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Range-wide molecular analysis of the western pond turtle (*Emys marmorata*): cryptic variation, isolation by distance, and their conservation implications

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Abstract

We analysed phylogeography and population genetic variation across the range of the western pond turtle (*Emys marmorata*) using rapidly evolving mitochondrial and nuclear DNA sequence data. Nuclear DNA sequences from two unlinked introns displayed extremely low levels of variation, but phylogenetic analyses based on mtDNA recovered four well-supported and geographically coherent clades. These included a large Northern clade composed of populations from Washington south to San Luis Obispo County, California, west of the Coast Ranges; a San Joaquin Valley clade from the southern Great Central Valley; a geographically restricted Santa Barbara clade from a limited region in Santa Barbara and Ventura counties; and a Southern clade that occurs south of the Tehachapi Mountains and west of the Transverse Range south to Baja California, Mexico. An analysis of molecular variance (AMOVA) based on regional hydrographic units revealed that populations from the Sacramento Valley north to Washington were virtually invariant, with no evidence of population substructure among northern river drainage basins. In other areas, *E. marmorata* contains considerable unrecognized variation, particularly in central and southern California and in northern Baja California, Mexico. Our northern clade is congruent with the distribution of the subspecies *Emys marmorata marmorata* (Washington–central California). However, no clade is congruent with the distribution of the southern subspecies *Emys marmorata pallida* from central California–Baja. Thus, recognition of the current subspecies split is not warranted, based on the available genetic evidence. Our AMOVA and phylogenetic results, in conjunction with a growing comparative database for other codistributed aquatic taxa, confirm the occurrence of genetic breaks across the Tehachapi Mountains and Transverse Range bounding the southern end of the Great Central Valley, and point to southern California as a rich source of cryptic genetic variation.

Keywords: *Actinemys*, *Clemmys*, control region, *Emys marmorata*, *GAPDH*, isolation by distance, *ND4*, partial Mantel test, R35 nuclear intron

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Introduction

The evolutionary history and dynamics of 'amphibious' taxa constitutes an important challenge to evolutionary genetics and landscape-level management. Unlike purely aquatic organisms (fishes, for example), amphibious taxa are linked to aquatic habitats, but can also traverse intervening terrestrial habitats. Thus, generating landscape-level predictions concerning population substructure is particularly difficult: Do river drainage catchments define the units of evolution, or is isolation by distance (IBD) across the terrestrial landscape a defining feature for such taxa? Do all semiaquatic species respond to the mix of aquatic habitats in the terrestrial matrix in the same ways, leading to similar patterns of differentiation and speciation? Particularly in arid terrestrial landscapes like western North America, understanding how these partly aquatic, partly terrestrial taxa interact with their patchy, often rare aquatic habitats is a critical element of their evolutionary

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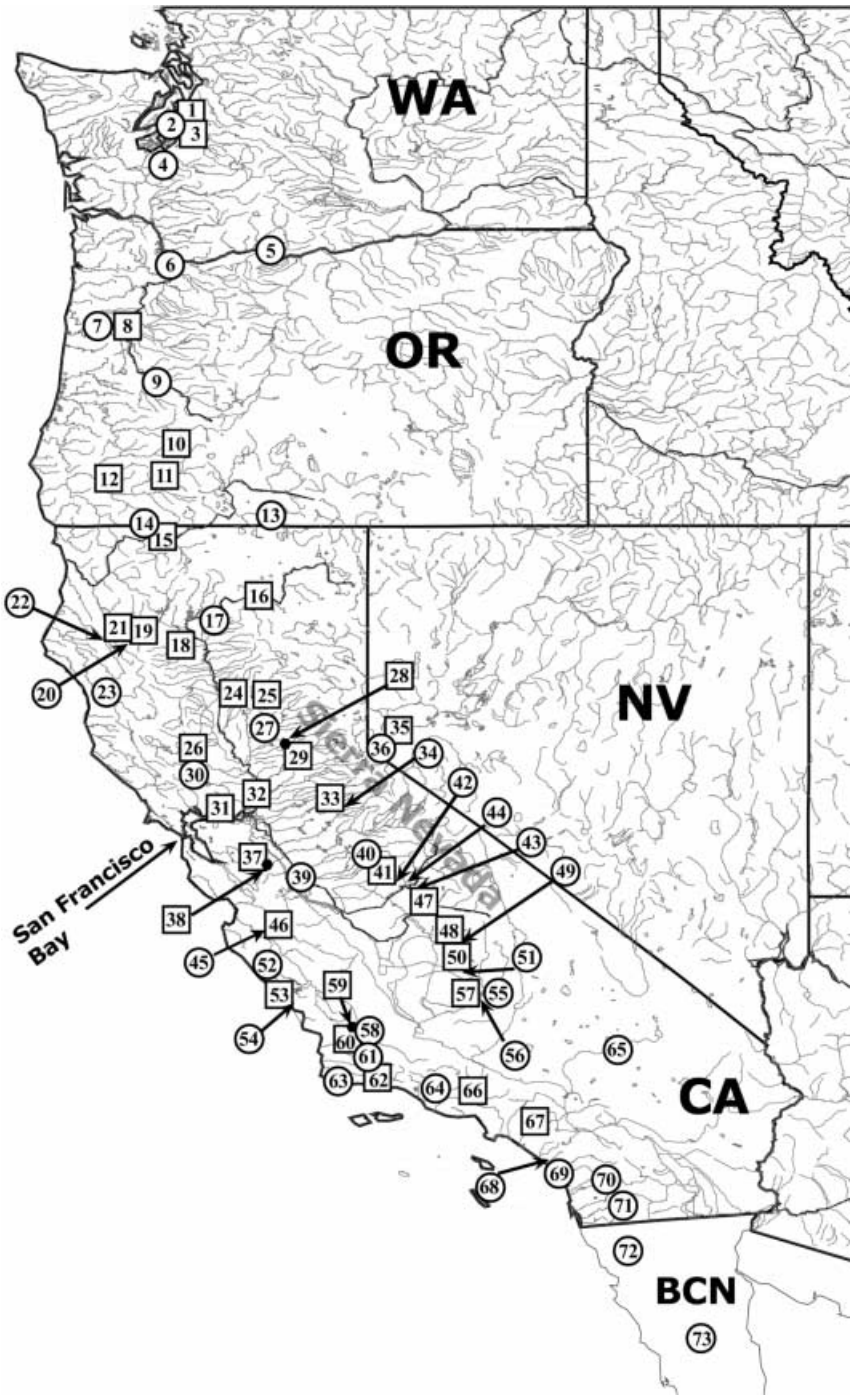


Fig. 1 Map showing major rivers of western North America and sample localities for *Emys marmorata*. In some instances, samples collected in close proximity to one another were combined into a single site. Circles indicate sites where both mtDNA and nDNA were collected, and squares indicate sites where mtDNA only was collected (Appendix). WA, Washington; OR, Oregon; NV, Nevada; CA, California; and BCN, Baja California Norte, Mexico.

history and a key component of their future management.

The western pond turtle (*Emys marmorata*) (formerly *Clemmys marmorata*: see Feldman & Parham 2002) is the only freshwater turtle that is restricted to western North America. The species is highly aquatic, inhabiting ponds, streams, rivers, and marshes (Holland 1991; Stebbins 2003) including some urban waterways (Germano & Bury 2001; Spinks *et al*. 2003). Deeper understanding of the importance of landscape-level aquatic habitat variation on population differentiation in *E. marmorata* is important in at least two regards. First, as the only native freshwater turtle over most of western North America, the origins and dispersal history of this isolated species constitute an important problem in both biogeography and landscape ecology. Second, *E. marmorata* has been a long-standing conservation concern. It was proposed (but rejected) for range-wide

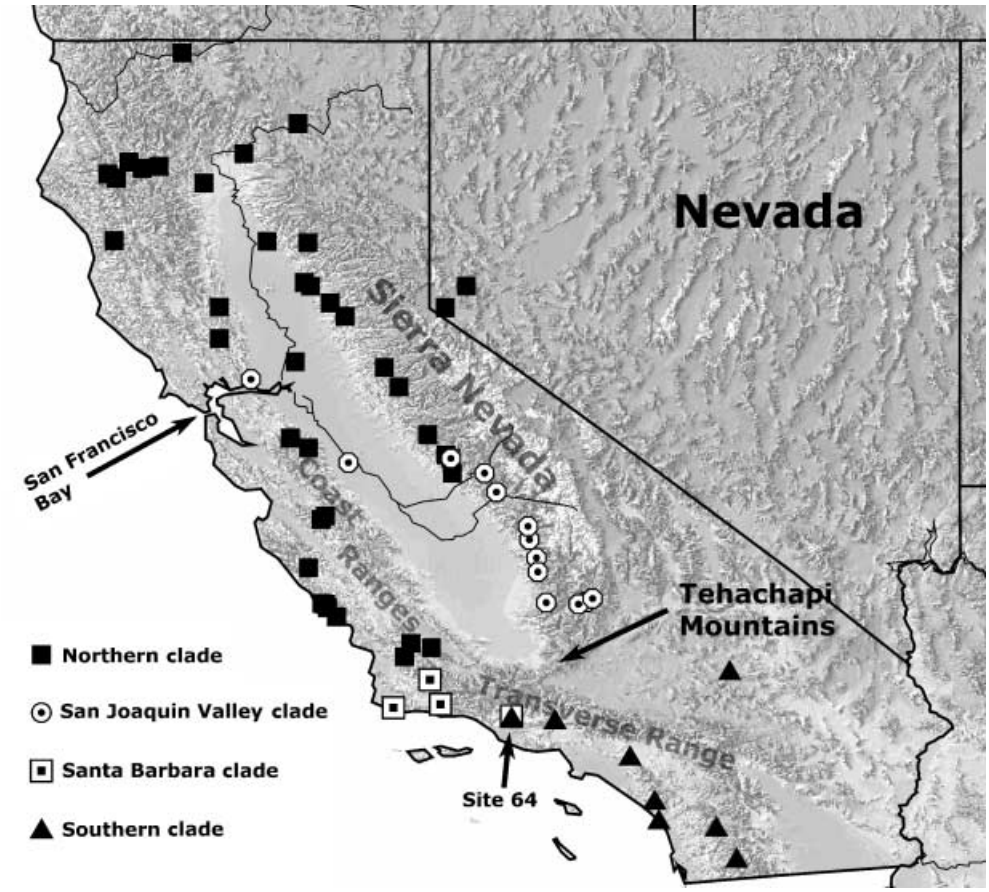


Fig. 2 Map showing major topographic features of California and distribution of mtDNA haplotypes of *Emys marmorata* determined from the phylogenetic analysis (Fig. 3). All samples from Oregon and Washington had Northern haplotypes whiles samples from BCN had Southern haplotypes. Site 64 (Santa Paula Creek, Ventura County) contained both Southern and Santa Barbara clade haplotypes.

listing under the US Endangered Species Act (US Fish and Wildlife Service 1992, 1993), and is currently afforded limited protection in Washington (State listed as endangered, Hays *et al*. 1999) and California (Species of Special Concern, http://www.dfg.ca.gov/hcpb/cgi-bin/read_one.asp?specy=reptiles&idNum=8). Given its wide geographical and ecological range (Figs 1 and 2), an analysis of genetic variation across the species has been called for as an important element of range-wide management and conservation (Gray 1995).

Taxonomically, *E. marmorata* is currently composed of two subspecies, the northwestern pond turtle (*Emys marmorata marmorata*) and the southwestern pond turtle (*Emys marmorata pallida*). *Emys marmorata marmorata* is distributed from Washington south through Oregon and northern California to the San Francisco Bay area, from the Pacific coast to the west slope of the Sierra/Cascade mountain crest. An isolated population east of the Sierra Nevada in extreme western Nevada has been suggested to be a human-mediated introduction (Cary 1887), although this has never been formally examined. *Emys marmorata pallida* is distributed from the San Francisco Bay area south to Baja California Norte (BCN), Mexico, including an isolated population in the Mojave Desert of southern California (Figs 1 and 2). The current interpretation is that the two subspecies have a large region of intergradation in the San Joaquin Valley and adjacent foothills of the Sierra Nevada of central California (Stebbins 2003; Appendix). The two subspecies have historically been recognized by two distinguishing morphological features; *E. m. marmorata* has dull neck markings and a pair of triangular inguinal plates, while *E. m. pallida* usually has lighter neck markings than *E. m. marmorata* and small or absent inguinal plates (Seeliger 1945; Stebbins 2003).

Previous genetic work within *E. marmorata* includes preliminary work with DNA fingerprinting (Gray 1995) and mitochondrial DNA (mtDNA) sequence variation (Janzen *et al*. 1997). Using DNA fingerprinting based on specimens from nine localities (six clustered in Washington and adjacent Oregon and three from southern California with an *c*. 1500 km gap in between), Gray (1995) concluded that gene flow is restricted between northern and southern populations

of *E. marmorata* and that there is a severe lack of genetic variability in northern populations. The preliminary mtDNA cytochrome *b* (cyt *b*) analysis of Janzen *et al*. (1997) incorporated broader sampling, including turtles from Baja California, central and northern California, and more extensive sampling from Oregon. Although Janzen *et al*. (1997) only examined a very short (180–307 bp) gene fragment, their data were consistent with previous morphological work (Seeliger 1945) in suggesting a north/south split over the range of the species. However, their cyt *b* data were relatively invariant, and only weak inferences could be made regarding variation among populations and regions.

Although detailed population sampling with appropriately variable genetic markers has yet to be performed, we can make some initial predictions as to how genetic variation might be structured within this species. *Emys marmorata* is a freshwater aquatic turtle. Thus, one reasonable expectation is that genetic diversity might be structured according to regional drainage patterns because this species occupies aquatic habitats in a relatively arid landscape. Gray (1995) found indications of among-drainage differentiation in Washington, and the importance of drainages in structuring differentiation within and between species has been emphasized in other aquatic turtles (Lamb *et al*. 1994; Georges & Adams 1996). However, *E. marmorata* also disperses widely across the terrestrial landscape (Holland 1994), and it may be that a pure isolation-by-distance (IBD) model best explains variation in the species. These are not mutually exclusive hypotheses, and both among drainage differentiation and IBD may contribute to species-level patterns of variation.

Our main goals in this study were to determine if there are distinct phylogenetic lineages within *E. marmorata*, and if so, whether this phylogeographical structure is congruent with regional drainage patterns. Our approach was to use phylogenetic trees, analysis of molecular variance (AMOVA), and IBD analyses based on rapidly evolving DNA sequence data and thorough range-wide sampling. Mitochondrial DNA has been the workhorse for phylogeographical analyses for over two decades (Avise 1998), and we base most of our conclusions on *ND4* and control region sequences. However, it is now widely recognized that mtDNA is essentially a single locus and therefore may provide a limited perspective on the evolutionary history of a species. In response, attention has now turned to the nuclear genome as an additional source of data for phylogeographical and population genetics analyses (Hare 2001; Brumfield *et al*. 2003; Zhang & Hewitt 2003; Ballard & Whitlock 2004; Morin *et al*. 2004). Accordingly, we complemented our mtDNA data set with *c*. 1 kb of nucleotide sequence data from two unlinked single-copy nuclear introns in an attempt to provide an additional nuclear-gene perspective on the evolutionary history of this species.

Materials and methods

Taxon and gene sampling

Dan Holland provided most of the tissue samples used in this analysis (Holland 1992). For tissues that we collected, turtles were captured by hand and in traps (see Spinks *et al*. 2003 for trapping methods). Our mtDNA sampling included 135 individuals from 73 localities distributed throughout the range of the species (Fig. 1, Appendix). Our Nevada samples are noteworthy because it is not known if the Nevada population of *Emys marmorata* is a disjunct relict or an introduced population (Holland 1991; Lovich & Meyer 2002). The European pond turtle (*Emys orbicularis*) and Blanding's turtle [*Emys* (formerly *Emydoidea*) *blandingii*] were included as outgroups because these species are the closest living relatives of *E. marmorata* (Bickham *et al*. 1996; Lenk *et al*. 1999; Holman & Fritz 2001; Feldman & Parham 2002; Stevens & Wiens 2003).

Cytochrome *b* is relatively invariant within *E. marmorata* (Janzen *et al*. 1997). Thus, we assessed nucleotide sequence variation within two relatively fast-evolving segments of mtDNA: the control region and the nicotinamide adenine dinucleotide dehydrogenase subunit 4 (*ND4*) gene. The control region is generally thought to be the most variable region of the mitochondrial genome, and it is more variable than *ND4* in the painted turtle, *Chrysemys picta* (Starkey *et al*. 2003). However, *ND4* is more variable than the control region in the common snapping turtle (*Chelydra serpentina*) (Shaffer *et al*., unpublished). Thus, we sequenced partial segments of both control region and *ND4* for a panel of seven individual turtles from across the range of the species to determine which gene was most variable in *E. marmorata*. Among these seven individuals, maximum sequence divergence for *ND4* was greater (3.1% uncorrected) than control region (1.9%) but mean sequence divergence was similar (*ND4* mean among all pairwise comparisons = 1.3%, control region mean = 0.95%). Because *ND4* and the control region were roughly equivalent in overall sequence divergence, we sequenced both gene segments for all individuals in our study.

For our nuclear DNA (nDNA) data, we included sequence data from a 452 bp fragment of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene (Friesen *et al*. 1997) and a 521 bp fragment of intron 1 of the fingerprint protein 35 (R35) (Friedel *et al*. 2001; Fujita *et al*. 2004) for a subset of 45 and 51 individuals, respectively (Appendix). We generally collected both nDNA sequences from the same individual, although occasionally we had to use two individuals from the same population (Appendix). For each of four mitochondrial clades (see next section) we sequenced representative turtles from across the geographical range of the clade; this also yielded nuclear gene coverage from across the range of the species.

Table 1 Primers for the control region, *ND4* gene and nuclear introns

Primer	Sequence (5′–3′)	Gene	Length	Source
DES-1	GCATTCATCTATTTTCCGTTAGCA	control region	629 bp	Starkey *et al*. (2003)
DES-2	GGATTTAGGGGTTTGACGAGAAT	control region	629 bp	Starkey *et al*. (2003)
Leu	CATTACTTTTACTTGGATTTGCACCA	*ND4* + $tRNA^{His}$	742 bp	Arevalo *et al*. (1994)
ND4672	TGACTACCAAAAGCTCATGTAGAAGC	*ND4* + $tRNA^{His}$	742 bp	Engstrom *et al*. (2002)
R35 Ex1	ACGATTCTCGCTGATTCTTGC	R35	521 bp	Fujita *et al*. (2004)
R35 Ex2	GCAGAAAACTGAATGTCTCAAAGG	R35	521 bp	Fujita *et al*. (2004)
GapdL890	ACCTTTAATGCGGGTGCTGGCATTGC	*GAPDH*	452 bp	Friesen *et al*. (1997)
GapdH950	CATCAAGTCCACAACACGGTTGCTGTA	*GAPDH*	452 bp	Friesen *et al*. (1997)

DNA extraction, amplification and sequencing

Tissue samples included blood, tail tips, liver, and skeletal muscle. Samples were frozen and maintained at –80 °C or stored in 95% ethanol at –20 °C. Genomic DNA was extracted from tissue using a standard salt extraction protocol (Sambrook & Russell 2001) and sequences were collected for the mitochondrial control region and *ND4* gene as well as the nuclear introns using 25 μL volume *Taq*-mediated polymerase chain reactions (PCRs) and primers listed in Table 1. Initial amplification conditions for mitochondrial genes were 2 min at 95 °C followed by 40 cycles of 0.75 min denaturing at 94 °C, 0.75 min annealing at 55 °C and 1.5 min extension at 72 °C. These same conditions were used for *GAPDH* and R35 except that the annealing temperature was increased to 61 °C. PCR products were sequenced on ABI 3100 or 3730 automated sequencers at the University of California, Davis, Division of Biological Sciences sequencing facility (http://dnaseq.ucdavis.edu/). The protein-coding *ND4* sequences were converted into amino acid sequences using GENEJOCKEY (Biosoft) to check for stop codons (none were found). The mtDNA sequence data were concatenated into single haplotypes for each individual. Because both the mitochondrial and nuclear data displayed relatively low levels of variation, alignments were made by eye in PAUP* version 4.0b10 (Swofford 2002). Gaps were coded as '–' and missing data were coded as '?'. GenBank Accession nos are AY904892–AY905262 (Appendix).

Analysis

Phylogenetic trees were estimated using maximum-parsimony (MP), maximum-likelihood (ML) and Bayesian inference. Maximum-parsimony and ML analyses were performed using PAUP*4.0b10 (Swofford 2002). For MP, 10 random-stepwise heuristic searches were performed with tree-bisection–reconnection (TBR) branch-swapping and default settings, except that searches were constrained to 10^6 rearrangements each. Statistical reliability of the resulting trees was assessed using nonparametric bootstrapping with 1000 pseudoreplicates (Felsenstein 1985). We consider bootstrap proportions ≥ 70% to be indicative of well-supported nodes (Hillis & Bull 1993), and those ≥ 95% to represent strongly supported nodes. Uninformative characters were excluded from calculations of consistency indices (CI) and retention indices (RI). Decay indices were calculated using AUTODECAY 4.0.2′ PPC (Eriksson 1998) and visualized using TREEVIEW version 1.5 (Page 1998). Maximum-likelihood searches employed SPR branch-swapping and model parameters estimated using MODELTEST version 3.06 PPC (Posada & Crandall 1998). Bayesian inference was performed using MRBAYES version 3.0b4 (Huelsenbeck & Ronquist 2001) with the sequence data divided into four partitions: three for each codon position of the protein coding *ND4* gene and one for the control region. The nuclear data were excluded from these as well as the population genetic analyses (see below). We ran three independent analyses each with four chains and each with 10^7 generations, saving the current tree every 10^3 generations. –ln *L* scores were plotted against generations and –ln *L* scores obtained prior to the chains reaching stationarity were discarded as burn-in.

To test the importance of regional drainage systems in structuring populations, we ran two separate AMOVAS including a 'drainage' analysis and an 'alternate' analysis. For the drainage analysis, we defined 12 a priori units (hereafter referred to as 'drainages') based on regional hydrology or a combination of hydrology and geographical barriers, and partitioned our samples among those 12 units. In the alternate analysis, we again defined 12 units, but these units were defined to span, rather than be contained within, the 12 drainages (Appendix, see next discussion). Thus, the drainage AMOVA should provide a quantitative assessment of the fraction of the genetic variation attributable to river catchments, whereas the alternate AMOVA provides similar information for the same 12 geographical regions, but for units that span river catchments. For both the drainage AMOVA and alternate AMOVA, we did not impose any structure among or within units.

For the drainage AMOVA, drainages from Washington south to about central California were based on regional hydrology while those from about central California south (including the Nevada population) were mostly determined by geographical barriers (Appendix, Figs 1 and 2). At the northern limit of the species' range, the Puget Sound drainage contained five samples collected from four sites that ultimately drain into the Puget Sound in northern Washington. The Columbia River drainage contained 19 samples collected from two sites along the Columbia River on the Washington/Oregon border, and three sites from the Williamette River drainage (northern Oregon) which empties into the Columbia River near Portland, Oregon. The North Coast drainage of southern Oregon/northwestern California contained 17 samples from 12 sites all of which flow westwards into the Pacific Ocean. The Carson River drainage on the eastern side of the Sierra Nevada crest contained six samples from two sites along the Carson River in southwestern Nevada. The Sacramento Valley drainage in north-central California contained 17 samples from eight sites which ultimately drain into the northern San Francisco Bay. The Napa Valley drainage in west-central California contained six samples from two sites. These sites also drain into the northern San Francisco Bay, but are in a different hydrologic unit than the Sacramento Valley drainage to the east. The Monterey drainage in central-coastal California contained four samples collected from four different sites. One site drains into the southern San Francisco Bay while the remaining three drain into Monterey Bay. Samples from the southern half of the Great Central Valley (the San Joaquin Valley) were divided into two drainages including the San Joaquin Basin (San Francisco Bay area south to northern Fresno County) and the Tulare Basin (northern Fresno County south to the Tehachapi Mountains). Geographic barriers do not separate the San Joaquin and Tulare basins, but each is a distinct hydrologic unit (Gronberg *et al*. 1998). The San Joaquin Basin drainage (13 samples, nine sites) drains northwards into the San Francisco Bay while the Tulare Basin drainage (15 samples, eight sites) terminates in the Tulare Lake Bed. The San Joaquin Basin sites are separated from the Monterey sites by the intervening Coast Ranges, and the Tulare Basin sites in the southernmost San Joaquin Valley are separated from more southerly sites by the Tehachapi Mountains. The coastal California and Mexico sites from San Luis Obispo (SLO) County south to Baja California all drain westward into the Pacific Ocean. We divided these sites across the Santa Ynez (SY) Mountains such that samples from the Santa Ynez Mountains north to San Luis Obispo County were allocated to the SLO-SY drainage (12 samples, eight sites) while samples from the Santa Ynez Mountains south to Baja were allocated to the SY-BCN drainage (17 samples, nine sites). Finally, the Mojave River drainage in inland southern California contained three samples from the Mojave River which terminates in the Mojave Desert. The Mojave drainage is separated from northerly sites by the Tehachapi Mountains and from westerly sites by the Transverse Ranges (Figs 1 and 2). One sample (HBS39774) was excluded from population genetic analysis because it was collected at the confluence of the Sacramento and San Joaquin river drainages and therefore could not be assigned to either drainage (Appendix).

For the alternate AMOVA, we partitioned samples across our a priori drainage units in order to assess the potential impacts of the landscape in structuring and maintaining genetic diversity within this species. We took roughly half of the samples from adjacent sides of a pair of drainages and created a new unit that spanned drainages or hypothetical geographical barriers. For example, we combined half of the Puget Sound drainage with half of the Columbia River drainage to create a new unit, and combined the remaining Columbia River samples with some north Coast drainage samples. Our goal was to create 12 new, geographically contiguous units that were roughly equal to our drainage units in geographical size, proximity, and sample sizes. If the among-drainage F_{ST} values were larger than the alternate F_{ST}'s, then this should indicate that at least some of our hydrologic units or geographical barriers play a role in determining the genetic structure within this turtle species. If there is no difference, then we conclude that the 'among-drainage' F_{ST}'s reflect general geographical isolation rather than isolation among hydrological basins. We recognize that this is not a strict statistical test of alternative hypotheses, but instead use it as a heuristic tool for examining our a priori biogeographical arrangements. Estimates of population variation and other descriptive statistics within and among drainages were calculated using ARLEQUIN version 2.0 (Schneider *et al*. 2000) with uncorrected pairwise sequence distances and 10^4 permutations.

To determine whether variation among drainages was pure IBD, pure among-drainage variation, or a combination of the two, we estimated the correlation between mtDNA sequence divergence and geographical distance with the program IBD version 1.5 (Bohonak 2002) using log-transformed genetic and log-transformed geographical distances (Slatkin 1993). We used the latitude and longitude coordinates of the point-of-capture for each individual specimen as input data, allowing us to calculate isolation by distance (IBD) using both partial and full Mantel tests with 10^4 randomizations (Bohonak 2002). We present P values both for uncorrected and for sequential Bonferroni corrected values (Rice 1989). In interpreting these values, we make no direct inferences concerning the number of migrants exchanged among populations (Slatkin 1993; Hellberg 1994). Rather, we treat them as descriptive tools to gain insights into the role of IBD in shaping genetic differentiation.

Results

mtDNA sequence variation

We collected up to 1372 bp of mtDNA sequence data including 672 bp of *ND4*, 70 bp of the flanking $tRNA^{His}$ (hereafter collectively referred to as *ND4*) and 630 bp of the control region for 135 *Emys marmorata* and the two outgroups. Within the mtDNA ingroup data set, no insertions or deletions (indels) were detected but there was one indel between the ingroup and outgroups. Of the 137 concatenated mtDNA sequences, 54 were identical and were excluded from phylogenetic analyses (Appendix) leaving 83 unique sequences (81 *E. marmorata* and the two outgroups) in the mtDNA-only data set. Of the 1372 characters, 1180 were invariant and 83 were parsimony informative. Within the *ND4* ingroup data 666 characters were constant while 41 were parsimony informative (maximum uncorrected pairwise sequence divergence was 3.23%). Variation within the control region ingroup data was similar; 588 characters were constant while 20 were parsimony informative (maximum uncorrected pairwise sequence divergence was 3.34%).

Visual inspection of the sequence chromatograms indicated that four individuals displayed sequence heterogeneity (C/G) for a nonsynonomous substitution at position 278 of the *ND4* sequences (Appendix). These samples were re-extracted and re-amplified for *ND4*. The resulting PCR products plus PCR products derived from individuals that displayed sequence homozygosity (G) at position 278 were then subjected to a restriction digest using *StyD4*I (New England BioLabs), an enzyme which would cleave the suspected heterozygous DNA but not the homozygous DNA. The gel-banding patterns confirmed that all four individuals were heterozygous with a single nucleotide polymorphism (SNP) at site 278, while the controls were homozygous. We are currently working to determine if this SNP is the result of heteroplasmy in the mitochondrial genome or if it is a nuclear mitochondrial pseudogene (numt). For our analyses, the SNP was coded as 'S' (C/G) and included in the data set since excluding this character had very minor effects on our results. In all other respects, our mtDNA behaved like typical mtDNA. Nucleotide composition was A-T biased (63.4%), and the coding region reading frame was conserved. In addition, 28% of sites at the third codon position were variable compared to first and second codon positions (12% and 4.5%, respectively) reflecting the greater variation at first and third codon positions typical of coding sequences. Thus, we are confident our analyses were based on authentic mtDNA.

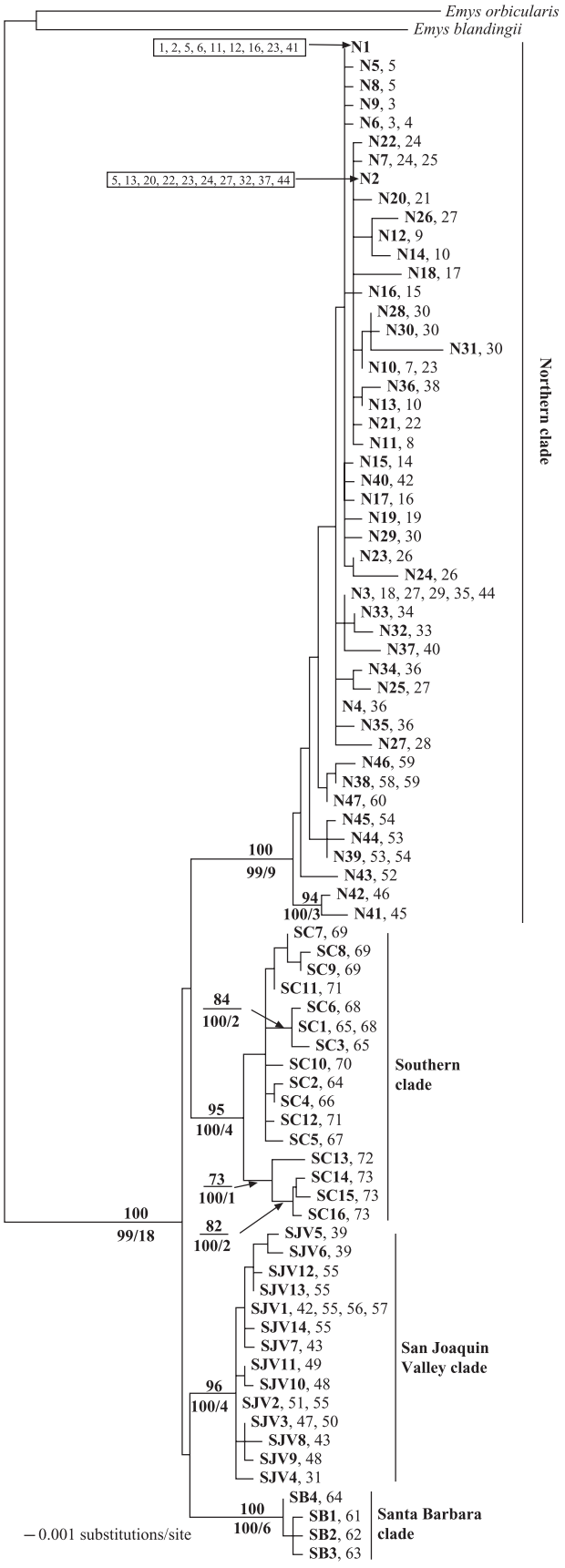
mtDNA phylogenetic analysis

Maximum-parsimony analysis of the concatenated 1372 bp data set recovered 1981 trees (length = 276 steps, CI = 0.549, RI = 0.917). Maximum-likelihood model parameters conform to the TVM + I + Γ model of nucleotide sequence evolution. ML analysis recovered 124 equally likely trees, and the lowest –log-likelihood (–ln *L*) score = 3745.1844. In all three Bayesian analyses, –ln *L* scores reached stationarity at or prior to 117 000 generations, and trees saved prior to stationarity were discarded as burn-in. Figure 3 shows the ML tree with decay indices, MP bootstrap proportions and Bayesian posterior probabilities (BPP).

ML, MP and Bayesian analyses all recovered the same set of strongly supported (MP bootstraps ≥ 95%, BPP = 100, DI ≥ 1), largely allopatric clades (Fig. 3). Geographically, these clades fall into: (i) a Northern clade composed of populations from San Luis Obispo County and San Benito County, California, north to Washington and including the Nevada population; (ii) a San Joaquin Valley clade including populations east of the Coast Ranges and west of the Sierra Nevada from the lower Central Valley of California south to the Tehachapi Mountains; (iii) a Santa Barbara clade including populations from the Santa Ynez River and Santa Paula Creek (a tributary of the Santa Clara River; Ventura County); and (iv) a Southern clade which includes sequences from the Santa Paula Creek site as well as populations from the southern slope of the Santa Ynez/Tehachapi Mountains and Transverse Ranges southward through the Mojave Desert to BCN (Fig. 2). There was limited geographical overlap between the Northern and San Joaquin Valley clades in the mid-Central Valley of California, and between the Santa Barbara and Southern clades at site 64 in Ventura County, California (Fig. 2, Appendix).

Nuclear data

We generated 973 bp of nDNA sequence data from the two introns including 452 bp from *GAPDH* (397 bp of intron XI and 55 bp of the flanking exons) for 45 individuals, and 521 bp from intron 1 of the R35 fingerprint protein for 51 individuals. We collected nDNA sequence data for 35 turtles from the Northern clade, 9 from the San Joaquin Valley clade, 3 from the Santa Barbara clade, and 6 from the Southern clade. There was relatively little variation in either intron. For *GAPDH*, 34 of 45 individuals were identical, and 450 of 452 characters were invariant (one parsimony informative), yielding four unique haplotypes including one individual with a 7 bp deletion. Results were much the same for the R35 data where 41 of 51 individuals were identical. Of the 521 characters, 517 were invariant (one parsimony informative), yielding five unique haplotypes for this locus (Appendix). Because our nuclear sequences showed so little variation, we excluded them from further quantitative analyses, although they do contribute some qualitative information to our analysis.



Population genetics: drainage vs. alternate AMOVAS

In the drainage vs. alternate AMOVA comparison, the geographical proximity of samples was largely maintained, but the samples were reorganized to test for an explicit among-drainage component of variation. The proportion of among population variation (V_a) was about 20% greater in the drainage AMOVA where samples were organized according to our a priori drainages (68.54%) than in the alternate AMOVA where samples were arranged to span hydrologic units/geographical barriers (57.74%). Consistent with this, the average pairwise F_{ST} values among all 12 units decreased from 0.50 in the drainage AMOVA (Table 2) to 0.40 in the alternate AMOVA (Table 3).

Drainage amova

Pairwise F_{ST} values among the 12 drainages ranged from a nonsignificant –0.04 (Sacramento Valley–Carson River) to a highly significant 0.982 (Columbia River–Mojave River), and show a clear pattern of differentiation along a north–south geographical gradient (Table 2). For example, pairwise F_{ST} values among the five northernmost drainages (Sacramento Valley north to Washington and including the Carson River, Nevada) were generally low and nonsignificant. The single exception to this pattern may be the Puget Sound drainage, which displayed a significant pairwise F_{ST} with both the Columbia River and North Coast comparisons at the $\alpha = 0.05$ level only (Table 2). Pairwise comparisons between the northern drainages (groups 1–5) and southern drainages (groups 6–12) were mostly significant, with 33/35 comparisons significant at $\alpha = 0.05$ and 20/35 comparisons significant after sequential Bonferonni correction ($\alpha = 0.00076$). Among southern groups, all comparison except three involving the Mojave and Monterey drainages were significant at the $\alpha = 0.05$ level (18/21 comparisons), while 9/21 comparisons (those involving the Tulare Basin and SY-BCN drainages) were significant at the $\alpha = 0.00076$ level (Table 2). However, inferences about the Mojave River and Monterey drainages suffer from

Fig. 3 One of 124 equally likely trees from the ML analysis. This reconstruction is based on 81 unique *Emys marmorata* and two outgroup mtDNA haplotypes (1372 bp). –ln L = 3735. 1957. Estimated ML parameters conform to the TVM + I + Γ model of sequence evolution. Rate matrix: A-C = 0.7057, A-G = 3.3094, A-T = 0.4302, C-G = 0.9509, C-T = 3.3094 and G-T = 1. Proportion of invariable sites (I) = 0.6541, Γ = 0.9564. Base frequencies are: A = 0.33, C = 0.24, G = 0.13, T = 0.30. Numbers above branches are MP bootstrap proportions. Numbers below branches are Bayesian posterior probabilities/decay indices. Terminal names are haplotype in bold followed by collection locality (site) numbers where that haplotype was found (Fig. 1, Appendix). In several instances the same haplotype was recovered from multiple sites. For example, haplotype N1 was recovered from nine different sites.

Table 2 Matrix of pairwise F_{ST} values for the drainage AMOVA. * indicates comparisons that were significant at the $\alpha = 0.05$ level and those in bold were significant after sequential Bonferroni correction ($\alpha = 0.00076$)

	Puget Sound	Columbia River	North Coast	Carson River	Sacramento Valley	Napa Valley	Monterey	San Joaquin Basin	SLO-SY	Tulare Basin	Mojave River
Columbia River	0.2265*										
North Coast	0.1255*	0.0086									
Carson River	0.0734	0.0662	−0.0024								
Sacramento Valley	0.0181	0.0133	−0.0019	−0.0431							
Napa Valley	0.1987*	**0.4233***	**0.3514***	0.1086	**0.2519***						
Monterey	0.4764*	0.7094*	0.6235*	0.4567*	0.4816*	0.3391*					
San Joaquin Basin	0.1580	**0.3624***	**0.3321***	0.1974*	**0.3058***	0.2178*	0.1549				
SLO-SY	0.2418*	**0.4623***	**0.4277***	0.2842*	**0.3967***	0.3002*	0.1057	0.1658*			
Tulare Basin	**0.9245***	**0.9528***	**0.9413***	**0.9244***	**0.9166***	**0.8812***	**0.8907***	**0.5179***	**0.7072***		
Mojave River	0.9629*	**0.9822***	0.9679*	0.9544*	0.9317*	0.8508*	0.8896*	0.5589*	0.6457*	0.9014*	
SY-BCN	**0.7637***	**0.8512***	**0.8364***	**0.7767***	**0.8170***	**0.7483***	**0.7172***	**0.5410***	**0.6124***	**0.7067***	0.1705

SLO, San Luis Obispo County; SY, Santa Ynez Mountains; BCN, Baja California Norte, Mexico.

Table 3 Matrix of pairwise F_{ST} values for the alternate AMOVA. * indicates comparisons that were significant at the $\alpha = 0.05$ level and those in bold were significant after sequential Bonferroni correction ($\alpha = 0.00076$)

	1	2	3	4	5	6	7	8	9	10	11
1. Puget Sound/Columbia River											
2. Columbia River/North Coast	0.0571										
3. North Coast/Sacramento Valley	0.0289	0.0165									
4. Carson River/Sacramento Valley	0.2251*	0.1045	0.0793								
5. North Coast/Napa Valley	**0.1076***	0.0213	0.0410	−0.0755							
6. Sacramento Valley/Napa Valley	0.0457*	−0.0102	0.0083	0.0034	0.0127						
7. Monterey/San Joaquin Basin	**0.3388***	0.2220*	0.2293*	0.1254	**0.2083***	**0.2306***					
8. San Joaquin Basin/SLO-SY	**0.5005***	**0.3758***	**0.3688***	0.2567*	**0.3324***	**0.3726***	−0.0157				
9. SLO-SY/Tulare Basin	**0.5923***	0.4773*	**0.4758***	0.3771*	**0.4567***	**0.4836***	0.0691	0.0684			
10. SLO-SY/SY-BCN	**0.8728***	**0.8035***	**0.7947***	0.7103*	**0.7528***	**0.7936***	**0.4490***	0.4662*	0.3473*		
11. Tulare Basin/SY-BCN	**0.8066***	**0.7458***	**0.7430***	**0.6904***	**0.7229***	**0.7455***	**0.439***	**0.4647***	0.2784*	0.3392*	
12. Mojave River/SY-BCN	**0.8982***	**0.8541***	**0.8463***	**0.8001***	**0.8091***	**0.8434***	**0.5776***	**0.6111***	**0.5106***	0.4642*	0.3101*

small sample sizes ($N = 3$ and $N = 4$, respectively). Pairwise F_{ST} values for the all but one of the Mojave River comparisons were extremely high (average $F_{ST} = 0.8646$), but only one comparison (Columbia River) was significant at the $\alpha = 0.00076$ level. Likewise, pairwise F_{ST} values from two Monterey drainage comparisons were relatively high (0.6235 and 0.7094) but nonsignificant at the $\alpha = 0.00076$ level (Table 2). We view these results as potentially indicative of population subdivision, but more sampling is necessary before any firm conclusions can be drawn. Nucleotide diversity was also lowest in the northern drainages and highest in the San Joaquin Basin and SLO-SY coastal drainages (Table 4).

Insights from allelic distributions

Of the 81 unique mtDNA haplotypes, two were fairly widespread over the northern portion of the range. Haplotype N1 was fixed among 18 individuals sequenced from nine sites from central California (Madera County) north through Oregon to Puget Sound, Washington, while haplotype N2 was fixed among 14 individuals sequenced from 10 sites from Madera County in central California north to southern Washington (Klickitat County) (Appendix). This mitochondrial pattern of genetic uniformity in the north was mirrored in the R35 nuclear gene data. One of the five unique R35 haplotypes (R1) was recovered from 34 sites encompassing the entire range except BCN, and was fixed for all turtles sampled from the northern mtDNA group (Appendix). The remaining R35 haplotype diversity (three additional R35 haplotypes) was concentrated in the San Joaquin Valley samples (San Joaquin Basin + Tulare Basin), with limited nuclear diversity scattered across the BCN, Mojave River, San Diego County and southern Monterey County sites (Appendix). *GAPDH* was less variable, and showed slightly more variation across the entire range;

Table 4 Estimates of nucleotide diversity for 12 drainages

Puget Sound	=	0.0007
Columbia River	=	0.0004
North Coast	=	0.0009
Carson River	=	0.0013
Sacramento Valley	=	0.0015
Napa Valley	=	0.005
Monterey	=	0.007
San Joaquin Basin	=	0.009
SLO-SY Coast	=	0.009
Tulare Basin	=	0.001
Mojave River	=	0.001
SY-BCN Coast	=	0.005

SLO, San Luis Obispo; SY, Santa Ynez Mountains; BCN, Baja California Norte, Mexico.

otherwise, a few rare SNPs were distributed haphazardly across individuals (Appendix).

Isolation by distance

To gain a clearer picture of the role of pure IBD in shaping genetic differentiation, we conducted a series of IBD analyses based on the mtDNA. First, we used individual observations within drainages, based on straightline 'as the crow flies' distances between points calculated in the program R 4.0 (available at http://www.fas.umontreal.ca/BIOL/Casgrain/en/labo/R/v4/progress.html) to quantify the relationship between genetic and geographical distance within drainages for which we had more than 10 sequences. We also conducted partial Mantel tests for all pairwise comparisons among drainages with significant (uncorrected) F_{ST} values using three input matrices; the genetic distance between individuals, the straightline geographical distance between individuals, and an indicator (0,1) matrix that identifies the drainage of each individual. The genetic–geographical distance correcting for indicator drainage variable (GG/I) partial Mantel test then tests for IBD alone, while the genetic-indicator test correcting for geography (GI/G) provides a test of differentiation among drainages corrected for IBD. Because we were not provided with precise locality information for the Puget Sound and Columbia River individuals, these drainages were excluded from all IBD analyses (Appendix).

Within drainages, we generally found a significant association of genetic and geographical distance (Table 5), although most of our population estimates are based on the more southerly drainages for which we have larger sample sizes. In pairwise drainage comparisons, IBD corrected for drainages (GG/I, Table 5) was significant for 27/38 of the uncorrected pairwise comparisons, and 16/38 of the Bonferroni-corrected ($\alpha = 0.0013$) comparisons, suggesting that IBD is a significant component of variation contributing to many pairwise F_{ST} values. However, there was an even stronger signal of among-drainage differentiation corrected for IBD, where 33/38 of the uncorrected pairwise comparisons, and 20/38 of the Bonferonni-corrected pairwise comparisons were significant. This pattern was particularly strong for comparisons involving the Tulare Basin and SY-BCN populations, where every GI/G comparison (except the Mojave River/SY-BCN comparison) was highly significant (Table 5).

Discussion

Phylogeography and systematics

Our results are somewhat concordant with those from previous genetic research, and with Seeliger's (1945) subspecies descriptions. Like Gray (1995) and Janzen *et al*. (1997) we found relatively low levels of genetic variation within *Emys marmorata*. Almost all Washington, Oregon, Nevada and northern California populations were not significantly different from one another in the AMOVA (Table 2) and these populations were also recovered as a single clade in the phylogenetic analyses (Fig. 3). Nucleotide diversity was lowest in the northernmost drainages and highest in the San Joaquin Basin and SLO-SY coastal drainages (Table 4). Based on these results, *E. marmorata* may have colonized the northern part of their current distribution sometime after the last glacial maxima about 20 000 years BP (Guyton 1998), possibly from the relatively diverse Sacramento River watershed. This pattern of an expansive northern clade with little genetic diversity has been recovered from both the dusky-footed woodrat (*Neotoma fuscipes*, Matocq 2002) and the California mountain kingsnake (*Lampropeltis zonata*, Rodríguez-Robles *et al*. 1999), and in both instances the authors proposed a recent northward expansion to account for the lack of genetic diversity among northern populations.

The currently recognized subspecies split in *E. marmorata* is also somewhat congruent with our results, but does not adequately reflect major genetic subdivisions within the species. In the phylogenetic analyses, populations from Washington to northern California were recovered in the Northern clade, a group that is virtually coincident with the range of *Emys marmorata marmorata* (Fig. 2, Appendix). The only difference between their respective ranges is that our Northern clade extended south along the Coast Ranges to San Luis Obispo County, while the range of *E. m. marmorata* terminates about 378 km to the north, near San Francisco. Populations throughout most of the intergrade zone recognized by Seeliger (1945) comprise the phylogenetically distinct San Joaquin Valley clade based on mtDNA, rather than a mixture of northern and southern haplotypes as might be expected from the subspecies descriptions. Populations within the range of *Emys marmorata pallida* fell

Table 5 Matrix of *r* (top cell entry) and *P* (bottom cell entry) values from IBD partial Mantel tests of comparisons that were significant in the F_{ST} analysis (Table 2). Above diagonal is the correlation of genetic/geographic distance correcting for indicator drainage variable (GG/I) while cells below the diagonal show the genetic-indicator drainage variable test correcting for geography (GI/G). Diagonals are *r* (top cell entry) and *P* (bottom cell entry) values from intrapopulation IBD analyses ($\alpha = 0.05$). Values in bold are significant after Bonferroni correction ($\alpha = 0.0013$), and ns, not significant in the drainage AMOVA

	North Coast	Carson River	Sacramento Valley	Napa Valley	Monterey	San Joaquin Basin	SLO-SY	Tulare Basin	Mojave River	SY-BCN
North Coast	0.1353 0.0881	ns	ns	–0.0979 0.8458	0.1132 0.1021	0.0353 0.2583	0.1516 0.0099	**0.1709** **0.0007**	0.1454 0.0601	**0.2338** **0.0001**
Carson River	ns	—	ns	ns	0.2272 0.1815	0.3050 0.0095	**0.4126** **0.0007**	0.1657 0.0433	–0.2672 0.9641	**0.5416** **0.0001**
Sacramento Valley	ns	ns	0.2140 0.0247	0.0513 0.2624	0.1969 0.0159	0.1274 0.0107	**0.2427** **0.0005**	**0.2271** **0.0001**	0.2315 0.0075	**0.3315** **0.0001**
Napa Valley	0.4659 0.0015	ns	0.3579 0.0060	—	0.1418 0.1891	0.1410 0.0770	0.2617 0.0117	–0.0280 0.5928	0.2468 0.1086	**0.4378** **0.0001**
Monterey	0.3983 0.0115	0.2643 0.0351	0.2947 0.0332	0.1946 0.0568	—	ns	ns	0.2474 0.0023	**0.8316** **0.0001**	**0.5548** **0.0001**
San Joaquin Basin	0.1625 0.0081	–0.1065 0.8048	0.1035 0.0259	0.0339 0.3152	ns	0.1818 0.0649	**0.2774** **0.0003**	0.1675 0.0021	0.3318 0.0020	**0.3685** **0.0001**
SLO-SY	**0.4336** **0.0001**	0.0145 0.3991	**0.3308** **0.0001**	0.1708 0.0763	ns	0.1269 0.0197	0.4544 0.0052	**0.3064** **0.0001**	**0.4971** **0.0004**	**0.4649** **0.0001**
Tulare Basin	**0.8535** **0.0001**	**0.8292** **0.0001**	**0.8159** **0.0001**	**0.8355** **0.0002**	**0.8069** **0.0003**	**0.3236** **0.0001**	**0.6807** **0.0001**	0.2355 0.0199	0.2401 0.0112	**0.3909** **0.0001**
Mojave River	**0.8011** **0.0013**	0.7720 0.0075	**0.7129** **0.0006**	0.5926 0.0063	0.5147 0.0112	0.3245 0.0017	0.3803 0.0050	**0.7871** **0.0010**	—	ns
SY-BCN	**0.7287** **0.0001**	**0.6231** **0.0001**	**0.7068** **0.0001**	**0.7187** **0.0001**	**0.6812** **0.0003**	**0.5335** **0.0001**	**0.5920** **0.0001**	**0.6556** **0.0001**	ns	0.5561 0.0001

into the Santa Barbara and Southern clades, with limited geographical overlap in Ventura County, California (site 64, Fig. 2). We found no support for the monophyly of the combined Santa Barbara + Southern clades, and thus no support for the recognition of *pallida* as a taxonomic unit. The AMOVA and pairwise F_{ST} results also indicated that considerable subdivision exists within *E. m. pallida* but not *E. m. marmorata* (Table 2).

Taken together, our data are consistent with the interpretation that four phylogenetic taxa may exist within what is currently recognized as *E. marmorata*. Of these four, three were identified previously based on morphological grounds (Seeliger 1945) either as subspecies or as a zone of intergradation, suggesting that they are not artefacts of mtDNA gene trees. In addition, the San Joaquin Valley and Santa Barbara clades have coincident distributions with deep mitochondrial divisions for the California tiger salamander (*Ambystoma californiense*) from the same landscape (Shaffer *et al*. 2004a). Codistributed clades from different taxa provide further evidence that these gene tree lineages represent true organismal lineages maintained by common historical causes; likely cases include the mountainous topography of central and southern California, and the history of marine embayments of the southern San Joaquin Valley (discussed below). Although these data are consistent with the interpretation of four phylogenetic species contained within *E. marmorata*, we prefer to wait for additional nuclear data before reaching a final determination of the number of species and their geographical distribution in the western pond turtle species complex.

Our partial Mantel tests provide insights into the role of drainages as a feature structuring genetic subdivision in *E. marmorata*. AMOVA and pairwise F_{ST} results clearly indicate that among-drainage differentiation is often large and significant, particularly for southern drainages. Based on partial Mantel tests, drainages appear to be a highly significant factor structuring these populations, even after correcting for straight-line IBD (the GI/*G* tests in Table 5). Even between adjacent drainages, this effect is sometimes extremely strong, particularly on the xeric landscape of southern California. For example, all comparisons involving the Tulare Basin in the southern San Joaquin Valley are highly significant, including comparisons with the San Joaquin Basin immediately to its north (GI/G $r = 0.32$,

$P = 0.0001$). Similarly, all comparisons involving the Mojave River (uncorrected only) are significant, as are all but one involving coastal populations from the Santa Ynez Mountains south to Baja California (corrected and uncorrected values, Table 5). Thus, it appears that drainages often are important components of population subdivision, even in the face of potential overland migration in these animals.

The Great Central Valley

The geographical subdivision of *E. marmorata* in the Great Central Valley of California contributes to our growing understanding of this critically important hydrological region of the American west. Two features of the pattern seen in *E. marmorata* merit discussion: the east-to-west differentiation of turtle populations in the southern San Joaquin Valley, and the precise placement of the contact zone on the eastern side of the Great Central Valley.

The phylogenetic analysis indicated a sharp genetic break between turtle populations on the east and west sides of the San Joaquin Valley (Fig. 2). The history of marine inundation of the San Joaquin Valley (Dupré 1990), including a large, north–south orientated embayment 0.6–0.72 million years ago (Ma) (Dupré *et al*. 1991) is consistent with the observed genetic differentiation on the east and west side of the San Joaquin Valley that we found in *E. marmorata*. A similar pattern has been observed in *A. californiense* (Shaffer *et al*. 2004a), *N. fuscipes* (Matocq 2002) and *L. zonata* (Rodríguez-Robles *et al*. 1999). This consistent east–west split implies that the marine embayment may have been a common cause (e.g. Avise 1998) for this diverse array of species, and suggests that other taxa inhabiting the southern San Joaquin Valley may harbour similar cryptic genetic variation.

Interestingly, the contact zone between the Northern and San Joaquin Valley clades on the eastern side of the Great Central Valley is not at the confluence of the Sacramento and San Joaquin rivers in the San Francisco Bay delta, as one might predict for these aquatic turtles. Rather, the break is at the Fresno River (site 42, Appendix), a tributary of the San Joaquin River. This is precisely coincident with a similarly deep phylogeographical break in *A. californiense* (Shaffer *et al*. 2004a), a vernal pool specialist that breeds in seasonal aquatic habitats (Shaffer & Trenham in press). Although the Pleistocene history of California's Great Central Valley (reviewed in Shaffer *et al*. 2004a) is reasonably well known, there are no known current or past barriers to gene flow in this region of the San Joaquin Valley. However, this precise concordance for two aquatic species implies that the break at the Fresno River is not an artefact of the coalescent process (Irwin 2002), but rather reflects a real historical break in organismal gene flow.

Southern California

The deep split that we found between San Joaquin Valley populations and those from southern California south to BCN (Fig. 3, Table 2) implies that the Tehachapi Mountains/Transverse ranges are important barriers for *E. marmorata* in southern California. Recent research on several California taxa has demonstrated a similar pattern, suggesting that the Transverse Ranges are one of the major phylogeographical boundaries along the Pacific coast of North America (Calsbeek *et al*. 2003).

The genetic isolation of the Santa Barbara clade has only been suggested for two other species. In the California tiger salamander, a distinct mitochondrial clade exists in Santa Barbara County (Shaffer *et al*. 2004a) that is codistributed with the Santa Barbara clade of *E. marmorata*. The California red-legged frog (*Rana draytonii*) has a range through southern California and northern Baja California, Mexico, that is virtually identical to *E. marmorata*. Although most southern California populations of the frog are now extinct, recent molecular studies have demonstrated that the remaining southern populations in Riverside County, California and BCN form a distinct mtDNA clade from coastal populations to the north in Santa Barbara and Ventura counties (Shaffer *et al*. 2004b). Although no Santa Barbara clade has been found in *R. draytonii*, the meeting point of the southern California/Baja and more northern clades near the Ventura/Los Angeles county line is only a few kilometres from the meeting point of the *E. marmorata* Southern and Santa Barbara clades at site 64 (Fig. 2). Once again, this geographical concordance in clade boundaries implies a common historical cause in the region.

Thus, the Tehachapi Mountains/Transverse Range appear to be emerging as a major biogeographical boundary for both terrestrial and aquatic taxa in southern California.

At least for some aquatic taxa, the coastal region of Santa Barbara and Ventura counties forms an additional refuge for deep genetic lineages that are distinct from those further south in coastal California and Mexico. The intense anthropogenic activity in this area for agriculture and urban land uses has led to declines of many species (Davidson *et al*. 2002), and our genetic results emphasize that southern California may be a repository of cryptic genetic diversity worthy of conservation attention.

The Nevada population

Based on both AMOVA and phylogenetic analyses, the Nevada samples were virtually indistinguishable from other northern samples of *E. marmorata*. If the Nevada populations are native, our results suggest that the Sierra Nevada does not pose a barrier to this turtle; this interpretation is at odds with our expectations based on both natural history and the species' distribution. However, the issue is confounded

by the possibility that *E. marmorata* was introduced into Nevada in the late 1880s (Cary 1887). A recently introduced population should have mtDNA that is virtually identical to its founder(s), while a relictual population would be expected to be divergent from all others. One individual sampled from Nevada shared haplotype N3 with turtles from central and northern California (Appendix). Five out of six turtles sampled from Nevada had haplotypes not found west of the Sierra Nevada, but which differed only slightly from other northern clade haplotypes (N4, N34, N35, Appendix). These haplotypes may be present west of the Sierras and we failed to recover them with our sampling, or they may represent haplotypes unique to the eastern slope of the Sierra Nevada. Although little is known about the historical distribution of this species, fossil *E. marmorata* have been found as far east as Idaho (Zug 1969). Thus, the species could have been present east of the Sierra Nevada and then forced south to the Carson River (the source of our samples) during the Wisconsin glacial interval when much of the Great Basin experienced severe drying (Bartlein *et al*. 1998). In our minds, the issue of whether or not the Nevada population is natural or introduced remains unresolved and requires increased sampling of turtles from Nevada and from drainages of the northwest Sierra Nevada, perhaps coupled with hypervariable nuclear data for final resolution.

Conclusions

Although mitochondrial DNA forms the cornerstone of many phylogeographical analyses, incorporating nDNA data can provide additional evolutionary perspectives that help distinguish gene tree and organismal tree phenomena. While this idea is certainly valid, recent work indicates that, at least for turtles, it may be extremely difficult to find informative nuclear gene sequences at the intraspecific level. For example, Caccone *et al*. (2004) collected *c*. 4 kb of nDNA sequence data from eight introns as well as the ribosomal DNA (rDNA) internal transcribed space (ITS-1) and found little variation within or among species of Galápagos tortoises. Our analyses also revealed very low levels of nucleotide sequence variation for *c*. 1 kb of sequence data from two introns for *E. marmorata*. Thus, while mtDNA is but 'a single perspective' (Ballard & Whitlock 2004) on the history of a species, it appears that it may necessarily be the dominant perspective for some taxa. As more cost-effective sequencing or SNP discovery and genotyping methods are developed for decidedly nonmodel systems like turtles, we will continue to look to these tools to probe important questions in historical population biology. However, for now, mtDNA seems to remain the tool of choice.

Our results highlight the importance of phylogenetic analyses for conservation and management of threatened or vulnerable taxa like *E. marmorata*. Management recommendations from the California Department of Fish and Game state that, 'The systematic status of the various historical units that are represented by *C. marmorata* in California must be determined to establish whether different units need to be treated separately' (http://www.dfg.ca.gov/hcpb/cgi-bin/read_one.asp?specy=reptiles&idNum=8). Our mtDNA and nDNA results indicate that most northern populations are genetically extremely similar, and appear to form a single management unit. However, populations from about San Francisco south to BCN are much more subdivided than is apparent in the current subspecies designations, with most major drainage systems showing significant variation from each other. These among-drainage differences are not simply a function of accumulated IBD, as revealed by our IBD partial Mantel tests. Rather, drainages are generally distinct. The central and southern California populations in particular should receive increased conservation attention since these populations contain a large proportion of the genetic variation found in the species, are thought to be in decline (Holland 1991), and are threatened by habitat modification and loss over much of their range.

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This work is part of Phil Spinks's dissertation research on conservation of freshwater turtles. Phil Spinks is currently a postdoc in the Shaffer lab where he is continuing his research on phylogenetics and population genetics of reptiles and amphibians. The authors share a common interest in using molecular genetic tools to understand evolutionary processes ranging from landscape ecology to deep phylogenetic history. Much of their current work focuses on the application of molecular data to the conservation of amphibians and reptiles.

Appendix

Locality, sample identification, and GenBank accession numbers for all samples used in this study. Site numbers refer to Figure 1. WA, Washington; OR, Oregon; CA, California; NV, Nevada; BCN, Baja California Norte, Mexico. Latitude/longitude are in decimal degrees (WGS84). HBS = tissue collection of H. Bradley Shaffer, CA = California Academy of Sciences. Subspecies determinations are based on Seeliger (1945) and Stebbins (2003). Drainage and alternate ANOVA: Columbia R., Columbia River; Sac. Valley, Sacramento Valley; Carson R., Carson River; Mojave R., Mojave River; S. J. Basin, San Joaquin Basin; SLO, San Luis Obispo; SY, Santa Ynez Mountains. Haplotypes: N, Northern; SJV, San Joaquin Valley; SB, Santa Barbara; SC, Southern. Genes: ND4, nicotinamide adenine dehydrogenase subunit 4; R35, intron 1 of fingerprint protein 35; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Outgroups: *Emys blandingii*, HBS23408, Indiana, Kosciusko County, DeWart Lake; *Emys orbicularis*, HBS41824, no locality. Specific localities for some samples (indicated with an *) were not provided because these populations are extremely fragile and susceptible to poaching and other human disturbances (Ed DeGrauw pers. comm.). Samples in **Bold** displayed sequence heterogeneity at position 278 of the ND4 gene.

Site	State	County	Locality	Latitude/ longitude	Sample no.	Subspecies	Drainage AMOVA	Alternate AMOVA	Combined control region/*ND4* mtDNA haplotype	R35 haplotype	*GAPDH* haplotype	Control region accession nos	*ND4* accession nos	*GAPDH* accession nos	R35 accession nos
1	WA	King	*	*	HBS39824	*marmorata*	Puget Sound	Puget Sound/Columbia River	N1	—	—	AY904902	AY905084	—	—
2	WA	Kitsap	*	*	HBS39826	*marmorata*	Puget Sound	Puget Sound/Columbia River	N1	R1	G1	AY904904	AY905086	AY905030	AY905213
3	WA	Pierce	*	*	HBS39823	*marmorata*	Puget Sound	Puget Sound/Columbia River	N9	—	G1	AY904901	AY905083	AY905029	—
3	WA	Pierce	*	*	HBS39825	*marmorata*	Puget Sound	Puget Sound/Columbia River	N6	—	—	AY904903	AY905085	—	—
4	WA	Thurston	*	*	HBS39832	*marmorata*	Puget Sound	Puget Sound/Columbia River	N6	R1	—	AY904910	AY905092	—	AY905215
5	WA	Klickitat	*	*	HBS39816	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904894	AY905076	—	—
5	WA	Klickitat	*	*	HBS39817	*marmorata*	Columbia River	Puget Sound/Columbia River	N5	R1	—	AY904895	AY905077	—	AY905212
5	WA	Klickitat	*	*	HBS39818	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904896	AY905078	—	—
5	WA	Klickitat	*	*	HBS39819	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904897	AY905079	—	—
5	WA	Klickitat	*	*	HBS39820	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904898	AY905080	—	—
5	WA	Klickitat	*	*	HBS39821	*marmorata*	Columbia River	Puget Sound/Columbia River	N8	—	—	AY904899	AY905081	—	—
5	WA	Klickitat	*	*	HBS39822	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904900	AY905082	—	—
5	WA	Klickitat	*	*	HBS39830	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904908	AY905090	—	—
5	WA	Klickitat	*	*	HBS39831	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904909	AY905091	—	—
5	WA	Klickitat	*	*	HBS39875	*marmorata*	Columbia River	Puget Sound/Columbia River	N2	R1	G2	AY904992	AY905174	AY905064	AY905251
5	WA	Klickitat	*	*	HBS39851	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	R1	G2	AY905004	AY905186	AY905069	AY905258
5	WA	Klickitat	*	*	HBS39876	*marmorata*	Columbia River	Puget Sound/Columbia River	N2	—	—	AY905005	AY905187	—	—
5	WA	Klickitat	*	*	HBS39877	*marmorata*	Columbia River	Puget Sound/Columbia River	N5	—	—	AY905006	AY905188	—	—
6	OR	Multnomah	*	*	HBS39827	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	R1	—	AY904905	AY905087	—	AY905214
6	OR	Multnomah	*	*	HBS39828	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904906	AY905088	—	—
6	OR	Multnomah	*	*	HBS39829	*marmorata*	Columbia River	Puget Sound/Columbia River	N1	—	—	AY904907	AY905089	—	—
7	OR	Wasco	Moser Pond	44.6959°N, 123.6475°W	HBS39860	*marmorata*	Columbia River	Columbia River/North Coast	N10	R1	G2	AY905021	AY905203	AY905072	AY905261
8	OR	Benton	Willamette drainage	44.6926°N, 123.2455°W	HBS39881	*marmorata*	Columbia River	Columbia River/North Coast	N11	—	—	AY905027	AY905209	—	—
9	OR	Lane	Elijah Bristow State Park	43.9430°N, 122.8468°W	HBS39722	*marmorata*	Columbia River	Columbia River/North Coast	N12	R1	G1/G2	AY905019	AY905201	AY905071	AY905260
10	OR	Douglas	South Fork Umpqua River	43.0226°N, 122.8169°W	HBS39852	*marmorata*	North Coast	Columbia River/North Coast	N13	—	—	AY905020	AY905202	—	—
10	OR	Douglas	Carmen Lake	43.1169°N, 122.5853°W	HBS39721	*marmorata*	North Coast	Columbia River/North Coast	N14	—	—	AY905023	AY905205	—	—
11	OR	Douglas	Elk Creek	42.6793°N, 122.7395°W	HBS39853	*marmorata*	North Coast	Columbia River/North Coast	N1	—	—	AY905024	AY905206	—	—
12	OR	Josephine	Grave Creek	42.6450°N, 123.5039°W	HBS39854	*marmorata*	North Coast	Columbia River/North Coast	N1	—	—	AY905022	AY905204	—	—
13	OR	Klamath	Lost River	42.1378°N, 121.3041°W	HBS39859	*marmorata*	North Coast	Columbia River/North Coast	N2	R1	G1	AY905025	AY905207	AY905073	AY905262
14	OR	Jackson	Little Squaw Lake	42.0303°N, 123.0136°W	HBS39756	*marmorata*	North Coast	Columbia River/North Coast	N15	R1	G2	AY904934	AY905116	AY905038	AY905225
15	CA	Siskiyou	Klamath River	41.8569°N, 122.7728°W	HBS39844	*marmorata*	North Coast	Columbia River/North Coast	N16	—	—	AY904976	AY905158	—	—
16	CA	Shasta	Tule River	41.0621°N, 121.4717°W	HBS39804	*marmorata*	Sacramento Valley	North Coast/Sac. Valley	N1	—	—	AY904972	AY905154	—	—
16	CA	Shasta	Tule River	41.0621°N, 121.4717°W	HBS39805	*marmorata*	Sacramento Valley	North Coast/Sac. Valley	N17	—	—	AY904973	AY905155	—	—
17	CA	Shasta	Little Cow Creek	40.7268°N, 122.0774°W	HBS39734	*marmorata*	Sacramento Valley	North Coast/Sac. Valley	N18	R1	G4	AY904974	AY905156	AY905056	AY905242
18	CA	Shasta	Cottonwood Creek	40.3956°N, 122.5260°W	HBS39882	*marmorata*	Sacramento Valley	North Coast/Sac. Valley	N3	—	—	AY904916	AY905098	—	—
19	CA	Trinity	Upper Hayfork Creek	40.5841°N, 123.0284°W	HBS39870	*marmorata*	North Coast	North Coast/Sac. Valley	N19	—	—	AY904935	AY905117	—	—
20	CA	Trinity	Middle Hayfork Creek	40.5539°N, 123.2181°W	HBS39842	*marmorata*	North Coast	North Coast/Sac. Valley	N2	R1	G1	AY904936	AY905118	AY905039	AY905226
21	CA	Trinity	Lower Hayfork Creek	40.6272°N, 123.3696°W	HBS39840	*marmorata*	North Coast	North Coast/Sac. Valley	N20	—	—	AY904937	AY905119	—	—
22	CA	Humboldt	Van Duzen River	40.4909°N, 123.6048°W	HBS39873	*marmorata*	North Coast	North Coast/Sac. Valley	N2	—	—	AY904982	AY905164	—	—
22	CA	Trinity	Mad River	40.4503°N, 123.5049°W	HBS39874	*marmorata*	North Coast	North Coast/Sac. Valley	N2	R1	G2	AY904983	AY905165	AY905059	AY905246
22	CA	Humboldt	Van Duzen River	40.4909°N, 123.6048°W	HBS39737	*marmorata*	North Coast	North Coast/Sac. Valley	N21	R1	G1/G2	AY904985	AY905167	AY905061	AY905248
22	CA	Trinity	Mad River	40.4503°N, 123.5049°W	HBS39767	*marmorata*	North Coast	North Coast/Sac. Valley	N2	—	—	AY904989	AY905171	—	—

Appendix *Continued*

Site	State	County	Locality	Latitude/ longitude	Sample no.	Subspecies	Drainage AMOVA	Alternate AMOVA	Combined control region/*ND4* mtDNA haplotype	R35 haplotype	*GAPDH* haplotype	Control region accession nos	*ND4* accession nos	*GAPDH* accession nos	R35 accession nos
23	CA	Mendocino	Ten Mile Creek	39.7505°N, 123.5335°W	HBS39741	*marmorata*	North Coast	North Coast/Napa Valley	N1	R1	G1	AY904984	AY905166	AY905060	AY905247
23	CA	Mendocino	Ten Mile Creek	39.7505°N, 123.5335°W	HBS39770	*marmorata*	North Coast	North Coast/Napa Valley	N2	—	—	AY904986	AY905168	—	—
23	CA	Mendocino	Ten Mile Creek	39.7505°N, 123.5335°W	HBS39769	*marmorata*	North Coast	North Coast/Napa Valley	N10	—	—	AY904987	AY905169	—	—
24	CA	Butte	Bidwell Park, Chico	39.7422°N, 121.8158°W	**HBS39781**	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N2	—	—	AY904911	AY905093	—	—
24	CA	Butte	Bidwell Park, Chico	39.7422°N, 121.8158°W	HBS39782	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N2	—	—	AY904912	AY905094	—	—
24	CA	Butte	Bidwell Park, Chico	39.7422°N, 121.8158°W	**HBS39783**	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N22	—	—	AY904913	AY905095	—	—
24	CA	Butte	Bidwell Park, Chico	39.7422°N, 121.8158°W	**HBS39785**	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N7	—	—	AY904914	AY905096	—	—
25	CA	Butte	Plumas National Forest	39.7227°N, 121.3624°W	CA209927	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N7	—	—	AY904915	AY905097	—	—
26	CA	Colusa	Bear Creek	39.0042°N, 122.3553°W	HBS39735	*marmorata*	Napa Valley	North Coast/Napa Valley	N23	—	—	AY904991	AY905173	—	—
26	CA	Colusa	Bear Creek	39.0042°N, 122.3553°W	HBS39802	*marmorata*	Napa Valley	North Coast/Napa Valley	N24	—	—	AY905026	AY905208	—	—
27	CA	Yuba	Dry Creek	39.2760°N, 121.3976°W	HBS39751	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N25	—	—	AY904967	AY905149	—	—
27	CA	Yuba	Dry Creek	39.2760°N, 121.3976°W	HBS39795	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N26	R1	G1	AY904969	AY905151	AY905055	AY905241
27	CA	Yuba	Sierra Foothills Research Center	39.2365°N, 121.3285°W	HBS39776	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N2	—	—	AY904917	AY905099	—	—
27	CA	Yuba	Sierra Foothills Research Center	39.2365°N, 121.3285°W	HBS39778	*marmorata*	Sacramento Valley	Sac. Valley/Napa Valley	N3	R1	G1	AY904918	AY905100	AY905031	AY905216
28	CA	Nevada	Wolf Creek	39.0515°N, 121.1085°W	HBS39754	*marmorata*	Sacramento Valley	Carson R./Sac. Valley	N27	—	—	AY904970	AY905152	—	—
29	CA	El Dorado	Penobscott Creek	38.8984°N, 120.9422°W	HBS39793	*marmorata*	Sacramento Valley	Carson R./Sac. Valley	N3	—	—	AY904968	AY905150	—	—
29	CA	El Dorado	Penobscott Creek	38.8984°N, 120.9422°W	HBS39792	*marmorata*	Sacramento Valley	Carson R./Sac. Valley	N3	—	—	AY904971	AY905153	—	—
30	CA	Napa	Pope Creek	38.6450°N, 122.3540°W	HBS39757	*marmorata*	Napa Valley	Sac. Valley/Napa Valley	N28	R1	G1	AY904978	AY905160	AY905057	AY905244
30	CA	Napa	Pope Creek	38.6450°N, 122.3540°W	HBS39760	*marmorata*	Napa Valley	Sac. Valley/Napa Valley	N29	R1	G1	AY904980	AY905162	AY905058	AY905245
30	CA	Napa	Pope Creek	38.6450°N, 122.3540°W	HBS39759	*marmorata*	Napa Valley	Sac. Valley/Napa Valley	N30	—	—	AY904981	AY905163	—	—
30	CA	Napa	Pope Creek	38.6450°N, 122.3540°W	HBS39761	*marmorata*	Napa Valley	Sac. Valley/Napa Valley	N31	R1	G1/G2	AY904988	AY905170	AY905062	AY905249
31	CA	Solano	Suisun Marshes	38.1926°N, 121.9966°W	HBS39774	*marmorata*	not assigned	not assigned	SJV4	—	—	AY905018	AY905200	—	—
32	CA	Sacramento	Stone Lakes	38.3866°N, 121.5060°W	**HBS39787**	*marmorata*	Sacramento Valley	Carson R./Sac. Valley	N2	—	—	AY904919	AY905101	—	—
33	CA	Calaveras	North Fork Calaveras River	38.3240°N, 120.5072°W	HBS39755	intergrade	San Joaquin Basin	Carson R./Sac. Valley	N32	—	—	AY904966	AY905148	—	—
34	CA	Tuolumne	Rose Creek	38.1056°N, 120.3398°W	HBS39872	intergrade	San Joaquin Basin	Carson R./Sac. Valley	N33	R1	G1	AY904965	AY905147	AY905054	AY905240
35	NV	Lyon	Carson River	39.2372°N, 119.5879°W	HBS39865	*marmorata*	Carson River	Carson R./Sac. Valley	N3	—	—	AY904929	AY905111	—	—
36	NV	Douglas	Carson River	38.9913°N, 119.8240°W	HBS39866	*marmorata*	Carson River	Carson R./Sac. Valley	N34	—	—	AY904930	AY905112	—	—
36	NV	Douglas	Carson River	38.9913°N, 119.8240°W	HBS39867	*marmorata*	Carson River	Carson R./Sac. Valley	N4	R1	—	AY904931	AY905113	—	AY905222
36	NV	Douglas	Carson River	38.9913°N, 119.8240°W	HBS39868	*marmorata*	Carson River	Carson R./Sac. Valley	N4	R1	G1	AY904932	AY905114	AY905037	AY905223
36	NV	Douglas	Carson River	38.9913°N, 119.8240°W	HBS39869	*marmorata*	Carson River	Carson R./Sac. Valley	N35	R1	—	AY904933	AY905115	—	AY905224
36	NV	Douglas	Carson River	38.9913°N, 119.8240°W	HBS39846	*marmorata*	Carson River	Carson R./Sac. Valley	N4	R1	—	AY904975	AY905157	—	AY905243
37	CA	Alameda	Arroyo Mocho	37.5270°N, 121.5543°W	HBS39724	intergrade	Monterey	Monterey/S. J. Basin	N2	—	—	AY904994	AY905176	—	—
38	CA	Stanislaus	Del Puerto Creek	37.4191°N, 121.3559°W	HBS39847	intergrade	San Joaquin Basin	Monterey/S. J. Basin	N36	—	—	AY904979	AY905161	—	—
39	CA	Merced	Kesterson NWR	37.2572°N, 120.9058°W	HBS39806	intergrade	San Joaquin Basin	Monterey/S. J. Basin	SJV5	R1/R5	G1	AY904920	AY905102	AY905032	AY905217
39	CA	Merced	Kesterson NWR	37.2572°N, 120.9058°W	HBS39800	intergrade	San Joaquin Basin	Monterey/S. J. Basin	SJV6	R1	G1	AY904921	AY905103	AY905033	AY905218
40	CA	Mariposa	Sherlock Creek	37.5721°N, 120.0153°W	HBS39808	intergrade	San Joaquin Basin	Monterey/S. J. Basin	N37	R2	—	AY904993	AY905175	—	AY905252
41	CA	Madera	Chowchilla River	37.3450°N, 119.8101°W	HBS39773	intergrade	San Joaquin Basin	Monterey/S. J. Basin	N1	—	—	AY904996	AY905178	—	—
42	CA	Madera	Fresno River	37.3042°N, 119.7605°W	HBS39838	intergrade	San Joaquin Basin	Monterey/S. J. Basin	SJV1	—	—	AY904949	AY905131	—	—
42	CA	Madera	Fresno River	37.3042°N, 119.7605°W	HBS39766	intergrade	San Joaquin Basin	Monterey/S. J. Basin	N40	R1/R5	—	AY904950	AY905132	—	AY905232
43	CA	Fresno	Jose Creek	37.1390°N, 119.3796°W	HBS39730	intergrade	San Joaquin Basin	S. J. Basin/SLO-SY	SJV7	R1	G1	AY904947	AY905129	AY905043	AY905230
43	CA	Fresno	Jose Creek	37.1390°N, 119.3796°W	HBS39731	intergrade	San Joaquin Basin	S. J. Basin/SLO-SY	SJV8	R1	G1	AY904948	AY905130	AY905044	AY905231
44	CA	Madera	Coarsegold Creek	37.1356°N, 119.7383°W	HBS39729	intergrade	San Joaquin Basin	S. J. Basin/SLO-SY	N2	R3	G1	AY904998	AY905180	AY905066	AY905254
44	CA	Madera	Coarsegold Creek	37.1356°N, 119.7383°W	HBS39833	intergrade	San Joaquin Basin	S. J. Basin/SLO-SY	N3	R1	—	AY904999	AY905181	—	AY905255
45	CA	San Benito	Tres Pinas Creek	36.6563°N, 121.1628°W	HBS39814	*pallida*	Monterey	Monterey/S. J. Basin	N41	R1	G1	AY904997	AY905179	AY905065	AY905253
46	CA	San Benito	San Benito River	36.6146°N, 121.2106°W	HBS39810	*pallida*	Monterey	Monterey/S. J. Basin	N42	—	—	AY904995	AY905177	—	—
47	CA	Fresno	Big Creek	36.9297°N, 119.2453°W	HBS39803	intergrade	Tulare Basin	SLO-SY/Tulare Basin	SJV3	—	—	AY904941	AY905123	—	—
48	CA	Tulare	North Fork Kaweah River	36.5443°N, 118.8966°W	HBS39878	intergrade	Tulare Basin	SLO-SY/Tulare Basin	SJV9	—	—	AY905010	AY905192	—	—
48	CA	Tulare	North Fork Kaweah River	36.5443°N, 118.8966°W	HBS39858	intergrade	Tulare Basin	SLO-SY/Tulare Basin	SJV10	—	—	AY905017	AY905199	—	—
49	CA	Tulare	Kaweah River	36.3916°N, 118.8746°W	HBS39836	intergrade	Tulare Basin	SLO-SY/Tulare Basin	SJV11	R1	G1	AY904940	AY905122	AY905041	AY905228

Appendix *Continued*

Site	State	County	Locality	Latitude/ longitude	Sample no.	Subspecies	Drainage AMOVA	Alternate AMOVA	Combined control region/*ND4* mtDNA haplotype	R35 haplotype	*GAPDH* haplotype	Control region accession nos	*ND4* accession nos	*GAPDH* accession nos	R35 accession nos
50	CA	Tulare	Sycamore Creek	36. 1861°N, 118.7978°W	HBS39835	intergrade	Tulare Basin	SLO-SY/Tulare Basin	SJV3	—	—	AY904946	AY905128	—	—
51	CA	Tulare	Tule River	36.0299°N, 118.7843°W	HBS39762	intergrade	Tulare Basin	SLO-SY/Tulare Basin	SJV2	R1/R5	G1	AY904938	AY905120	AY905040	AY905227
52	CA	Monterey	San Antonio River	36.0726°N, 121.3522°W	HBS39772	*pallida*	Monterey	Monterey/S. J. Basin	N43	R4	G1	AY904990	AY905172	AY905063	AY905250
53	CA	San Luis Obispo	Pico Creek	35.6231°N, 121.1415°W	HBS39809	*pallida*	SLO-SY	S. J. Basin/SLO-SY	N39	—	—	AY905007	AY905189	—	—
53	CA	San Luis Obispo	Oak Knoll Creek	35.6703°N, 121.2033°W	HBS39863	*pallida*	SLO-SY	S. J. Basin/SLO-SY	N44	—	—	AY905008	AY905190	—	—
54	CA	San Luis Obispo	Broken Bridge Creek	35.6663°N, 121.1678°W	HBS39861	*pallida*	SLO-SY	S. J. Basin/SLO-SY	N45	R1	G1	AY905003	AY905185	AY905068	AY905257
54	CA	San Luis Obispo	Perry Creek	35.5207°N, 121.0380°W	HBS39862	*pallida*	SLO-SY	S. J. Basin/SLO-SY	N39	—	—	AY905016	AY905198	—	—
55	CA	Kern	Mariposa Pond	35.6903°N, 118.2386°W	HBS39740	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV12	—	—	AY904922	AY905104	—	—
55	CA	Kern	Mariposa Pond	35.6903°N, 118.2386°W	HBS39738	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV13	R1	G1	AY904923	AY905105	AY905034	AY905219
55	CA	Kern	Bloomfield Ranch	35.7325°N, 118. 1714°W	HBS39839	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV1	—	—	AY904943	AY905125	—	—
55	CA	Kern	Bloomfield Ranch	35.7325°N, 118. 1714°W	HBS39733	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV2	—	—	AY904944	AY905126	—	—
55	CA	Kern	Bloomfield Ranch	35.7325°N, 118. 1714°W	HBS39732	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV14	—	—	AY904945	AY905127	—	—
56	CA	Kern	South Fork Kern River	35.6721°N, 118.3276°W	HBS39728	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV1	R1	G1	AY904942	AY905124	AY905042	AY905229
56	CA	Kern	South Fork Kern River	35.6721°N, 118.3276°W	HBS39855	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV1	R1	G1	AY905009	AY905191	AY905070	AY905259
57	CA	Kern	Cedar Creek	35.6898°N, 118.6866°W	HBS39799	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV1	—	—	AY904924	AY905106	—	—
57	CA	Kern	Cedar Creek	35.6898°N, 118.6866°W	HBS39768	intergrade	Tulare Basin	Tulare Basin/SY-BCN	SJV1	—	—	AY904939	AY905121	—	—
58	CA	San Luis Obispo	Barrett Creek	35. 1780°N, 119.9803°W	HBS39739	*pallida*	SLO-SY	SLO-SY/Tulare Basin	N38	R1	G1	AY904928	AY905110	AY905036	AY905221
59	CA	San Luis Obispo	Upper Alamo Creek	35.2237°N, 120.2091°W	HBS39856	*pallida*	SLO-SY	SLO-SY/Tulare Basin	N46	—	—	AY905028	AY905210	—	—
59	CA	San Luis Obispo	Upper Alamo Creek	35.2237°N, 120.2091°W	HBS39879	*pallida*	SLO-SY	SLO-SY/Tulare Basin	N38	—	—	AY905014	AY905196	—	—
59	CA	San Luis Obispo	Upper Alamo Creek	35.2237°N, 120.2091°W	HBS39880	*pallida*	SLO-SY	SLO-SY/Tulare Basin	N38	—	—	AY905015	AY905197	—	—
60	CA	San Luis Obispo	Lower Alamo Creek	35.0754°N, 120.2740°W	HBS39850	*pallida*	SLO-SY	SLO-SY/Tulare Basin	N47	—	—	AY905013	AY905195	—	—
61	CA	Santa Barbara	Manzana Creek	34.8238°N, 119.9971°W	HBS39864	*pallida*	SLO-SY	SLO-SY/SY-BCN	SB1	R1	G1	AY904962	AY905144	AY905052	AY905238
62	CA	Santa Barbara	Santa Ynez River	34.5497°N, 119.8742°W	HBS39841	*pallida*	SLO-SY	SLO-SY/SY-BCN	SB2	—	—	AY904963	AY905145	—	—
63	CA	Santa Barbara	Jalama Creek	34.5036°N, 120.4045°W	HBS39871	*pallida*	SLO-SY	SLO-SY/SY-BCN	SB3	R1	G1	AY904964	AY905146	AY905053	AY905239
64	CA	Ventura	Santa Paula Creek	34.4089°N, 119.0825°W	HBS39837	*pallida*	SY-BCN	SLO-SY/SY-BCN	SC2	—	G1	AY904954	AY905136	AY905047	—
64	CA	Ventura	Santa Paula Creek	34.4089°N, 119.0825°W	HBS39843	*pallida*	SY-BCN	SLO-SY/SY-BCN	SB4	R1	G1	AY904955	AY905137	AY905048	AY905235
65	CA	San Bernadino	Camp Cady	34.9304°N, 116.6314°W	HBS39727	*pallida*	Mojave River	Mojave R./SY-BCN	SC1	—	—	AY905000	AY905182	—	—
65	CA	San Bernadino	Camp Cady	34.9304°N, 116.6314°W	HBS39725	*pallida*	Mojave River	Mojave R./SY-BCN	SC1	R1/R5	G1/G2	AY905001	AY905183	AY905067	AY905256
65	CA	San Bernadino	Camp Cady	34.9304°N, 116.6314°W	HBS39726	*pallida*	Mojave River	Mojave R./SY-BCN	SC3	—	—	AY905002	AY905184	—	—
66	CA	Los Angelos	Pico Creek	34.3770°N, 118.5894°W	HBS39849	*pallida*	SY-BCN	SLO-SY/SY-BCN	SC4	—	—	AY905011	AY905193	—	—
67	CA	San Bernadino	Gordon Ranch	33.9669°N, 117.7534°W	HBS39736	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC5	—	—	AY904977	AY905159	—	—
68	CA	San Diego	San Mateo Creek	33.4698°N, 117.4727°W	HBS39807	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC6	R1	G3	AY904951	AY905133	AY905045	AY905233
68	CA	San Diego	San Mateo Creek	33.4698°N, 117.4727°W	HBS39801	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC1	—	—	AY904952	AY905134	—	—
68	CA	San Diego	San Mateo Creek	33.4698°N, 117.4727°W	HBS39857	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC1	—	—	AY905012	AY905194	—	—
69	CA	San Diego	Cocklebur Creek	33.2515°N, 117.4276°W	HBS39798	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC7	R4	G1	AY904925	AY905107	AY905035	AY905220
69	CA	San Diego	Cocklebur Creek	33.2515°N, 117.4276°W	HBS39765	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC8	—	—	AY904926	AY905108	—	—
69	CA	San Diego	Cocklebur Creek	33.2515°N, 117.4276°W	HBS39796	*pallida*	SY-BCN	Tulare Basin/SY-BCN	SC9	—	—	AY904927	AY905109	—	—
70	CA	San Diego	Scholder Creek	33. 1733°N, 116.7872°W	HBS39813	*pallida*	SY-BCN	Mojave R./SY-BCN	SC10	R1	G1	AY904953	AY905135	AY905046	AY905234
71	CA	San Diego	Pine Valley Creek	32.8205°N, 116.5601°W	HBS39763	*pallida*	SY-BCN	Mojave R./SY-BCN	SC11	—	—	AY904956	AY905138	—	—
71	CA	San Diego	Pine Valley Creek	32.8205°N, 116.5601°W	HBS39812	*pallida*	SY-BCN	Mojave R./SY-BCN	SC12	—	G1	AY904957	AY905139	AY905049	—
72	Mexico	BCN	Vallecitos	32.2100°N, 116.4900°W	HBS39848	*pallida*	SY-BCN	Mojave R./SY-BCN	SC13	R4	G1	AY904958	AY905140	AY905050	AY905236
73	Mexico	BCN	Arroyo Del Rancho Portrero	31.0333°N, 115.5042°W	HBS39811	*pallida*	SY-BCN	Mojave R./SY-BCN	SC14	—	—	AY904959	AY905141	—	—
73	Mexico	BCN	Arroyo Del Rancho Portrero	31.0333°N, 115.5042°W	HBS39753	*pallida*	SY-BCN	Mojave R./SY-BCN	SC15	R4	G1	AY904960	AY905142	AY905051	AY905237
73	Mexico	BCN	Arroyo Del Rancho Portrero	31.0333°N, 115.5042°W	HBS39771	*pallida*	SY-BCN	Mojave R./SY-BCN	SC16	—	—	AY904961	AY905143	—	—

Outgroups = *Emys blandingii* HBS23408, Indiana, Kosciusko County, DeWart Lake. *Emys orbicularis*, HBS41824, no locality.

*Specific localities for these samples were not provided because these populations are extremely fragile and susceptible to poaching and other human disturbances (Ed DeGrauw pers. comm.). Sample numbers: HBS = tissue collection of H. Bradley Shaffer, CA = California Academy of Sciences. Samples in **Bold** displayed sequence heterogeneity at position 278 of the ND4 gene.