OR; 44.405 N, 122.565 W	Willamette Valley	na
2	HBS 32986	Chetco River, ~1.5 km upriver from Loeb State Park off of N. Bank Chetco Rd., Curry Co., OR; 42.125 N, 124.187 W	North Coast	na
3	HBS 32971, 32975, 32977	South Umpqua River, ~2 km upriver (along S. Umpqua Rd.) from Tiller Trail Highway, Douglas, Co., OR; 42.935 N, 122.939 W	North Coast	na
4	GMF R109a-2	Smith River near mouth of Cedar Creek, Del Norte Co., CA; 41.819 N, 124.102 W	North Coast	na
5	HBS 29121, 29214	Smith River near junction of Hwy 199 and South Fork Rd, Del Norte Co., CA; 41.799 N, 124.059 W	North Coast	na
6	HBS 30451, 30452, 30453, 30454	Redwood Creek, near Tall Trees via Tom McDonald Creek, 7.1 km from M-Line intersection, Humboldt Co., CA; 41.209 N, 124.014 W	North Coast	na
7	HBS 37105, 37106	Deep Creek near confluence with Pit River, Shasta Co., CA 40.971 N, 121.849 W	Sacramento	Upper Sacramento
8	HBS 37102	Little Cow Creek, along Hwy 299, 3.5 km (along road) southwest of turn-off to Oak Run Rd., Shasta Co., CA; 40.746 N, 122.071 W	Sacramento	Upper Sacramento
9	GMF WT002a-11, 14, 17, 23	Brandy Creek at picnic area, Whiskeytown Recreation Area, Shasta Co., CA; 40.618 N, 122.573 W	Sacramento	Upper Sacramento
10	HBS 29238, 29242	Mattole River, near mouth of Big Finley Creek, Humboldt Co., CA; 40.093 N, 124.003 W	North Coast	na
11	GMF L052d-1	Deer Creek from Little Pine Creek upstream to bridge crossing of USFS Road 28N29, Ishi Wilderness, Lassen National Forest, Tehama Co., CA; 40.075 N, 121.728 W	Sacramento	Upper Sacramento
12	HBS 27042	Thistle Glade Creek at Rice Creek Rd./M3 Crossing, Lake Co., CA; 39.396 N, 122.866 W	North Coast	na
13	KM-1,2,3	Bean Creek, near crossing of USFS road 25N17, Plumas National Forest, Plumas Co., CA; 39.958 N, 121.068 W	Sacramento	Bear-Feather
14	HBS 35054	Spanish Creek (tributary to East Branch of NF Feather River), ~0.8 km downstream (along Bucks Lake Rd) from turn-off to Snake Lake, Plumas Co., CA; 39.947 N, 121.033 W	Sacramento	Bear-Feather
15	HBS 35042, 35046	Oregon Creek, 100–150 m upstream of Log Cabin Reservoir, Middle Fork Yuba River drainage, off Hwy 49, Yuba Co., CA; 39.443 N, 121.058 W	Sacramento	Yuba

Table 5 continued

Locality number	Specimen numbers	Locality description	Hydrologic regions, (for AMOVA)	Sacramento–San Joaquin river basin (for AMOVA)
16	HBS 37180	Shady Creek, ~0.4 km above Purdon Rd. crossing., Nevada Co., CA; 39.355 N, 121.060 W	Sacramento	Yuba
17	HBS 37194	Spring Creek, ~250 m upstream of mouth (South Fork Yuba River), Nevada Co., CA; 39.333 N, 120.990 W	Sacramento	Yuba
18	HBS 37199	South Fork Yuba River, upstream and near mouth of Spring Creek, Nevada Co., CA; 39.331 N, 120.986 W	Sacramento	Yuba
19	HBS 37260	"Shubert Creek" in Shubert Watershed, ~0.4 km upstream of mouth (Yuba River), at University of California Foothills Research and Extension Center, Yuba Co., CA; 39.235 N, 121.287 W	Sacramento	Yuba
20	HBS 27565, 27566, 27567	Missouri Canyon, tributary to Bear River, Nevada Co., CA; 39.217 N, 120.917 W	Sacramento	Bear-Feather
21	HBS 18998, 18999	Small Stream in Shirt Tail Canyon, along Shirt Tail Canyon Rd. 1.1 km west of intersection with Yankee Jims Rd., Placer Co., CA; 39.045 N, 120.900 W	Sacramento	American
22	HBS 35669, 35671	Middle Fork American River, upstream of junction with USFS road 23, Tahoe National Forest, Placer Co., CA; 39.005 N, 120.732 W	Sacramento	American
23	HBS 15138	South Fork Gualala River, 1 km east of Sea Ranch Airport., Sonoma Co., CA; 38.708 N, 123.423 W	North Coast	na
24	HBS 18078, 18084	Esperanza Creek, Calaveras Co., CA; 38.298 N, 120.522 W	San Joaquin	San Joaquin
25	HBS 30441, 30442, 30444	Halleck Creek, Marin Co., CA; 38.078 N, 122.670 W	San Francisco Bay	na
26	HBS 35066, 35072	Arroyo Mocho Creek, ~4.7 km southeast along Mines Rd. from junction of Mines Rd. and Del Valle Rd., Alameda Co., CA; 37.603 N, 121.670 W	San Francisco Bay	na
27	AJL-128, 136	East Branch of Soquel Creek, near bridge along Hihn's Mill Rd., ~200 m downstream from mouth of Amaya Creek, Santa Cruz Co., CA; 37.073 N, 121.927 W	Central Coast	na
28	GMF S464-1, 2, 3, 4	Jose Creek, tributary to San Joaquin River off Italian Bar Rd. off Jose Basin Rd., Fresno Co., CA; 37.142 N, 119.381 W	San Joaquin	San Joaquin
29	HBS 30512, 30513, 30517, 30522	Arroyo Leona Creek, Interstate-5, Derrick-Halan exit, 2.1 km south, 17.5 km on dirt road, Fresno Co., CA; 36.398 N, 120.538 W	Tulare	na
30	HBS 37164, 37166	Clear Creek at "ORV staging area 2", Road R001, 5.5 km east of Coalinga Rd., San Benito Co., CA; 36.371 N, 120.741 W	Central Coast	na
31	HBS 37154, 37158	San Benito River at Sawmill Creek confluence, San Benito Co., CA; 36.343 N, 120.659 W	Central Coast	na
32	HBS 37100, 37177, 37178, 37179	3rd unnamed tributary to Kern River north of Rincon Trailhead at Sherman Pass Rd., Rincon Roadless Area, Sequoia National Forest, Tulare Co., CA; 36.023 N, 118.457 W	Tulare Lake	na
33	AJL-144, 146, 148, 150	Los Burros Creek, near downstream road crossing of unnamed road, Fort Hunter Liggett, Monterey Co., CA; 35.867 N, 121.289 W	Central Coast	na

Table 5 continued

Locality number	Specimen numbers	Locality description	Hydrologic regions, (for AMOVA)	Sacramento–San Joaquin river basin (for AMOVA)
34	HBS 37109, 37110, 37111, 37112	Dutra Creek, ~0.5 km above confluence with San Carpoforo Creek., Monterey Co., CA; 35.802 N, 121.294 W	Central Coast	na
Rana sierrae				
13	KM-8	Bean Creek, near crossing of USFS road 25N17, Plumas National Forest, Plumas Co., CA; 39.958 N, 121.068 W	na	na

Localities are arranged approximately north to south and numbered sequentially. Latitudes and longitudes are in decimal degrees. All specimens are housed in the H.B. Shaffer lab at the University of California, Davis. Specimen numbers represent local catalog numbers and/or original collectors as follows: *HBS* H.B. Shaffer, *GMF* G.M. Fellers, *AJL* A.J. Lind, *KM* K. Mathews. The following map (Fig. 3) shows detailed locations of sample sites for the Sacramento–San Joaquin river basin analysis

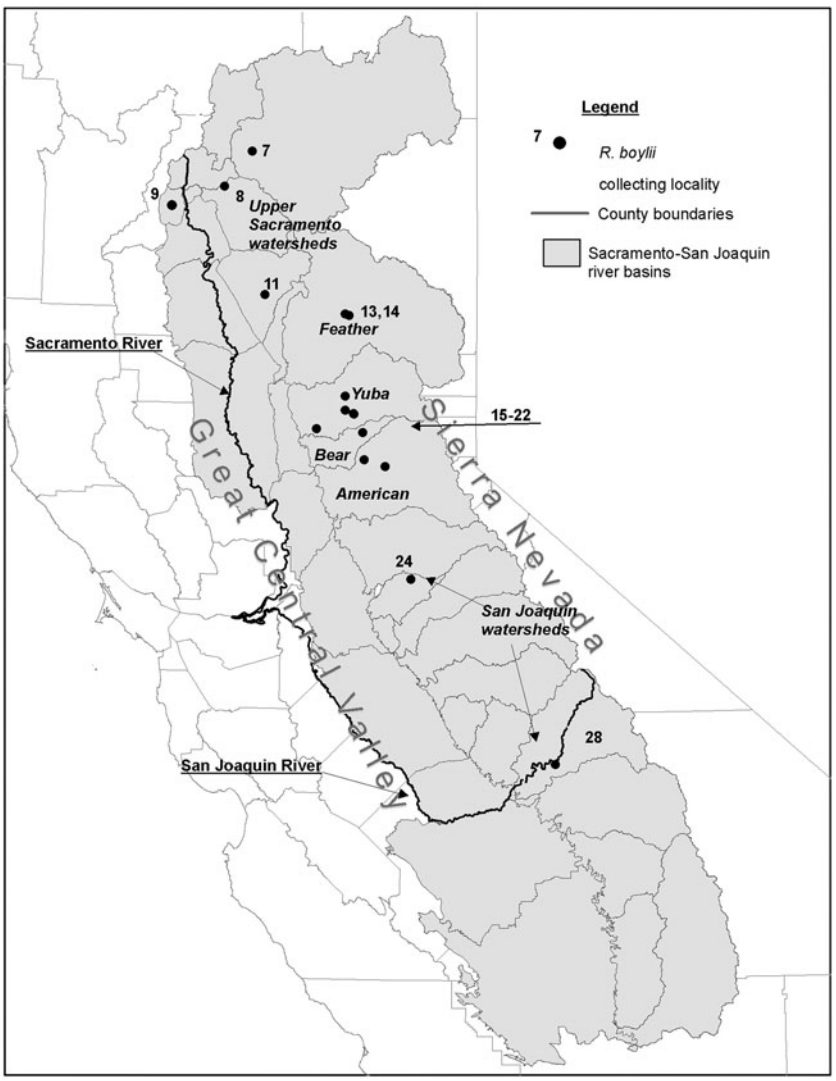

Fig. 3 Detailed map of collecting localities showing Sacramento–San Joaquin river basins used in AMOVA river basin scale analyses. River basin labels are placed within the appropriate watershed/river basin (e.g., American) or adjacent to the set of localities assigned to each river basin grouping (e.g., Upper Sacramento watersheds)

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