Discovery of a New, Disjunct Population of a Narrowly Distributed Salamander (Taricha rivularis) in California Presents Conservation Challenges

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ABSTRACT. A newly discovered population of Red-Bellied Newts (Taricha rivularis) in the Stevens Creek watershed in Santa Clara County, California, represents a significant southerly range extension of this species, by approximately 130 km from the nearest records in Sonoma County, California. To investigate the origin of this population we sequenced two mitochondrial genes (ND1, cytochrome b) and one nuclear exon (POMC) from the Santa Clara County population and from the main portion of the range including Sonoma, Mendocino, and Humboldt Counties. Phylogenetic relationships, historical demography, and genetic diversity were used to infer the origin of the newly discovered population and to elucidate the evolutionary history of the species. This species exhibits the lowest genetic diversity of any salamander in coastal California, and we infer unique signatures of population expansion not found in sympatrically occurring salamander species. The newly discovered population, characterized by a ubiquitous mtDNA haplotype found throughout the main range, is not genetically divergent. Although we were unable to determine whether the Santa Clara population is natural or introduced, we consider it to be of potential conservation significance and to warrant management protection. Although it may be unconventional to protect a population that is possibly introduced, this newly discovered population might be a critical assurance colony that will aid in the long-term persistence of this declining species. Because T. rivularis lacks genetic variation, has a small geographic range, and has experienced high levels of habitat disturbance, we recommend that it receive protection throughout its range.

Given the worldwide trend of amphibian population decline and extinction, it is imperative to protect isolated or peripheral populations of amphibians because they may harbor unique genetic variation, may be insulated from threats to central populations, and therefore have higher extinction risks than central populations (Lesica and Allendorf, 1995). As the climate and environment change, this standing genetic variation will likely be invaluable in allowing species to adapt to changing conditions (Frankel and Soule`, 1981; Moritz, 2002). Low genetic variation within species has been linked to depressed fitness, lowered resistance to disease and parasites, and diminished flexibility to cope with environmental challenges (Lacy, 1997; Amos and Balmford, 2001; Hedrick, 2001). Although not all peripheral populations contain unique genetic variation, as is the case with recent range expansions or high gene flow between populations, these populations retain value because they give the species a better chance for long-term persistence. However, the possibility that a newly discovered, disjunct population or metapopulation of an otherwise rare or declining species may be the result of human introduction presents a quandary for conservation agencies and planners tasked with protecting threatened species. When confronted with such a situation, the general course of action is to investigate the populations empirically, generally using genetic tools, in an effort to determine the origin (natural or introduced) of the newly discovered population. Here we employ this approach in an effort to elucidate the source of a newly discovered salamander population widely separated from the central range of this species.

Human-mediated dispersal has been documented in many amphibian species, and is most often revealed through the use of molecular data (Slade and Moritz, 1998; Bonett et al., 2007; Brown et al., 2010; Holsbeek et al., 2010). In western North America, the introduction of the salamander Aneides vagrans to Vancouver Island was discovered using mitochondrial sequence and allozyme data (Jackman, 1998). Similarly, introduced populations of another salamander species, Ambystoma tigrinum, to central California were discovered using mitochondrial and nuclear sequence data (Riley et al., 2003). These cases of recent human introductions typically exhibit predictable genetic signatures, such as a reduction in genetic diversity as compared to the central portion of the species range. Molecular data can also determine the source of the introduced population if substantial molecular variation occurs across the known range of the founding population, and geographic sampling is adequate to capture that variation. Haplotypes in introduced populations are expected to be very similar to or identical to haplotypes from a portion of the central range. Conversely, cases of naturally occurring disjunct populations can be expected to evolve unique, shared mutations given that sufficient time has passed and that gene flow is absent or greatly restricted.

Red-Bellied Newts, Taricha rivularis, inhabit the northern coastal region of California including northern Sonoma and Lake Counties, Mendocino County, and southern Humboldt County (Stebbins, 2003). These stream/river-dwelling newts breed in flowing water from late February until May in close association with coastal woodlands, especially those including the Coast Redwood, Sequoia sempervirens (Stebbins, 2003). Previous studies on the migratory and homing capabilities of T. rivularis demonstrated that this species is capable of dispersing up to a few kilometers, yet they are highly philopatric to breeding sites (Twitty et al., 1964; Twitty, 1966). Kuchta and Tan (2006) examined genetic diversity across just four localities of T. rivularis using allozyme data and a small mitochondrial data set (6 total sequences of ~350 bp) and determined that levels of genetic variation and genetic divergence between populations were low compared to other California salamanders. Kuchta and Tan (2006) proposed either high levels of gene flow (despite being highly philopatric) or a recent population expansion as explanations and suggested that denser sampling with additional molecular data might resolve these two hypotheses.

Recently, a population of T. rivularis was discovered in the Santa Cruz Mountains, Santa Clara County, California. The new
Although many salamander species naturally occur in disjunct populations in the Santa Cruz Mountains, it is unclear whether the newly discovered population of *T. rivularis* is a naturally occurring population or the result of a recent human introduction. To resolve this question, we examined the genetic diversity of *T. rivularis* across both the main portion of its range and the newly discovered Santa Cruz population using mitochondrial and nuclear DNA sequence data. We investigated population structuring and historical demography within *T. rivularis* and attempted to identify the genetic affinities of the newly discovered population. Based on our findings, we offer our recommendation for the appropriate conservation status of this population.

**MATERIALS AND METHODS**

**Neot Surveys and Genetic Sampling.**—Visual surveys of the upper Stevens Creek Watershed were conducted to collect *T. rivularis* samples. Additional insight into the status of the population was gained by observing their relative abundance, and by searching for evidence of breeding in the main stem of Stevens Creek as well as suitable tributaries in the drainage. We used 40 samples of *T. rivularis* from the frozen tissue collection at the Museum of Vertebrate Zoology (University of California Berkeley) that were collected from the known range (Sonoma, Mendocino, and Humboldt Counties), as well as two specimens from Stevens Creek (Fig. 1; Table 1). Previously published sequences of *cytochrome b* (*cyt b*) for Taricha granulosa, *Taricha sierra*, and *Taricha torosa* were obtained to compare estimates of genetic diversity (GenBank accession numbers: L22714, L22771–879, L22880–81, AY627899–912, DQ196241–308).

**Molecular Data.**—Whole genomic DNA was extracted from liver tissue using the DNeasy tissue kit (Qiagen, Valencia, California). The mitochondrial encoded NADH dehydrogenase 1 (*ND1*) region and a portion of the *cyt b* regions were sequenced, as well as the nuclear pro-opiomelanocortin gene (*POMC*). The *ND1* region and its flanking tRNAs were amplified using primers LX16S1 and Met3850H (Zhang et al., 2008). A portion of the *cyt b* region was amplified using primers MVZ15 (Kocher et al., 1989) and Cytb2 (Moritz et al., 1992). *POMC* was amplified using primers POMC_DRV_F1 and POMC_DRV_R1 (Vieites et al., 2007).

PCR reactions were carried out in 25-µL volume reactions containing 10–40 ng of genomic DNA, 18.3 µL water, 2.5 µL 10X PCR buffer, 1.5 µL magnesium chloride solution, 1.5 µL of DNTPs (2 µM), 0.5 µL of each primer at 10 µM, and 0.2 µL Taq polymerase. PCR products were sequenced using ExoSAP-IT (USB, Cleveland, Ohio) and fluorescent-dye labels were added following standard cycle sequencing protocols (PE Applied Biosystems, Foster City, California). The cycle sequencing products were purified using ethanol precipitation and sequenced using an ABI 3730 automated sequencer. All sequence data have been deposited in GenBank (accessions KF550306-KF550406).

**Phylogenetic Analyses.**—We used both maximum-likelihood and Bayesian methods to analyze the molecular data. A selection of mitochondrial genes can be considered to comprise a single nonrecombining locus; therefore, *ND1* and *cyt b* were combined for analysis. The nuclear marker *POMC* was found to be invariable and, thus, excluded from analyses. Given the low level of variability in the mtDNA data, no partitions were used, and the best-fit model of evolution was found using the Akaiki Information Criterion (AIC) in jModelTest v0.1.1 (Posada, 2008).
Maximum-likelihood analysis was performed using the GTR + Ψ substitution model in the program GARLI v0.96 (Zwickl, 2006). Analyses were conducted three times to ensure the algorithm was not trapped on local optima. For each analysis, 500 nonparametric bootstrap replicates were performed, and a 50% majority-rule consensus tree was created. Bayesian analyses were conducted on each data set using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Parallel runs were performed with random starting trees and allowed to run for 2 × 10^6 generations, with Markov chains sampled every 1,000 generations. Stationarity was assessed using the program Tracer v1.5.0 (Rambaut and Drummond, 2009). The first 25% of the total number of generations were discarded as burn-in, leaving 15,000 trees for each parallel run. The resulting post-burn-in trees from the two parallel runs were combined, and a 50% majority-rule consensus tree was calculated from a total of 30,000 trees. Additionally, a mitochondrial haplotype network was created using statistical parsimony with a 95% connection significance in the program TCS v1.21 (Clement et al., 2000).

Historical Demography and Genetic Diversity.—To investigate historical demography, mismatch distributions, Tajima’s D, and Fu’s Fs-values were calculated for the mitochondrial data set using Arlequin v3.11 (Excoffier and Schneider, 2005). For mismatch distribution analysis, the observed distribution of pairwise differences of the mitochondrial data was compared to data simulated under the sudden expansion model. Multimodal or ragged distributions suggest a historically stable population size, whereas smooth or unimodal distributions imply population expansion (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). Sum of square deviations (SSD) and Harpending’s raggedness index (HRI) were used to assess the fit of the data to the sudden expansion model, with significant P-values allowing rejection of the expansion hypothesis. Tajima’s D can be used to detect selection or changes in population size, with negative values indicating population expansion or positive selection and positive values indicating population contraction or balancing selection (Tajima, 1989). Fu’s Fs-value is useful for detecting population growth or genetic hitchhiking (Fu, 1997). In both tests, populations that have been stable are expected to have values near zero. Additionally, the nucleotide diversity (π) and average number of within-population pairwise distances (K) were also estimated using Arlequin v3.11 (Excoffier and Schneider, 2005). Uncorrected pairwise sequence divergences were estimated using Mega v5.03 (Tamura et al., 2011).

RESULTS

Neot Survey.—Diurnal newt surveys were conducted in the Stevens Creek watershed in an area that is part of the Monte Bello Open Space Preserve. The first survey was conducted on 10 February 2010 and yielded 4 T. rivularis, all found along the Grizzly Creek trail on the western side of Stevens Creek. All four newts were found within 15 m of each other, and two adults (MVZ 264098–99) were collected (Fig. 2A, B). We observed a large number (>100) of California Newts (T. torosa) and Rough-Skinned Newts (T. granulosa), all moving actively during the survey. The second survey, conducted on 9 May 2010, produced 6 more T. rivularis and the discovery of 2 separate T. rivularis egg masses. All six newts were found along the Grizzly Creek trail, and two additional juvenile T. rivularis were collected (MVZ 264100–01). The two egg masses (Fig. 2C) were identified easily and distinguished by their small disk-like shape and their placement on rocks in the rapidly flowing portions of the main fork of Stevens Creek (Fig. 2D). No T. rivularis egg masses were found in the smaller tributaries feeding into Stevens Creek.

Phylogenetic and Network Analyses.—The final alignment consisted of 1,347 bp for ND1 (11 variable, 2 parsimony informative sites), 422 bp for cyt b (1 variable and parsimony informative site), and 477 bp for POMC (invariable). There were 11 total combined mitochondrial haplotypes identified among the 40 individuals analyzed, with the number of individuals per haplotype ranging between 1 and 28 (Table 2). Four of the variable sites within the ND1 gene are nonsynonymous mutations. The phylogenies resulting from both the Bayesian and maximum-likelihood methods were identical, showing no significant genetic structuring across the range of T. rivularis. The haplotype network better captures the low level of variation in the mtDNA data, and the 11 haplotypes are separated from each other by no more than three substitutions in total. The T. rivularis samples collected from the Santa Cruz Mountains population share the most common haplotype found in the group with 26 other samples from across the main range possessing this haplotype (Fig. 3, Haplotype 1). The overall star-
shaped appearance of the network indicates that most substitutions are autapomorphic and that there are few shared derived mutations among the samples included (Fig. 3).

Historical Demography and Genetic Diversity.—The mismatch distribution of the combined mtDNA data does not allow rejection of the sudden expansion model for *T. rivularis* (SSD = 0.0017, *P* = 0.866; HRI = 0.0726, *P* = 0.849; Table 3). The results of Tajima's *D* (−2.23, *P* < 0.01) and Fu's *Fs*-value (−9.29, *P* < 0.01) also lend further support to a historical population expansion event in *T. rivularis* (Table 3). Based on available mtDNA data, other species of *Taricha* do not show strong evidence of historical population expansion (Table 3).

Levels of nucleotide diversity (*π*) and the average number of within-population pairwise distances (*K*) in *T. rivularis* are extremely low compared to other species in the genus (Table 3, Fig. 4). Both nucleotide diversity and within-population pairwise distances are two orders of magnitude lower in *T. rivularis* than in *T. granulosa*, *T. sierrae*, and *T. torosa*, which are more comparable to one another. Although the mean number of within population pairwise differences (*K*) across the entire range of *T. rivularis* for 1,769 bp of mtDNA data is 0.78; comparable numbers for *T. granulosa*, *T. sierrae*, and *T. torosa* within a 773 bp fragment of *cyt b* fragment are 21.67, 29.96, and 30.80 (Fig. 4). Nucleotide diversity in *T. rivularis* (Fig. 4) is exceptionally low (*π* = 0.0004) as compared to *T. granulosa* (*π* = 0.0282), *T. sierrae* (0.0387), and *T. torosa* (*π* = 0.0401).

**DISCUSSION**

*Taricha rivularis* lacks geographically structured mitochondrial lineages and exhibits extremely low levels of overall genetic diversity. The nuclear marker POMC is invariable, and less than 0.2% divergence in mtDNA is found across the approximate 200 km geographic range of the species, remarkably low for salamanders in this region, and for salamanders in general. Although POMC shows no variation within *T. rivularis*, it has

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**TABLE 2.** Uncorrected pairwise mtDNA sequence divergences among *Taricha granulosa* and *Taricha rivularis* haplotypes recovered. Haplotype numbers correspond to those in Figure 3. Totals in parentheses indicate the number of samples assigned to a particular haplotype.

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been used to characterize population-level genetic structure in other salamander species with similar range sizes. A previous allozyme study (Kuchta and Tan, 2006) confirmed that there is variation in nuclear genes, and further DNA sequencing of multiple nuclear loci may clarify the historical biogeography of *T. rivularis*. The only salamander we know of that has lower genetic diversity is the narrowly distributed *Salamandra lanzai* (Riberon et al., 2002). Other salamander species with similar coastal distributions exhibit substantially greater levels of mtDNA variation or geographic structuring, including *A. macrodactylum* (Savage, 2008), *Ambystoma californiense* (Shaffer et al., 2004), and *Ambystoma tigrinum* (Gibbs et al., 2007).

**FIG. 3.** A maximum-likelihood mitochondrial phylogram and haplotype network of the ND1 and cyt b genes. Numbers above branches are Bayesian posterior probabilities, whereas numbers below are maximum-likelihood bootstrap percentages. Scale bar is representative of substitutions/site for the phylogram. Main range localities are depicted by grey circles and the Santa Clara County specimens by white circles. The mitochondrial allele network was constructed with statistical parsimony with a 95% connection significance. Unique haplotypes are separated by one mutational step, and black circles along connecting lines represent additional mutational steps. Shades of haplotypes correspond to the main range (grey) and Santa Clara County population (white).

**FIG. 4.** The nucleotide diversity (π) and average number of within-population pairwise differences (K) of mtDNA data across four species in the genus *Taricha*. Error bars represent confidence intervals for each parameter. Calculations are based on a 773 bp fragment of cyt b for *Taricha granulosa*, *T. sierrae*, and *T. torosa*, whereas calculations for *T. rivularis* are based on 1347 bp of ND1 and 422 bp of cyt b.

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<th>Species</th>
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<th>π</th>
<th>K</th>
<th>Tajima’s D</th>
<th>Fu’s Fs</th>
<th>SSD</th>
<th>HRI</th>
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<td>ND1, cyt b</td>
<td>0.0004 ± 0.0003</td>
<td>0.78 ± 0.58</td>
<td>−2.23</td>
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<td>0.0387 ± 0.0207</td>
<td>29.96 ± 14.20</td>
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<td><em>T. torosa</em></td>
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<td>0.0401 ± 0.0207</td>
<td>30.80 ± 14.26</td>
<td>1.03</td>
<td>−2.92</td>
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et al., 2004). _A. flavipunctatus_ (Rissler and Apodaca, 2007), _B. attenuatus_ (Martinez-Solano et al., 2007), _D. ensatus_ (Steele et al., 2005), _E. eschscholtzii_ (Kuchta et al., 2009), _T. granulosa_ (Kuchta and Tan, 2005), and _T. torosa_ (Tan and Wake, 1995). The contrast in genetic diversity between _T. rivularis_ and co-occurring species cannot be explained by differences in life history or ecology. Generally, species with stricter habitat requirements or reduced vagility can be expected to show more geographic structuring relative to generalized species. Geographic structuring in plesodontid salamanders is likely driven by the ecological consequences of terrestrial breeding (such as in _A. flavipunctatus_, _B. attenuatus_, and _E. eschscholtzii_) and may not be an appropriate comparison for aquatic-breeding salamanders (species such as _A. macrodactylum_, _A. californiense_, _D. ensatus_, _T. granulosa_, and _T. torosa_). However, even if only aquatic-breeding salamanders are considered, _T. rivularis_ exhibits remarkably low genetic diversity (Table 3, Fig. 4). This pattern is especially surprising considering that _T. rivularis_ is philopatric and has specialized habitat requirements for breeding compared to other aquatic-breeding salamander species. Given these ecological characteristics, we would predict that _T. rivularis_ exhibits highly structured genetic lineages associated with independent watersheds if the range limits and population dynamics have been historically stable. This pattern of geographically structured genetic lineages is seen in other aquatic-breeding salamander species, including the sympatrically occurring newt species _T. granulosa_ (arguably the most generalized species of all the coastal aquatic-breeding salamanders) but not for the more specialized _T. rivularis_.

The apparent lack of geographic genetic variation and structured lineages in _T. rivularis_ was also detected by Kuchta and Tan (2006) using allozyme data. Kuchta and Tan (2006) hypothesized high levels of gene flow or a recent population expansion could be responsible for the patterns in _T. rivularis_. Based on both molecular and ecological evidence, we infer that population expansion following a bottleneck is responsible for these patterns. All tests for historical population expansion were statistically significant (Tajima’s D, Fu’s Fs, mismatch distributions; Table 3), supporting the hypothesis of recent expansion in _T. rivularis_. This signature of expansion is not detected in other species in the genus _Taricha_ (Table 3) and is not documented in other salamander species occurring in sympathy with _T. rivularis_. The historical processes responsible for producing these patterns in _T. rivularis_ are unclear. Although historical events, such as reduction in suitable habitat or climatic fluctuations, may have affected all salamander species along coastal California, the responses of individual species could have differed.

**Origin and Status of the Santa Cruz Population.**—The Santa Cruz Mountains mark the southern range limit of many of the amphibian species found in the redwood forest ecosystem, which approaches its southern limit in this area (redwoods are found nearly to the San Luis Obispo County line in coastal Monterey County, south of Carmel). Red-Bellied Newts have been thought to be associated with the redwood forest region, north of San Francisco Bay. Although there are no redwoods in the upper Stevens Creek watershed, extensive redwood forests occur within a few kilometers of the collection site. The disjunct distributional pattern of _T. rivularis_ is shared by several other salamander species, including _A. macrodactylum_, _A. californiense_, _A. flavipunctatus_, _B. attenuatus_, _D. ensatus_, and _T. granulosa_. These species exhibit a range of genetic divergences between the Santa Cruz Mountains and populations north of San Francisco Bay, ranging from less than 0.4% mtDNA divergence (Steele et al., 2005) to up to 9% mtDNA divergence (Rissler and Apodaca, 2007). In general, the aquatic breeders (ambystomatid and salamandrid species) show lower levels of divergence across San Francisco Bay than the terrestrial breeders (plethodontid species). Although levels of divergence across San Francisco Bay may be low for aquatic breeders, the disjunct populations of _D. ensatus_, _A. californiense_, and _T. granulosa_ each possess unique mtDNA haplotypes not found in the northern parts of their ranges. The presence of unique haplotypes in these populations demonstrates that they have been isolated for substantial periods of time and that they are naturally occurring. The Santa Cruz population of _T. rivularis_ possesses the most common haplotype found across the main range (haplotype 1, N = 28; Fig. 3, Table 2), making this population genetically indistinguishable from other populations across the main range.

Is the newly discovered population of _T. rivularis_ in the Santa Cruz Mountains a naturally occurring population, or the result of human introduction? Both naturally occurring and introduced populations can be investigated by comparing haplotypes of the population in question to the standing genetic variation within the main portion of the species range (Bonett et al., 2007). Clear cases of salamander introductions involve haplotypes of the introduced populations derived from genetic variation within the main range of the species range (Jackman, 1998; Riley et al., 2003; Bonett et al. 2007), whereas naturally occurring isolated salamander populations tend to exhibit unique haplotypes and some degree of divergence from the main range (Kuchta and Tan, 2005; Steele et al., 2005; Martinez-Solano et al., 2007; Rissler and Apodaca, 2007). Although a variety of molecular, population level, or ecological processes could produce exceptions to these patterns, this framework is still generally useful for assessing the status of questionable populations. Our data demonstrate that the newly discovered population of _T. rivularis_ does not possess unique mtDNA haplotypes and is not genetically divergent from other populations in the main range. Although this would appear to be a signature of human introduction, populations in the main range of _T. rivularis_ exhibit uncharacteristically low levels of genetic diversity, a complete lack of geographic structuring, and our analysis shows that the species has undergone a recent, rapid expansion that could well have led to establishment of the new population. Thus, we cannot reject the possibility that this population is natural.

Although equivocal, the Santa Cruz population of _T. rivularis_ could have arrived in a relatively recent natural dispersal event from the main range into the Santa Cruz Mountains. The area between the two populations contains multiple barriers to gene flow such as San Francisco Bay (currently containing saltwater that would kill a newt very quickly) and the southern portion of Sonoma County (also known as the “Sonoma gap”), which consists of expansive grasslands unsuitable for _Taricha_. However, only 20,000 years ago, when sea level was much lower, San Francisco Bay was little more than a broad river valley with the Sacramento River flowing through it (Sloan, 2006). At this point, the coastline of California extended an additional 40–50 km past the Farallon Islands (Sloan, 2006), creating a large coastal corridor for taxa to disperse across the freshwater Sacramento River. This would have rendered the area that is now San Francisco Bay a minor obstacle to dispersal for aquatic breeding amphibians at that time. This short time frame of approximately 20,000 yr could be insufficient for accumulation of fixed mutations in the isolated population given the size of the
genetic loci examined and our knowledge of DNA mutation rates.

Our molecular data allow no robust inference of the origins of the Santa Cruz population, but other lines of evidence are useful for assessing this population. Because *T. rivularis* has such specialized breeding requirements (clean, rapidly flowing streams), one might predict that it is a poor candidate for introduction. There are no museum collections of any herpetofauna from the upper Stevens Creek watershed, currently a preserve, and without a record of the species found there in the past, it is difficult to determine how long *T. rivularis* has been present. The lack of sampling/surveying of the area could help to explain why this population remained undiscovered for so long, if indeed it is natural. The V.C. Twitty laboratory at Stanford University studied newts in the 1960s, and newt releases associated with this research could have resulted in the presence of newts with the age structure that we observed. However, conversations with former members of that laboratory as well as then-graduate students in herpetology at Stanford, revealed no known newt releases in the Santa Cruz Mountains during that time (A. Jacobson, J. M. Savage, A. Leviton, pers. comm.). Furthermore, although within about 15 km of the Stanford campus, upper Stevens Creek is surprisingly remote and access to is difficult. Considering these factors, introduction of this relatively uncommon species appears to be unlikely.

Soon conservation geneticists will have access to large quantities of genomic data as next-generation sequencing becomes more affordable. These types of genomic data sets can be used to inform conservation policy (Allendorf et al., 2010), and it is possible that additional informative molecular data (microsatellites, genome scans) may ultimately resolve the origins of the Santa Cruz population of *T. rivularis*.

**Conservation Status of *Taricha rivularis*** Because metapopulations are groups of populations linked by immigration and emigration (Gotelli, 2001), we consider this species to be represented by one relatively large metapopulation and one isolated population, given that migration between central Sonoma and Santa Clara Counties is currently unrealistic. Because the Santa Cruz Mountain population is small, with no opportunities for recruitment from other populations, it has a high extinction risk. However, the habitat in which the population lives is excellent and well protected.

Low genetic variation within a species or population has been a benchmark for assigning increased protection (Schultz et al., 2008; Ishtiaq et al., 2011; Ricanova et al., 2011), and this premise largely rests on the notion that this lack of genetic variation correlates with a lack of adaptive variation. A lack of adaptive variation in a species with a small range size, or specialized habitat requirements (such as *T. rivularis*), means that this species has low potential for an adaptive response to new environmental challenges such as a new disease, competition from an introduced species, or climate change (Hedrick, 2001). We detected four variable sites within the *ND1* mitochondrial gene that result in an amino acid substitution, although further study is needed to confirm whether these substitutions are correlated with recent local adaptation. Although extremely low levels of variation were detected in the central range of *T. rivularis*, no variation was detected in the Santa Cruz population. Given these findings, we predict that the long-term persistence of *T. rivularis* will face many challenges from introduced species, disease, and a rapidly changing climate and land use.

Because no ecological studies or focused population surveys have been conducted on this species in the past few decades, the conservation status of *T. rivularis* is poorly understood. The species has a restricted and patchy distribution in the mountains of northwest California, and pressure attributable to human activity has intensified considerably over much of its range. Very little of the central range of *T. rivularis* is protected, and much of this range is owned by logging companies that practice clear cutting. Clear cutting alters temperature, sediment load, and physical structure of rivers and streams and the surrounding microhabitat, such that the landscape become less hospitable to resident species (Giusti and Merenlender, 2002). Clear cutting of coastal forests in western North America has been linked to the decline of many aquatic breeding amphibians (Bury, 1983; Corn and Bury, 1989; deMaynadier and Hunter, 1995; Curtis and Taylor, 2004), and suitable breeding conditions for *T. rivularis* are almost certainly affected when the erosion of exposed soil fills in the rocky bottom of streams (to which *T. rivularis* attaches its egg masses). Although long-term population studies are lacking for this species (Petranka, 1998), the repeated clear cutting of the forest throughout their range may be responsible for the observed low population densities in *T. rivularis* (SBR, pers. obs.) when compared to historical estimates (Twitty, 1966). In areas that are not owned by logging companies, the conversion of grasslands and forests to vineyards and rural housing divisions has permanently altered the habitat, especially in southern Mendocino and Sonoma Counties. And finally, the increased fragmentation of their range by roads and vehicular traffic has resulted in increased mortality of newts (AmphibiaWeb, 2012).

A recent review of the conservation status of *T. rivularis* suggests that the species should be elevated to a Priority 2 Species of Special Concern, a recommendation based on the small range of the species combined with the increased levels of habitat loss and fragmentation in recent decades. The total priority score given to the species is actually the highest SSC score of any salamander in California and places *T. rivularis* in the range of scores given to Priority 1 Species of Special Concern (unpubl. CDFW report; R. C. Thomson et al.). Given that *T. rivularis* is not abundant and has a small range in an area that is experiencing high levels of habitat disturbance, the newly discovered population in the Santa Cruz Mountains may represent an important assurance colony for the species and should be protected, even if it ultimately proves to be an introduced population. In the Stevens Creek habitat, it occurs in sympathy with the aquatic breeding species with which it co-occurs in Sonoma and southern Mendocino County, and even the terrestrial co-occurring salamander species are largely the same. The community gives the impression of integration and stability, and this population of *T. rivularis* will likely be restricted from expanding by a lack of suitable habitat outside of the Stevens Creek area.

In view of the extremely low genetic diversity within *T. rivularis*, its small geographic range, low population densities, and its specialized breeding requirements, we recommend changing the IUCN threat status from Least Concern (LC) (IUCN, 2010) to Near Threatened (NT). Currently *T. rivularis* has no protection under California law despite being listed for consideration as a species of special concern by Jennings and Hayes (1994); and we strongly recommend that the California Department of Fish and Game list *T. rivularis* as a Priority 1 Species of Special Concern. Protection may also facilitate growth of population size, expansion of the geographic range, and the
accumulation of additional genetic variation with time. Given the results of our study, we also recommend that the Santa Cruz population of _T. rivularis_ be provided protection to better ensure its survival. Additional survey work is critically needed.

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