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Multilocus genealogies reveal multiple cryptic species and biogeographical complexity in the California turret spider *Antrodiaetus riversi* (Mygalomorphae, Antrodiaetidae)

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Abstract

***Antrodiaetus riversi* (Araneae, Antrodiaetidae) is a dispersal-limited, habitat specialized mygalomorph spider species endemic to mesic woodlands of northern and central California. This species occupies a disjunct distribution, with populations in the Sierra Nevada and Coast Ranges, separated by the inhospitable Central Valley. Previous studies of morphological and allozyme variation have suggested that these populations may constitute cryptic species. We investigated the phylogeography of *A. riversi* using both nuclear and mitochondrial DNA sequences, collected for a comprehensive population sample. These data reveal the presence of at least five species in the *A. riversi* complex — these species are deeply diverged, and genealogically exclusive in both nuclear and mitochondrial genomes. Each of these species is characterized by extreme population subdivision and deep phylogeographical structuring, consistent with minimal gene flow across the dissected Californian landscape. Three species are restricted to the Coast Ranges, one to high altitudes of the central Sierran Nevada, and one species is found in both ranges. These species have allopatric distributions, although species parapatry is hypothesized to occur in several areas. Species diversification appears to have pulsed in the Late Miocene/Early Pliocene, a timing consistent with biogeographical reconstructions for many Californian taxa, and a time of turbulent geological activity in the region.**

Keywords: California biogeography, genealogical congruence, parapatry, phylogeography, population subdivision, speciation

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Introduction

California is a well-known hotspot for biological diversity, with more native plant and animal species than any comparable area in North America (see Lapointe & Rissler 2005). This diversity reflects a complex geological, topographic and climatic setting that has shaped the phylogenetic and biogeographical history of its inhabitants. Important historical processes include the uplift of the Sierra Nevada and Coast Range mountains, the presence of inland seaways, fluctuating climatic regimes, and dramatic plate movements (Harden 1997; Wakabayashi & Sawyer 2001; Furlong & Schwartz 2004; Jacobs *et al*. 2004). Because of this high diversity and endemicity, California has become a 'model area' for modern phylogenetic and phylogeographical analyses, and recent comparative studies reveal strong concordance in genetic breaks of codistributed taxa (see Calsbeek *et al*. 2003; Lapointe & Rissler 2005; Feldman & Spicer 2006; Rissler *et al*. 2006). Moreover, the comparative data sets assembled for Californian taxa have inspired development of novel biogeographical methods (Lapointe & Rissler 2005; Rissler *et al*. 2006).

Despite the numerous studies performed to date, our understanding of the biogeographical history of California is both taxonomically biased, and limited to a small fraction of the native diversity. In particular, there have been very few modern phylogeographical and/or phylogenetic studies of native terrestrial arthropods (e.g. Bond *et al*. 2001; Segraves & Pellmyr 2001; Law & Crespi 2002; Bond 2004), despite the fact that this fauna is tremendously rich. For example, several endemic arachnid lineages (classified

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as genera or species groups) comprise large species radiations, including amaurobiid (Leech 1972) and tengellid (Platnick & Ubick 2001) spiders, phalangodid opilionids (e.g. Ubick & Briggs 1989), and many others. For most of these radiations, the recognized species have small, typically allopatric, geographical distributions. This fine-grained diversification is compelling evidence for biogeographical signal in these taxa, but because these taxa have been understudied from a phylogenetic perspective, this biogeographical information is not yet fully accessible.

This study focuses on the diversity and biogeographical history of the mygalomorph spider *Antrodiaetus riversi* (O.P. — Cambridge 1883). Mygalomorph spiders include such spiders as tarantulas, trapdoor spiders, and purseweb spiders, which together form a clade of early diverging spiders. The mygalomorph spider fauna of California is one of the richest in the world, and includes the highest familial, generic and species-level diversity in the United States. Mygalomorphs make excellent model systems for biogeographical analysis — mygalomorph lineages (e.g. species, genera, families) are relatively ancient (e.g. Penney *et al*. 2003; Hendrixson & Bond 2007), and many mygalomorph species are long-lived, habitat-specialized, sedentary animals (summarized in Bond *et al*. 2006). Furthermore, in California, many mygalomorph taxa are comparable to other well-studied vertebrate taxa (e.g. *Ensatina* salamanders, Wake 1997) in that they show similar distributions, and share several biological features in common (e.g. low vagility, mesic-habitat specialists, with male-mediated gene flow). These characteristics make Californian mygalomorphs especially useful for comparison in a broader context.

Antrodiaetus riversi is an antrodiaetid mygalomorph, recently transferred from the genus *Atypoides* to the genus *Antrodiaetus* (Hendrixson & Bond 2007). This species is endemic to California, and has had an apparently long, isolated history in this region. Hendrixson & Bond (2007) used relaxed molecular clock methods to estimate that *A. riversi* diverged from its sister species *Antrodiaetus hadros* (now found in Missouri and Illinois) between 80 million and 100 million years ago (Ma). *A. riversi* occupies a disjunct distribution in California, with groups of populations found in both the central Sierra Nevada and in the central and north Coast Ranges (Fig. 1). Except for a single population found at the island-like Sutter Buttes, the Central Valley represents an inhospitable environment, and an obvious modern-day barrier between coastal and Sierran populations. These spiders are moisture-sensitive microhabitat specialists, often restricted to north-facing ravines within otherwise continuous habitat (Coyle 1971). In favourable microhabitats, these spiders live in silk-lined subterranean burrows, and build conspicuous silken entrance constructs that resemble small 'turrets'. Vincent (1993) conducted a life history analysis of a Sierran *A. riversi* population, and documented extremely low and sex-limited dispersal, finding only wandering adult males in pitfall traps placed directly adjacent to *A. riversi* burrow aggregations. Vincent (1993) also estimated that adult female *A. riversi* may live to at least 16 years old.

Two prior studies have suggested that *A. riversi* may represent a 'species complex', rather than a single species, thus also implying a possibly more complex regional history for the group. Coyle (1968) revised the genus (then *Atypoides*), and noted considerable morphological divergence in both genitalic and somatic characters between coastal and Sierran populations of *A. riversi*. Although he ultimately considered the coastal and Sierran population groups to be conspecific, he suggested that these groups might represent 'incipient species' (Coyle 1968, 1971). More recently, Ramirez & Chi (2004) tested Coyle's incipient speciation hypothesis through analysis of allozyme variation, with spiders collected from eight *A. riversi* localities in the coast Ranges, the Sierra Nevada, and at Sutter Buttes (see Fig. 1). Genetic distance analyses suggest the recognition of multiple population groups, but again, these authors chose to maintain the current taxonomy, referring to the genetically divergent populations as 'distinct entities, which as a complex comprise *At. riversi*'.

In this study, we extend the analyses of Coyle (1968) and Ramirez & Chi (2004) to further explore biogeographical patterns and species limits in *A. riversi*. We employ a multilocus phylogeographical approach, using both mitochondrial DNA (mtDNA) and nDNA sequences, with samples encompassing the entire species' range. While mtDNA has received widespread use in studies of phylogeography and species limits (see Avise 2000), mtDNA divergence patterns may be unreliable in some cases and should be complemented with nuclear data (see Jockusch & Wake 2002; Zhang & Hewitt 2003; Ballard & Whitlock 2004; Rubinoff & Holland 2005). Although gathering nuclear gene tree data that is informative near or below the species level is nontrivial, especially in nonmodel systems, such data are becoming more prevalent in studies of species boundaries (e.g. Broughton & Harrison 2003; Zhang *et al*. 2005). The integration of gene tree data from multiple loci allows for a more complete picture of population demographic history (e.g. rather than female demographic history), provides more precise estimates of divergence times (Thorne & Kishino 2002; Yang & Yoder 2003), and allows one to assess genealogical conflict and congruence. Genealogical congruence is particularly important in revealing long-standing biogeographical barriers to gene exchange (Kuo & Avise 2005), and represents a fundamental empirical criterion for species recognition under many species concepts (Avise & Ball 1990; reviewed in Sites & Marshall 2004).

Our research is aimed at addressing the following primary questions: (i) What does DNA sequence data suggest about the population genetic structure of *A. riversi*? Are

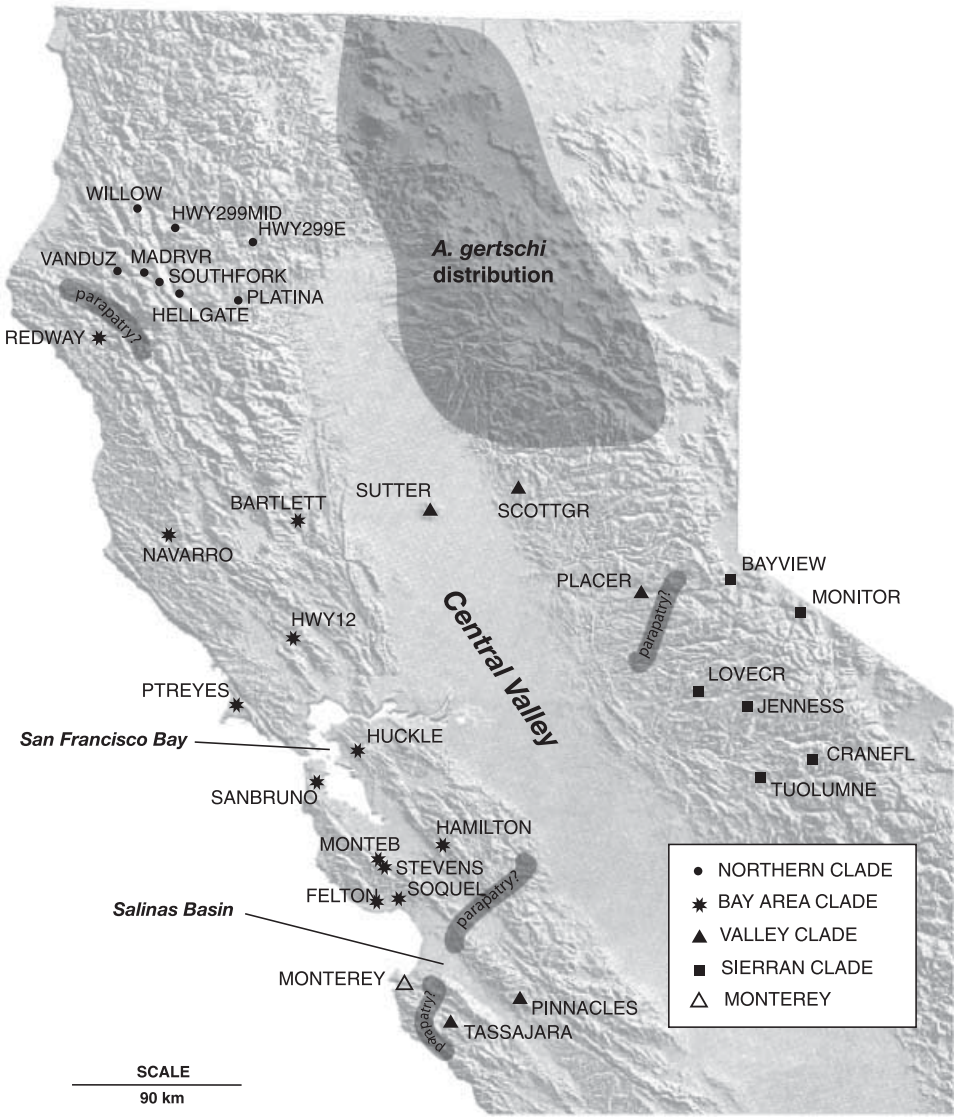

Fig. 1 Map of central and northern California, with *Antrodiaetus riversi* sampling sites. Population acronyms are listed in the Appendix. The following sites correspond exactly to six of the sites sampled by Ramirez & Chi (2004): Bayview, Monitor, Jenness, Sutter Buttes, Point Reyes and Monterey. In addition, Ramirez & Chi (2004) sampled at two sites in the Santa Cruz Mountains, near our Felton and Montebello sites. Sites are grouped into genetic clades, following results of phylogenetic analyses (see text and Fig. 5). Hypothesized areas of parapatry, and geographical features mentioned in the text are highlighted. Geographical distribution of *Antrodiaetus gertschi* is from Coyle (1968).

genetic patterns consistent with limited dispersal abilities, as suggested by field data? If so, what is the spatial scale of such genetic structuring, and does such structuring apply to both sexes? (ii) Do congruent genealogical patterns emerge from multilocus DNA data, and if so, what do these patterns suggest about the number and geographical limits of species? (iii) Are observed biogeographical patterns in the *A. riversi* complex consistent with patterns found in other well-studied Californian taxa? What are the inferred ages of biogeographical events, and are these estimated ages consistent with known events in the geological and climatic history of California?

Materials and methods

Population and outgroup sampling

Specimens were collected from 32 sites (Fig. 1), providing a comprehensive coverage of the known range of *Antrodiaetus riversi* (following Coyle 1968; map 1). At each location, a series of individuals was collected, consisting mostly of adult females, but sometimes also including immature spiders. A number of new populations were discovered, including populations in the north Coast range as well as from south of Monterey, extending the known southern

range of this species. Eight sites were near (or correspond exactly to) those sampled by Ramirez & Chi (2004). Even though our sampling efforts are fairly comprehensive, an obvious gap exists in the north coast range (Fig. 1) — our attempts to find spiders in this region have failed thus far, although we believe that the species is likely found in this region. The species does not likely occur further north in the Sierra Nevada, where the congeneric species *Antrodiaetus gertschi* appears to replace *A. riversi* (see Coyle 1968). Collecting efforts at more southern locations (e.g. southern Sierra Nevada) have yet to uncover *A. riversi* populations.

Outgroup taxa were sampled following the systematic findings of Miller & Coyle (1996) and Hendrixson & Bond (2007). Sequences were gathered from both basal and derived species of *Antrodiaetus*, and from members of the genus *Aliatypus*, the likely sister genus to *Antrodiaetus* (Coyle 1971). More distant outgroups included representatives of the mygalomorph families Atypidae and Ctenizidae, following findings of Hedin & Bond (2006). All specimens used in this study have been assigned a unique specimen identification (MY) number (see Appendix). Upon completion of our on-going studies, these voucher specimens will be deposited in the California Academy of Sciences, San Francisco, California.

Data collection

Most spiders were transported live back to the laboratory, where entire legs were removed from freshly sacrificed specimens and preserved in 100% EtOH at –80 °C. Voucher specimens were stored separately in 80% EtOH at –20 °C. Genomic DNA was extracted from leg tissue using the cetyltrimethyl ammonium bromide (CTAB) protocol of Shahjahan *et al*. (1995), or DNeasy kits (QIAGEN). To confirm sequence accuracy, and assess population variation, we typically gathered data from more than one individual per collection site. Three separate gene fragments were amplified via polymerase chain reaction (PCR), including two fragments from the mitochondrial genome (cytochrome oxidase I (COI), ~1 kb; 12S, ~1kb), and an approximately 1 kb fragment of nuclear 28S (LSU) rRNA. PCR primers and conditions are detailed in Table 1. PCR products were purified on polyacrylamide gels, and sequenced directly using ABI Big Dye chemistry on an ABI 377 machine. COI and 28S templates were sequenced in both directions to confirm sequence accuracy. The 12S sequences were determined in only a single direction, but collection of two individuals per site allowed for sequence confirmation. MACCLADE 4.0 (Maddison & Maddison 2000) was used to edit, manually align, and manipulate sequences.

Sequence alignment

The protein-coding COI sequences contained no insertions, deletions or stop codons, and were aligned manually using MACCLADE. Conversely, considerable length variation was found in both the 12S and 28S data. We used several alignment methods to accommodate this length variation, including secondary structure informed, CLUSTALX (Thompson *et al*. 1997), and manual alignments. Conducting multiple alignments allowed us to gauge the sensitivity of phylogenetic results to alignment method, and we were ultimately most confident in those clades recovered across all alignments. Alignments were conducted as follows: (i) Secondary-structure informed (12SSSI) — the model for animal 12S rDNA proposed by Hickson *et al*. (1996) — was used to create secondary structure and gap penalty masks in CLUSTALX. Aligned sequences were then imported into MACCLADE where the sequences were inspected by eye and further insertion/deletion events were inferred where necessary; (ii) CLUSTALX (12SCLU,

Table 1 PCR primers and amplification protocol

Target gene	PCR primers	AT
COI	*C1-J-1718, 5′-GGAGGATTTGGAAATTGATTAGTTCC-3′	44°
	*C1-J-1751SPID, 5′-GAGCTCCTGATATAGCTTTTCC-3′	
	*C1-J-1751RIV, 5′-GAGTTCCTGATATRGCTTTTCC-3′	
	*C1-N-2568, 5′-GCTACAACATAATAAGTATCATG-3′	
	*C1-N-2776, 5′-GGATAATCAGAATATCGTCGAGG-3′	
12S	LR-J-13328, 5′-TGATTATGCTACCTTAGCAC-3′	44°
	LR-J-13417, 5′-ATGTTTTTGTTAAACAGG-3′	
	*SR-N-14588, 5′-AGGATTAGATACCCTATTAT-3′	
28S	*28SN, 5′-GTGAGACCGATAGCAAACAAG-3′	50°
	*28SC, 5′-GGTTCGATTAGTCTTTCGCC-3′	

PCR experiments were conducted as follows: 92 °C initial denaturation for 30 s, 30 cycles of 92 °C for 30 s, AT (annealing temperature) at +0.2°C for 45 s deg/cycle, 72 °C for 1 min 30 s, final extension at 72 °C for 5 min. Primer references as follows: 1751SPID, 2568, 2776 (Hedin & Maddison 2001); 1718, 13328, 13417, 14588 (Simon *et al*. 1994); 1751RIV, 28SN, 28SC (this study). Primers marked with an asterisk were used in sequencing reactions.

28SCLU) — the computer program CLUSTALX (Thompson *et al*. 1997) was used to align the data using a traditional progressive alignment approach. We used 16/6 gap-opening/gap-extension multiple alignment parameters and default DNA transition weights. Upon completion of an initial alignment, we searched for areas of ambiguous alignment, and used the 'realign selected residue range' function until regional alignments stabilized; (iii) Manual (12SMAN, 28SMAN) — sequences were aligned manually in MACCLADE, with taxon labels recoded ('hidden') to minimize potential alignment bias due to preconceived notions of relationship. We found it difficult to manually align 28S ingroup sequences with outgroup sequences, and therefore excluded outgroups from the 28SMAN alignment.

Phylogenetic analyses

We conducted phylogenetic analyses on all three data partitions in isolation, plus an analysis of a combined data matrix. We were interested in separate analyses for two reasons. First, although the linked mtDNA genes should share the same genealogical history, and therefore be congruent, the alignment issues apparent in 12S might preclude such congruence. In essence, we conducted separate analyses on the mtDNA genes to help judge the accuracy of our 12S alignments. Separate analyses were conducted on the nuclear vs. mitochondrial partitions to explicitly visualize and assess genealogical congruence, as such congruence is fundamental in our arguments regarding species status (following Avise & Ball 1990; Sites & Marshall 2004; Kuo & Avise 2005).

A combined data matrix (28SCLU +12SSSI + COI) was assembled that consisted of approximately 2.5 kb of sequence data for a single representative per sampled site. For *A. riversi*, concatenated sequences were generated from the same specimen in all but one instance (see Appendix I). For outgroup taxa, sequences were combined for congeneric spiders, or were from the same specimen (see Appendix I).

Parsimony analyses were conducted using heuristic searches [(tree-bisection–reconnection) TBR branch-swapping, 1000 random addition sequence replicates] as implemented in PAUP* 4.0b8-b10 (Swofford 2002). Relative support of reconstructed clades was evaluated using the nonparametric bootstrap (Felsenstein 1985), based on analyses comprising 1000 pseudoreplicates (heuristic TBR branch-swapping, five random addition sequence replicates per pseudoreplicate). For the length-variable data, parsimony analyses were conducted treating gaps as both missing and as a 'fifth state'. Gaps were treated as missing in combined data analyses.

Bayesian analyses were conducted using MRBAYES version 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Sequence evolution models were chosen using MODELTEST (Posada & Crandall 1998), based on the Akaike information criteria (AIC) as suggested in Posada & Buckley (2004). The COI data were analysed using a partitioned strategy (see Ronquist & Huelsenbeck 2003; Nylander *et al*. 2004; Brandley *et al*. 2005), where a separate model was applied to each COI codon position. Ribosomal DNA sequences were not partitioned (e.g. stem vs. loop, etc.) because of shortness of sequences (12S) and ambiguities in loop identification (28S). The same sequence models used in separate analyses were used in a partitioned analysis of the combined data. For partitioned COI and combined analyses, estimated parameters (revmat, statefreq, gamma shape, pinvar) for each partition were 'unlinked'. Default cold and heated chain parameters were used in all analyses. Two independent searches were run for 1–2 million Markov chain Monte Carlo (MCMC) generations; we considered the sampling of the posterior distribution to be adequate when the average standard deviation of split frequencies (compared across two independent searches) dropped below 0.01 (Ronquist *et al*. 2005). Approximately 40% of all sampled trees were excluded as burn-in, with the remaining used to generate a majority rule consensus tree.

Divergence time analysis

To estimate divergence times without assuming a molecular clock, we used the penalized likelihood method (Sanderson 2002), implemented in R8S version 1.71 (Sanderson 2003). A range of rate smoothing parameter values were used, with both additive and logarithmic penalty functions. Each run included five searches from random starts (num_time_guesses = 5), and five restarts after each search (num_restarts = 5). The following commands were used to implement this search strategy: set smoothing = 1/100/1000, penalty = add/log, checkgradient = yes, num_time_guesses = 5, num_restarts = 5; divtime method = pl algorithm = tn;). Similar R8S analyses were run on trees derived from both parsimony and Bayesian analyses of the combined data.

We considered two options for calibration of relaxed clocks (i.e. deriving absolute estimates of time). First, we used a 'secondary calibration' derived from Hendrixson & Bond (2007), who estimated the divergence time of *A. riversi* and *A. hadros* at between 80 Ma and 100 Ma. These authors used the fossil antrodiaetid *Cretacattyma raveni*, known from the lower Cretaceous (~110 Ma; Eskov & Zonshtein 1990), to derive their original time estimates. Alternatively, we used an internal biogeographical calibration to fix the age of a single node within *A. riversi* phylogenies. This calibration was based on the phylogenetic isolation of the Monterey population (see Results). We hypothesize that this divergence results from the formation and persistence of the Salinas seaway. This

large marine embayment extended inland from Monterey Bay southeast to the southern Central Valley (Fig. 1), and appears to have been intact from ~5–2.5 Ma (Harden 1997; Fig. 11.17; Jacobs *et al*. 2004; Fig. 1). Since it is the presence of the seaway that is the proposed cause of vicariance, rather than its absence, we used fixage = 5 as a calibration point. We note that a similar Mio-Pliocene vicariance scenario has been hypothesized for the salamander genus *Ensatina* (Wake 1997; Fig. 6).

Results

The Appendix provides sequence lengths and GenBank Accession numbers for all gene fragments, and summarizes the outgroup taxonomic composition of each data matrix. Sequences of COI (~1000 bp), 12S (~450 bp), and 28S (~900 bp) were gathered for 60, 61, and 72 *A. riversi* individuals, respectively. Except for the lack of 12S data for the Stevens site, at least one sequence was gathered for all 32 sites for all gene fragments. Table 2 provides a summary of phylogenetic analysis statistics for both individual and combined matrices (e.g. alignment lengths, parsimony statistics, models, burn-in, etc.).

COI phylogeny

A partitioned Bayesian analysis strongly supports the monophyly of *A. riversi* (Fig. 2). Within *A. riversi*, four strongly supported clades (PP > 0.99) are revealed that reflect deep, geographically coherent, genetic divergences. A 'Northern' clade includes haplotypes from eight sites north and east of the Eel River. A 'Bay Area' clade includes haplotypes from 10 sites surrounding the San Francisco Bay, ranging from near Santa Cruz north to the Navarro River. A 'Sierran' clade includes spiders from six mid- to high-altitude sites (all above 1000 m above sea level), ranging from Lake Tahoe south to Yosemite. Finally, a 'Valley' clade includes sequences from the Sutter Buttes, two sites in the western foothills of the Sierra Nevada, and two sites south and east of Monterey. Haplotypes from three 'rogue populations' (Monterey, Bartlett, Redway) do not group with any of the four major clades, despite the geographical proximity of some rogue sites to other sampled sites (e.g. Monterey and Tassajara, Redway and Mad River, see Fig. 1). The rogue populations, Bay Area and Sierran clades together form a well-supported clade (PP = 0.96), but otherwise, relationships among the four major clades are largely unresolved. COI sequences sampled from the same site form exclusive genetic clades in all but one case. This instance involves the geographically adjacent Crane Flat and Tuolumne sites, which are fixed for the same COI haplotype. These sites also represent the southernmost known records for *A. riversi* in the Sierra Nevada (Fig. 1).

A strict consensus of most-parsimonious COI trees is essentially identical to the Bayesian consensus tree, but is less resolved at deeper levels. The consensus tree (not shown) includes four genetic clades plus three rogue populations as above, which together form a seven-clade polytomy within a well-supported *A. riversi* clade. Parsimony bootstrap values are generally lower than posterior probability

Table 2 Summary of phylogenetic analyses

	Parsimony				Bayesian		
Matrix	Aligned length	PIC	PT	PL	Model	Burn-in	HML
COI	1008	338	N = 36	L = 1699	GTR + I + G, pos 1 & 2, GTR + G, pos 3	8000 (20000)	–7978.04, –7970.25
12SSSI G5	450	233	N = 9	L = 1068			
12SSSI GM	'	214	N = 30	L = 915	GTR + I + G	8000 (20000)	–4396.24, –4398.45
12SMAN G5	446	231	N = 4	L = 1109			
12SMAN GM	'	215	N = 2	L = 915			
12SCLU G5	447	220	N = 7	L = 1041			
12SCLU GM	'	208	N = 22	L = 895			
28SMAN G5	915	194	N = 1232	L = 452			
28SMAN GM	'	133	N = 687*	L = 288*			
28SCLU G5	1076	378	N = 408	L = 1419			
28SCLU GM	'	265	N = 21*	L = 922*	GTR + I + G	8000 (20000)	–5582.29, –5575.22
Combined GM	2528	740	N = 2	L = 3437	As above	6000 (18984)	–17461.58, –17464.73

G5, gaps scored as fifth state; GM, gaps scored as missing; PIC, number of parsimony-informative characters; PT, number of most-parsimonious trees; PL, length of most-parsimonious trees; Burn-in, number of trees/parameters excluded from initial MCMC chains (total number of trees/parameters sampled *per independent run* in parentheses); HML, post burn-in harmonic mean likelihood values for each of two independent runs. *To facilitate parsimony and bootstrap analysis of the 28SMAN and 28SCLU GM matrices, we included only a single *A. riversi* sequence per sampling site.

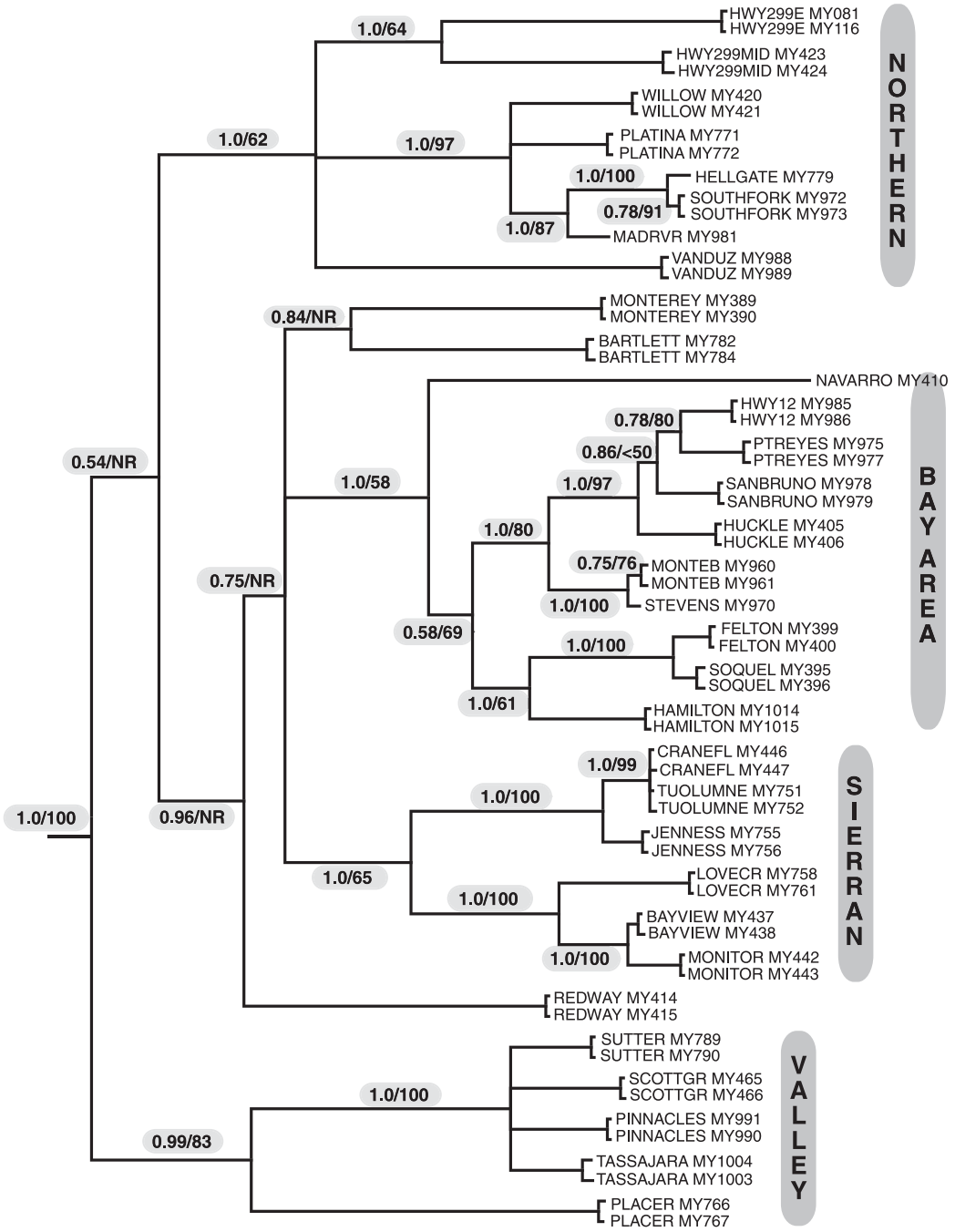

Fig. 2 COI Bayesian consensus phylogram, showing both posterior probability and parsimony bootstrap support values (PP/bootstrap). Branch lengths are averaged from across the posterior distribution (post burn-in). Support values for the monophyly of haplotypes sampled from the same site equals PP > 0.95/BS > 95, unless indicated otherwise. For bootstrap values, NR denotes clade not recovered in strict consensus of most-parsimonious trees. Outgroup relationships are not shown.

values at deeper nodes in the tree, but equivalent and high at shallow nodes (Fig. 2).

As summarized in Table 3, COI divergence values within and among major clades are quite high. Most interclade genetic distances hover around 10% average pairwise divergence, with average intraclade distances ranging from 5 to 8%. Sequences from Monterey, Redway and Bartlett are noticeably divergent. There exists little to no sequence diversity within sampled sites [maximum Kimura 2-parameter (K2P) distance = 0.003).

Table 3 Genetic distances

COI	North	Bay	Valley	Sierran	Monterey	Bartlett	Redway
North	0.0787	0.1127	0.1224	0.1240	0.1130	0.1066	0.1171
Bay	—	0.0587	0.1121	0.0955	0.0898	0.0804	0.0984
Valley	—	—	0.0691	0.1107	0.1107	0.0984	0.1324
Sierran	—	—	—	0.0553	0.0904	0.0887	0.0955
Monterey	—	—	—	—	—	0.0696	0.1015
Bartlett	—	—	—	—	—	—	0.0878
Redway	—	—	—	—	—	—	—
Within-site	min = 0.0, mean = 0.0003, max = 0.0033						
12S							
	North	Bay	Valley	Sierran	Monterey	Bartlett	Redway
North	0.0781	0.1526	0.1529	0.1649	0.1383	0.1519	0.1524
Bay	—	0.0716	0.1526	0.1316	0.1260	0.1164	0.1100
Valley	—	—	0.0875	0.1644	0.1530	0.1582	0.1297
Sierran	—	—	—	0.0710	0.1311	0.1357	0.1218
Monterey	—	—	—	—	—	0.1382	0.1131
Bartlett	—	—	—	—	—	—	0.1122
Redway	—	—	—	—	—	—	—
Within-site	min = 0.0, mean = 0.0002, max = 0.0024						
28S							
	North	Bay*	Valley	Sierran	Monterey		
North	0.0345	0.0941	0.0949	0.0848	0.0944		
Bay	—	0.0100	0.0389	0.0568	0.0326		
Valley	—	—	0.0111	0.0551	0.0178		
Sierran	—	—	—	0.0186	0.0468		
Monterey	—	—	—	—	—		
Within-site	min = 0.0, mean = 0.0002, max = 0.0023						

All values are reported as average Kimura 2-parameter (K2P) distances (Kimura 1980), calculated using a single, randomly chosen haplotype per site (except for within-site values). For the 12S data, the 12SSSI alignment was used. For the 28S data, Bay Area clade values include Bartlett and Redway sites, and were calculated using the 28SMAN alignment.

12S phylogeny

Because of general consistency in parsimony results across 12S alignments, we restricted Bayesian analysis to a single matrix (12SSSI). Bayesian analysis of this matrix supports the monophyly of *A. riversi*, and recovers four major clades (Fig. 3), as seen for the COI data. Again, the three rogue populations do not group within any larger clade, and furthermore, their 12S placement differs from that recovered in COI analyses. The basal placement of a Northern clade is moderately supported, and similar to the COI Bayesian results, Sierran, Bay Area and rogue sites together form a clade, exclusive of Northern and Valley clades (PP = 0.99). Except for the Crane Flat/Tuolumne population pair, all 12S haplotypes are confined to a single site (i.e. each site is genealogically exclusive).

Although tree topologies are not reported here, clade recovery and parsimony bootstrap values for each of the six different 12S alignments are summarized in Fig. 3. Parsimony analysis of most alignments results in the recovery of four major clades as follows: Northern (all alignments), Sierran (all alignments), Valley (SSI and MAN) and Bay Area (SSI and MAN). Phylogenetic placement of the Monterey, Redway and Bartlett populations is never strongly supported, and varies across alignments (Fig. 3).

Patterns of 12S divergence are qualitatively similar to those seen for the COI data, but generally higher in absolute divergence (Table 3). Interclade K2P distances range from 12 to 16% average pairwise divergence, with average intraclade distances ranging from 7 to 8%. Again, there exists little to no 12S sequence variation within sites.

28S Phylogeny

The 28SCLU alignment included several regions characterized by long insertions found in outgroup taxa only; these regions (comprising 122 of 1076 aligned bases) were excluded from all subsequent phylogenetic analyses. Because Bayesian analysis of the 28SCLU alignment results in an unexpected root placement (see below), we first discuss results of 28S parsimony analyses in the context of a 28SCLU parsimony phylogram (Fig. 4). These analyses result in a phylogenetic picture that is generally congruent with mtDNA results. Three clades are strongly supported

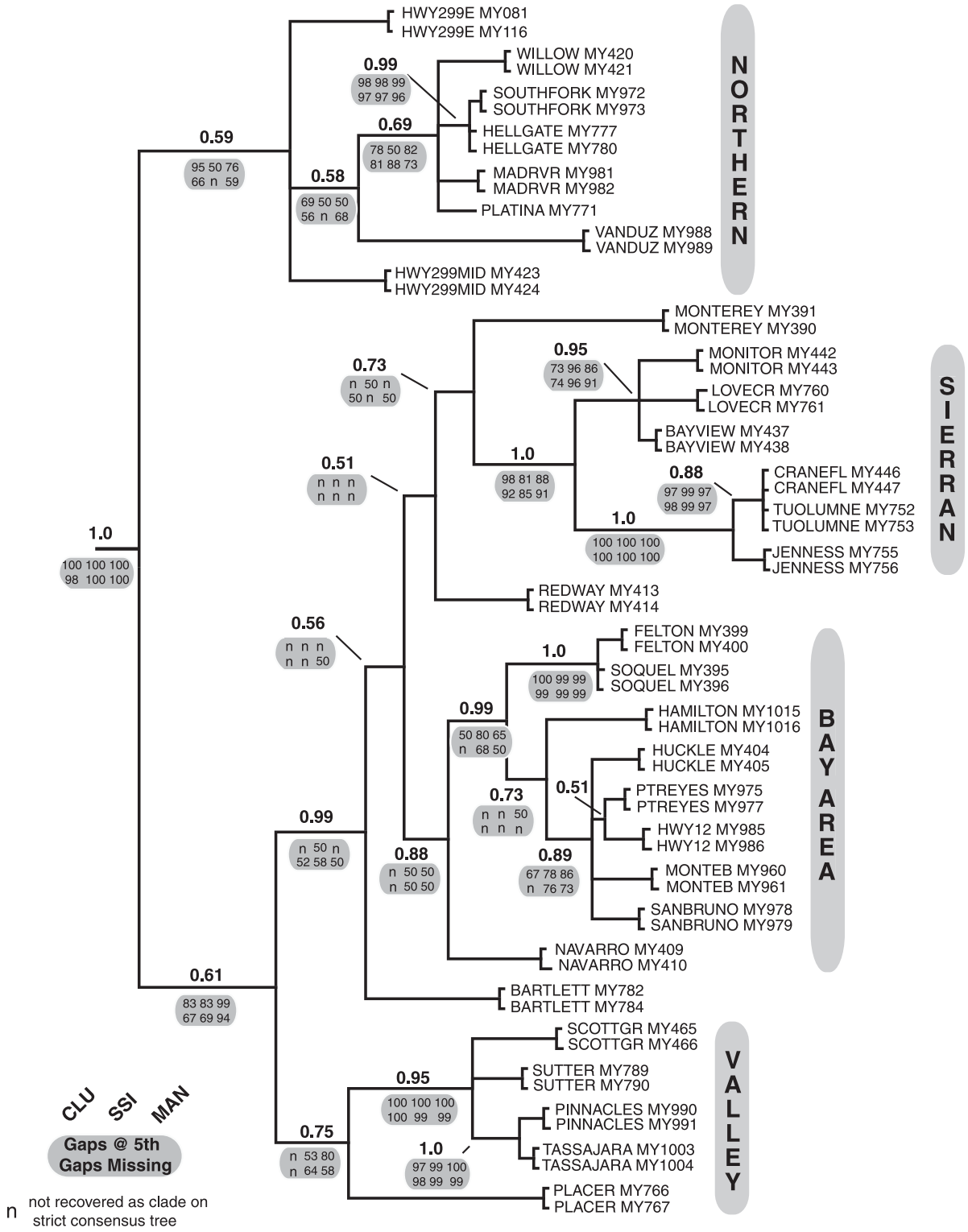

Fig. 3 12S Bayesian consensus phylogram (12SSSI alignment), with posterior probability values. Branch lengths are averaged from across the posterior distribution (post burn-in). Posterior probability values for clades of haplotypes sampled from the same site > 0.95, except for Bayview site (PP = 0.89). Also shown is the presence and bootstrap support for clades recovered on a strict consensus parsimony tree for each individual 12S alignment; bootstrap values < 50% are displayed as '50'. Parsimony bootstrap values for clades of haplotypes sampled from the same site are not shown. Outgroup relationships are not shown.

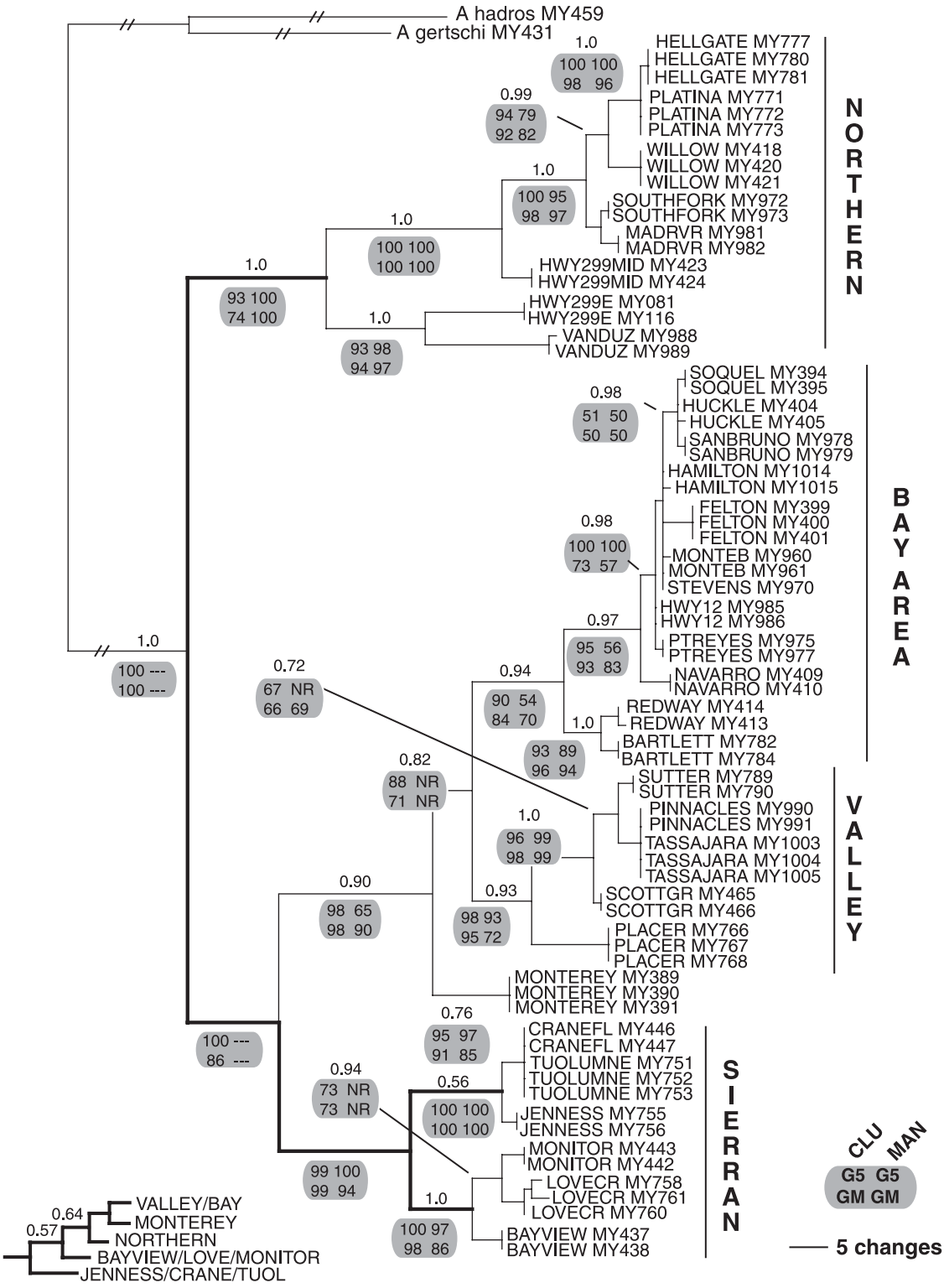

Fig. 4 Randomly chosen most parsimonious tree derived from analysis of 28SCLU alignment, gaps as fifth state, with branch lengths drawn proportional to length. Also shown is bootstrap support for clades recovered on the strict consensus parsimony tree for each individual 28S alignment (G5, gaps as fifth state; GM, gaps as missing); bootstrap values < 50% are displayed as '50'; NR denotes not recovered on strict consensus tree. Above branches are posterior probability values derived from Bayesian analysis of the 28SCLU alignment; lower inset includes alternative tree topology recovered in this analysis. Support for multiple-site clades not shown: SOUTHFORK + MADRVR (PP = 0.99, bootstrap > 63), PINNACLES + TASSAJARA (PP = 0.99, bootstrap > 82). Support values for clades of haplotypes sampled from the same site not shown. Illustrated branches leading to outgroup antrodiaetid taxa are truncated; deeper outgroup relationships are not shown.

(bootstrap values > 70), including Northern, Valley and Sierran clades (Fig. 4). A Bay Area clade is also recovered, but with lower support. An important difference between 28S and mitochondrial trees is the 28S placement of Redway and Bartlett populations as sister to the Bay clade. This result is not inconsistent with the mtDNA data, as the placement of these populations is essentially unresolved for mtDNA genes. Similar to mtDNA results, the position of Monterey remains ambiguous in 28S trees, and the Northern clade is sister to remaining populations. Only three groups of geographically proximate sites share 28S haplotypes, including Tuolumne/Crane Flat, Tassajara/Pinnacles, and Hamilton/Montebello/Stevens (Figs 1 and 4). Other parsimony results (i.e. 28SMAN gaps as fifth, 28SMAN gaps as missing, 28SCLU gaps as missing) are consistent with the results summarized above (Fig. 4).

Bayesian analyses of the 28SCLU alignment result in trees that are mostly consistent with parsimony results, but differ importantly in root placement. The root is placed on a branch leading to a subclade of the Sierran clade, rendering the Sierran clade paraphyletic, and basal to all other clades (Fig. 4). This root placement is weakly supported (PP < 0.70), and moreover, is never found in mtDNA trees, 28S parsimony trees, or combined data trees. We suggest that this unexpected result is an artifact of the 28SCLU alignment, and the extreme divergence between ingroup and outgroup sequences.

Levels of 28S divergence within and across clades are unexpectedly high (Table 3), with limited diversity found within populations. The gene 28S is a relatively conserved gene, typically used in resolving deeper phylogenetic relationships in animals (e.g. Hillis & Dixon 1991; Mallatt *et al*. 2004), and we are aware of relatively few animal studies that have used this gene at the population and/or species level (e.g. Monaghan *et al*. 2005). We hypothesize that the observed extreme levels of divergence seen in *A. riversi* reflect both accelerated rates of nuclear rDNA evolution in the family Antrodiaetidae (Hedin & Bond 2006; Hendrixson & Bond 2007), and a relatively deep age for this species complex.

Combined data analysis

Parsimony and partitioned Bayesian analysis of the combined data result in phylogenetic trees that are similar to each other in both topology and relative branch lengths (Fig. 5). These trees are also largely consistent with individual gene analyses, and provide very strong support for four major clades, plus an isolated Monterey population. Consistent with the 28S results, the Bay Area clade extends northwards to include the Bartlett and Redway sites (see Fig. 1). The rogue nature of Monterey is illustrated by the lack of strong positional support in either Bayesian or parsimony analysis; the phylogenetic position of Monterey also varies in position across combined analyses (Fig. 5). Consistent with other analyses, the Northern clade appears as a sister group to all remaining clades.

Divergence time analysis

Combined data trees from parsimony and Bayesian analyses (see Fig. 5), with associated branch lengths, were used as input trees in r8s analyses. Although two most-parsimonious trees result from parsimony analyses, these trees differ only in the placement of a single population (Mad River), and imply very similar divergence time estimates (results not shown). In general, time estimates based on Bayesian input trees are 'shallower' than estimates from parsimony trees, but this pattern does not apply at all nodes (Table 4). Absolute time estimates based on the 'secondary calibration' (fixage = 80 at node separating *A. hadros* and *A. riversi*) are 2–3 times higher than time estimates based on the biogeographical calibration (Table 4), and are considered to be unrealistically ancient. Alternatively, the Salinas seaway calibration results in time estimates (Table 4, Fig. 6) that are consistent both with biogeographical reconstructions for other Californian taxa, and with geological/climatic data (see below).

Discussion

Our studies of divergence dynamics within *Antrodiaetus riversi* have uncovered high species diversity and biogeographical complexity that is masked by current taxonomy. This cryptic diversity and complexity was foreshadowed by studies of Coyle (1968) and Ramirez & Chi (2004), who suggested the potential for multiple species in this group. In fact, we hypothesize that *A. riversi* represents a complex of at least five deeply diverged, allopatric species. Each of these species is characterized by extreme population subdivision, apparent at small spatial scales in both male and female-based genetic systems. The biogeographical relationships of these taxa, and the inferred time depth of speciation events, are partially mirrored in other well-studied vertebrates from California, particularly dispersal-limited salamanders. In sum, the novel insights provided by multilocus genealogical data drastically change our perception of evolution and diversity in these spiders.

Species limits

Many different empirical criteria can be used to delimit species (reviewed in Sites & Marshall 2004). Of these, we favour tree-based methods, which are used to recognize species as historical lineages (De Queiroz 1998), and also favour methods that rely upon multiple lines of evidence. This mixed-evidence philosophy is particularly important in a low gene flow system like the *A. riversi* complex, where

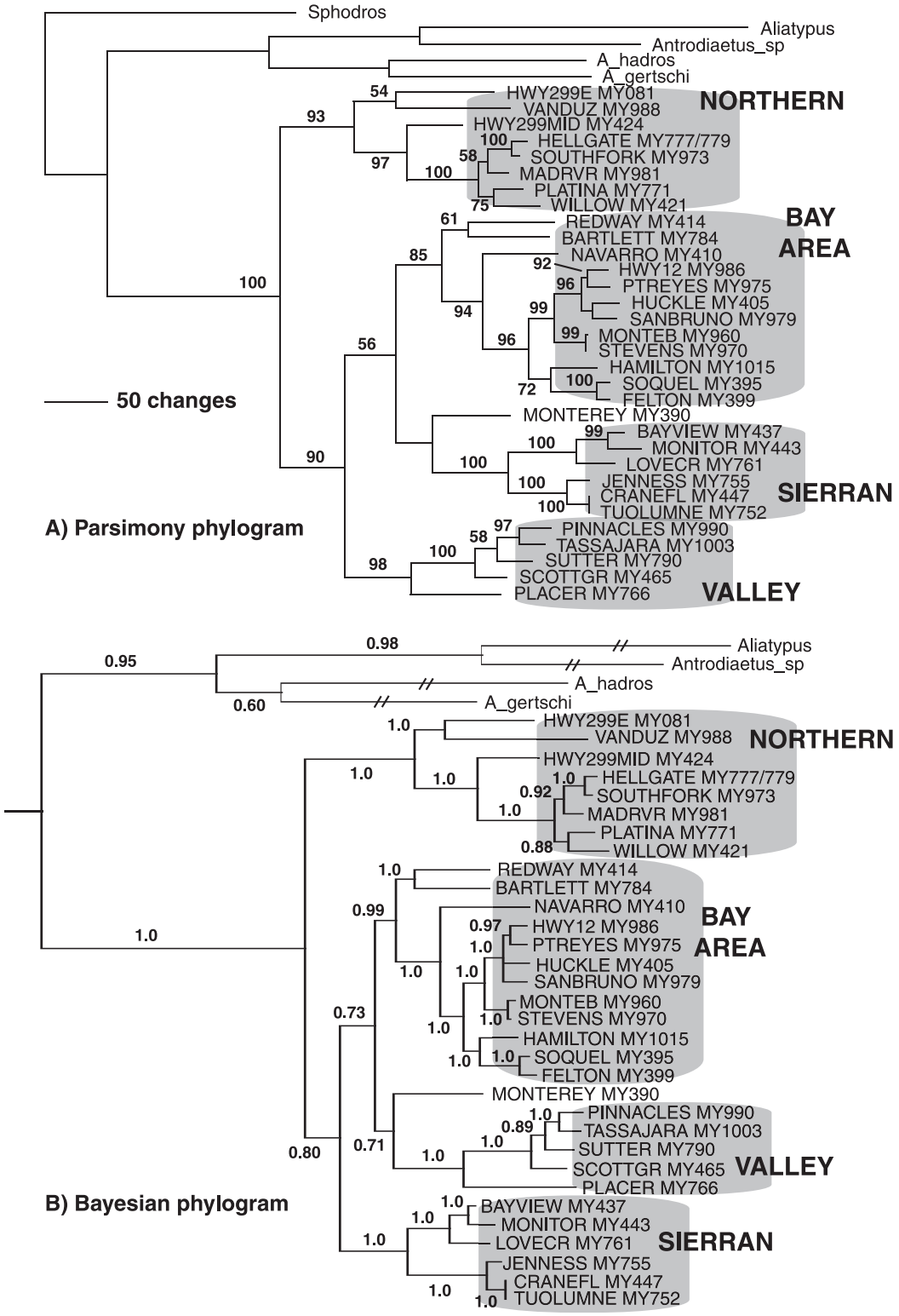

Fig. 5 Results of combined data analysis. (A) Parsimony phylogram (branch lengths drawn proportional to length), shown for one of two most-parsimonious trees. Bootstrap values above 50% shown. (B) Bayesian phylogram, with posterior probability values. Branch lengths are averaged from across the posterior distribution (post burn-in). Illustrated branches leading to outgroup antrodiaetid taxa are truncated.

Table 4 Penalized likelihood divergence time estimates

	Secondary calibration		Salinas calibration	
Node	ParsTree	BayesTree	ParsTree	BayesTree
1	46.04–51.87	22.52–25.09	9.62–11.85	8.38–8.68
2	28.63–36.08	13.68–14.95	6.03–7.8	4.95–5.28
3	20.19–26.03	10.67–11.74	4.26–5.56	3.87–4.17
4	17.41–23.27	7.78–9.04	3.68–4.67	2.82–3.28
5	6.62–9.35	2.53–3.49	1.59–1.87	0.92–1.33
6	4.84–6.93	1.83–2.51	1.14–1.36	0.66–0.96
7	1.32–2.04	0.51–0.77	0.34–0.41	0.18–0.3
8	4.48–6.53	1.78–2.62	1.15–1.36	0.65–1.01
9	33.70–40.50	17.76–20.07	7.51–8.65	6.80–7.17
10	25.14–31.88	14.41–17.07	6.18–6.59	5.56–5.76
11	18.03–23.66	11.60–14.21	4.72–4.83	4.49–4.82
12	13.99–18.39	8.87–11.32	3.53–3.67	3.44–3.94
13	12.34–16.87	7.78–9.47	3.24–3.6	3.02–3.13
14	7.40–10.70	5.59–7.82	1.94–2.49	2.17–2.23
15	4.60–6.99	3.56–5.62	1.21–1.72	1.37–1.42
16	2.39–4.93	2.06–2.49	0.63–1.05	0.74–0.83
17	1.85–3.81	1.44–1.77	0.49–0.81	0.51–0.59
HU/SB	1.64–3.65	—	0.43–0.78	—
18	0.26–0.39	0.50–0.62	0.07–0.08	0.19–0.21
19	5.03–8.38	4.23–5.11	1.32–1.78	1.63–1.69
20	1.06–1.95	1.05–1.30	0.28–0.42	0.38–0.43
MONT	19.08–25.23	12.95–15.18	—	—
21	20.64–25.93	8.22–9.75	4.41–5.17	2.94–3.35
22	8.13–11.35	2.94–3.88	2.04–2.19	1.05–1.4
23	5.24–7.71	2.02–2.84	1.31–1.57	0.72–1.04
24	2.85–4.40	1.17–1.76	0.71–0.94	0.42–0.66
25	10.21–14.83	9.33–11.55	2.67–3.15	3.6–4.13
26	3.99–7.02	3.90–5.29	1.04–1.49	1.51–1.89
27	1.63–3.37	1.83–2.53	0.43–0.72	0.71–0.9
28	2.74–3.94	1.56–1.96	0.7–0.82	0.61–0.69

For each node (corresponding to those numbered in Fig. 6), a minimum–maximum time estimate (in millions of years) is shown for (i) each calibration (secondary or biogeographical), and (ii) each input tree topology (parsimony or Bayesian — ParsTree, BayesTree). Min–max values derived from analyses that varied rate smoothing parameter values (1/100/1000), with both additive and logarithmic penalty functions (add/log). For analyses that used the secondary calibration, *Sphodros* was used to root the *A. riversi*/*A. hadros* clade, but was then pruned (following the r8s manual). For analyses using the biogeographical calibration, *A. hadros* was used to root the *A. riversi* tree, but was subsequently pruned. All other outgroups were pruned from trees prior to analysis. Composite haplotypes from Crane Flat were identical to those of Tuolumne (see Fig. 5), and were therefore excluded from clock analyses.

deep phylogeographical breaks can arise stochastically (see Kuo & Avise 2005), and reliance upon the topology of a single gene tree (e.g. Fig. 2) to define species is clearly problematic. Instead, we have used multiple gene trees to assess genealogical congruence, which we use as primary evidence for recognizing species limits (Avise & Ball 1990; Baum & Shaw 1995). Genealogical congruence describes the presence of concordant genealogical breaks in independently segregating loci, and is expected only when lineages have been separated for long periods of time (relative to population size), and have maintained this evolutionary separation (i.e. no gene flow after divergence) (Avise & Ball 1990; Baum & Shaw 1995; Kuo & Avise 2005).

At least five species in the *A. riversi* complex are recognizable under a genealogical congruence framework. These correspond to the Bay Area, Northern, Sierran and Valley clades, each of which includes multiple populations (Figs 1 and 5). We also consider the phylogenetically isolated Monterey population to represent a geographically restricted species. Formal taxonomic description of these taxa will be published elsewhere; here, we continue to refer to these species/clades by informal geographical names. The Northern, Sierran, and Valley species are consistently and strongly recovered in analyses of all genes (Figs 2–4). The Bay Area species including Bartlett and Redway populations is an exception (Fig. 1), because these latter populations are divergent in mtDNA analyses. However, the placement of these populations is well-supported by the 28S data, and is not strongly contradicted by the mtDNA data. Such a phylogenetic pattern has been referred to as 'genealogical nondiscordance' by Dettman *et al*. (2003). We believe that the isolation of Redway and Bartlett mtDNA haplotypes is an artefact of high genetic divergence with sparse geographical sampling, and predict that further sampling in the north Coast Ranges will ultimately strengthen the mitochondrial cohesion of the Bay Area clade. Nevertheless, it is clear that considerable north to south phylogeographical structure exists in this Bay Area species (e.g. Fig. 5).

Other lines of evidence, including genetic divergence and geographical information, as well as prior morphological (Coyle 1968) and genetic research (Ramirez & Chi 2004), are consistent with a multiple-species hypothesis. Levels of genetic divergence between these species are quite high (Table 3), suggesting relatively old divergences (Table 4). All five species, except for the Valley clade, occupy cohesive geographical distributions (Fig. 1). Even the Valley clade, which includes populations that are disjunct across the Central Valley, shows a distribution that is generally similar to distributions found in other Californian taxa (see below). Although the species' distributions appear to be largely allopatric, we believe that further geographical sampling will actually reveal species parapatry. The predicted regions of parapatry, and possible contact, are highlighted in Fig. 1. An interesting aspect of this parapatry hypothesis is that, in all cases, species predicted to be in contact are not obviously sister taxa. Moreover, in most cases, these regions do not involve conspicuous biogeographical barriers. For example, the habitat separating the very divergent Tassajara (Valley clade) and Monterey populations (Fig. 1) is essentially continuous, and we should

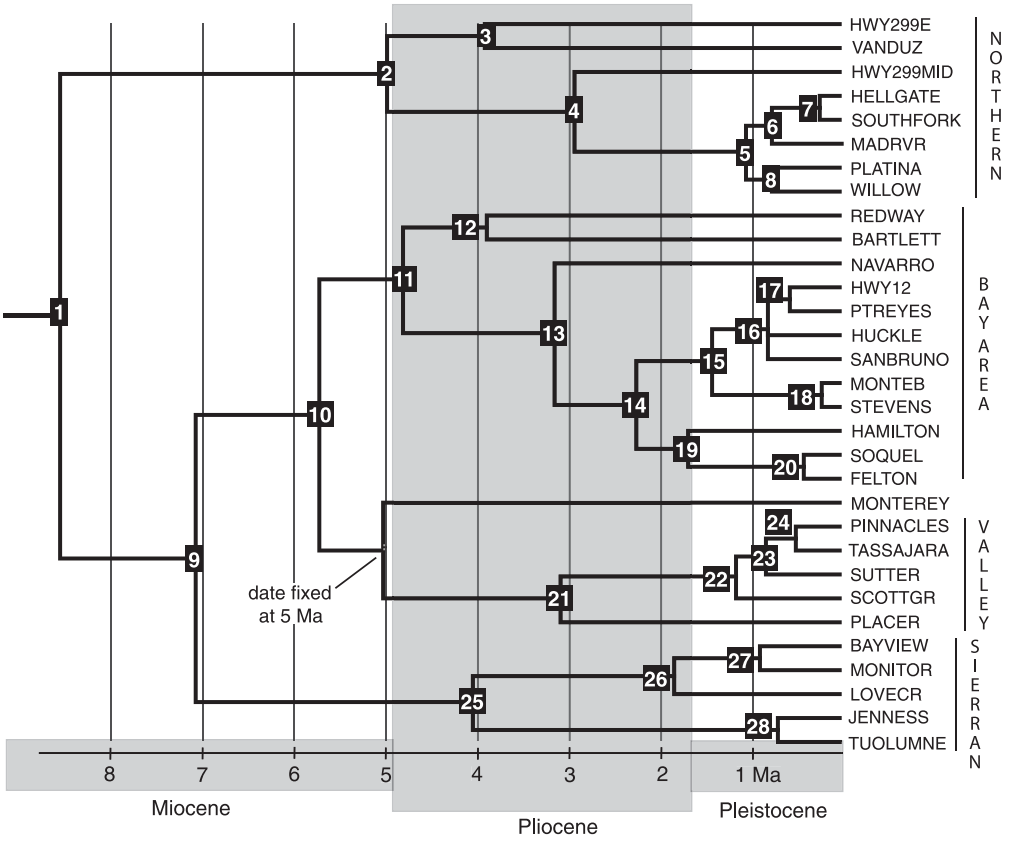

Fig. 6 Chronogram derived from penalized likelihood analysis (biogeographical calibration, Bayesian input tree, S = 100, penalty = log). Composite haplotypes from Crane Flat were identical to those of Tuolumne (see Fig. 5), and therefore excluded from all clock analyses.

be able to 'close the gap' in this region. A similar argument holds for habitats east of Placerville, separating Valley and Sierran species, and the region northeast of Redway, separating Bay Area and Northern species (Fig. 1). The distributions and biogeographical relationships of all species are discussed more extensively below.

Coyle (1968) clearly recognized morphological divergence within *A. riversi*, particularly between coastal vs. Sierran populations. He observed that for a majority of his character measurements, univariate 'distances' were smaller within the two geographical groups than between, with some measurement ratios providing obvious discrimination of these groups (e.g. Fig. 24, Coyle 1968). Of course, this dominant east/west divergence does not fully capture the dynamics in the *A. riversi* complex, because three species are actually found in the Coast Ranges; one is restricted to the Sierra Nevada, and one species occurs in both regions. However, Coyle (1968) was able to foresee even these groups. For example, he states that '… specimens collected low in the western foothills of the Sierras are less different from the coastal populations than are most of the higher Sierran specimens …', suggesting evidence for morphological differentiation between the Valley and Sierran species along the western face of the Sierra Nevada (Fig. 1). Also, Coyle (1968) states that 'the only instance of striking intracoastal geographical variation is the relatively long tibia I (and femur I) of sample A (= Miranda)', a comparison between his only Northern sample (Miranda) and other Bay Area samples. Coyle (1968) lacked samples from Monterey, but otherwise, he provided evidence for at least some morphological divergence for all species in the complex. We find this rather remarkable, particularly since mygalomorph spiders are notorious for being morphologically conservative (Hedin & Bond 2006; references therein).

Using a multidimensional scaling analysis of genetic distances from seven variable allozyme loci, Ramirez & Chi (2004) recognized four genetically divergent groups in *A. riversi*. One group corresponds to the Bay Area clade, and two others correspond to subclades of the Sierran species. The authors found that the Sierran Jenness Camp population was particularly divergent, fixed for two private alleles not found in northern Sierran populations (Bayview and Monitor Pass). We found this same north-to-south split in the Sierran clade for all genes (see Figs 2–4), suggesting that we may be underestimating species level

diversity in this region. Analyses of Ramirez & Chi (2004) also grouped Sutter Buttes and Monterey populations together, but this grouping appears to be driven by allelic fixation at a single locus (Sod). These two populations are fixed for alternative alleles at one locus (Mpi), and the other variable loci suggest multiple possible affinities. Again, we found no consistent relationship between the Monterey and Valley clades.

The species hypotheses articulated above can and should be tested in several ways. The several hypothesized regions of contact between species (Fig. 1) are obvious targets for denser geographical sampling. Finding genetic breaks over very short distances, species sympatry, or even evidence for hybridization in these regions would provide further opportunities for studies of species status. Also, more research needs to be conducted on both the morphology and ecology of these species. For example, although male secondary sexual characteristics typically provide the majority of diagnostic character variation in congeneric mygalomorph taxa, adult males are only known for the Sierran and Bay Area species (Coyle 1968). The collection and morphological study of adult males for all species will be extremely important. Finally, the hypothesized species reside in regions of California that differ considerably in several ecological parameters (e.g. vegetation cover, elevation, precipitation, etc.). All populations of the Sierran clade, for example, are found at higher altitudes than any other species in the group. More ecological research, including perhaps ecological niche modelling (e.g. Bond *et al*. 2006), is needed in this species complex.

Population genetic structure

Ramirez & Chi (2004) also used allele frequency data to study population genetic structure in the *A. riversi* complex. As it turns out, their sample of eight populations represents at least four species, with only two species (Bay Area & Sierran) represented by multiple-site samples (see Fig. 1). Overall, and within these two taxa, these authors found minimal genetic variation within sampled sites (observed heterozygosity = 0–5.7%, n = 8–16 spiders/site), and generally high differentiation between sites. Only geographically adjacent sites (two sites in the Santa Cruz Mountains) revealed genetic evidence for gene flow. These authors concluded that 'opportunities for genetic exchange in *At. riversi* will be maximized by the existence of interpopulation forest corridors and *will be essentially nonexistant* when these do not exist or are interrupted by nonforest habitat'.

Our nuclear genealogical data are consistent with the findings of Ramirez & Chi (2004), and moreover, patterns seen in the mitochondrial data indicate that extreme population subdivision applies to both genomes. Basically all sampled sites, for all species in the complex, are characterized by an exclusive set of private alleles for the genes sequenced (see Figs 2–4). Even in cases where nuclear and mtDNA sequences are shared across space (i.e. Tassajara and Pinnacles, Tuolumne and Crane Flat), these site pairs are found at the southwestern and southeastern periphery of species' ranges, respectively (Fig. 1). As such, the genetic similarity seen in these regions might reflect shared ancestral variation, rather than on-going gene flow (see Larson *et al*. 1984). A similar argument might apply to the south Bay region, where sites are genealogically exclusive for mtDNA variation, but some sites share nuclear haplotypes (Fig. 4). Again, this south Bay region seems relatively derived (i.e. most recently colonized), such that shared ancestral polymorphism cannot be ruled out.

In essence, we have found no conclusive genetic evidence for on-going gene flow in these spiders. Denser geographical sampling will be needed to distinguish shared polymorphism from occasional gene flow and to fully characterize the spatial scale at which genetic subdivision occurs, but our current sample clearly indicates that genetic differences arise and persist at small spatial scales. Populations separated by more than 30 km will almost certainly be genetically distinct, and in many cases, populations closer than this are distinct. This finding of microgeographical genetic structuring has been found in other mygalomorph spiders (e.g. Bond *et al*. 2001, 2006; Bond 2004), including other *Antrodiaetus* species (Hendrixson & Bond 2005), and is consistent with the known biology of spiders in the *A. riversi* complex. These spiders live a sedentary, fossorial existence, closely tied to favourable mesic microhabitats (Coyle 1968, 1971; Vincent 1993). Subadult spiders that leave maternal burrows are small and prone to dessication, are not known to balloon, and typically settle close to maternal burrows. Adult males appear to represent the only conduit for potential gene flow, but even here, the spatial scale of male wandering is likely limited. This combination of life history and habitat preference traits results in a spatial 'clumping' of animals across the highly dissected Californian landscape, and this clumping is clearly revealed in the genetic structure of these spiders. California salamanders in the genus *Batrachoseps* and *Ensatina* are like these spiders in being highly sedentary, mesic-habitat specialists, and as expected, show similar patterns of extreme population genetic and phylogeographical structuring (e.g. Wake 1997; Jockusch *et al*. 2001; Jockusch & Wake 2002).

Timing and geography of species diversification

The biogeographical history of the *A. riversi* complex is clearly complicated. There has been diversification both along north/south and east/west axes in California, the age of divergence events appear to be relatively old, and hypothesized zones of species parapatry are more

consistent with secondary, rather than primary, contact. These distributional patterns are similar to those found in other Californian taxa, and agree generally (both spatially and temporally) with known events in the geological and climatic history of California. In drawing the comparisons made below, we have paid particular attention to biogeographical patterns seen in *Ensatina* and *Batrachoseps* salamanders. These salamanders are biologically similar to *Antrodiaetus* spiders, and as such, might be expected to share aspects of evolutionary and biogeographical history in common. For example, in both *Ensatina* and *Batrachoseps*, the general evolutionary dynamic seems to be one of ancestral species expansion, followed by isolation and differentiation, and subsequent secondary contact with or without gene flow (Wake 1997; Jockusch *et al*. 2001; Jockusch & Wake 2002). Although more studies are clearly needed, the data available for the *A. riversi* complex are at least consistent with such a scenario.

Penalized likelihood clock analyses suggest two very different timeframes for *A. riversi* species diversification, depending upon calibration used (Table 4). The older estimated dates (Table 4) seem unrealistic, as few (if any) other California lineages are known to have such ancient species. Conversely, calibration based on the hypothesized isolating effects of the Salinas seaway results in time estimates that more closely match inferred ages for other lineages, and also coincides with known geological events (see below). In general, we appreciate the uncertainty involved in any clock analysis (see Rutschmann 2006), and interpret our results with caution — we view the clock analyses as useful, but clearly not as bullet-proof.

We hypothesize that the *A. riversi* complex is of northern origin. The Northern species is strongly supported as the earliest diverging member of the complex (Fig. 5). This species carries high levels of intraclade genetic diversity (Table 3), and all Northern sites are genealogically exclusive, regardless of geographical proximity. Phylogeographical relationships within the region are complex, and not always predicted by geographical proximity. These observations suggest that Northern *Antrodiaetus* have been able to persist in this topographically complex region of moist redwood and Douglas fir forests for long periods of time. *Ensatina* also has proposed northern origins, with early diverging subspecies in this region characterized by extreme amounts of deep genetic variation, suggesting an ancient, large metapopulation (Moritz *et al*. 1992; Wake 1997).

The basal divergence between the Northern species and all other species in the complex is estimated to have occurred in the Late Miocene, perhaps at least 8 Ma (Table 4; Fig. 6). There was clear palaeorelief in both the Sierra Nevada (Wakabayashi & Sawyer 2001; Fig. 11) and Coast Ranges (Harden 1997) during the Late Miocene (5–10 Ma), providing potential upland habitats for more southerly populations. We also hypothesize that coastal populations would have occurred south of Monterey Bay and the Salinas Basin at this time. Subsequently, southern populations of *A. riversi* were fragmented into four lineages, these being centred in the North Coast ranges (likely north of San Francisco Bay), the central Sierra Nevada, and near Monterey; as discussed below, the geographical origin of the Valley clade is unclear. Lack of phylogenetic resolution suggests that these primary isolation plus divergence events may have been closely spaced in time, and clock analyses suggest a general timeframe centred from 4 to 7 Ma (Table 4; Fig. 6). Interestingly, Calsbeek *et al*. (2003) stated that '… molecular divergence dates for animal groups suggest that a period of molecular differentiation began in California about 7 million years BP'. This time period appears to have been a relatively turbulent time in the geological history of California. In the Sierra Nevada, there has been an apparent pulse of uplift, westward tilting, and stream incision since about 5 Ma (Wakabayashi & Sawyer 2001). The Coast Ranges have been characterized by similar active uplift, associated with plate movements, since about 3–5 Ma (Harden 1997; Jacob *et al*. 2004).

Obviously, since these initial hypothesized divergence events, species' ranges have expanded, contracted, and moved across the landscape to varying degrees. Consideration of the phylogeographical signal within each species, in the light of current geographical distributions, allows us to reconstruct (in general terms) these biogeographical changes. The earliest divergences in the Bay Area clade date to the Early Pliocene (Table 4). From a phylogeographical perspective, these early divergences involve northern populations, which imply a general northern ancestry, with southern Bay Area populations more derived. The southern boundary of this species corresponds rather closely to the northern edge of the Monterey Bay, which is a well-known biogeographical barrier in multiple amphibian species (see Wake 1997). Currently, this region may separate three different lineages in *A. riversi*, including Monterey, Valley, and Bay Area species (Fig. 1). In the north, the boundary between Northern and Bay Area species lies inland of the Mendocino Triple Junction, which is the meeting place of the Gorda, North American, and Pacific plates (Furlong & Schwartz 2004). This is a region of active historical and ongoing tectonic and volcanic activity, which has influenced the surface landscape tremendously (e.g. drainage and fault zone directionality, volcanic eruptions, etc.); potential modern-day barriers include several large northwest-tending rivers, including the Eel River. This general region is also known to correspond with both phylogeographical and species-level distributional breaks in many other vertebrate taxa (see Shaffer *et al*. 2004).

Within the Sierran clade, there exists a deep north/south phylogeographical division (Fig. 5; also Ramirez & Chi 2004), which is dated to ~4 Ma, near the time of renewed

Sierran uplift (Wakabayashi & Sawyer 2001). In general, phylogeographical splits along a north/south axis in the central Sierra Nevada are a common feature of many vertebrate taxa (see Macey *et al*. 2001; Rissler *et al*. 2006). For example, Macey *et al*. (2001) used a molecular clock to date north/south Sierran splits within a frog species, and estimated times (1.5–2.2 Ma.) consistent with effects of Pleistocene glaciation. In Sierran *Antrodiaetus*, we suggest that a combination of both renewed uplift (older) and climatic fluctuation (more recently) is influencing phylogenesis in this species. Sampling is currently sparse, but the modern north–south divergence may coincide with the Stanislaus River.

The Valley species is perhaps the most biogeographically interesting, but also confusing, species in the complex. We are less certain about the geographical origin and boundaries of this species than any other in the complex. The fact that this species spans the Central Valley is unsurprising, as similar distributional patterns are seen in many taxa (see LaPointe & Rissler 2005), including other spiders (e.g. Coyle 1971). For example, such a 'transvalley leak' is seen in *Ensatina eschscholtzii xanthopicta* (Wake 1997), and in *Batrachoseps attenuatus* (Jockusch & Wake 2002). In both of these cases, the 'leak' is centred at latitudes near or north of the San Francisco Bay, phylogeographical directionality seems to be from west (Coast Range) to east (Sierra Nevada), and timing appears to be relatively recent (i.e. mid-Late Pleistocene). Although the Valley *Antrodiaetus* has relatively deep origins (~3–5 Ma, Table 4), clock analyses indicate that the 'leak' occurred relatively recently (late Pleistocene, Fig. 6), consistent with the salamander data. However, in *Antrodiaetus,* basal populations (Placerville) are eastern, and an east to west connection occurs further south, as western populations have only been found southwest of the Monterey Bay (Fig. 1). This would suggest that the geography of the 'leak' is different between spiders and salamanders. We acknowledge, however, that there are many places to look for additional populations of Valley *Antrodiaetus*, including along the eastern edge of the coastal Diablo Range.

Conclusions

This study has opened a window into an unexpectedly diverse system that carries more species richness and biogeographical complexity than anticipated. This newly discovered diversity is important from both a taxonomic and conservation perspective (e.g. the Monterey species is currently known only from a single site). Also, this species complex is a potentially unique system in which to understand the interplay between population structure, phylogeographical divergence and species limits; the findings of this study provide an obvious starting point for further work in this area. Finally, the data from this study contribute to a general picture of the multifarious historical factors that have shaped Californian biodiversity, and add to our knowledge of species and phylogeographical 'hotspots' in the state. We are convinced that the arthropod fauna of California is rich in such 'cryptic' systems, and look forward to further studies of this largely unexplored fauna.

Acknowledgements

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James Starrett conducted this research while an undergraduate at San Diego State University, as part of an NSF REU project. Jim continues his studies of spider diversity and molecular evolution at the University of California, Riverside. Marshal Hedin is an Associate Professor of Biology at SDSU where he teaches courses in organismal biology and systematics, and conducts research focused on diversity and diversification in spiders and other arachnid groups.

Appendix

Location and voucher information, population acronym, and GenBank Accession numbers for taxon sample; N, north; S, south; W, west; E, east; Alt., altitude; m a.s.l., metres above sea level

Species	Location	MY #	Acronym	COI	12S	28S
Sphodros abboti Walckenaer, 1835	FL: Alachua County, Gainesville, vic. University of Florida campus N29.6462, W82.3581	MY 026	—	AF303528	NA	DQ898772
Bothriocyrtum californicum (O.P.-Cambridge, 1874)	CA: San Diego County, San Diego State University campus N32.7788, W117.0727	MY 066	—	NA	DQ898809	NA
Aliatypus torridus Coyle, 1974	CA: San Bernardino County, ~4.8 km. E Apple Valley N34.4829, W117.1283	MY 038	—	NA	DQ898810	NA
Aliatypus erebus Coyle, 1974	CA: Santa Clara County, Los Altos, corner of Magdalena Rd & Foothill Expressway N37.3607, W122.0991	MY 462	—	NA	DQ898811	NA
Aliatypus californicus (Banks, 1896)	CA: Colusa County, Bear Creek Road N39.0110, W122.3893	MY 106	—	DQ898845	NA	DQ898773
Antrodiaetus apachecus Coyle (1971)	AZ: Apache County, S of Greer N33.9977, W109.4642	MY 118	—	DQ898846	NA	DQ898774
Antrodiaetus pacificus (Simon, 1884)	CA: Sonoma County, ~4.8 km. of Guerneville N38.4795, W123.0013	MY 383	—	NA	DQ898812	NA
Antrodiaetus hadros (Coyle 1968)	MO: St Genevieve County, Pickle Springs NA N37.8029, W90.2962	MY 459	—	DQ898847	NA	DQ898775
Antrodiaetus gertschi (Coyle 1968)	OR: Jackson County, Ashland, Lithia Park N42.1738, W122.7143	MY 431	—	DQ898848	DQ898813	DQ898776
Antrodiaetus riversi (O.P.–Cambridge, 1883)	CA: Trinity County, Hwy 299, E Grass Valley Creek crossing N40.6740, W122.8270 Alt. ~650 m a.s.l.	**MY 081**, MY 116	**HWY299E**	MY 081 — DQ898849, MY 116	MY 081 — DQ898814, MY 116	MY 081 — DQ898777, MY 116
Antrodiaetus riversi	CA: Trinity County, Highway 299, W Del Loma N40.7901, W123.4535 Alt. 330 m a.s.l.	MY 423, **MY 424**	**HWY299MID**	MY 423, MY 424 — DQ898850	MY 423, MY 424 — DQ898815	MY 423, MY 424 — DQ898778
	CA: Trinity County, Hell Gate CG, E Forest Glenn N40.3692, W123.3111 Alt. 730 m a.s.l.	MY 777, MY 779, MY 780, MY 781	**HELLGATE**	**MY 779 —** DQ898851	**MY 777 —** DQ898816, MY 780	**MY 777 —** DQ898779, MY 780, MY 781
	CA: Trinity County, South Fork, Mountain, Horse Ridge Lookout Road N40.3949, W123.4357 Alt. 1230 m a.s.l.	MY 972, **MY 973**	**SOUTHFORK**	MY 972, MY 973 — DQ898852	MY 972, MY 973 — DQ898817	MY 972, MY 973 — DQ898780
	CA: Trinity County, Mad River Rock Rd, 1.3 km. S Highway 36 N40.4425, W123.4937 Alt. 760 m a.sl.	**MY 981,** MY 982	**MADRVR**	MY 981 — DQ898853 MY 982	MY 981 — DQ898818, MY 982	MY 981 — DQ898781,
	CA: Shasta County, White Rock Rd. CG, ~2.2 km. W Platina N40.3480, W122.9603 Alt. 840 m a.s.l.	**MY 771,** MY 772, MY 773	**PLATINA**	MY 771 — DQ898854, MY 772	MY 771 — DQ898819	MY 771 — DQ898782, MY 772, MY 773
	CA: Humboldt County, Highway 36, 2.6 km. E of bridge over South Fork Van Duzen River N40.4581, W123.6524 Alt. 830 m a.s.l.	**MY 988,** MY 989	**VANDUZ**	MY 988 — DQ898855, MY 989	MY 988 — DQ898820, MY 989	MY 988 — DQ898783, MY 989
	CA: Humboldt County, E Fork Willow Creek CG, off Highway 299 N40.9071, W123.7069 Alt. 480 m a.s.l.	MY 418, MY 420, **MY 421**	**WILLOW**	MY 420, MY 421 — DQ898856	MY 420, MY 421 — DQ898821	MY 418, MY 420, MY 421 — DQ898784
	CA: Humboldt County, W Redway N40.1224, W123.8424	MY 413, **MY 414,**	**REDWAY**	MY 414 — DQ898857,	MY 413, MY 414 —	MY 413, MY 414 —

Appendix *Continued*

Species	Location	MY #	Acronym	COI	12S	28S
	Alt. 100 m a.s.l.	MY 415		MY 415	DQ898822	DQ898785
	CA: Mendocino County, along Navarro River N39.0856, W123.4864 Alt. 50 m a.s.l.	MY 409, **MY 410**	**NAVARRO**	MY 410 — DQ898858	MY 409, MY 410 — DQ898823	MY 409, MY 410 — DQ898786
	CA: Sonoma County, 0.5 km. on Cavedale Road, e up Trinity Rd. off Hwy 12 N38.3904 W122.4788 Alt. 530 m a.s.l.	MY 985, **MY 986**	**HWY12**	MY 985, MY 986 — DQ898859	MY 985, MY 986 — DQ898824	MY 985, MY 986 — DQ898787
	CA: Lake County, Bartlett Springs Rd, E Indian Valley Res. N39.1671 W122.5070 Alt. 530 m a.s.l.	MY 782, **MY 784**	**BARTLETT**	MY 782, MY 784 — DQ898860	MY 782, MY 784 — DQ898825	MY 782, MY 784 — DQ898788
	CA: Marin County, Point Reyes NS, Mt Vision Rd, N38.0959, W122.8873 Alt. 300 m a.sl.	**MY 975,** MY 977	**PTREYES**	MY 975 — DQ898861, MY 977	MY 975 — DQ898826, MY 977	MY 975 — DQ898789, MY 977
	CA: Contra Costa County, Oakland, Huckleberry Botanic Preserve N37.8421, W122.1948 Alt. 400 m a.s.l.	MY 404, **MY 405,** MY 406	**HUCKLE**	MY 405 — DQ898862, MY 406	MY 404, MY 405 — DQ898827	MY 404, MY 405 — DQ898790
	CA: Santa Clara County, 0.16 km. on Peacock Ct., up Montebello Rd, N37.2952, W122.0940 Alt. 290 m a.s.l.	**MY 960,** MY 961	**MONTEB**	MY 960 — DQ898863, MY 961	MY 960 — DQ898828, MY 961	MY 960 — DQ898791, MY 961
	CA: Santa Clara County, Stevens Canyon Rd, 0.8 km. S Montebello Rd, N37.2909, W122.0819 Alt 180 m a.s.l.	**MY 970**	**STEVENS**	DQ898864	NA	DQ898792
	CA: Santa Clara County, Highway 130, 5.8 km. E of Clayton Rd, up W Quimby Rd N37.3539, W121.7408 Alt. 575 m a.s.l.	MY 1014, **MY 1015,** MY 1016	**HAMILTON**	MY 1014, MY 1015 — DQ898865	MY 1015 — DQ898829, MY 1016	MY 1014, MY 1015 — DQ898793
	CA: San Mateo County, NE side San Bruno Mountain, end of Paul Rd N37.6789, W122.4075 Alt. 140 m a.s.l.	MY 978, **MY 979**	**SANBRUNO**	MY 978, MY 979 — DQ898866	MY 978, MY 979 — DQ898830	MY 978, MY 979 — DQ898794
	CA: Santa Cruz County, N Soquel Center on Soquel Old San Jose Rd N37.0383,W121.9446 Alt. 60 m a.s.l.	MY 394, **MY 395,** MY 396	**SOQUEL**	MY 395 — DQ898867, MY 396	MY 395 — DQ898831, MY 396	MY 394, MY 395 — DQ898795
	CA: Santa Cruz County, Highway 9, N Felton, N37.0176, W122.0639 Alt. 140 m a.s.l.	**MY 399,** MY 400, MY 401	**FELTON**	MY 399 — DQ898868, MY 400	MY 399 — DQ898832, MY 400	MY 399 — DQ898796, MY 400, MY 401
	CA: Monterey County, Monterey, near intersection Vieja/Valenzuela Rds N36.5757 W121.8991 Alt. 130 m a.s.l.	MY 389, **MY 390,** MY 391	**MONTEREY**	MY 389, MY 390 — DQ898869	MY 390 — DQ898833, MY 391	MY 389, MY 390 — DQ898797, MY 391
	CA: San Benito County, road to Pinnacles NM N36.4862, W121.2233 Alt. 500 m a.s.l.	**MY 990,** MY 991	**PINNACLES**	MY 990 — DQ898870, MY 991	MY 990 — DQ898834, MY 991	MY 990 — DQ898798, MY 991
	CA: Monterey County, Tassajara Rd, 6 km. W off Carmel Valley Rd (G16) N36.3665, W121.5896 Alt. 530 m a.s.l.	**MY 1003,** MY 1004, MY 1005	**TASSAJARA**	MY 1003 — DQ898871, MY 1004	MY 1003 — DQ898835, MY 1004	MY 1003 — DQ898799, MY 1004, MY 1005
	CA: Sutter County, Sutter Buttes, Dean Place. N39.2176 W121.7843 Alt. 400 m a.s.l.	MY 789, **MY 790**	**SUTTER**	MY 789, MY 790 — DQ898872	MY 789, MY 790 — DQ898836	MY 789, MY 790 — DQ898800
	CA: Yuba County, Scott Grant Rd, 1.6 km. N of jnct. w/Marysville Rd off Highway 20 N39.3220, W121.3781 Alt. 185 m a.s.l.	**MY 465,** MY 466	**SCOTTGR**	MY 465 — DQ898873, MY 466	MY 465 — DQ898837, MY 466	MY 465 — DQ898801, MY 466
	CA: El Dorado County, Highway 193,	**MY 766,**	**PLACER**	MY 766 —	MY 766 —	MY 766 —

Appendix *Continued*

Species	Location	MY #	Acronym	COI	12S	28S
	N of Placerville N38.7559, W120.8186 Alt. 360 m a.s.l.	MY 767, MY 768		DQ898874, MY 767	DQ898838, MY 767	DQ898802, MY 767, MY 768
	CA: El Dorado County, Lake Tahoe, vic. Bayview CG N38.9435, W120.0994 Alt. 2100 m a.s.l.	**MY 437,** MY 438	**BAYVIEW**	MY 437 — DQ898875, MY 438	MY 437 — DQ898839, MY 438	MY 437 — DQ898803, MY 438
	CA: Mono County, Highway 89, E Monitor Pass N38.6546, W119.5935 Alt. 1980 m a.s.l.	MY 442, **MY 443**	**MONITOR**	MY 442, MY 443 — DQ898876	MY 442, MY 443 — DQ898840	MY 442, MY 443 — DQ898804
	CA: Calaveras County, along Love Creek, SE of Avery N38.2203, W120.3361 Alt. 1130 m a.s.l.	MY 758, MY 760, **MY 761**	**LOVECR**	MY 758, MY 761 — DQ898877	MY 760, MY 761 — DQ898841	MY 758, MY 760, MY 761 — DQ898805
	CA: Tuolumne County, N Fork Tuolumne River, Jenness Park Baptist Camp N38.1404, W120.0559 Alt. 1480 m a.s.l.	**MY 755,** MY 756	**JENNESS**	MY 755 — DQ898878, MY 756	MY 755 — DQ898842, MY 756	MY 755 — DQ898806, MY 756
	CA: Mariposa County., Yosemite NP, Highway 120, E Crane Flat N37.8489, W119.6027 Alt. 2440 m a.s.l.	MY 446, **MY 447**	**CRANEFL**	MY 446, MY 447 — DQ898879	MY 446, MY 447 — DQ898843	MY 446, MY 447 — DQ898807
	CA: Mariposa County, Yosemite NP, near Tuolumne Grove. N37.7579, W119.8041 Alt. 1880 m a.s.l.	MY 751, **MY 752,** MY 753	**TUOLUMNE**	MY 751, MY 752 — DQ898880	MY 752 — DQ898844, MY 753	MY 751, MY 752 — DQ898808, MY 753

Data for the following outgroup taxa were used in combined analysis: *Sphodros* — COI and 28S, no 12S; *Aliatypus* — COI and 28S for MY106 (*A. californicus*) combined with 12S for MY038 (*A. torridus*); *Antrodiaetus* — COI and 28S for MY118 (*A. apachecus*) combined with 12S for MY383 (*A. pacificus*); *A. hadros* — COI and 28S, no 12S; *A. gertschi* — all data from specimen MY431. *Antrodiaetus riversi* specimens used in combined analysis are in bold.