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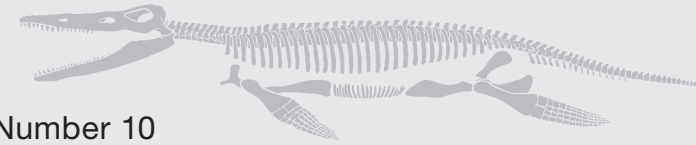
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Photo on the front cover:

Hydromantes wintu from the type locality, preserved as a paratype in the MCZ collection (MCZ A-151700).
Photo: Robert W. Hansen

PHYLOGEOGRAPHY AND SPECIES BOUNDARIES IN THE *HYDROMANTES SHASTAE* COMPLEX, WITH DESCRIPTION OF TWO NEW SPECIES (AMPHIBIA; CAUDATA; PLETHODONTIDAE)

ROBERT E. BINGHAM,¹ THEODORE J. PAPPENFUSS,¹ LEN LINDSTRAND III,² AND DAVID B. WAKE^{1,3}

ABSTRACT. The Shasta salamander, *Hydromantes shastae*, is a geographically restricted lungless salamander (Plethodontidae) exhibiting remarkable evolutionary diversification at small spatial scales. Tissue samples were sequenced for the mitochondrial cytochrome *b* and 16S genes. Bayesian phylogenetic analyses revealed five statistically supported clades with large divergences between lineages. In three different pairwise comparisons showing high levels of genetic differentiation, neighboring samples located a minimum of 3.5 km apart were at least 4.5% divergent in the cytochrome *b* gene. Allozyme data were analyzed using multidimensional scaling and population structure software. With relatively high values of Nei's genetic distance and low levels of gene flow for 18 allozyme loci, these nuclear data support the hypothesis that significant isolation and diversification at small spatial scales warrants recognition of additional species. Morphometric analysis finds three groups, in agreement with analyses of molecular data. The combined concordant results from analyses of mitochondrial genes and nuclear markers show the *Hydromantes shastae* complex to be highly structured across its restricted range. Accordingly, *H. shastae* is rediagnosed and restricted to the eastern portion of its former range, and two new allopatric species are named.

Key words: Allozymes, Conservation, *Hydromantes samueli*, *Hydromantes wintu*, Mitochondrial DNA, Morphometrics, New species

INTRODUCTION

Hydromantes shastae is one of the three species of *Hydromantes* located in North America. It is a strictly terrestrial, web-footed plethodontid salamander endemic to the southeastern Klamath Ranges around the current Shasta Lake, Shasta County, in northern California. Populations of the species are disjunctly distributed across its small geographic range. Reproduction is by means of large, yolky eggs that are laid on land and develop directly into miniatures of the adult. Historically, the species was considered a limestone obligate found among rock outcrops in the region where the McCloud and Pit rivers joined the Sacramento River. Recent surveys have uncovered some individuals in a broader range of habitats away from limestone, including some other types of rock outcrops, and even habitats with no rock outcrop associations (Lindstrand, 2000; Nauman and Olson, 2004; Lindstrand et al., 2012). Still, the known localities for this species are spread widely within its limited geographic range, resulting in spatially variable densities. A minimum convex polygon outlining the four corners of the species range, as represented by samples studied in this paper, has a latitudinal axis (north to south) of 50.43 km and a longitudinal axis (east to west) of 37.40 km. These dimensions yield an estimate for the species range of approximately 850 km² and is somewhat larger if sight records to the west are

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included, but still very small for vertebrate species. However, the potentially habitable portion of this area is even smaller because the surface of Shasta Lake, almost fully enclosed in the polygon, covers about 119 km². Although little is known about the ecology of this species, one study of movements over a 2-year period showed a mean cumulative distance moved of 15 m, with several longer movements as great as 104 m (Herman, 2003). The low vagility of these salamanders necessarily affects genetic divergence of widely separated populations. Because of the combination of restricted distribution and apparent habitat specialization, *H. shastae* is listed by IUCN (Red List) as Near Threatened, and collecting is regulated by the California Department of Fish and Wildlife. This species was listed as Rare by the California Fish and Game Commission in 1971 and grandfathered into the California Endangered Species Act of 1985 as Threatened. The species is listed as a Sensitive species by the Region 5 U.S. Forest Service and the California Region Bureau of Land Management. Based on a petition submitted by the Center for Biological Diversity, in 2015, the U.S. Fish and Wildlife Service included *H. shastae* as a Candidate Species for federal protection as Threatened or Endangered.

It came as a surprise when an early combined allozyme and immunological study (Wake et al., 1978) found *H. shastae* to display substantial genetic variation expressed geographically within its small distributional limits. For allozymes, values of Nei *D* among the five populations of *H. shastae* sampled ranged as high as 0.275, leading the authors to suggest that the most divergent sample might represent a distinct species. These results led Highton (2000) to argue that two species were present, but no new species were described. Here, we present a combined analysis of previously published and new data from mitochondrial DNA (mtDNA), allozymes, and morphometrics and describe two new species.

MATERIALS AND METHODS

Molecular Phylogenetic Analysis

For the phylogenetic analysis of mitochondrial DNA, we obtained tissue samples from one or two vouchered individuals of *Hydromantes shastae* from each of 18 populations around Shasta Lake, California (Table 1). Although more samples per locality would have been preferable for calculations of genetic divergence, two individuals per population is the maximum number permitted because of the threatened status of the species. The populations sampled encompass most of the range of the species known at the time of the study (Bingham, 2007), including a new northern locality (Ash Camp, population 10). We lack samples from the westernmost occurrences (Lindstrand et al., 2012). We also include sequences from single specimens of two outgroup taxa of *Hydromantes*.

Genomic DNA was extracted from frozen liver tissue using Qiagen DNeasy extraction kits (Qiagen Inc., Valencia, California). The targeted mitochondrial DNA fragment included the cytochrome *b* (*cyt b*) and 16S genes. Polymerase chain reaction was performed in a total volume of 25 μ l, including 0.125 μ l *Taq* polymerase (5 U/ μ l), 1.25 μ l of each primer (10 μ mol/L), 0.47 μ l deoxyribonucleotide triphosphates (dNTPs, 40 mM), 2.5 μ l of 10 \times buffer, and 6.25 μ l of sample DNA (10 ng/ μ l). Polymerase chain reaction consisted of 36 cycles with a denaturing temperature at 94 $^{\circ}$ C (1 minute), annealing at 53 $^{\circ}$ C (1 minute; *cyt b*) and 45.5 $^{\circ}$ C (1 minute; 16S), and extension at 72 $^{\circ}$ C (1.5 minutes). Polymerase chain reaction products were purified using ExoSAP-IT (USB Inc., Cleveland, Ohio) and sequenced on an ABI 3730 automated DNA sequencer with two overlapping sets of internal primer pairs. DNA sequences were edited and aligned unambiguously using SequencherTM version 4.2 (Gene Codes Corp., Ann Arbor, Michigan). All divergence values are uncorrected *p* distances.

TABLE 1. COLLECTION LOCALITIES FOR *HYDROMANTES SHASTAE* AND THE NUMBER OF SAMPLES FROM EACH USED IN THE MITOCHONDRIAL, ALLOZYME, AND MORPHOLOGICAL ANALYSES.

Locality	Latitude	Longitude	mtDNA <i>n</i>	Allozyme <i>n</i>	Morphology <i>n</i>
1. Ash Camp	41.11811	-122.05030	1	—	—
2. Samwel Cave	40.92028	-122.23778	1	—	11/14
3. Dekkas Rock Creek	40.87072	-122.21905	2	18	—
4. Salt Creek	40.87736	-122.13836	2	—	—
5. Ellery Creek	40.91529	-122.25231	1	13	—
6. Dekkas Rock Camp	40.87434	-122.24122	1	—	—
7. Mammoth Mine	40.75577	-122.44638	2	21	—
8. Backbone Ridge	40.78203	-122.41537	2	30	13/12
9. Campbell Creek	40.84906	-122.23597	2	12	5/8
10. Curl Creek	40.81753	-122.27150	2	—	—
11. Potter Creek	40.77972	-122.28406	2	26	11/13
12. Gray Rock Quarry	40.74611	-122.29947	2	15	—
13. McClure Gulch	40.77054	-122.18919	1	—	—
14. Reynolds Creek	40.77039	-122.16286	1	—	—
15. Dead Horse Creek	40.76462	-122.14284	1	—	—
16. Brock Mountain	40.81126	-122.11354	1	9	9/10
17. Low Pass Creek	40.85842	-122.10550	2	—	—
18. Ingot	40.77708	-122.00540	2	6	—

Bayesian phylogenetic analyses were implemented with MrBayes version 3.04b (Huelsenbeck and Ronquist, 2001). We ran the Bayesian analysis for 20 million generations and sampled every 1,000 generations using four chains and default priors. Stationarity was determined by plotting the likelihood values against generation time (Leaché and Reeder, 2002), and a conservative 10 million generations were discarded as burn-in. The remaining 10 million generations (10,000 samples) were analyzed in PAUP version 4.0b10 (Swofford, 2002) to reconstruct the topology and calculate posterior probabilities for each node.

Allozyme Study

For the multilocus nuclear dataset, we used from 6 to 30 individuals (average $n = 16$; Table 1) from each of nine populations for 18 allozyme loci. The original electrophoretic data (Wake et al., 1978) were supplemented by additional samples gathered later and studied using the original methods. Specific allozymes discussed are identified by Enzyme Commission (EC) numbers.

Calculations of Nei's genetic distance (D_N ; Nei, 1972) and Cavalli-Sforza's chord distance (D_{CE} , Cavalli-Sforza and Edwards, 1967) were made by the program PHYLIP version 3.6 (Felsenstein, 2005). To understand relationships among populations using these distance measures for the allozyme loci, values of D_N were used to create an unweighted pair group method with arithmetic mean (UPGMA) tree, and D_{CE} values were used to create a neighbor-joining tree in the program MEGA (Kumar et al., 2004). Multidimensional scaling (MDS) of D_N was performed in the statistical package R (Ihaka and Gentleman, 1996). Multidimensional scaling allows visualization of clusters of related groups on the basis of pairwise distance measures. To compare with the clustering of populations on the basis of nuclear genetic distances, uncorrected pairwise genetic distances from the mitochondrial loci were also visualized with MDS.

To assess population structure on the basis of allozyme loci, the Bayesian clustering method in the program Structure version 2.2 (Pritchard et al., 2000) was implemented. Structure uses multilocus genotypes to estimate likelihoods for different numbers

of populations, K . High likelihood scores identify the number of genetically distinct clusters that maximize the probability of the data, and Structure accordingly plots the estimated membership coefficients for each individual in each cluster. Although the K value with the highest likelihood score may be the most appropriate number of populations statistically, careful analysis of admixture results can lead to a slightly different value of K that is more biologically relevant. Three simulations for K between 2 and 7 were run, with 100,000 iterations after a burn-in period of 30,000 iterations. Initial runs indicated that values of alpha, likelihood, and $\ln P(D)$ scores stabilized well before the burn-in.

Morphometric Analyses

Morphological measurements were taken from 106 fluid-preserved specimens of *Hydromantes shastae* cataloged at the Museum of Vertebrate Zoology. The specimens were from five localities (one from each mitochondrial clade), with an average of 10 males and 11 females from each (Table 1). All specimens were sexually mature as determined by gonadal inspection, by a minimum snout-to-vent length (SVL) of 42 mm, or both. The 17 morphometric characters (Fig. 1) for multivariate analysis are: SVL, tail length (TL), axilla-groin length (AXG), head width (HW), snout-to-gular fold length (SGF), chest width (CW), forelimb length (FL), hindlimb length (HL), foot width (FW), length of longest toe (LLT), length of fifth toe (L5T), intercanthal distance (ID), internarial distance (ND), orbitonarial distance (OD), eye diameter (ED), total number of maxillary and premaxillary teeth (MPT), and number of vomerine teeth (VT). All body size measurements (SVL, TL, AXG, HW, SGF, CW, FL, HL) were made to the nearest 0.01 mm with digital calipers by REB. All foot and face measurements (FW, LLT, L5T, ID, ND, OD, ED) were made to the

nearest micron with a microscope by TJP. All teeth (MPT, VT) were counted by DBW. Symmetrical characters were scored on each specimen's right side.

Principal components analysis (PCA; JMP v.6, SAS Institute Inc.) was used to study morphometric traits in five populations. PCA reduces the dimensionality of the morphological data set by creating independent composite variables from the original correlated traits, and functions to describe the total variation represented in the data. The principal components were then used in a multivariate discriminant function analysis (DFA; JMP v.6) to determine which morphological traits maximized differentiation between the a priori populations and to assess whether these multivariate differences were statistically significant. Classification matrices describing the number of incorrectly assigned individuals in the DFA were also computed. All morphometric analyses were run separately for the two sexes subsequent to the determination of significant sexual dimorphism.

For species descriptions, color names and numbers are from Köhler (2012). All measurements are in millimeters.

RESULTS

Mitochondrial DNA Phylogeny

The final alignment of sequence data included 788 nucleotide positions of the *cyt b* gene and 530 nucleotides of the 16S ribosomal gene for a total of 1,318 nucleotides. Three major and five minor clades were identified with >95% posterior probabilities. Minor clades A, B, and C comprise one major clade; these have shallower divergences than either clade D or clade E in the phylogram (Fig. 2). The uncorrected pairwise genetic distance within each of these five clades was <2% for the *cyt b* gene, whereas the pairwise genetic distance between clades D and E ranged from 3.8% to 4.5% (Fig. 3a). Comparable genetic distances from D and E to nearest neighbor

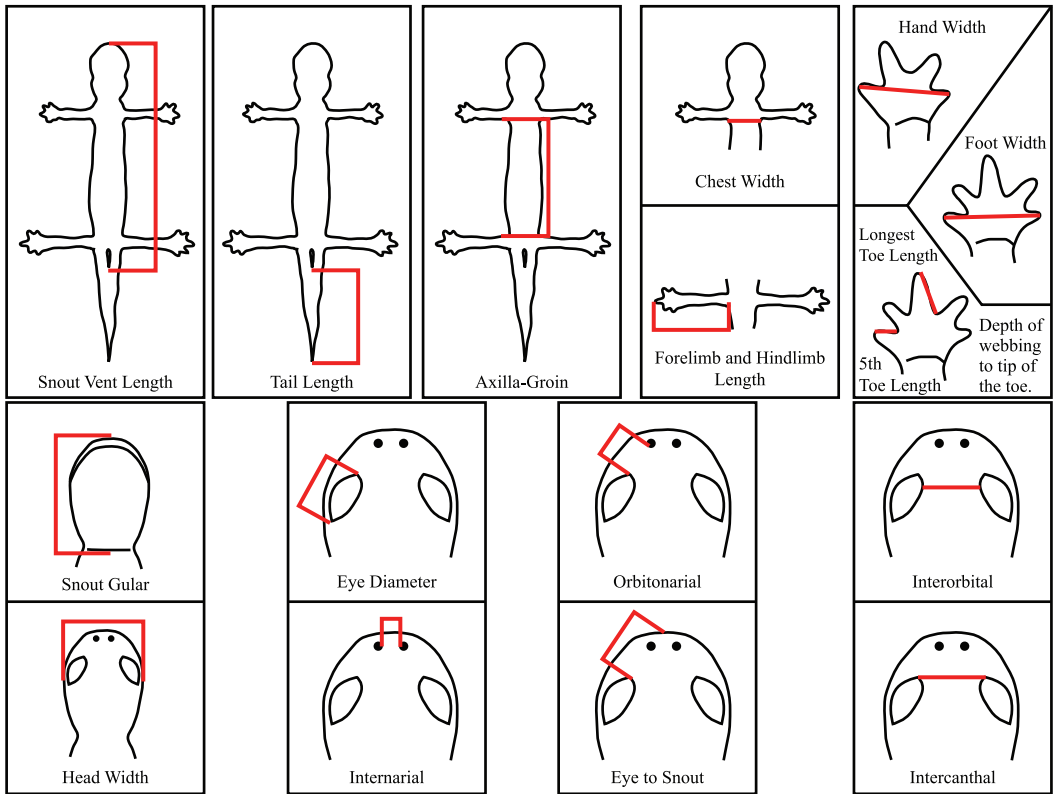


Figure 1. How to measure a preserved salamander.

populations of combined clade A-B-C are between 4.2% and 4.9%. Genetic distances to the *Hydromantes platycephalus* outgroups ranged from 7% to 9%.

Three statistically supported clades A, B, and D have relatively wide geographic distributions and span potential riverine, habitat, and elevation barriers, whereas clades C and E are geographically restricted (Fig. 2). Clade A (populations 1–4) is found east of the McCloud River Arm and west of the Squaw Creek Arm and includes a recently discovered population (population 1) that lies 27.5 km to the northeast of population 2 along the upper McCloud River. Clade B (populations 5–8) lies west of the McCloud River Arm. It crosses the Sacramento River Arm and includes the westernmost populations sampled. Clade C

comprises two populations (9 and 10) that occur in a small area along the east side of the McCloud River Arm. Clade D includes the southeastern populations (12–18) and lies east of the Squaw Creek Arm, spanning the Pit River Arm, including the type locality of *H. shastae*, Low Pass Creek (population 17). Clade E is restricted to the small Marble and Potter creek drainages at the southern end of the east side of the McCloud Arm; we treat these as a single population (11).

Allozyme Variation

Allozymes are available from nine of the 18 populations and represent all five mitochondrial clades. Calculations of D_N reveal high levels of divergence (Table 2, Fig. 3b). The highest values are for population 11

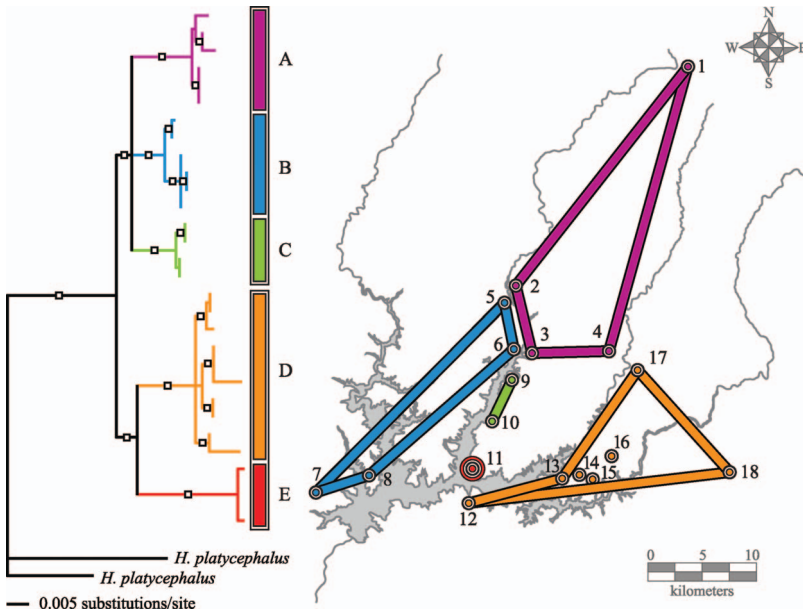


Figure 2. Collection localities of *Hydromantes shastae* complex around Shasta Lake, Shasta County, California. Bayes mtDNA phylogram. Nodes with squares represent >95% posterior probabilities. Clades are labeled A–E, and populations are labeled 1–18. Shasta Lake reservoir and tributaries are shown in gray.

(mitochondrial clade E; 0.18–0.21, except 0.11 to population 9). Populations 5, 7, and 8 (mitochondrial clade B) and 12, 16, and 18 (mitochondrial clade D) were essentially identical (0.00–0.01). Population 3 (mitochondrial clade A) is very similar to population 5 (0.007) but more distant from its other neighbors (0.07 to population 16; 0.08 to population 9). Population 9 (mitochondrial clade C) has values of 0.08–0.17 (to nearest geographical neighbors), and populations 5, 7, 8, and 9 differ from populations 12, 16, and 18 by 0.06–0.17. Each of the 18 loci was variable, but some distinctive patterns emerged. At locus *Icd-2* (EC 1.1.1.41; Fig. 4a), five populations are fixed for one allele, three populations are fixed for the other allele, and one population (11) shares both alleles. Locus *Sod* (EC 1.1.1.14; Fig. 4b) shows a connection between the three central populations (3, 9, 11). The pattern at locus *PGD* (EC 1.1.1.49; Fig. 4c) shows striking evidence for endemism and isolation of population 11,

which has a fixed difference from all populations. Private alleles (those found in only a single population) were widespread; populations 5, 7, 11, and 16 each had three, population 3 had two, populations 12 and 18 had one, and only populations 8 and 9 had none. Only population 8, represented by a large sample of 30 individuals, showed no variation. The habitat at this site (Backbone Ridge) is limited to a small sinkhole, and the population is one of the most isolated, so it may be inbred.

Both the UPGMA phylogenetic tree of D_N (Fig. 5a) and the neighbor-joining tree of D_{CE} (Fig. 5b) show consistent groupings of mitochondrial clades A + B and D (Table 2). Populations 9 and 11 have different relationships from each other and the other taxa, although they are always outside the cluster that includes clades A + B and D. The outgroups show complex relations to the *Hydromantes shastae* complex, with population 11 (Potter Creek) consistently lying outside the crown *shastae* populations

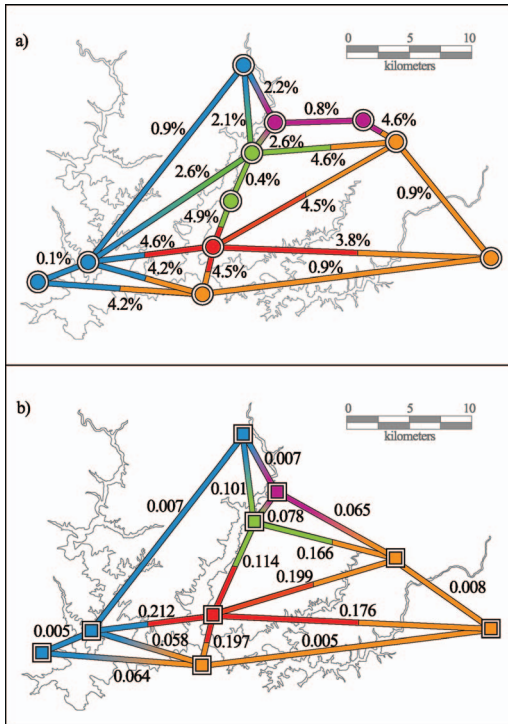


Figure 3. (a) Divergence (uncorrected p values) in mtDNA between all nearest neighbor populations. Solid color bars indicate divergences <1%, blended color bars represent divergences between 1% and 3%, and split color bars represent higher levels of divergence. (b). D_N on the basis of 18 allozyme loci between all nearest neighbor populations, color coded by mtDNA affiliation as in (a). Solid color bars indicate D_N between populations in the same clade; blended color bars represent distances between populations in different clades, ranging from 0.007 to 0.101; and split color bars represent higher levels of divergence.

(i.e., the three populations of *platycephalus* are nested inside the *shastae* complex in these phenetic analyses). Population 11 clusters with the geographically closest population of *H. platycephalus* from Smith Lake, El Dorado County, California.

Population Structure

The nine populations sampled for the 18 allozyme loci show the *shastae* complex to be highly structured across its small range, as evaluated in the program Structure. Likelihood scores rose steeply toward a value of $K = 4$ and then tapered to an

asymptote at $K = 7$, suggesting little support for values of $K > 4$ (Fig. 6). The admixture of population 3 shows that values of $K > 4$ are overestimates of population structure; they only increase the splitting of individual ancestries and not the number of populations. The value $K = 4$ is the most biologically and geographically appropriate interpretation of the Structure results and reflects genetic relationships also seen in the mitochondrial clades (compare Figs. 2 and 7). Again, population 11 is a distinct entity, while populations 12, 16, and 18 are a distinct cluster, and populations 3, 5, 7, and 8 are a group of populations closely connected to population 9. Although population 9 is classified as its own group, we detect admixture between it and population 3, as evidenced by 7 of 18 individuals with significant proportions of their ancestry attributable to population 9.

Multidimensional Scaling

Multidimensional scaling of D_N reveals four distinct, well-separated groupings along dimensions 1 and 2 (Fig. 8, left). These include a group of four populations (3, 5, 7, and 8), a group of three populations (12, 16, and 18), and two populations (9 and 11), each of which is distinct. In comparison, multidimensional scaling of the mitochondrial uncorrected genetic distances shows three distinct groupings (Fig. 8, right). These include one group containing all populations in clades A, B, and C in the mitochondrial phylogeny, one group of populations in clade D, and one population forming clade E. The only discordance between the two datasets in this analysis is in the placement of population 9. Allozymic data suggest that this population is sufficiently distinct to be its own cluster, whereas mtDNA shows it to be sufficiently similar to clades A and B to group with them.

TABLE 2. PAIRWISE GENETIC DISTANCES BETWEEN ALL POPULATIONS AND OUTGROUPS CALCULATED FROM 18 ALLOZYME LOCI. *HYDROMANTES SHASTAE* COMPLEX POPULATIONS FROM TABLE 1. *HYDROMANTES PLATYCEPHALUS* POPULATIONS: (A) SMITH LAKE, EL DORADO CO., CA, (B) SONORA PASS, TUOLUMNE CO., CA, (C) KINGS CANYON NATIONAL PARK, FRESNO CO., CA. *HYDROMANTES BRUNUS* POPULATION (D): TOPOTYPIC SPECIMEN FROM BRICEBURG, MARIPOSA CO., CA. NEI'S GENETIC DISTANCE IS BELOW DIAGONAL AND CAVALLI-SFORZA AND EDWARDS CHORD DISTANCE IS ABOVE.

	<i>Hydromantes shastae</i>									<i>Hydromantes platycephalus</i>				<i>brunus</i> (d)
	3	5	7	8	9	11	12	16	18	(a)	(b)	(c)		
	3		0.03	0.04	0.02	0.10	0.24	0.09	0.11	0.11	0.10	0.12	0.22	
5	0.01		0.05	0.03	0.14	0.26	0.10	0.10	0.13	0.10	0.09	0.20	0.30	
7	0.01	0.01		0.02	0.14	0.28	0.10	0.11	0.12	0.10	0.12	0.19	0.37	
8	0.00	0.01	0.00		0.12	0.26	0.08	0.09	0.10	0.08	0.10	0.20	0.36	
9	0.08	0.10	0.10	0.10		0.17	0.20	0.21	0.18	0.09	0.18	0.28	0.43	
11	0.19	0.20	0.21	0.21	0.11		0.25	0.26	0.22	0.18	0.29	0.29	0.37	
12	0.06	0.07	0.06	0.06	0.16	0.20		0.02	0.02	0.16	0.18	0.28	0.32	
16	0.07	0.07	0.07	0.06	0.17	0.20	0.00		0.04	0.17	0.19	0.30	0.31	
18	0.07	0.07	0.07	0.06	0.14	0.18	0.00	0.01		0.13	0.19	0.30	0.33	
(a)	0.06	0.06	0.06	0.06	0.06	0.14	0.12	0.12	0.10		0.10	0.21	0.35	
(b)	0.08	0.07	0.08	0.07	0.12	0.23	0.13	0.14	0.13	0.07		0.14	0.30	
(c)	0.16	0.14	0.14	0.15	0.21	0.23	0.22	0.23	0.22	0.15	0.08		0.34	
(d)	0.30	0.26	0.30	0.30	0.36	0.31	0.26	0.24	0.25	0.30	0.24	0.29		

Morphometric Differentiation

To examine morphometric differentiation within the complex, we performed a PCA that highlighted strong sexual dimorphism between sexes. A DFA on the PCA results revealed that the trait that maximized differentiation between the sexes was total number of maxillary and premaxillary teeth, females having roughly three times as many as males. We present results of the DFA on the separate PCA for each sex in Figure 9, which shows that we are able to discriminate among samples representing the three major mitochondrial clades for both males and females. The DFA correctly classified 91.8% of males and 91.2% of females to the five a priori population groupings (Table 3a, b). Of the nine individuals that were misclassified, eight were incorrectly assigned among the three populations (2, 8, 9, all members of the same mitochondrial major clade) that do not statistically differ in morphology. If we treat these three populations as a single group, then the DFA correctly classified 100% of male salamanders and

98.2% of female salamanders to the three morphological population groupings. The high success rate of the DFA indicates that these three statistically different morphological groupings represent distinct and diagnosable phenotypes. The discontinuities in phenotype observed between the groups coincide with the results of both the mitochondrial and allozyme analyses.

DISCUSSION

Congruence of Molecular and Morphometric Datasets

Molecular data show significant breaks between the three major lineages comprising the small range of this complex (Figs. 3, 4, 8). The degree of differentiation suggests temporally deep divergences despite current geographic juxtaposition of clades.

Molecular methods have led to a surge in the discovery of cryptic species of salamanders (Wake, 2017). Many species have gone unnoticed because of morphological convergence or stasis. At the same time,

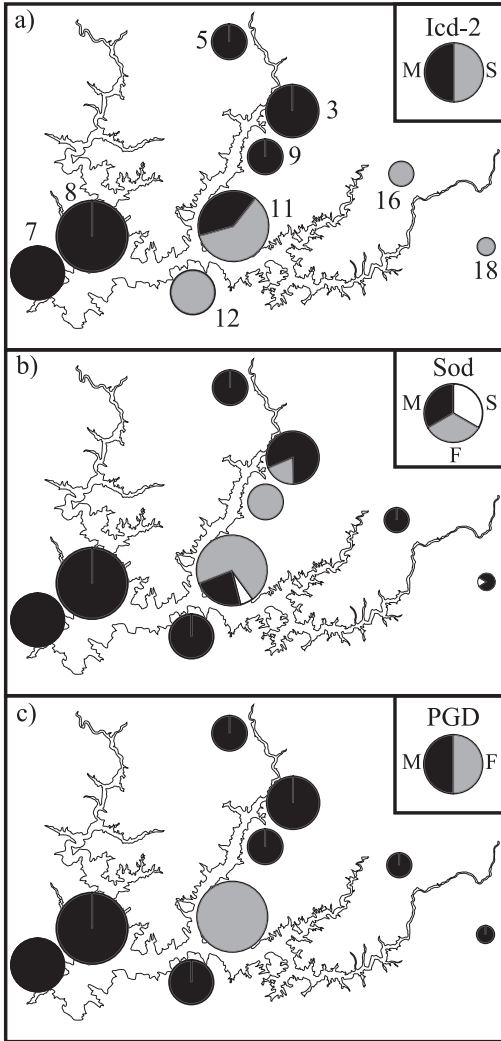


Figure 4. Allele frequency patterns for three allozyme loci. Sizes of pie charts are proportional to numbers of individuals sampled (see Table 1).

presumptions about the size or continuity of a species range have led workers to overlook the degree of environmental heterogeneity and its relation to opportunities for diversification, which are greatly increased in the absence of gene flow. Factors such as low vagility and habitat specificity have changed our perceptions on the fractal nature of differentiation within species. As we test for

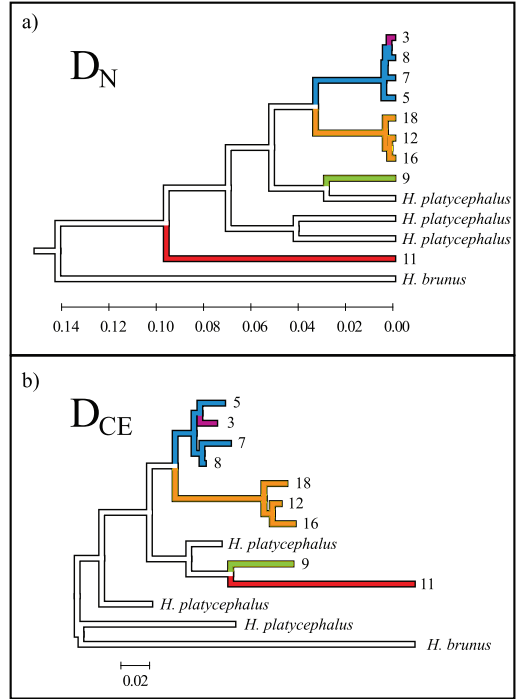


Figure 5. (a) UPGMA phylogenetic tree of D_N . (b). Neighbor-joining tree of D_{CE} . Both measures are based on allozyme loci. Numbers refer to population localities in Table 1.

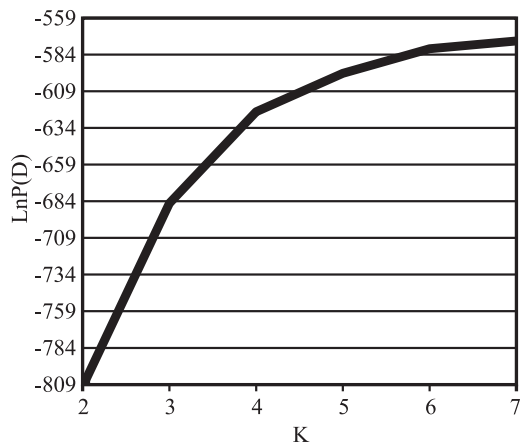


Figure 6. Plot of likelihood scores for number of optimal populations K as determined in the structure analysis. Optimal level of $K = 4$.

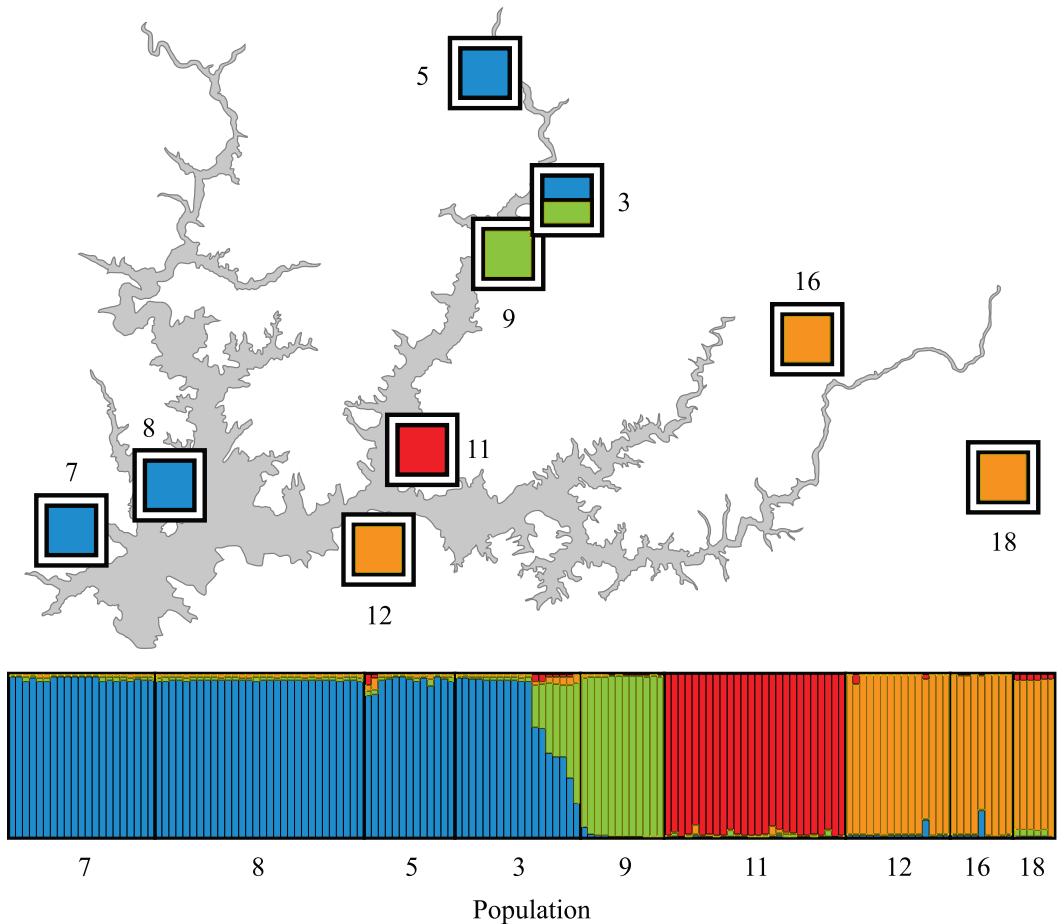


Figure 7. Plot of admixture results from structure analysis showing individuals as vertical bars, grouped in a priori populations on the basis of sampling. Colors in bars represent proportion of ancestry or population affiliation for that individual. The mapped localities are colored by identifications from the structure analysis, with the split square showing population admixture.

divergences between taxa, we are finding significant differences at finer and finer spatial scales.

The clades identified using molecular markers correspond to units identified in the morphometric study. The concordant results from all three types of markers provide robust evidence for three strongly divergent clades within what we now label the *Hydromantes shastae* complex, including three species-level taxa (see below).

Levels and Timing of Molecular Divergence

The maximum geographic distance between any two localities within a clade is 30.6 km between populations 1 and 3 in Clade A. Although population 1 is a disjunct, peripheral population, it is only 0.53% (mtDNA) diverged from population 3. In strong contrast to the genetic similarity of widely separated populations within clade A, three pairwise comparisons between clades show high genetic divergence (E to D, 4.49%; E to B, 4.94%; E to C, 4.61%) over small geographic distances (4.2, 4.0,

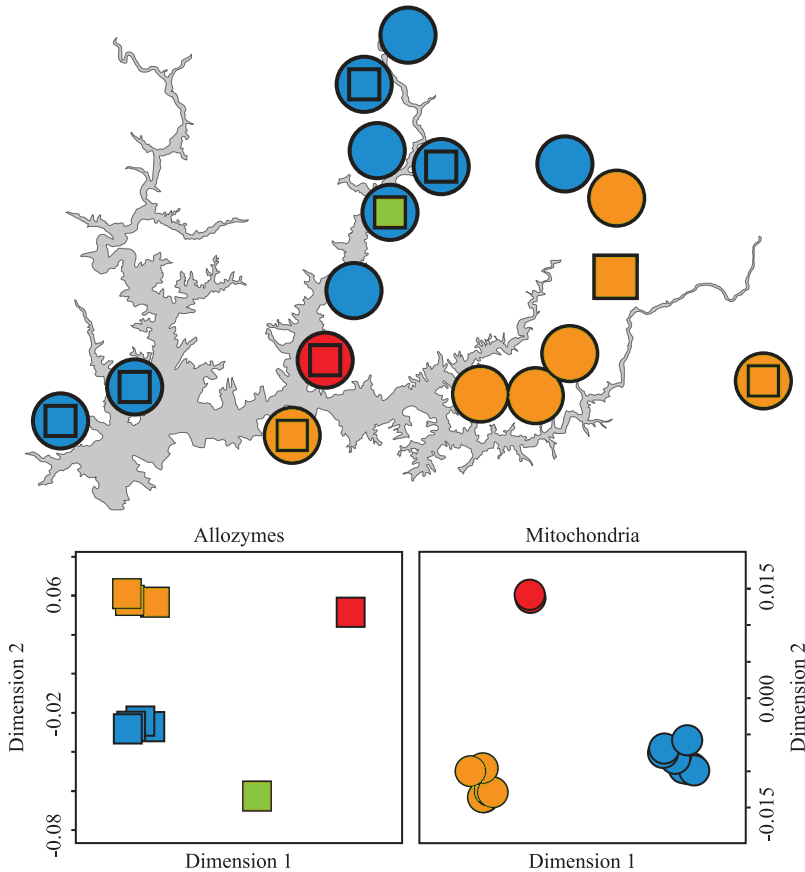


Figure 8. (a) Multidimensional scaling of allozyme (square) and mitochondrial (circle) data along dimensions 1 and 2. (b) Map showing population affiliations.

and 3.5 km, respectively; Fig. 3a). We are reluctant to conduct a formal analysis of times of divergence for this complex because of insufficient data. A salamander species complex that also displays strong geographic and genetic structure, *Batrachoseps attenuatus* (Martínez-Solano et al., 2007), is even more differentiated and accumulated these differences on the order of about 2 million years. Yet, in the *Hydromantes shastae* complex, such estimates apply to populations separated by as little as 4.0 km of discontinuous habitats across contiguous geography.

Criteria for Species Recognition and the Taxonomic Status of the *Hydromantes shastae* Complex

The scattered populations of salamanders surrounding the confluence of rivers in the southeastern Klamath Ranges have evolved over a long period of time and have differentiated genetically to the point that taxonomic revision needs to be considered. This is a classic case of divergence in allopatry. The geographic distances are small, but significant, because these salamanders are habitat limited and have low dispersal ability. The closest relatives of the California *Hydromantes* are a small clade of

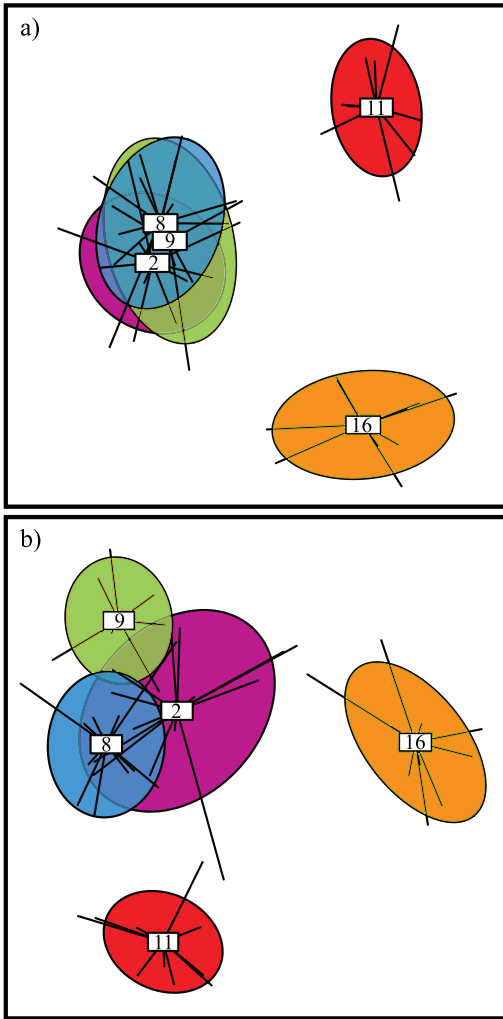


Figure 9. (a) Plot of the discriminant function analysis for males (a) and females (b). Population codes are at the centroids, with sampled individuals connected by black lines. Ellipses represent 95% confidence limits for each population and are color coded by mitochondrial clade.

salamanders (*Hydromantes* subgenera *Atylodes* and *Speleomantes*) occurring on Sardinia and the Italian–French mainland. Across the vast, remarkably disjunct distribution, taxa of *Hydromantes* typically are geographically limited and display great differentiation (Carranza et al., 2008; Rovito, 2010). Although many so-called species

TABLE 3. CLASSIFICATION MATRICES FOR (A) MALES AND (B) FEMALES FROM THE DISCRIMINANT FUNCTION ANALYSIS. NUMBERS IN ROWS REPRESENT THE A PRIORI POPULATION DESIGNATION ON THE BASIS OF MITOCHONDRIAL CLADE, AND NUMBERS IN COLUMNS REPRESENT THE PREDICTED AFFILIATION ON THE BASIS OF MULTIVARIATE MORPHOMETRICS. ELEMENTS ON THE DIAGONAL ARE CORRECTLY ASSIGNED INDIVIDUALS, WHEREAS OFF-DIAGONAL NUMBERS ARE MISCLASSIFIED SALAMANDERS.

	Clade A	Clade B	Clade C	Clade D	Clade E
(a) Males					
Clade A	8	2	1	—	—
Clade B	—	13	—	—	—
Clade C	1	—	4	—	—
Clade D	—	—	—	9	—
Clade E	—	—	—	—	11
(b) Females					
Clade A	10	3	—	—	1
Clade B	1	11	—	—	—
Clade C	—	—	8	—	—
Clade D	—	—	—	10	—
Clade E	—	—	—	—	13

delimitation methods have been proposed, we favor a multidimensional, integrative approach that recognizes the continuous nature of species formation and seeks to identify apparently stabilized segments of clades that have achieved identity as a result of congruent patterns of variation in molecular and morphological markers and geographic limitation (see Kuchta et al., 2016; Kuchta and Wake, 2016).

The taxonomic status of the population at Potter Creek (11) has been unresolved since the late 1970s when its genetic divergence was first studied (Wake et al., 1986). The average N_D for all pairwise comparisons to Potter Creek is 0.189 with a range from 0.114 to 0.215; accordingly, Highton (2000) argued in favor of elevating Potter Creek to full species status. The lowest values of D_N were observed for pairwise comparisons of Potter to populations 9 and 18 ($D_N = 0.11$ and $D_N = 0.14$, respectively), which are populations that cluster with more northern and eastern populations in the mtDNA analysis.

The Potter Creek salamanders are a distinct evolutionary lineage geographically

restricted in the middle of the range of the complex, and apparently have differentiated in situ, although peripheral populations in other lineages have not asymmetrically diverged from their respective clades over the same evolutionary time. Environmental factors affecting one clade of salamanders would also affect the other clades that are distributed in such contemporary geographic proximity, negating the effect of possible historical refugia and secondary contact. Salamanders are not rare in the Potter Creek region, and there is no reason to think that the population is of a size at which small population phenomena become important. Furthermore, we recorded 23 alleles at four of the 18 allozyme loci sampled, showing local drift is unlikely to have been a major factor in genetic differentiation. Although we know little about the historic distribution of the form currently restricted to the immediate vicinity of Potter Creek, especially with respect to climate and geology, the history of *Hydromantes* has evidently been complicated, particularly given the larger biogeographic questions related to this clade (Wake, 2013).

Endemism, Isolation, and Peripheral Populations

The context in which a focal taxon has evolved is important. Comparative phylogeography may reveal coincident phylogenetic breaks in multiple species and highlight specific geographic features (e.g., barriers to gene flow, glacial refugia). Comparing the species ranges of codistributed taxa can also highlight areas of interest. The southeastern Klamath Ranges, including the region surrounding Shasta Lake, are geologically old (Potter, 1966) and neither subject to glaciation nor overlain by volcanic material as were adjacent regions (Lindstrand and Nelson, 2006). Additionally, this is an area of high annual precipitation at relatively low elevations, producing a com-

bination of mesic conditions and relatively mild temperatures. The combinations of these geologic and climatic factors result in conditions favorable for a diverse flora and fauna. Numerous taxa are either endemic, have isolated disjunct populations, or have distributions with peripheral populations in the area. Endemism within this region is indicated by the presence of several endemic plant and wildlife species, including Shasta eupatory, *Ageratina shastensis* (Taylor and Stebbins, 1978); Shasta snowwreath, *Neviusia cliftonii* (Shevock et al., 1992); and the Shasta and Wintu sideband snails, *Monadenia troglodytes troglodytes* and *M. t. wintu* (Roth, 1981). The documentation of regional endemic taxa continues with recent descriptions of four plant species including Shasta limestone monkeyflower, *Erythranthe taylori* (Nesom, 2013); Shasta maidenhair fern, *Adiantum shastense* (Huiet et al., 2015); Shasta fawn lily, *Erythronium shastense* (York et al., 2015); and Shasta huckleberry, *Vaccinium shastense shastense* (Nelson and Lindstrand, 2015).

Other taxa in the southeastern Klamath Ranges include isolated inland populations of amphibian species generally distributed along the Pacific coastal zone or in the Sierra Nevada, including the California slender salamander *B. attenuatus*, the black salamander *Aneides flavipunctatus*, the Pacific giant salamander *Dicamptodon tenebrosus*, and the tailed frog *Ascaphus truei*.

We choose to recognize three species within the *Hydromantes shastae* complex, two of them new. Populations in our mitochondrial clade D include the type locality of *H. shastae* and accordingly retain the name *Hydromantes shastae*. Populations in a major mitochondrial clade, including members of minor clades A, B, and C, are placed in the first new species, and clade E is the second new species (see below).



Figure 10. MVZ 269780, adult female *Hydromantes shastae* from the type locality (Low Pass Creek, Shasta County, California).

SYSTEMATICS

Redescription of *Hydromantes shastae*

Hydromantes shastae
Gorman and Camp, 1953
Shasta Salamander
Figure 10

Holotype. MVZ 52314, an adult female from “under a small mossy log at the entrance to limestone caves at the edge of Flat Creek Road in the narrows of Low Pass Creek (0.7 mi. east of Squaw Creek Road, 18.4 mi. north and 15.3 mi. east of Redding), Shasta County, California. Elevation 1500 ft.” (Gorman and Camp, 1953: 39).

Diagnosis. “A plethodontid salamander with pedunculate tongue, blunt toes with webs extending more than halfway to tips, and a blunt tail; readily distinguishable from all California salamanders except *Hydromantes platycephalus*. It differs from *H. platycephalus* (Camp, 1916) in its larger more protrusive eyes, narrower head and prominent supranasal ridge (canthus rostralis), more cylindrical body, and blunter toes” (Gorman and Camp, 1953: 39). The species differs from its close genetic and geographic congeners (described below) in allozyme and mtDNA sequence differences (detailed earlier in this paper). These are

morphologically cryptic species, which nonetheless differ in multivariate morphometric features (see DFA in this paper; Fig. 9). The longest digits of the pes are longer in *H. shastae* than in either of its morphologically cryptic relatives.

Redescription. The holotype was poorly preserved. It is contorted and impossible to measure, but Gorman and Camp (1953) report measurements of the holotype, the male paratype (MVZ 52318), and 10 additional members of the species taken from anaesthetized specimens. The holotype, in particular, is soft, and the skin is badly deteriorated. Accordingly, we have used measurements of nine males and 10 females from the Brock Mountain region (measurements of six individuals from the site are recorded by Gorman and Camp). Coloration was studied in detail both in life and in preservative for one recently collected specimen (MVZ 269780) from the type locality.

This species is smaller than both *H. platycephalus* and *Hydromantes brunus*. Standard length (mean) in males 55.3–62.2 (59.0); females 50.3–63.6 (58.3). Tails are short, strongly tapered and generally blunt-tipped: males 27.7–34.1 (30.8); females 24.3–33.2 (29.7). Heads are broad and flattened, but less so than in *H. platycephalus*: males 9.8–10.4 (10.1); females 8.5–10.1 (9.6). In adult, sexually active males the maxillary bones are rotated outward to some degree so that enlarged teeth protrude slightly from the side of the head. The trunk has a generally stocky form, rather quadrangular in shape, and somewhat flattened. There is a neck region that is slightly narrower than the body. The very elongate epibranchials and associated muscular wrapping extend from the throat region dorsally and over the shoulder; these extend far posteriorly along the dorsolateral portion of the body, rendering it broad, with a sharp lateral margin. Costal grooves are evident and extend onto the ventral surfaces; they typically number 13 (14 trunk

vertebrae, verified in radiographs). Limbs are long, and manus and pes are large and broad. Limbs overlap by as much as one costal fold when appressed to the side of the body. Combined limb length divided by axilla–groin length is 1.1–1.3 in males and 1.1–1.2 in females. Basal webbing is moderate, but the digits all extend well out from the webbing. Digits are relatively stout with broad, almost knoblike tips that enclose T-shaped terminal phalanges (evident in radiographs). Digits in order of increasing length: 1-4-2-3; 1-5-2-4-3.

Measurements of MVZ 269780 (mm). Head width 9.8; snout to gular fold (head length) 14.6; head depth at posterior angle of jaw 4.4; eyelid width 2.7; eyelid length 4.5; orbitonarial 3.1; eye to snout 4.3; horizontal orbit diameter 3.0; intercanthal 4.4; interorbital distance 3.7; distance between nuchal groove and gular fold 2.6; snout to forelimb 16.5; internarial 3.3; snout projection beyond mandible 0.4; snout to posterior angle of vent (standard length) 56.2; snout to anterior angle of vent 52.0; axilla to groin 30.2; chest width 6.2; number of costal interspaces between appressed limbs –2; tail length 31.5; tail width at base 3.4; tail depth at base 3.3; forelimb length (to tip of longest finger) 16.9; hindlimb length 17.6; hand width 5.6; foot width 7.1; length of fifth toe 1.2; length of third toe 2.1.

Coloration in Life. MVZ 269780 (topotypic female, Fig. 10). Top of head, dorsal and lateral ground color Hair Brown (277) extensively mottled with highlights of Ground Cinnamon (270). Dorsum of tail with bright highlights (Yellow Ochre, 14). Manus and pes with silvery highlights (Pale Neutral Gray, 296). Upper part of iris Light Orange Yellow (77).

Coloration in Preservative. MVZ 269780 (topotypic female). Coloration close to that in life but duller and more subdued. Dorsal surfaces Hair Brown (277) with mottling of Ground Cinnamon (270). Ventral surfaces Cinnamon (255) and True Cinnamon (260).



Figure 11. MCZ A-151701, adult male paratype *Hydromantes samweli*, collected 50 m below entrance to Samwel Cave, type locality. Photos by Robert W. Hansen.

Descriptions of New Species

Hydromantes samweli new species
 urn:lsid:zoobank.org:act:B8C4EE0D-
 3B4A-4162-97D5-45D2CC3227B1
 Samwel Shasta Salamander
 Figures 11 and 12

Holotype. MVZ 170650, an adult female, from below Samwel Cave, E side McCloud Arm of Shasta Lake, 2.2 mi (3.5 km) SSE (by road) McCloud River Bridge, Shasta County, California, 40.9203°, –122.2378°, 434 m elevation, collected by T. J. Papenfuss on 10 March 1978.

Paratypes. All E side McCloud Arm of Shasta Lake, Shasta County, California: MVZ 84929, 170633, 170635–49, 170651, 170653–55, 220896, 227721–23, same as holotype; 140697–98, 140700, Samwel Cave area between cave and river; MCZ A-151701 (Fig. 11), 46 m W (airline) of mouth of Samwel Cave, Shasta County, California, 40.9169°, –122.2387°, 390 m elevation.



Figure 12. Samwel Cave limestone outcrop showing habitat at the type locality (center right) of *Hydromantes samweli*.

Diagnosis. A morphologically cryptic member of the *Hydromantes shastae* species complex that differs from *H. shastae* in having a shorter long digit (third) on the pes, and from both it and *H. wintu* in DFA of morphometric traits (Fig. 9). It differs further from both species in allozymes and in mtDNA sequences (see text for details; Fig. 8).

Description. This species has no readily discernable morphological differences from *H. shastae*. Like that species, *H. samweli* is smaller than both *H. platycephalus* and *H. brunus* and has a narrower head. Standard length in males 49.2–60.7 (55.3); females 54.0–61.5 (57.4). Tails are short, strongly tapered, and generally blunt-tipped: males 24.2–31.9 (27.6) females 25.6–31.5 (28.0). Heads are broad and flattened, but less than in *H. platycephalus*: males 8.2–10.1 (9.4), females 9.0–10.2 (9.6). In adult, sexually active males the maxillary bones are rotated outward to some degree so that enlarged teeth protrude slightly from the side of the head. The trunk has a generally stocky form, rather quadrangular in shape, and somewhat flattened. There is a neck region that is slightly narrower than the body. The very elongate epibranchials and associated muscular wrapping extend from the throat region dorsally and over the shoulder and

extend far posteriorly along the dorsolateral portion of the body, rendering it broad, with a sharp lateral margin. Costal grooves are evident and extend onto the ventral surfaces; they typically number 13 (14 trunk vertebrae, verified in radiographs). Limbs are long, and manus and pes are large and broad. Limbs overlap by as much as one costal fold when appressed to the side of the body. Combined limb length divided by axilla–groin length is 1.1–1.3 in males and 1.1–1.2 in females. Basal webbing is moderate, but the digits all extend well out from the webbing. Digits are relatively stout with broad, almost knoblike tips that enclose T-shaped terminal phalanges (evident in radiographs). Digits in order of increasing length: 1-4-2-3; 1-5/2-4-3.

Measurements of Holotype (mm). Head width 10.9; snout to gular fold (head length) 14.5; head depth at posterior angle of jaw 4.6; eyelid width 2.3; eyelid length 3.4; orbitonarial 2.8; eye to snout 4.3; horizontal orbit diameter 2.8; intercanthal 3.0; interorbital distance 2.7; distance between nuchal groove and gular fold 4.5; snout to forelimb 19.0; internarial 3.4; snout projection beyond mandible 1.3; snout to posterior angle of vent (standard length) 67.8; snout to anterior angle of vent 62.1; axilla to groin 36.3; chest width 9.0; number of costal interspaces between appressed limbs –0.5; tail length 31.6; tail width at base 3.6; tail depth at base 4.0; forelimb length (to tip of longest finger) 16.7; hindlimb length 17.3; hand width 5.5; foot width 7.0; length of fifth toe 1.0; length of third toe 2.2. Numbers of teeth: premaxillary + maxillary 79; vomerine 29

Coloration of Holotype in Alcohol. Ground color of head, body, tail, and lateral surfaces True Cinnamon (260). Ventral surface Medium Fawn (257).

Coloration of Paratype MCZ A-151701 (Topotypic Male) in Life. Dorsal and lateral surfaces of head, body, limbs, and tail a subdued mottling of darker Warm Sepia (40) and somewhat brighter Chestnut (30).



Figure 13. MVZ 269778, adult male paratype *Hydromantes wintu* from type locality, Potter Creek area, Shasta County, California. Photo by Sean M. Rovito.

Dorsal surfaces of hands and feet lighter than other surfaces, Fawn Color (258).

Coloration of Paratype MCZ A-151701 (Topotypic Male) in Preservative. Top of head and dorsal and lateral ground color Dark Neutral Gray (299). Ventral ground color Medium Neutral Gray (298).

Etymology. The species is named for its original discovery site, Samwel Cave, a well-known fossil locality since the days of Eustace Furlong (e.g., Blois et al., 2010), sometimes written Samwell. Samwel is derived from the Wintu language word Sa-Wal, meaning Grizzly Bear, which was considered sacred by the Wintu people, and *samweli* is used as a genitive of the Latinized name. The Wintu language was



Figure 14. MVZ 269779, adult female paratype *Hydromantes wintu* from type locality, Potter Creek area, Shasta County, California. Photo by Sean M. Rovito.



Figure 15. MCZ A-151700, adult male *Hydromantes wintu* from small lower entrance to Potter Creek Cave, Shasta County, California. Photos by Robert W. Hansen.

used by the Native Americans (Winnemem Wintu Tribe) who inhabited this area.

Hydromantes wintu new species
urn:lsid:zoobank.org:act:C6B10B7A-DC2C-40A1-AEBA-7F3B53812EE4

Wintu Shasta Salamander

Figures 13–16



Figure 16. Habitat of *Hydromantes wintu* along south-facing slope above Potter Creek.

Holotype. MVZ 170610, an adult female from former Shasta Iron Mine Quarry, slope above S side Potter Creek at SE end McCloud Arm, Shasta Lake, Shasta County, California, 40.7770°, -122.2825°, 527 m elevation, collected by T. J. Papenfuss on 10 March 1978.

Paratypes. All from SE end McCloud Arm, Shasta Lake, Shasta County, California: MVZ 143066, 143067, 143069, 143071-73, 143075, 170611, 170630, 170631, 170674, 170676-84, 170686, same as holotype; 141998 S side Marble Creek 40.7873° -122.2782°; 143061 Potter Creek; 268778 (Fig. 13), 269779 (Fig. 14), just below Potter Creek Cave; MCZ A-151700 (Fig. 15), 50 m SSE (airline) of mouth of Potter Creek Cave, Shasta County, California, 40.7829°, -122.2826°, 415 m elevation.

Diagnosis. A morphologically cryptic member of the *Hydromantes shastae* species complex that differs from *H. shastae* in having a shorter long digit (3) on the pes, and from both it and *H. samueli* in DFA of morphometric traits (Fig. 9). It differs further from both species in allozymes and in mitochondrial DNA sequences (see text for details; Fig. 8).

Description. This species has no readily discernable morphological differences from *H. shastae* or *H. samueli*. Like those taxa, *H. wintu* is smaller than both *H. platycephalus* and *H. brunus* and has a narrower head. Standard length in males 53.4-62.0 (57.4); females 55.9-67.8 (60.2). Tails are short, strongly tapered, and generally blunt-tipped: males 24.6-31.5 (28.0); females 26.7-31.6 (29.6). Heads are broad and flattened, but less than in *H. platycephalus*: males 9.4-10.6 (9.8), females 9.4-10.9 (9.9). In adult, sexually active males the maxillary bones are rotated outward to some degree so that enlarged teeth protrude slightly from the side of the head. The trunk has a generally stocky form, rather quadrangular in shape, and somewhat flattened. There is a neck region that is slightly narrower than the body. The very elongate epibranchials

and associated muscular wrapping extend from the throat region dorsally and over the shoulder and extend far posteriorly along the dorsolateral portion of the body, rendering it broad, with a sharp lateral margin. Costal grooves are evident and extend onto the ventral surfaces; they typically number 13 (14 trunk vertebrae, verified in radiographs). Limbs are long, and manus and pes are large and broad. Limbs overlap by as much as one costal fold when appressed to the side of the body. Combined limb length divided by axilla-groin length is 1.1-1.2 in males and 0.9-1.1 in females. Basal webbing is moderate, but the digits all extend well out from the webbing. Digits are relatively stout with broad, almost knoblike tips that enclose T-shaped terminal phalanges (evident in radiographs). Digits in order of increasing length: 1-4-2-3; 1-2-5-4-3.

Measurements of holotype (mm). Head width 10.9; snout to gular fold (head length) 14.5; head depth at posterior angle of jaw 4.6; eyelid width 2.3; eyelid length 3.4; orbitonarial 2.8; eye to snout 4.3; horizontal orbit diameter 2.8; intercanthal 3.0; interorbital distance 2.7; distance between nuchal groove and gular fold 4.5; snout to forelimb 19.0; internarial 3.4; snout projection beyond mandible 1.3; snout to posterior angle of vent (standard length) 67.8; snout to anterior angle of vent 62.1; axilla to groin 36.3; chest width 9.0; number of costal interspaces between appressed limbs -0.5; tail length 31.6; tail width at base 3.6; tail depth at base 4.0; forelimb length (to tip of longest finger) 16.7; hindlimb length 17.3; hand width 5.5; foot width 7.0; length of fifth toe 1.0; length of third toe 2.2. Numbers of teeth: premaxillary + maxillary 79; vomerine 29.

Coloration in Life. MVZ 269779 (female paratype, Fig. 14). Top of head and dorsal and lateral ground color Walnut Brown (27). Dorsum of tail Yellow Ochre (14). Manus and pes bright, mottled with Pale Neutral Gray (296) and Pale Bluff (1).

Upper iris bright, metallic Sulphur Yellow (80).

MVZ 269778 (male paratype, Fig. 13). Ground color of top of head and dorsal and lateral surfaces of body a mélange of Medium Tawny (60) and Amber (51). Dorsum of tail Dark Spectrum Yellow (78). Manus and pes lively Cream White (52). Upper iris bright, metallic Spectrum Yellow (79).

MCZ A-151700 (male paratype, Fig. 15). Dorsal surfaces of head and body and lateral surfaces of body ground color Jet Black (300). Ventral surface Dark Neutral Gray (299). Scattered ventrolateral chromatophores Pale Neutral Gray (296).

Coloration of Holotype in Alcohol. Ground color of head and dorsal and lateral surfaces of trunk True Cinnamon (260). Ventral surfaces Cinnamon (255). Dorsum of tail Pale Cinnamon (55).

Etymology. The original inhabitants of this region, the Winnemem Wintu Tribe, were displaced by early European inhabitants. An early fish hatchery established at the site named Baird has been inundated by Shasta Lake. The species epithet is a noun used in apposition to the generic name.

HISTORICAL BACKGROUND AND NATURAL HISTORY

In their description of *Hydromantes shastae*, Gorman and Camp (1953) note that Camp had long been aware of salamanders occurring in a cave on Brock Mountain in the Triassic limestone outcrops above Squaw Creek in Shasta County, California. He examined mummified dry specimens from the cave in 1915 when he was a student working in the Museum of Vertebrate Zoology (MVZ), University of California at Berkeley. These specimens had been collected in 1902 or 1903 by Eustace L. Furlong, preparator of fossil vertebrates for John C. Merriam at the Department of Paleontology. Furlong and the expedition's sponsor, Annie Alexander,

collected 43 Mesozoic reptile fossils in limestone outcrops. It is not known whether Furlong or Alexander actually found the salamanders in the Brock Mountain cave.

On 18 July 1915, Charles Camp, then a 22-year-old student working with Joseph Grinnell at MVZ, caught a pair of salamanders in a snap trap intended for mammals at the base of Lyell Glacier in Yosemite National Park. Camp, an undergraduate student, was participating in a vertebrate survey of Yosemite. He wrote in his field notes, "In a mouse trap set at the entrance of a small hole (size of a gofer [sic]) beside a large rock outcropping in a patch of heather (100 ft in dia.) on a steep hillside (east facing slope) above the Donohue pass trail at 10,800 ft two remarkable salamanders were found this morning. Both had been caught by the middle of the back in the same trap. One was alive and the other dead and they had been walking out of the hole when caught. Altho this heather patch lies directly in the sun all most [sic] all day, there is still snow about it and it is partially surrounded by rock slides on a bare rock slope. I think the salamanders are entirely new. They seem of the *Plethoda* [sic] group and possibly represent an arrested development stage of *Aneides*. Several traps in the heather had been sprung without catching anything" (Camp, 1914–22: 481).

Camp described *Speleperpes* (now *Hydromantes*) *platycephalus* in 1916. He did not describe the Brock Mountain specimens because of their mummified condition (Gorman and Camp, 1953). After completion of his doctoral studies at Columbia University in 1922, Camp joined the University of California Museum of Paleontology, where he spent his entire professional career. In 1924, he searched Brock Mountain but failed to find Furlong's salamander cave. On 12 June 1950, an MVZ graduate student, Joe Gorman, discovered a population of Furlong's salamanders at Low Pass Creek in an area of limestone outcrops and small caves. The holotype of *H. shastae*

(MVZ 52314) was collected under a mossy log at the entrance of a limestone cave. Gorman returned to the site with Charles Camp on 13 October of that year.

Camp writes, “I prospected under rocks along the road 100 yds E of the cave—where rocks lie beside road in soft red earth—decaying lime—with many fissures + crevices. Here I found a nice big *Hydromantes* with a flush of brick red over top of its tail. Eyes rather large I should say + head rather narrow compared with Lyell form—also web not so pronounced on front feet” (Camp, 1950: 3957–3971).

At the time of its description *H. shastae* was known only from three localities: Low Pass Creek, McCloud River at Baird and Potter Creeks (likely the current Potter and Marble creeks), and Brock Mountain. Field work by Bury et al. (1969) documented a fourth locality 2 miles south of the McCloud River Bridge on Forest Service Road 34N00. Five salamanders were located during 15 person-hours of search. Because of the small number of salamanders found, the authors suggested that the species should be considered for protection as a rare species.

Surveys conducted in the Shasta Lake region during the 1970s added an additional nine localities to the four sites previously known (Papenfuss and Carufel, 1977; Bogener and Brouha, 1979; Papenfuss and Cross, 1980). These surveys were all conducted at sites where limestone outcrops were present. More recently, several additional populations have been found, some in habitats where neither limestone nor caves are present, but the total range of *Hydromantes* in Shasta County remains small (see map, Fig. 17; Lindstrand, 2000; Bingham, 2007; Lindstrand et al., 2012). Discovery of these new populations highlights the need for additional field and laboratory research on members of this complex.

The three species in the *Hydromantes shastae* complex have similar life history traits and habitat preferences (Olson, 2005; Wake and Papenfuss 2005). Some are found

on the surface during the rainy season from late autumn to early spring. Salamanders can be observed at night when they are active on wet limestone outcrops. During the spring, both adults and juveniles are found under rocks and logs. As the surface soil and outcrops dry, salamanders move underground. They have been observed on the damp walls of caves during the summer (TJP, LL, personal observations). Eggs apparently have been found twice, in late September and in early November, both in the same small cave on Brock Mountain (Gorman, 1956). In August 1978, >20 individuals were seen in one small cave (Wake and Papenfuss, 2005).

Densities can be high in preferred habitats of limestone outcrops within forested slopes. In a 2-year mark-recapture study (Herman, 2003), 306 salamanders were marked in a 2,750 m² grid near Samwel Cave and 77 were recaptured at least once. The farthest distance traveled was 104 m by a male. Specimens of *Hydromantes* have been found in association with metavolcanic or metasedimentary outcrops and occasionally in forest with no rock outcrops (Lindstrand, 2000; Lindstrand et al., 2012).

The general habitat of the area is a mosaic of hardwood, conifer, and hardwood-conifer forest with chaparral inclusions (see Lindstrand et al., 2012) on rugged landscape with many enormous limestone outcrops and other rock formations and soils. The salamanders occur from elevations of 240 to 1,661 m (Lindstrand et al., 2012). The region experiences cool, wet winters with snow on higher slopes. It has hot, dry summers, with five to seven dry months during which little or no rain falls. Thus, underground retreats are essential for salamander survival.

MANAGEMENT IMPLICATIONS

The three taxa constituting the *Hydromantes shastae* species complex are geo-

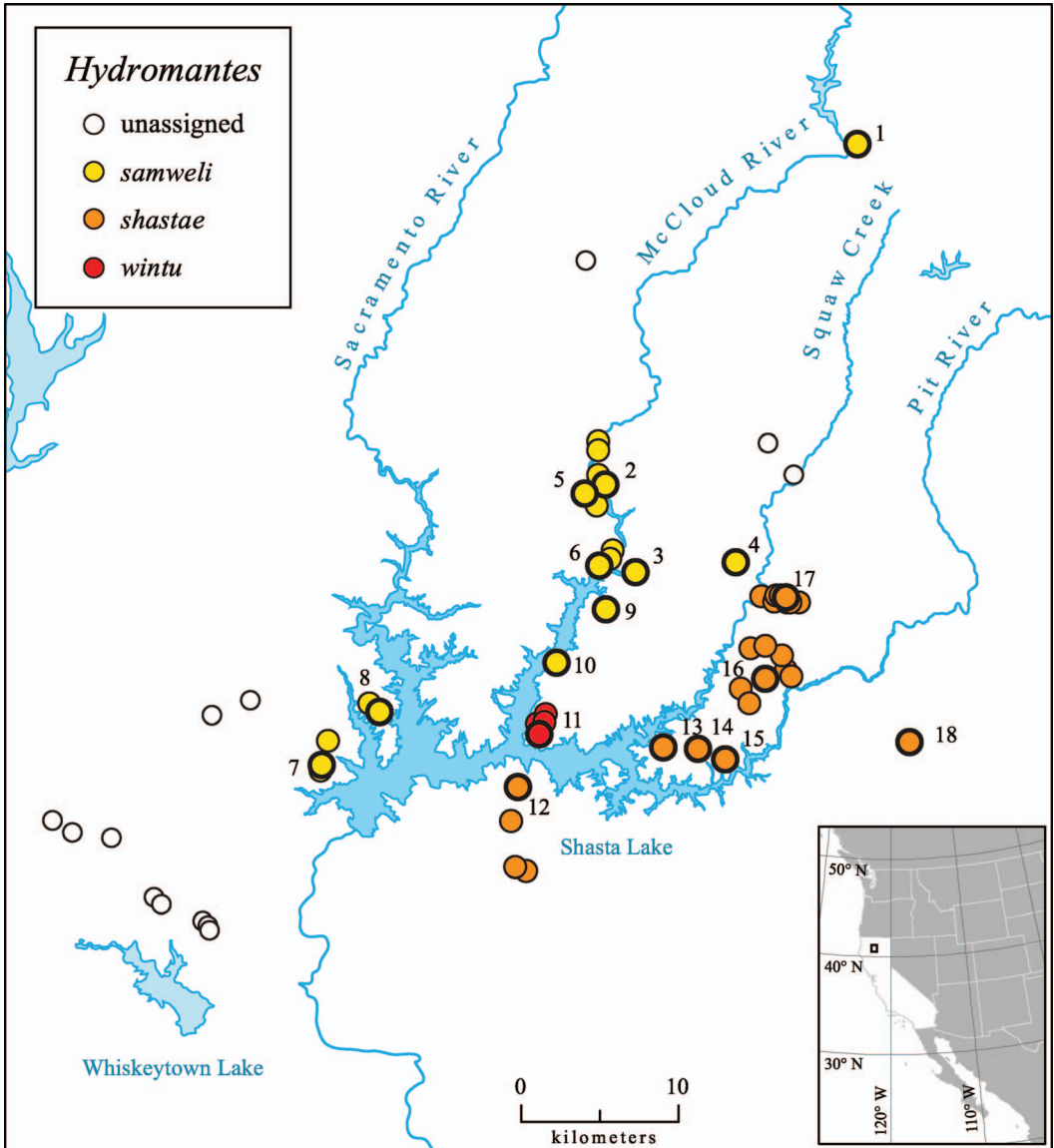


Figure 17. Known localities for members of the *Hydromantes shastae* complex. The 18 populations used for the current molecular analyses are and highlighted in bold. Type localities are as follows: 2, *H. samweli*; 11, *H. wintu*; 17, *H. shastae*. Recently discovered site localities to the west and south have not been studied.

graphically restricted clades that require attention from land and resource management agencies. The extent to which the creation of the Shasta Dam in the 1940s submerged pre-existing populations of this species is unknown, but during locally

favorable weather conditions, salamanders can be found immediately above and even below the high-water mark of the reservoir under cover objects. Doubtless some habitat was lost by the creation of the lake. Shasta Lake provides flood control and hydroelec-

tricity, serves many agricultural and industrial water users, and provides numerous recreation activities. Plans are being made to raise the level of Shasta Dam by 18.5 feet from its current water level at an elevation of 1,070 feet (328 m), which will affect some salamander populations. Quarrying of limestone and other mining activities directly degrade habitat. Additional potential threats to habitat include roadway and public utility infrastructure maintenance and expansion, both prescribed and natural wildfires, suburban development, and forest resource management. The majority of lands in the region are federally owned, with numerous potential irreversible and semipermanent threats in place, but sites are somewhat protected by the steep, rugged nature of the terrain (Fig. 12). Recognition of these evolutionary lineages as distinct species will highlight their uniqueness and contribute to their preservation.

DATA DEPOSITION

GenBank Accession Numbers

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