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Journal of Genetics
DOI 10.1007/s12041-017-0823-6

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RESEARCH ARTICLE

Genetic variability and structure of an isolated population of *Ambystoma altamirani*, a mole salamander that lives in the mountains of one of the largest urban areas in the world

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Received 17 October 2016; revised 8 December 2016; accepted 6 January 2017

Abstract. Amphibians are globally threatened by habitat loss and fragmentation; species within the order Ambystoma are not the exception, as there are 18 species of mole salamanders in México, of which 16 are endemic and all species are under some national or international status of protection. The mole salamander, *Ambystoma altamirani* is a microendemic species, which is distributed in central México, within the trans-Mexican volcanic belt, and is one of the most threatened species due to habitat destruction and the introduction of exotic species. Nine microsatellite markers were used to determine the genetic structure, genetic variability, effective population size, presence of bottlenecks and inbreeding coefficient of one population of *A. altamirani* to generate information which might help to protect and conserve this threatened species. We found two genetic subpopulations with significant level of genetic structure ($F_{ST} = 0.005$) and high levels of genetic variability ($H_o = 0.883$; $H_e = 0.621$); we also found a small population size ($N_e = 8.8$), the presence of historical ($M = 0.486$) and recent bottlenecks under IAM and TPM models, with a low, but significant coefficient of inbreeding ($F_{IS} = -0.451$). This information will help us to raise conservation strategies of this microendemic mole salamander species.

Keywords. population genetics; conservation genetics; microsatellites; microendemic species; threatened species.

Introduction

Amystoma altamirani is an endemic mountain mole salamander that lives in small, permanent streams which flows in the high mountains in central Mexico in *Pinus* and *Abies religiosa* forests. This species occur in isolated populations of the valley of Mexico, in the trans-Mexican volcanic belt (TMVB), at altitudes from 2700 to 3450 masl (meters above sea level) (Lemos-Espinal 2003; Shaffer *et al.* 2008). The *Abies–Pinus* forest and the streams in the surrounding area of Mexico city, where this species live, are severely changed, leading to a severely degraded habitat fragmented by agriculture and urban settlements (Lemos-Espinal *et al.* 1999; Griffiths *et al.* 2004; Frías-Álvarez *et al.* 2008; Contreras *et al.* 2009; Lemos-Espinal *et al.* 2016; Sunny et al. 2017) and the wooded areas are subjected to illegal logging (FAO 2006; Ellis and Porter-Bolland 2008; Becker *et al.* 2016), stream pollution and sedimentation (Shaffer *et al.* 2008). Also, local people tend to eat molesalamanders (Casas-Andreu *et al.* 2004) and

Electronic supplementary material: The online version of this article (doi:10.1007/s12041-017-0823-6) contains supplementary material, which is available to authorized users.

introduce trout, carp and tilapia farms that predate and compete with mole salamanders, even in protected natural areas (Lemos-Espinal *et al.* 1999, 2016; Griffiths *et al.* 2004; Frías-Álvarez *et al.* 2008; Shaffer *et al.* 2008; Contreras *et al.* 2009; Farías-Álvarez *et al.* 2010). This has eliminated the species from many streams, and many populations showed a severe decline of >50% over the last three generations (Shaffer *et al.* 2008). The environmental vulnerability score of *A. altamirani* is 13, in a range of 3–19; this score positions it between medium and high vulnerabilities, primarily because of its restricted geographic and ecological distributions (Wilson *et al.* 2013; Lemos-Espinal *et al.* 2016). Therefore, this species is protected by Mexican law under the 'Special Protection' category and the IUCN categorized this species as endangered (Shaffer *et al.* 2008; Semarnat 2010). The isolated and fragmented populations by a matrix of agriculture and urbanization, can be considered islands in terms of genetic variability, gene flow and landscape connectivity (Kim *et al.* 1998; Sunny *et al.* 2014a). The fragmentation of the habitat and the introduction of exotic species at the landscape scale may reduce connectivity among amphibian populations leading to fragmentation and isolation, which could reduce gene flow and increase the loss of genetic variability through genetic drift and increase the chance of inbreeding (Lande 1998; Frankham *et al.* 2002, 2005; Beebee and Griffiths 2005; Storfer *et al.* 2009; Hedrick 2011), bottlenecks and diseases (Frankham *et al.* 2005) and the probability of local extinctions (Newman and Tallmon 2001; Johansson *et al.* 2006). Habitat change affects the populations of amphibians and reptiles more than other vertebrate taxa (Jäggi and Baur 1999; Woinarski and Ash 2002; Anadón *et al.* 2006; Castellano and Valone 2006; Ribeiro *et al.* 2009; Sunny *et al.* 2014a, 2015, 2017), owing to their small home ranges, high philopatry and low vagility (Huey 1982; Ribeiro *et al.* 2009). For these reasons, amphibians are a high priority vertebrate group for which conservation strategies must be carried out (Cushman 2006; Storfer *et al.* 2009; Allentof and O'Brien 2010; Nori *et al.* 2015). Therefore, we studied the genetic variability and structure, effective population size, inbreeding and genetic bottlenecks of a population of *A. altamirani* in a highly disturbed habitat. The study area is subjected to pressures, such as illegal logging introduction of exotic species such as trout, human settlements and pollution of the streams. Under this scenario, we expected that there will be high genetic structure patterns induced by landscape modification and because we studied a small population, isolated and subjected to anthropogenic pressures, this population would present low levels of genetic variability and effective population size, and presence of inbreeding and genetic bottlenecks. This information will help us to raise conservation strategies of this microendemic mole salamander species.

Materials and methods

Study site and population sampling

Population samplings were done from January to June 2014 in a small stream (±1 km^2) surrounded by induced and alpine grassland near a *Pinus hartwegii* and *Abies religiosa* forest and some rural houses in Tlazala, state of México (19°31′31″N, 99°26′09.52″ W with an altitude of 3185 masl; figure 1). This site is a private land, where ecotourism activities are carried out, along with trout farming, subsistence logging, and cattle and sheep grazing. We obtained 96 tissue samples, the mole salamanders were found in small ponds (transects) of ∼40 cm in depth and 1–2 m of area separated by ∼10−20 m. There were multiple trapping locations along the rivers, so that the sampling areas were chosen for the differences in the microenvironmental characteristics of the river; we found four types of substrates (mud, gravel, bedrock and sand). To represent all maturity stages of *A. altamirani* 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. The individuals were collected with fishing net and we sampled 2 mm^2 of tail clips. This methodology is a low-impact method that does not affect the survival or growth of the mole salamanders (Arntzen *et al.* 1999; Polich *et al.* 2013); tissue was preserved in 90% ethanol and then frozen at −20°C until processed. All mole salamanders were released immediately at the point of capture. Our study received the approval of the ethics committee of Universidad Autónoma del Estado de México (3047-2011E).

DNA extraction and microsatellite amplification

Genomic DNA was extracted using a commercial kit (Vivantis GF-1 tissue DNA extraction kit); we used nine microsatellite loci (At 52.2, At 52.10, At 52.143, At 60.3, At 52.115, At 52.6, At 52.34, A5 52.20 and At 52.1) specifically developed for Mexican *Ambystomas* and followed published protocols (Parra-Olea *et al.* 2007) for amplification. PCR reactions were performed in a Techne thermocycler, amplified products were multiplexed on an ABI Prism3730xl and sized with a ROX-500 as an internal size and then scored using PEAK SCANNER V1.0 (Applied Biosystems, Foster City, USA) software; allele sizes were measured and rounded with the software TANDEM (Matschiner and Salzburger 2009).

Microsatellite analysis

Potential scoring errors: Typing errors and the presence of null alleles were determined using the Micro-Checker software (Van Oosterhout *et al.* 2004).

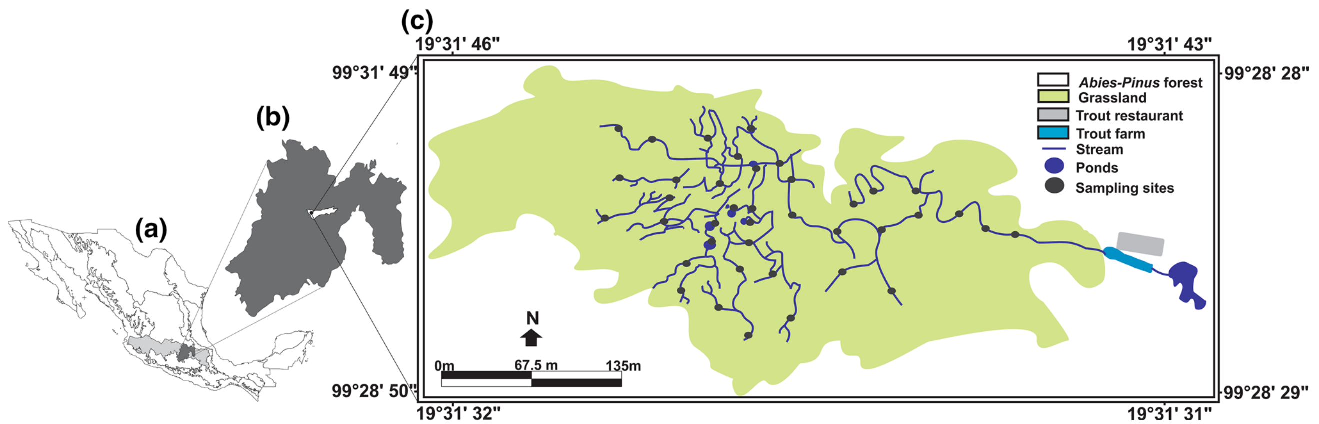

Figure 1. (a) Map of Mexico showing in gray, the TMVB and in dark gray, the State of Mexico. (b) State of Mexico showing in white, Isidro Fabela. (c) The sampling site in Tlazala, Isidro Fabela.

Genetic structure: The Structure 2.3.4 software (Pritchard *et al.* 2000) was used to infer population structure. First, to determine the degree of admixture, we tested $K = 1$ through $K = 8$ in 10 independent runs (10,000 Markov chains following a burning period of 1,000,000), a Dirichlet parameter was used. The evolution of allele frequencies in each genetic group was correlated with the allele frequencies of an ancestral population, and without prior information on population origin, so the most probable number of clusters which best represent our data was determined following the method in Evanno *et al.* 2005: the change 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 was considered, using the maximum value of ΔK, software Structure Harvester 0.6.92 (Earl and von Holdt 2012) was used.

Genetic variability: Linkage disequilibrium (LD) between all pairs of loci across all populations and Hardy–Weinberg equilibrium (HWE) between loci were determined with Genepop 4.2 (Raymond and Rousset 1995), using an exact test (10,000 dememorization steps, 1000 batches and 10,000 iterations), then, significance of data was analysed with a false discovery rate (FDR) test in R 2.8.1 Q-Value (R Development Core Team 2013).

Genealex was used to estimate the observed number of alleles (N_a), effective number of alleles per locus, observed heterozygosity (H_o) and expected heterozygosity (H_e); also, allelic richness (A) was obtained with FSTAT 2.9.3.2 (Goudet 2001). Additionally, different estimators of genetic population differentiation like: G_{ST_est}, G'_{ST_est} (Hedrick 2005; Jost 2008), Δ_{ST}, D and D_{est} (Jost 2008) were calculated in the SMOGD 1.2.5 (Crawford 2010) software considering 1000 replicates in the bootstrapped parameters. Finally, Nei's (1972) genetic distance between sampling localities and an analysis of molecular variance (AMOVA) was calculated. Also, Genealex 6.5 (Peakall and Smouse 2006) was used to perform AMOVA to analyse the distribution of the genetic variance between and within populations based on F_{ST} values, using 999 permutations, were estimated with Genalex 6.5 (Peakall and Smouse 2006).

Gene flow, effective population size, bottlenecks and relatedness: The gene flow was obtained with Genepop 4.2 (Raymond and Rousset 1995) using an exact test (10,000 dememorization steps, 1000 batches and 10,000 iterations) using private alleles method (Barton and Slatkin 1986). Examining LD with software Neestimator 2.01 software (Do *et al.* 2014), the effective population size (N_e) was determined. With the software Bottleneck 1.2.02 (Cournet and Luikart 1996; Piry *et al.* 1999), we tested for population bottleneck events, estimating the observed heterozygosity 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 default values (70% SMM, 30% IAM and 10% variance). Both settings were calculated with 10,000 replicates and an excess of heterozygosity was tested with a Wilcoxon test. Finally, historical bottlenecks were also tested; when M values are lower than the critical number, it indicates an historical population declines (Cournet and Luikart 1996; Garza and Williamson 2001). Therefore, we obtained the Garza-Williamson (M) index with Arlequin 3.1.1.2 software (Excoffier *et al.* 2005) and values of critical M (M_c) were obtained with Critical_M software (Garza and Williamson 2001), using 10,000 simulations and parameters from the two-phase mutation model, as described in Garza and Williamson (2001).

We obtained F_{IS} value in Genealex as an indicator of total inbreeding in the population and with the same software relatedness estimator of Queller and Goodnight (1989) (r_{qg}) was obtained to analyse relatedness among populations; significant differences between mean population relatedness were tested using 9999 permutations, which calculates the upper and lower 95% intervals for

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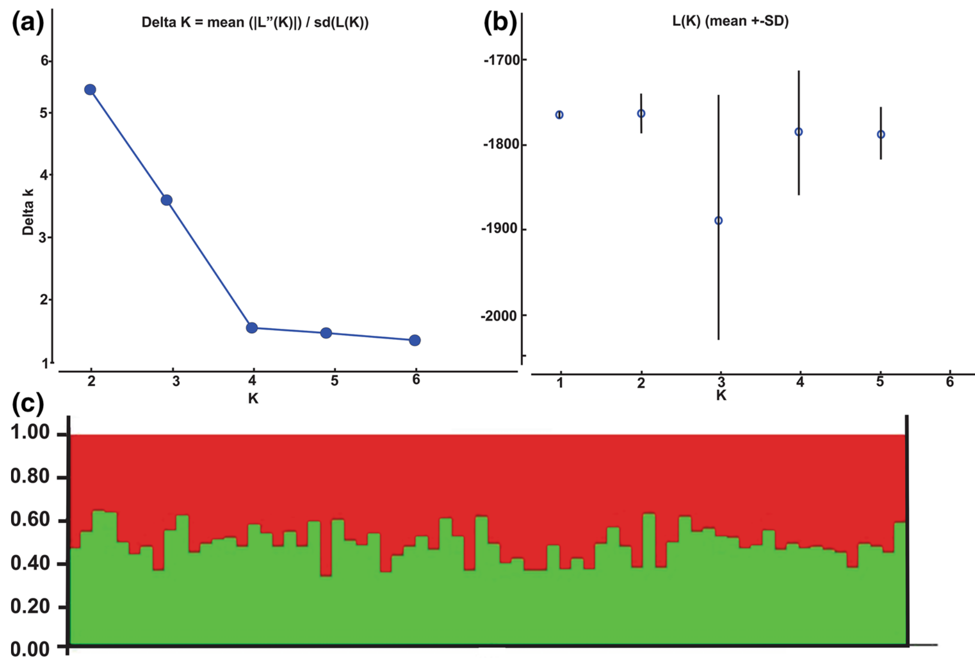

Figure 2. *A. altamirani* genetic structure: (a) population genetic structure partitioned into K components representing the ancestry fractions in lnPr ($K = 2$) $= -1407.2$ populations. (b and c) ΔK value of Evanno *et al.* (2005) plots for detecting the number of K groups that best fit the data.

the expected range of r_{qg} based on the populations. These intervals correspond to the range of r_{qg} that would be expected if reproduction was random across the populations. Also, we estimated confidence intervals to 95% by bootstrap resampling (9999) within population estimates of mean relatedness. Population r_{qg} values that fall above the 95% expected values from permutations indicate that processes such as inbreeding or drift are increasing the relatedness.

Results

Potential scoring errors

Based on a previous analysis, we decided to eliminate 26 tissues of larvae from the further analysis to avoid unbiased results (genotyping siblings). Finally, null alleles and other typing errors were not detected at any loci.

Genetic structure

The best log likelihood given by Structure was observed when $K = 2$ (lnPr ($K = 2$) $= -1407.2$; figure 2) and ΔK chose the best model considering two subpopulations (SUBP1: $N = 29$, SUBP2: $N = 41$). Therefore, we performed all analyses considering these two subpopulations. The genetic structure analysis (table 1) showed low genetic structure between subpopulations.

Genetic variability

FDR correction did not find any departures from HWE at any loci (table 1 in electronic supplementary material at), neither LD was detected at any loci. Across the nine loci in the 70 remaining samples, 34 alleles were identified, with a range of 2–8 (average 3.367) alleles per locus (table 2; figure 1 in electronic supplementary material). In the population, we found 68 genotypes (table 2 in electronic supplementary material); with a range of 2–5 (average 3.6) genotypes per locus for the SUBP1 population and with a range of 3–5 (average of 6.8) for the SUBP2 population. The SUBP1 population had 18 heterozygous genotypes and 15 homozygous genotypes with a total of 33 genotypes, and the SUBP2 had 19 heterozygous genotypes and 16 homozygous genotypes with a total of 35 genotypes (table 2 in electronic supplementary material). The SUBP1 and SUBP2 populations showed lower values expected than observed heterozygosity (table 2). The F_{ST} AMOVA results revealed that all the genetic variability is attributed to variation within individuals (100%; $P = 0.001$) with an F_{ST} value of 0.005 (table 3 in electronic supplementary material).

Gene flow, effective population size, bottlenecks and relatedness

With regard to genetic flow, the number of migrants (N_m) using private alleles method was 1.74. The N_e with the LD model and with allelic frequency of 0.05, was 8.8 for the

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Table 1. *A. altamirani* measures of genetic differentiation for the populations and for each locus.

Loci	F_{ST}	F_{IT}	F_{IS}	G_{ST_est}	G'_{ST_est}	Δ_{ST}	D	D_{est}
At52.2	0.004	−0.196	−0.202	−0.003	−0.011	1.006	0.012	−0.008
At52.10	0.000	−0.258	−0.258	−0.007	−0.054	1.000	0.000	−0.046
Atig52.143	0.001	−0.378	−0.379	−0.006	−0.033	1.002	0.004	−0.027
At60.3	0.007	−0.743	−0.756	0.000	0.000	1.007	0.015	0.000
Atig52.115	0.001	−0.642	−0.645	−0.006	−0.018	1.001	0.003	−0.012
At52.6	0.015	−0.372	−0.394	0.008	0.039	1.030	0.058	0.031
At52.34	0.000	−0.637	−0.637	−0.007	−0.028	1.000	0.000	−0.020
At52.20	0.001	−0.638	−0.640	−0.007	−0.025	1.001	0.002	−0.017
At52.1	0.013	−0.134	−0.149	0.006	0.065	1.065	0.123	0.059
Mean	0.005	−0.444	−0.451	−0.002	−0.007	1.012	0.024	−0.004

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 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; D_{est}, estimator of actual differentiation (Jost 2008).

Table 2. *A. altamirani* genetic diversity values in SUBP1 and SUBP2 populations.

	H_o	H_e	N	N_a	N_e	N_p	F_{IS}
SUBP1	0.870	0.621	29	3.556	2.864	0	−0.423
SUBP2	0.897	0.621	41	3.778	3.052	2	−0.479
Mean	0.883	0.621	35	3.667	2.958	0.500	−0.444

N, sample size; N_a, number of alleles; N_e, number of effective alleles; N_p, number of private alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, fixation index.

whole population and for subpopulations: SUBP1, N_e = 5.6 and SUBP2, $N_e = 7.2$.

The Bottleneck analysis detected genetic signs of recent demographic changes typical of bottleneck events, associated with a heterozygote excess at the two subpopulations under the IAM, SMM and TPM models (SUBP1: P = 0.00001, 0.0080, 0.00205; SUBP2: P = 0.00001, 0.00067, 0.00192; table 4 in electronic supplementary material). Critical M_c values were significantly higher (SUBP1: M_c = 0.9, SUBP2: $M_c = 0.8$) than M values (SUBP1: $M = 0.486$, SUBP2: $M = 0.474$) in the two subpopulations, indicating historical reductions in effective population size or historical bottlenecks. The F_{IT} statistic as an indicator of inbreeding for the whole population showed negative and high inbreeding values ($F_{IT} = -0.444$; table 1). We found that mean pairwise relatedness (r) within populations (figure 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). SUBP1 had low values of inbreeding (mean $r_{qg} = -0.036$, confidence interval (CI)=0.052–(−0.051)), while SUBP2 had highest value of inbreeding (mean r_{qg} =0.018, CI = 0.034–(−0.037)).

Discussion

The limited distribution and anthropogenic activities such as river pollution, deforestation and introduction of exotic species like trout, increase the possibility of *A. altamirani*

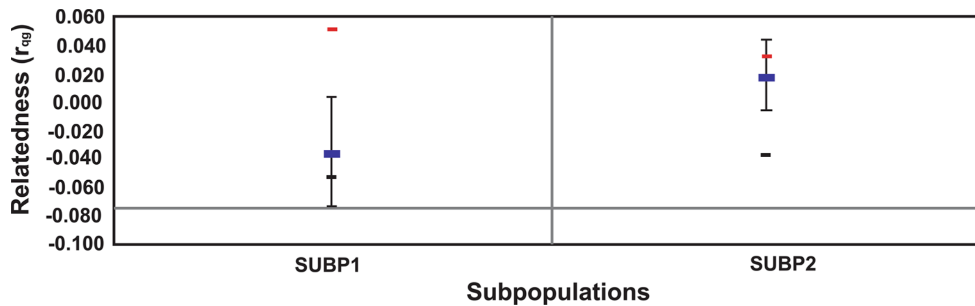

Figure 3. Mean within-populations pairwise relatedness coefficient, r_{qg} across the *A. altamirani* populations studied. The red bars are 95% upper expected values and the black bars are 95% lower expected values for a null distribution generated from 999 permutations of data from all populations, and enclose the values expected if breeding were panmictic across the populations. Blue bars represent the observed mean relatedness in each subpopulation, with the upper and lower bootstrap values for each subpopulation.

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populations to became endanger (Funk and Dunlap 1999; Shaffer *et al.* 2008). In the present study, we found high levels of genetic variability and different genetic groups as Structure suggest that the genetic structure captured appears to be an isolation by distance effect, while a large amount of admixture is occurring along this stream, but there is also signs of low effective population size and genetic bottlenecks. These results are important in terms of the conservation genetics perspective, because the high levels of genetic variability found, can be declining due to the bottlenecks and the low levels of effective population size.

Genetic structure

The structure analysis found two subpopulations (SUBP1 and SUBP2; figure 2, table 2) with very low genetic differentiation among them ($F_{ST} = 0.005$). The low genetic structure found could be due to an isolation by distance effect (IBD), with a large amount of admixture, these subpopulations are making a metapopulation system rather than isolated populations, the metapopulation pattern is common in amphibians (Rowe *et al.* 2000; Newman and Squire 2001; Palo *et al.* 2003; Funk *et al.* 2005; Jehle *et al.* 2005; Spear *et al.* 2005; Johansson *et al.* 2006; Giordano *et al.* 2007; Noël *et al.* 2007; Zamudio and Wieczorek 2007; Purrenhage *et al.* 2009; Consentino *et al.* 2012; Sunny *et al.* 2014a, b; Monroy-Vilchis *et al.* 2015). On the other hand, probably, the subpopulations could be starting an isolation process and this is reflected in our Structure test, there are physical factors that could isolate small groups and make them genetically different, changes in the stream like the closeness with the trout area, changes in water speed, oxygenation and depth; even substrate types are pointed out as factors that have repercussions in genetic structure of *Ambystomas* (Sunny *et al.* 2014b; Lemos-Espinal *et al.* 2016). *A. altamirani* prefers stream sites with certain peculiarities like places with a greater volume of water, higher dissolved oxygen levels, faster moving water, sites with emergent grass and forbs, and sites with black-coloured substrates like mud and sand (Sunny *et al.* 2014b; Lemos-Espinal *et al.* 2016); it avoids sites with no vegetation and those with gravel or bedrock bottoms in which it is easier to be observed, making it more exposed to predation. In the study site, mud and sand substrates are usually separated for every 10–20 m and this in fact congregate the individuals in ponds (separated 10–20 m), this may be causing isolation of small groups, which in our study are represented by SUBP1 and SUBP2, structuring the population and favouring the metapopulation pattern.

Genetic variability

The observed heterozygosity values were high and most of thegenotypes were heterozygous (table 2; table 2 in electronic supplementary material); high levels of genetic variability are not unusual in *Ambystoma* species (Goprenko *et al.* 2007; Greenwald *et al.* 2009; Sunny *et al.* 2014a; Heredia-Bobadilla *et al.* 2016; Percino-Daniel *et al.* 2016), despite with a limited distribution. The genetic variability found in this study was higher than that reported for *A. altamirani* from the Lagunas de Zempoala National Park (LZNP) (Parra-Olea *et al.* 2012), this may be due to the high anthropogenic pressures occurring in the LZNP, of the seven lagoons that inhabited *A. altamirani*, three are already completely dried and in the four other lagoons, the aquatic vegetation is disappearing and pollution is increasing and the deforestation and ecotourism activities are devastating the ecosystem (Islebe *et al.* 2003; Lemos-Espinal 2003; Parra-Olea *et al.* 2012). In the studied area, human activities are carried out, such as subsistence logging, cows and sheep grazing, ecotourism activities and recreational trout fishing, but because this area is not a touristic site, all these activities are on a small scale, which may be helped to preserve the genetic variability set-up in the habitat. Besides this, some *Ambystoma* characteristics could favour the high genetic variability set-up, like the high rates of breeding, multiple paternity and overlapping generations; also, it is reported that one *A. altamirani* female clutch can contain up to 10 or 21 eggs and can be larger, three years after the first clutch (Rodríguez-Reyes 2009), also the migration of one female can contribute to an increase in the genetic variability of the population (Tennessen and Zamudio 2003; Kinkead *et al.* 2006; Rodríguez-Reyes 2009; Iwao 2012; Lemos-Espinal *et al.* 2016). Unfortunately, we also found very low average numbers of alleles compared with other northern *Ambystoma* species like *A. tigrinum*, *A. maculatum* and *A. macrodactylym* (range $N_a = 3.5$–12.0; Giordano *et al.* 2007; Zamudio and Wieczorek 2007; Purrenhage *et al.* 2009) and lower than some Mexican *Ambystoma* species (*A. leorae*, *A. rivulare*, *A. velasci*; range $N_a = 4.0$–6.0 per locus; Parra-Olea *et al.* 2012; Sunny *et al.* 2014a), but similar values to the *A. altamirani* population of LZNP ($N_a = 3.5$; Parra-Olea *et al.* 2012). However, these results should be interpreted with caution because the number of alleles is a relative value as it depends on the number and type of loci studied, number of individuals and the population characteristics (Vázquez-Domínguez *et al.* 2013; Sunny *et al.* 2015). However, it is possible that the low number of alleles found in the clusters is a sign that genetic variability and allelic richness are declining as a result of habitat fragmentation and anthropogenic activities, which are leading to population declines and isolation which in turn could cause genetic drift (Rueda-Zozaya *et al.* 2016). The observed high heterozygosity values could be signs of retention of ancestral polymorphisms or standing genetic variation, which is pre-existing in the population (Kinkead *et al.* 2006; Weisrock *et al.* 2006; Recuero *et al.* 2010; Hedrick 2011).

Gene flow, effective population size, bottlenecks and relatednesss

The study population is isolated from other populations of *A. altamirani*, because the stream has no connection to other tributaries or rivers, consequently, gene flow only occurs between the pools that are within this river. We found low, but significant levels of gene flow ($N_m = 1.74$ means that in five generations, there are ≈8–9 migrants), this also explains the low genetic differentiation ($F_{ST} = 0.005$). There are some characteristics of the biology of the mole salamander that favours the low gene flow and also the genetic structure, like the philopatric tendencies and low vagility (Funk *et al.* 2005; Savage and Zamudio 2005; Spear *et al.* 2005; Gamble *et al.* 2007; Vences and Wake 2007; Calhoun and deMaynadier 2008; Semlitsch 2008; Wang *et al.* 2009; Wang and Summers 2010; Parra-Olea *et al.* 2012; Sunny *et al.* 2014a; Sunny *et al.* 2014b; Heredia-Bobadilla *et al.* 2016; Percino-Daniel *et al.* 2016). During the six months of sampling, we found the same individuals in the same pond, which reflects few migratory movements; although, this species can undergo metamorphosis, *A. altamirani* prefers to stay in the water (Lemos-Espinal *et al.* 1999; Shaffer *et al.* 2008), thus, it is unlikely that *A. altamirani* can be dispersed by land in this locality. Outside the stream, there are grasslands where human activities such as subsistence logging, cows and sheep grazing, ecotourism activities and recreational trout fishing are held, and occasionally, there are feral dogs and cats. All these features have made unfavourable microclimatic conditions necessary for *A. altamirani* to migrate. To make the mole salamanders to migrate, it is necessary to have forest coverage with enough vegetation and moisture; when these characteristics are not present, mole salamanders can be predated or die by desiccation (Naughton *et al.* 2000; Rhotermel and Luhring 2005; Spear *et al.* 2005; Cushman 2006; Noël and Lapointe 2010; Becker *et al.* 2016). Likewise, migration patterns occur mainly between neighbouring populations, which may suggest a stepping stone pattern and a metapopulation behaviour; the few migrants pattern also is a common trend in mole salamander populations and mainly in high mountain populations (Marsh and Trenham 2001; Kinkead *et al.* 2006; Savage *et al.* 2010; Parra-Olea *et al.* 2012; Sunny *et al.* 2014a).

The population size that is relevant for evolutionary matter is the number of breeding individuals; the effective population size of *A. altamirani* was very low, the low N_e values can be caused by genetic isolation, asymmetry in the proportion of males and females and differences in the reproductive success between individuals (Tennessen and Zamudio 2003; Wang *et al.* 2009; Hedrick 2011), generally, the mole salamanders have low N_e values (Wang *et al.* 2009; Savage *et al.* 2010; Parra-Olea *et al.* 2012; Sunny *et al.* 2014a; Percino-Daniel *et al.* 2016) due to high asymmetry in reproductive success among members of a population (Savage *et al.* 2010). If only a few individuals successfully breed one year, the variance in mating success may contribute strongly to low overall effective population sizes (Brandon and Altig 1973; Savage *et al.* 2010). The low N_e could also be explained by a bottleneck effect; and the bottlenecks found in our study can be explained because trout was recently introduced in the sampled stream for recreational fishing and now the mole salamanders compete with trout for food (Werner and Anholt 1996; Tyler *et al.* 1998), and are being predated upon in the early stages of development (Matthews *et al.* 2001; Pilliod and Peterson 2001; Welsh *et al.* 2006; Zambrano *et al.* 2010; Martín-Torrijos *et al.* 2016) and transfer of pathogens and some emerging infectious diseases (Blaustein *et al.* 2005; Johnson and Speare 2005; Fernández-Benéitez *et al.* 2008; Van den Berg *et al.* 2013; Sandoval-Sierra *et al.* 2014) causing embryonic mortality and several amphibian declines (Fernández-Benéitez *et al.* 2008), and also the *A. altamirani* individuals find it very difficult to recognize trout as a predator, because they do not have an evolutionary predator–prey history (Petranka *et al.* 1987; Funk and Dunlap 1999; Pilliod and Peterson 2001; Pearson 2004; Gall and Mathis 2010; Zambrano *et al.* 2010). All these features can lead the population to a process of genetic drift which in turn reduces genetic variability, making the population to lose fitness and the possibility to adapt to changes in the environment (Frankham *et al.* 2005). Some studies concluded that a minimum of one migrant per generation is sufficient to avoid consanguinity effects, but in small and fluctuating populations, 3–10 migrants per generation are necessary to maintain a particular level of inbreeding (Vucetich and Waite 2000). We found low to medium levels of inbreeding in our analyses, the r_{qg} values in the two subpopulations were above the 95% expected values from permutations, indicating that inbreeding or drift could be increasing relatedness, the r_{qg} values were lower than those found in the LZPN *A. altamirani* population, the r_{qg} values were higher ($r_{qg} = 0.620$; Parra-Olea *et al.* 2012), suggesting that in disturbed habitats the inbreeding increases.

Conservation implications

A. altamirani population studied are threatened by habitat fragmentation, deforestation, pollution of streams and introduction of trout. These are important to consider since amphibians are particularly sensitive to local habitat alterations (Castellano and Valone 2006; Ribeiro *et al.* 2009), because of their ecological low dispersal capacity and small home ranges (Huey 1982). The population studied has high heterozygosity values, alleles and genotypes, which are necessary to preserve, so that the species could have enough genetic variability and can adapt to changes in the environment. It is necessary to conserve unique genes, which could be essential for genetic rescue programmes of this mole salamander (Petit *et al.* 1996; Frankham *et al.* 2002). The population also has signs of inbreeding signs and reduced effective population size

caused probably by bottlenecks, this is important to consider because this phenomena could increase the genetic drift which in turn will cause a decline in the heterozygosity levels found and a decrease in the genetic variability found. Also, it is important to conserve the endangered *Abies–Pinus* habitat and the streams in this habitat to maintain the *A. altamirani* populations and other species of amphibians and reptiles that live in this type of habitat. Therefore, we propose to increase the natural protected areas because this strategy has been an effective way to protect biodiversity (Ledig 1988). In the study area, conifer woods are declining because of illegal and subsistence logging, as a measure to avoid this problem, the authorities are making reforestations, but this measure is not adequate since they are reforesting the *Abies* forests with other species such as: *Pinus pseudostrobus*, *Pinus patula* and *Pinus hartwegii*, this forest management plan changes the microclimate, environmental and habitat conditions necessary to this species and others to persist. Also, we propose to implement environmental education, not only to local communities, but also to the authorities to avoid bad forest management practices. As well, it is necessary to implement solar technologies in the communities, such as solar water heaters and solar stoves since the majority of the logging of the communities is to be able to heat the water and to cook. In addition, more studies are needed to create a complete genetic conservation programme of this *Ambystoma* species, but the genetic information provided in this study can be used as a first attempt and the basis for future research and conservation planning in *A. altamirani*.

Acknowledgements

We are deeply grateful to the students of CICBA for helping us in data collection and laboratory assistance. We thank the editor and two anonymous reviewers for their comments. RLHB is grateful to the graduate programme 'Doctorado en Ciencias Agropecuarias y Recursos Naturales' of the Autonomous University of the State of Mexico and for scholarships received from CONACYT and COMECYT (359990 and 16BTID0028). This work was funded by Autonomous University of the State of Mexico (3047-2011E).

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Corresponding editor: INDRAJIT NANDA