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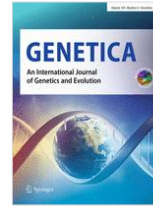
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Genetic structure and diversity in an isolated population of an endemic mole salamander (*Ambystoma rivulare* Taylor, 1940) of central Mexico

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Abstract Human activities are affecting the distribution of species worldwide by causing fragmentation and isolation of populations. Isolation and fragmentation lead to populations with lower genetic variability and an increased chance of inbreeding and genetic drift, which results in a loss of biological fitness over time. Studies of the genetic structure of small and isolated populations are critically important for management and conservation decisions. *Ambystoma rivulare* is a micro-endemic Mexican mole salamander from central Mexico. It is found in the most ecologically disturbed region in Mexico, the Trans-Mexican Volcanic Belt. The goal of this study of the population genetics of the micro-endemic mole salamander was to provide information to be used as a basis for future research and conservation planning of this species and other species of the *Ambystoma* genus in Mexico. The structural analysis found two subpopulations, one for each river sampled, with no signs of admixture and very high levels of genetic differentiation. Medium to high levels of heterozygosity and few alleles and genotypes were observed. Evidence of an ancestral genetic bottleneck, low values of effective population size, small inbreeding coefficients, and low gene flow were also found.

Keywords Mole salamander · Conservation genetics · Micro-endemic species · Microsatellites · Conservation

Introduction

Biodiversity of the planet is rapidly decreasing as a consequence of human exploitation of land resources. Consequences of decreased biodiversity include reduced species richness (Waltert et al. 2004; Ribeiro et al. 2009), a decline in genetic diversity (Frankham et al. 2005), and changes in the distribution of fauna resulting from habitat loss (Ribeiro et al. 2009; Sarukhán et al. 2009). Loss of habitat can result in small, isolated, and fragmented populations. These populations tend to have an increased chance of inbreeding as well as less genetic variability due to a loss of alleles through genetic drift (Frankham et al. 2005; Sunny et al. 2014a; Rueda Zozaya et al. 2016), reducing their biological fitness over time (Lande 1988; Jehle and Arntzen 2002). In order to maintain sufficient levels of genetic variability for small, fragmented populations and ensure their long term survival, studies of genetic variability must be conducted so that management strategies can be improved (Frankham et al. 2005; Palsbøll et al. 2007; Bradshaw et al. 2010).

Mexico is a biodiverse country which ranks fifth in the number of amphibian species (Parra-Olea et al. 1999; Frías-Alvarez et al. 2008) with 377 species (AmphibiaWeb 2016), of which 259 are endemic (Parra-Olea et al. 2014; Flores-Villela and García-Vázquez 2014; AmphibiaWeb 2016). However, Mexico also ranks fourth in world deforestation rates (FAO 2006; Ellis and Porter-Bolland 2008), which increases fragmentation and isolation of wildlife populations.

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The Trans-Mexican Volcanic Belt (TMVB) is one of the most ecologically disturbed regions in the country due to its nearness to highly urbanized cities (Sunny et al. 2015). Mexico also has 18 *Ambystoma* species, of which 16 are endemic (Parra-Olea et al. 2014). *Ambystoma rivulare* is a micro-endemic mountain mole salamander that inhabits slow-flowing streams within the TMVB, surrounded by *Pinus hartwegii* and *Abies religiosa* forest. It is found in streams located above 2800 m above sea level (masl) (Barriga-Vallejo et al. 2015).

This species is endangered, along with most of the mole salamanders of Mexico (SEMARNAT 2010; Parra-Olea et al. 2012; Sunny et al. 2014a; IUCN 2016), largely due to deforestation, pollution of rivers, and the introduction of exotic species (Casas-Andreu et al. 2004; Beebee and Griffiths 2005; Zambrano et al. 2010). However, there is little information on the current population trend and the status of genetic variability of this species, except within the Special Reserve of the Monarch Butterfly (SRMB) (Parra-Olea et al. 2012). The IUCN categorizes this species as data deficient (Shaffer et al. 2008). Additionally, this species is found in one of the most fragmented and disturbed areas of Mexico, where the environment has been heavily impacted by agriculture and urban settlements. In fact, some of the largest metropolitan areas in Mexico are in the distribution range of *A. rivulare* (CONAPO 2010; Bryson et al. 2014; Sunny et al. 2015).

The aim of this study was to assess two small, fragmented populations of *A. rivulare* found in the Nevado de Toluca Volcano (NTV) natural protected area, part of the TMVB, by examining their genetic diversity and structure, level of inbreeding, current effective population size, and evidence for bottlenecks. The NTV was declared a natural protected area in 1936, but lack of communication between the government and communal land holders prevented proper implementation of protection laws. Consequently, the presence of human settlements, parks, trout farming, illegal logging and other human activities has continued to affect the biodiversity of this area (Candeau and Franco 2007). Based on this species' life history and the poor condition of the natural protected area, we expected to find low genetic variability, a high degree of genetic structure, small effective population sizes, and low gene flow in these populations. These results will provide valuable information for making management decisions to help preserve *A. rivulare* in the NTV.

Materials and methods

Study site and population sampling

Population sampling was conducted in the NTV (18° 59′ N) in two small rivers surrounded by small alpine grassland, near a sacred fir and pine forest (*Abies religiosa* and *Pinus hartwegii*), at an altitude of 3200 masl. The sampling site is a recreational park with ecotourism activities, trout farming, and cattle and sheep grazing (Fig. 1). Tissue samples from 54 individuals were collected in the first river, and from 32 individuals in the second river. In order to represent all maturity stages of *A. rivulare* in the data, tissue was obtained from both adults and larvae. Sampling of larvae was limited (>10 %) and efforts were made to avoid sampling siblings. A total of 14 sampling sites were chosen after considering the characteristics of different micro-environments presented by the rivers (presence of ponds, flow rates, substrate type, amount of vegetation, etc.). Individuals were captured with a fishing net and a 2 mm^2 tail clip was collected. This methodology is low-impact and does not affect the survival or growth of the mole salamanders (Arntzen et al. 1999; Polich et al. 2013). Tissue samples were preserved in 90 % ethanol and then frozen at −20 °C until processing could be completed. All mole salamanders were released immediately at the point of capture.

DNA extraction and microsatellite amplification

DNA extraction was performed with a commercial kit (Vivantis GF-1 Tissue DNA extraction kit). Genomic DNA was used as a template for amplification of nine microsatellite loci: At 52.2, At 52.10, At 52.143, At 60.3, At 52.115, At 52.6, At 52.34, At 52.20, and At 52.1 (Parra-Olea et al. 2007). PCR reactions were performed in a Techne thermocycler. Amplified products were multiplexed on an ABI Prism3730xl and sized in PEAK SCANNER V1.0 (Applied Biosystems 2006) software using ROX-500 as an internal size standard. Allele sizes were measured and binned with the software TANDEM (Matschiner and Salzburger 2009).

Microsatellite analysis

Potential scoring errors and genetic structure

The presence of null alleles and other genotyping errors was determined using the software MICROCHECKER (Van Oosterhout et al. 2004).

We used the software STRUCTURE 2.3.4 (Pritchard et al. 2000) to infer the population structure. Due to the lack of genetic structure found in previous studies, the results of exploratory analyses of these data (K = 10, K = 8, K = 5, K = 3, K = 2 and K = 1; results not shown), and the results of a Delta K (ΔK) Evanno plot (see results), we decided to explore values of K from 1 to 8. The analysis was run ten times per K value in order to determine the maximum value of posterior likelihood [lnP(D)].
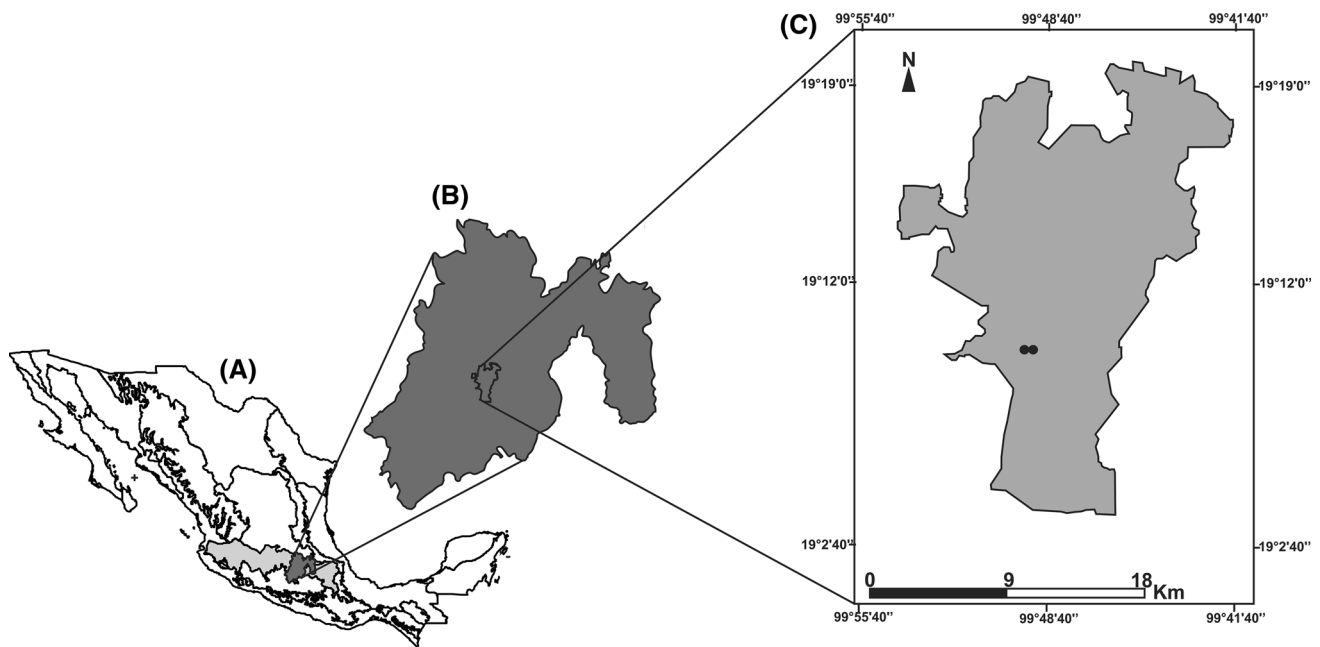
Fig. 1 a Map of Mexico showing the Trans-Mexican Volcanic Belt in *light gray* and in *dark grey* the State of Mexico. **b** State of Mexico, **c** Raíces, Zinacantepec, the *black dots* are the study sites

For each analysis we used 100,000 Markov chains following a burn-in period of 50,000 chains (Espindola et al. 2014).

A Dirichlet parameter was used to assess the degree of admixture; allele frequencies in each genetic group were correlated with the allele frequencies of an ancestral population, without prior information on population origin. We used the software STRUCTURE HARVESTER 0.6.92 (Earl and vonHoldt 2011) to determine the most probable number of clusters to best represent our data. Following the method of Evanno et al. (2005), we searched for the maximum value of ΔK (i.e. the ad hoc quantity related to the second order rate of change of the log probability of data) with respect to the number of clusters. We also used the software GENELAND 3.2.2 (Guillot et al. 2005) to infer population structure. GENELAND 3.2.2. implements a Bayesian algorithm through a Markov chain Monte Carlo procedure using genetic data and geographical coordinates. The program was run using the assumptions of a correlated allelic frequencies model and true spatial model (Guillot et al. 2005). We performed 10 independent runs of 1,000,000 iterations (thinning = 100, burn-in = 1000) using K = 10, K = 5, K = 3, K = 2, and K = 1 (Vázquez-Domínguez et al. 2012; Sunny et al. 2014a). Finally, we used an analysis of molecular variance (AMOVA) to analyze the distribution of the genetic variance between and within populations, based on F_{ST} values, with 10,000 permutations in the program ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010).

Genetic diversity

Linkage disequilibrium (LD) between all pairs of loci across all populations and conformance to Hardy–Weinberg equilibrium (HWE) expectations was evaluated using GENEPOP 4.2 (Raymond and Rousset 1995), which conducts exact tests (10,000 dememorization steps, 1000 batches and 10,000 iterations). A false discovery rate (FDR) test was performed using R 2.8.1 Q-VALUE (R Development Core Team 2013) in order to analyze the significance of data obtained. With GENALEX (Peakall and Smouse 2006), we estimated the observed (Na) and effective number (Ne) of alleles per locus as well as the observed (H_o) and expected heterozygosity (H_e). FSTAT 2.9.3.2 (Goudet 2002) software was used to obtain an estimate of allelic richness (A). In addition, we used SMOGD 1.2.5 (Crawford 2010) software, with 1000 bootstrap replications for each parameter, to calculate several estimators of genetic population differentiation such as G_{ST_est} (Nei et al. 1983), G'_{ST_est} (Hedrick 2005; Jost 2008), Δ_{ST}, D, and D_{est} (Jost 2008). We estimated Nei's genetic distance (Nei 1972) between sampling locations with GENALEX.

Gene flow, effective population size, inbreeding and bottlenecks

The gene flow was estimated with MIGRATE-N 3.6 (Beerli 2008), which utilizes Bayesian inferences. The Brownian motion model was used and we conducted five independent runs of four long chains of 10,000,000 genealogies, sampled

every 1000 steps, and a burn-in of 1,000,000 steps. The four heated chains had temperatures: T1 = 1.0, T2 = 1.5, T3 = 3.0 and T4 = 1,000,000. Default values were applied for the remaining parameters; effective immigration (M) rate and effective population size (Θ) were obtained.

To estimate the number of migrants per generation (N_{em}), we used the two populations defined by the STRUCTURE analysis M was multiplied by Θ (Beerli 2009, 2012). The effective population size (Ne) was determined by examining LD in the software NE ESTI-MATOR 2.01 (Do et al. 2014). As an inbreeding measure, we used the relatedness estimator (r_{qg}) of Queller and Goodnigh (1989), calculated by the software GENALEX. To test for significant differences among mean population relatedness, we calculated the upper and lower 95 % confidence intervals for the expected range of r_{qg} using 9999 permutations. These intervals correspond to the range of r_{qg} that would be expected if reproduction were random across the populations. Also, we calculated confidence intervals for estimates of mean relatedness within a population to 95 % by bootstrap resampling (9999 permutations). Population r_{qg} values that fall above the 95 % expected values indicate that processes such as inbreeding or genetic drift are increasing relatedness. Finally, we used the coefficient of inbreeding (F_{IS}), calculated by GENEALEX, as an indicator of total inbreeding in the population.

We used the software BOTTLENECK 1.2.02 (Cournet and Luikart 1996; Piry et al. 1999) to search for evidence of genetic bottlenecks events. We estimated the observed and expected heterozygosity under the infinite allele model (IAM), stepwise mutation model (SMM), and the two-phase model (TPM) with settings at 90 % SMM, 10 % IAM, and 10 % variance and also using the default values (70 % SMM, 30 % IAM, and 10 % variance). Both parameter settings were run with 10,000 replicates. Excess heterozygosity was tested with a Wilcoxon test. Finally, historical bottlenecks were tested with the Garza-Williamson (M) index, which was calculated with ARLEQUIN 3.5.1.2 software (Excoffier and Lischer 2010) and with CRITICAL M software (Garza and Williamson 2001). A critical M (M_c) value was obtained using 10,000 simulations and parameters from the two-phase mutation model, as described in Garza and Williamson (2001). M-values lower than the critical number, are indicative of historical population declines (Cournet and Luikart 1996; Garza and Williamson 2001).

Results

Potential scoring errors and genetic structure

Null alleles and other genotyping errors were not detected at any loci.

The highest log likelihood given by STRUCTURE was observed when K = 2 (LnPr = −1769.7); the ΔK method also chose two populations as the best model (Fig. 2). With GENELAND, three subpopulations were defined (LnPr = −2705.93). Since STRUCTURE assigned almost every individual to the river where it was collected, and the maximum value of ΔK (Evanno et al. 2005) analysis also suggested the presence of two subpopulations, we performed posterior analysis with two subpopulations. Population sampling was done in two independent streams, so using two subpopulations for analysis seemed to accurately represent the structure of A. rivulare at this study site. We defined these populations as Stream 1 (SUBP1: N = 54) and Stream 2 (SUBP2: N = 32). Other genetic structure analysis showed low genetic structure between the two populations (F_{ST} = 0.076, G_{ST_est} = 0.068, G'_{ST_est} = 0.300, Δ_{ST} = 1.258, D = 0.299, D_{est} = 0.285, $N_{ei's}$ = 0.328; Table 1 and 2). The F_{ST} calculated by AMOVA (F_{ST} = 0.076) revealed that most of the genetic diversity is attributed to variation among populations (72 %; p = 0.000), and only a small amount (21 %; p = 0.000) occurs among individuals or within populations.

Genetic variability

FDR correction found departures from HWE at loci At 52.10 and At 52.115 because of a heterozygote deficit (F_{IS} = −0.340). Linkage disequilibrium was not detected at any loci. Across the nine loci in the two populations, 68 alleles were identified, with a range of 2–7 (average 3.778) alleles per locus. SUBP1 had a total of 38 alleles, with 2–6 alleles per locus (mean = 4.222). SUBP2 had 2–5 alleles per locus (mean = 3.333) and a total of 30 alleles (Table 1; Fig. A2). Between the two populations, 91 genotypes were found (Table A3). There was a range of 2–10 (average 6.222) genotypes per locus for SUBP1 and 2–7 (average of 3.888) for SUBP2. Twenty-nine heterozygous genotypes and 27 homozygous genotypes were found in SUBP1, with a total of 56 genotypes. SUBP2 had a total of 35 genotypes, 18 heterozygous and 17 homozygous. SUBP1 showed lower observed and expected heterozygosity values (H_o = 0.761, H_e = 0.622) as compared to SUBP2 (Ho = 0.837, He = 0.591; Table 1).

Gene flow, effective population size, relatedness and bottlenecks

With regard to gene flow, the MIGRATE-N estimates of Θ for the two populations were 0.00273 for SUBP1 and 0.00412 for SUBP2. Migration rates per generation from SUBP1 to SUBP2 were 1.08 and from SUBP2 to SUBP1 were 0.137. With an allelic frequency of 0.05, the Ne in the
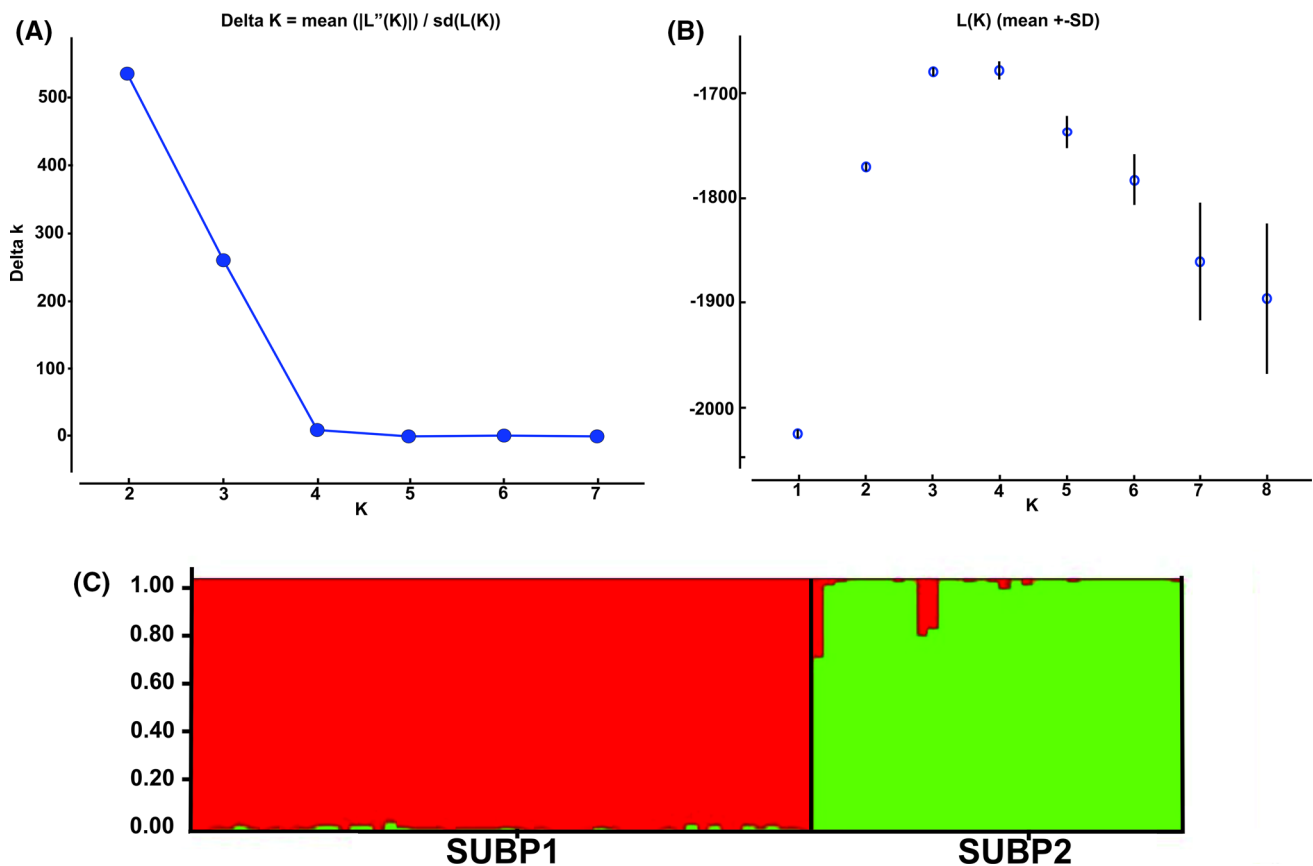
Fig. 2 *Ambystoma rivulare* genetic structure: **a**, **b** ΔK value of Evanno et al. ([2005](#)) plots for detecting the number of K groups that best fit the data. **c** Population genetic structure partitioned into K components representing the ancestry fractions in LnPr (K = 2) = −1769.7 populations

Table 1 *A. rivulare* genetic diversity values in the SUBP1 and in the SUBP2 populations

	N	Na	Ne	Np	Ho	He	F_{IS}
SUBP1	54	4.222	4.222	15	0.761	0.622	−0.225
SUBP2	32	3.333	3.333	7	0.837	0.561	−0.512
Total mean		3.778	2.605	11	0.799	0.591	−0.360

N sample size, *Na* number of alleles, *Ne* number of effective alleles, *Np* number of private alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, *FIS* fixation index

LD model was 19.9 for SUBP2 and 7.7 for SUBP2. The F_{IT} statistic for the whole population, used as an indicator of inbreeding, showed low inbreeding values (F_{IT} = −0.248; Table [2](#)). We found that mean pairwise relatedness (r_{qg}) within populations (Fig. [3](#)) was generally in accordance with that observed in other *Ambystoma* populations (Parra-Olea et al. [2012](#); Sunny et al. [2014a](#); Percino-Daniel et al. [2016](#)). The SUBP1 population had low values of inbreeding (mean r_{qg} = 0.098, confidence interval (CI) = 0.022–0.039) and SUBP2 had medium to high values (mean r_{qg} = 0.445, CI = 0.048–0.058). The

BOTTLENECK analysis did not detect any signs of the recent and/or sharp demographic changes which are typical of bottleneck events. However, critical M (M_c) values were significantly higher (SUBP1: M_c = 1.5 and SUBP2: M_c = 1.05) than M values (SUBP1 M = 0.47534 and SUBP2 M = 0.36772) in both subpopulations, indicating historical reductions in effective population size or historical bottlenecks.

Discussion

This is the first population genetics study of *A. rivulare* in the NTV. This species is very sensitive to environmental changes such as pollution of streams. Anthropogenic activities have already caused the loss of approximately 50 % of amphibian diversity worldwide (Marsh and Trenham [2001](#); Storfer et al. [2009](#)).

Genetic structure

Our genetic structure analysis found two subpopulations, one for each river sampled, with no signs of admixture and

Table 2 *A. rivulare* measures of genetic differentiation for the populations and for each locus

Locus	F_{IS}	F_{IT}	F_{ST}	GST_est	G'$_{ST\_est}$	Δ_{ST}	D	D_{est}
At 52.2	−0.586	−0.524	0.039	0.033	0.084	1.032	0.062	0.053
At 52.10	−0.402	−0.064	0.241	0.229	1.000	2.000	1.000	1.000
At 52.143	−0.354	−0.164	0.140	0.134	0.593	1.370	0.540	0.530
At 60.3	−0.314	−0.310	0.003	−0.003	−0.015	1.006	0.011	−0.012
At 52.115	−0.199	−0.191	0.007	0.002	0.009	1.014	0.028	0.007
At 52.6	−0.209	−0.209	0.000	−0.006	−0.017	1.000	0	−0.011
At 52.34	−0.500	−0.181	0.212	0.202	0.919	1.820	0.901	0.899
At 52.20	−0.216	−0.200	0.013	0.005	0.031	1.028	0.054	0.026
At 52.1	−0.431	−0.387	0.031	0.018	0.092	1.051	0.097	0.074
Mean	−0.360	−0.248	0.076	0.068	0.300	1.258	0.299	0.285

F_{IS}, F_{ST} and F_{IT}—fixation indices estimated according to Weir and Cockerham (1984), G_{ST_est}—nearly unbiased estimator of relative differentiation (Nei et al. 1983), G'$_{ST\_est}$—standardized measure of genetic differentiation (Hedrick 2005), Δ_{ST}—between-subpopulation component of diversity or the effective number of distinct subpopulations, D—actual differentiation and D_{est}—estimator of actual differentiation (Jost 2008)
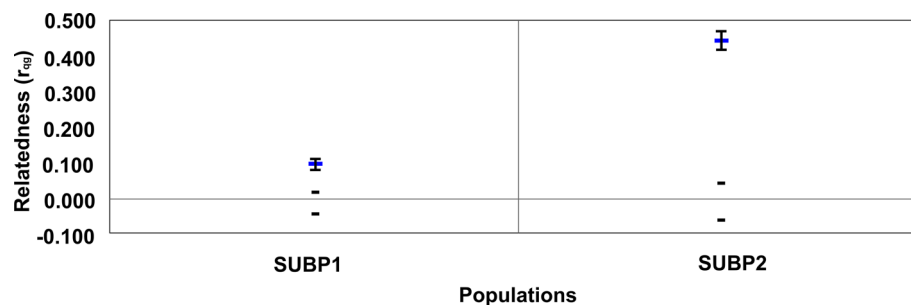

Fig. 3 Mean within-populations pairwise relatedness coefficient r_{qg} across the *Ambystoma* populations studied. The *black bars* are 95 % *upper* and *lower* expected values for a null distribution generated from 999 permutations of data from all populations, and enclose the values expected if breeding were panmictic across the populations; relatedness in the two populations fell outside of the range expected under panmixia. *Blue bars* represent the observed mean relatedness in each population or species, with the *upper* and *lower* bootstrap value for each population

a very high level of genetic differentiation. This is a trend which commonly occurs in mole salamanders (Savage et al. 2010; Parra-Olea et al. 2012; Sunny et al. 2014a, b) residing at high elevations. Mountain habitats are associated with severe topographical, climatic, and ecological conditions (Savage et al. 2010). The sampled rivers stem from different tributaries, are separated by low vegetation cover, and experience low air and water temperatures with different durations and intensities. Cows and sheep graze near these streams and other human activities such as tourism, horseback riding, and mountain biking take place in the area. All of these factors can significantly limit gene flow between populations (Naughton et al. 2000; Wang et al. 2009; Johnson et al. 2010; Savage et al. 2010; Sunny et al. 2014a) leading to high levels of genetic structure (Funk and Dunlap 1999; Tallmon et al. 2000). The biological and life history features that characterize amphibians in general, like the fine genetic structure over very short distances (Savage et al. 2010; Sunny et al. 2014a, b) resulting from female philopatry (Savage et al. 2010; Wang

and Summers 2010; Pauly et al. 2012) and low dispersal ability (Trenham and Shaffer 2005; Gamble et al. 2006, 2007; Searcy and Shaffer 2008; Summitt 2009; Savage et al. 2010; Sunny et al. 2014a; Percino-Daniel et al. 2016), favor the genetic structure observed. In fact, *A. rivulare* is a species which, even after metamorphosis, consistently remains in the same river (Shaffer et al. 2008).

Genetic variability

Two loci (At 52.10 and At 52.115) showed significant deviation from HWE because of a heterozygote deficiency. These deviations are common in threatened species with fragmented and isolated populations (Degne et al. 2007; Spear and Storfer 2010; Parra-Olea et al. 2012; Sunny et al. 2014a, 2015; Percino-Daniel et al. 2016). Analyses showed low levels of inbreeding and no presence of null alleles. It is also unlikely that a Wahlund effect is acting, since the two subpopulations were analyzed separately. So, the observed deviation from HWE could be the result of

genetic drift (Hedrick 2005). Genetic drift is considered to be the main cause of long-term loss of genetic variation and it leads to an increased chance of inbreeding, the foremost genetic factor threatening short-term survival of populations (Frankham et al. 2005; Vega et al. 2007; Sunny et al. 2014b).

The genetic variability found in this study was slightly higher than the figure reported for *A. rivulare* from the SRMB (Parra-Olea et al. 2012). This may be due to the constant anthropogenic pressures occurring in the SRMB (Vidal et al. 2014), where there are high rates of logging, agriculture, livestock production, fires, and ecotourism activities, despite its status as a natural reserve (WWF 2004; Champo-Jiménez et al. 2012). Over the course of four months, 87,337 people, from Mexico and around the world, visited the SRMB (Vidal et al. 2014). In the NTV, local people are leading the movement for implementation of improved conservation strategies for the forest and also for *A. rivulare* populations. This site is used for ecotourism activities, which benefit the local people, so by conserving the forest and the *Ambystomas* they ensure continuation of their income.

Despite the presence of anthropogenic pressures, and contrary to our expectations, the Ho values obtained for these populations were higher than those found for most of the other mole salamander species in Mexico (Parra-Olea et al. 2012; Percino-Daniel et al. 2016) and were similar to values observed in species that have been demographically stable (Goprenko et al. 2007; Dlugosh and Parker 2008; Greenwald et al. 2009; Purrenhage et al. 2009; Wang et al. 2009). However, the average number of alleles in these populations was very low. This is important to note because the genetic diversity of this species could be starting to decline due to habitat fragmentation, anthropogenic activities, and isolation (Noël and Lapointe 2010). Unfortunately, it is also very likely that the amount of logging and other human land use will increase in the near future because the government has recently changed the protection status of the NTV (DOF 2013; Mastretta-Yanes et al. 2014).

Gene flow, effective population size, inbreeding and bottlenecks

We found little evidence of gene flow, just one migrant per generation from SUBP1 to SUBP2. This is a common trend in mole salamander populations, especially in high mountain populations (Savage et al. 2010; Parra-Olea et al. 2012; Sunny et al. 2014a). This trend is likely the result of intrinsic biological characteristics of the species such as its limited dispersive capacity and highly philopatric tendencies (Savage et al. 2010). Some studies concluded that a minimum of one migrant per generation is sufficient to

avoid the effects of consanguinity, but in small and fluctuating populations, 3–10 migrants per generation are necessary to prevent inbreeding (Vucetich and Waite 2000). Given the levels of gene flow observed, these subpopulations could be in process of inbreeding. Low gene flow and the onset of inbreeding could contribute to the low number of alleles found in the subpopulations and the low values of Ne obtained. Other factors such as bottlenecks, genetic isolation, asymmetry in the proportions of males and females, and differences in reproductive success between individuals may also be involved in generating low Ne values (Tennessen and Zamudio 2003; Myers and Zamudio 2004; Semlitsch 2008; Wang 2009).

Mole salamanders typically have low Ne values (Spear et al. 2006; Wang 2009; Savage et al. 2010; Parra-Olea et al. 2012, Sunny et al. 2014a; Percino-Daniel et al. 2016) resulting from high asymmetry in reproductive success among the members of a population (Savage et al. 2010). If only a few individuals successfully breed each year, the variance in mating success may contribute strongly to low overall effective population sizes (Savage et al. 2010). We found low to moderate levels of inbreeding, possibly because we collected most of the individuals between March and August, when largely gilled larva and eggs are found (Sunny et al. 2014b; Monroy-Vilchis et al. 2015; Lemos-Espinal et al. 2016). The r_{qg} values observed in this study fell above the 95 % intervals for the expected values, which indicates that inbreeding or genetic drift are increasing the relatedness. The r_{qg} values found in the two subpopulations fell outside of the range expected under panmixia,, another possible explanation for the low Ne found. The inbreeding values of SUBP2 are very similar that those found in the *A. rivulare* population in the SRMB (Parra-Olea et al. 2012). Given the population history, lineage effects, and the environmental conditions encountered by the populations in this study, the amount of inbreeding is surprisingly low (Keller and Waller 2002; Parra-Olea et al. 2012).

Finally, we did not find evidence for a recent genetic bottleneck, perhaps indicating that the conservation plans currently being implemented by the local people are effective. However, we found signatures of ancestral genetic bottlenecks, possibly associated with a founder effect suffered when theses populations were separated from a larger ancestral population.

Conservation implications

All of the *Ambystoma* species of the TMVB (including *A. rivulare, A. altamirani, A. leorae, A. mexicanum, A. granulosum, A. andersoni, A. velasci* and *A. lermaense*) are threatened by habitat loss, fragmentation, and contamination of rivers and lakes. Amphibians are extremely

sensitive to local habitat changes (Castellano and Valone 2006; Ribeiro et al. 2009), more so than other vertebrate taxa (White et al. 1997; Ribeiro et al. 2009), because of their low dispersal capacities and small home ranges (Huey 1982). Therefore, to maintain populations of *A. rivulare*, it is necessary to implement informed conservation strategies to preserve the highly endangered habitat of this species. The NTV is a high mountain region of the TMVB, the most disturbed region in Mexico, with only 1346.9 km^2 (1.1 %) of *Abies* forest and 6507.7 km^2 (5.4 %) of *Pinus* forest remaining (Sunny et al. 2015). Most of the streams are contaminated and overexploited, a condition which has put most of the *Ambystoma* species in Mexico in a threatened situation. This study shows that the current conservation efforts being enacted by the local people are working, but, in order to improve protection of this species, it is necessary to communicate information to the local people. The genetic structure information from this study can be used as a basis for future research and conservation planning for the *Ambystoma* Genus. Furthermore, *A. rivulare* may be used as a proxy for other amphibian species in the region.

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