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Genetic diversity of two endemic axolotl species from Michoacán, Mexico

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Abstract

North America is the center of diversity for amphibian *Ambystoma* species, and half of the species from this genus are endemic to Mexico. Mexican species of *Ambystoma* are called axolotls and most of them are categorized as endangered or in risk of extinction. In particular, obligate neotenic species, which never transition through metamorphosis, are highly threatened because they are restricted to a single lake, and their habitats have been severely impacted by anthropogenic activities. This condition may impact their genetic diversity compromising their survival and making them key species for implementing urgent conservation strategies. In the present study, we used a double digest restriction-site associated DNA (ddRADseq) approach to compare the levels of genetic diversity and genetic differentiation between two critically endangered species, *Ambystoma andersoni* and *A. dumerilii*, which are endemic to Zacapu Lagoon and Patzcuaro Lake, respectively. We performed a reference-based SNP calling protocol in which the available genome of *A. mexicanum* was used to map reads, and retained 11,714 SNPs after filtering. We found that each species constituted a well-defined genetic cluster without evidence of gene flow between species. Both species showed similar levels of genetic diversity (H_E = 0.289 and H_E = 0.292, respectively), with heterozygosity excess (F_{IS} = -0.290 and F_{IS} = -0.331, respectively). Our results suggest that both species have undergone historical population declines, possibly associated to climatic changes during the late Pleistocene and early Holocene. Habitat protection and restoration are needed to promote persistence of these species in the short to middle term.

Introduction

Species with restricted distributions tend to have higher extinction risk than species with wide distributions, because they are usually found in small and isolated populations (Frankham, 1996; Castellanos-Morales et al., 2015). This is because of intrinsic biological factors, such as small population size and life history traits (i.e. low reproductive potential). Moreover, they are expected to have low levels of genetic variation (Frankham, 1996, 2005; Castellanos-Morales et al., 2015). In this context, species with restricted distributions could also be more affected by local threats, such as habitat loss and habitat fragmentation, which further increase their risk of extinction (Ceballos and Ehrlich, 2002; Cardillo et al., 2008).

In species conservation, population genomics can be used to estimate genetic diversity and structure, connectivity between populations, levels of inbreeding, and effective population size. These parameters are particularly critical in species conservation because small and geographically isolated populations are especially vulnerable to genetic drift and inbreeding, processes that can reduce fitness and erode adaptive genetic variation (Frankham, 1996; Keller and Waller, 2002; Spielman et al., 2004). As such genetic and demographic pressures accumulate, they may trigger a self-reinforcing decline in population viability, ultimately leading to an extinction vortex of which recovery becomes increasingly unlikely (Bouzat 2010; Olazcuaga et al., 2023). Additionally, genomic data may allow to identify genes important for local adaptation (Allendorf et al., 2010; Diez-del-Molino et al., 2018; Kardos et al., 2021). This

information is valuable for understanding species vulnerability and their potential to respond to habitat degradation, emerging diseases, and other environmental changes (Lacy, 1997; Spielman et al., 2004; Allentoft and O'Brien, 2010).

Amphibians are the most threatened vertebrate group worldwide, with more than 40% of species listed in a risk category according to the red list of the International Union for Conservation of Nature (IUCN) (Stuart et al., 2004; Luedtke et al., 2023). Population reductions and drastic decreases in their distribution areas are caused by climate change, natural habitat modifications due to anthropogenic activities, and to the introduction of invasive species and emerging diseases caused by the fungal pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) (Stuart et al., 2004; Parra-Olea et al., 2014; Mendoza-Almeralla et al., 2015; Fisher and Garner, 2020; Basanta et al., 2022; Luedtke et al., 2023).

Within amphibians, the axolotls (genus *Ambystoma*) found in Mexico are of particular conservation concern because they represent 51.5% of extant species of this genus worldwide (17 out of 33 species); besides, 16 out of 17 species that distribute in Mexico are endemic (SEMARNAT 2018). The populations of species belonging to the genus *Ambystoma* have decreased significantly, mainly due to habitat changes, the contamination of rivers and lakes, the introduction of invasive species, and the capture and commercialization of species (SEMARNAT, 2018; Ávila Akerberg et al., 2021). Few species of *Ambystoma* in Mexico have been studied, and the species with the largest number of published studies is the Mexican axolotl, *Ambystoma mexicanum* (Shaw and Nodder, 1798). In fact, the only available genome of the genus is that of *A. mexicanum*, which is characterized by being an extremely large ~ 32 Gbp diploid genome (Nowoshilow et al., 2018; Smith et al., 2019).

The genetic diversity of *Ambystoma* species has rarely been explored, and most studies have been based on nuclear microsatellite loci. For example, *A. leorae*, which has a restricted distribution and is critically endangered, showed high genetic diversity, but with signals of population reductions and a genetic bottleneck, as well as low levels of gene flow which suggested inbreeding (Sunny et al., 2014). In comparison, *A. altamirani*, a microendemic species, has a small effective population size but low inbreeding, and evidence of historical bottlenecks (Monroy-Vilchis et al., 2019). Information on genetic diversity in neotenic species restricted to a single lake (i.e., *A. dumerilii*, *A. andersoni*, and *A. taylori*) remains scarce, even though their restricted distributions and life-history traits may make them particularly vulnerable to anthropogenic impacts and genetic drift. In one of the few comparative studies available, Tracy et al. (2015) analyzed *A. andersoni*, *A. dumerilii*, *A. mexicanum*, *A. velasci*, and *A. altamirani*, finding that *A. altamirani* exhibited low nucleotide diversity and signals of positive selection in genes of the Major Histocompatibility Complex (MHC), relative to other species. Moreover, analyses of the karyopherin alpha 6 (*G1D6*) gene, which is considered a neutral region, and for the mitochondrial DNA cytochrome b (*cytb*) region, revealed consistently low nucleotide diversity across all five species (Tracy et al., 2015).

In this study, we analyzed two sister species of axolotls endemic to the central western part of the state of Michoacán, Mexico: *A. dumerilii* and *A. andersoni*. *Ambystoma dumerilii* Dugés 1870 is restricted to Lake Patzcuaro (7,460 hectares) (SEMARNAT 2018), whereas *A. andersoni* Krebs and Brandon 1984 is endemic to the Zacapu Lagoon (216.62 hectares) (Fig. 1). Both species are used as food and medicine, and both species are critically endangered according to the IUCN red list (IUCN SSC Amphibian Specialist Group, 2014, 2020) and are protected by Mexican legislation (NOM-059-SEMARNAT-2010). Information regarding these two species is scarce, although studies have been carried out describing their population dynamics, habitat complexity and conservation (Valencia-Vargas and Escalera-Vázquez, 2021; Mendoza, 2012; Huacúz Elias, 2001; Everson et al., 2021). Everson et al. (2021) reported signals of gene flow between these paedomorphic species, but their analyses could not differentiate between recent and historical gene flow. Here, we used a conservation genomics approach to evaluate the levels of genetic diversity present in *A. dumerilii* and *A. andersoni*, estimate the degree of inbreeding and recent gene flow between species. We also estimated effective population size for each species and implemented a coalescent approach to better-understand the demographic trends of these endemic species from Michoacán, in order to propose conservation guidelines for these critically endangered species. We expect to find low levels of genetic variation, low effective population size and high genetic differentiation given species' restricted distributions and isolation, as well signals of population decline.

Methods

Sampling and data collection.

Ambystoma samples were collected between April and July of 2021 in two localities: *Ambystoma andersoni* from Zacapu Lagoon and *A. dumerilii* from Patzcuaro Lake. Individuals of *A. dumerilii* from Pátzcuaro Lake, located in the western portion of the Transmexican Volcanic Belt (Fig. 1), were captured using 180 metallic cylinder traps with a conical inlet, set at the bottom of the lake. To identify these individuals, we used a microchip marking system, to avoid resampling. In the case of *A. andersoni*, individuals were captured with fish nets of 1.5 m wide per 50 m long at 2–5 m from the lake shore. The fish nets were collected the day after. All individuals sampled were classified as adults with a minimum snout-vent length (SVL) of 60 mm (Anderson and Worthington, 1971). Sampling was approved by SEMARNAT under sampling permit N ° SGPA/DGVS/02770/21.

Tissue samples were taken from the gills or tail of each individual, and a total of 45 individuals (30 from *A. andersoni* and 15 from *A. dumerilii*) were sampled. The tissue samples were placed in tubes with RNA-later (QIAGEN). The individuals were released at their capture sites after sampling. Tissue samples were transported to the Center for Genomic Sciences (CCG), National Autonomous University of Mexico (UNAM), where they were kept at − 80°C until processing. DNA was extracted with a DNAeasy Blood and Tissue Kit (QIAGEN), and DNA quality and concentration for library preparation were evaluated with nanodrop (Thermo Fisher Scientific) and Qubit Fluorometer using a dsDNA BR assay kit (Thermo Fisher Scientific). For genomic libraries construction and sequencing process, the DNA was sent to the Genomics Center at the University of Minnesota (https://genomics.umn.edu). We used a doble digest

restriction site associated DNA (ddRADseq) (Peterson et al., 2012) approach to obtain genomic libraries using restriction enzymes, *PstI* and *MspI*, with a selection of fragments of 200 to 300 bp. Paired-end sequences of 150 bp (~ 10 million sequences per sample) were obtained with the Illumina NovaSeq 6000 platform (Illumina, San Diego, California, United States). On average, 11,031,489 ± 1,202,922 reads per sample were obtained for *A. andersoni*, and 9,577,071 ± 651,678 reads per sample were obtained for *A. dumerilii.* Raw reads were deposited in NCBI SRA in the Bioproject PRJNA1163358.

The quality of the reads was evaluated with the FastQC v.0.11.9 program (Andrews, 2010). Illumina adapters (--nextera), trimmed low-quality data (--quality 30), and the first 3 bases of the 5' end of read 1 (--clip_R1) and read 2 (--clip_R2) using TrimGalore (v0.6.6; http//github.com/FelixKrueger/TrimGalore). The reads that passed the quality filters were mapped to the reference genome of *A. mexicanum* (AmbMex60DD; Nowoshilow et al., 2018) with the BWA-MEM command of BWA v 0.7.17-r1188 (Li and Durbin, 2009). The available genome of *A. mexicanum* has good quality and this species is closely related to our study subjects; therefore, we used default parameters to map reads to the reference genome (Rochette and Catchen, 2017). We sorted the BAM files with SortSam and MarkDuplicates from Picard 2.27.1 (Broad Institute, 2021). We performed a reference-based SNP calling with freebayes (Garrison and Marth, 2012), using parameters –use-best-n-alleles 4 –min-coverage 12. To retain the same loci for the two species, SNP calling was conducted with a single database that included the data for both species.

We performed quality filters for the SNP database with VCFtools v. 0.1.16 (Danecek et al., 2011) to eliminate possible biases and errors related to the SNP calling process (O'Leary et al., 2018). To eliminate SNPs of very low frequency that could be false-positives or sequencing errors we implemented a filter based on the minimum allele count (--mac 10), minimum allele frequency (--maf = 0.1), and maximum and minimum number of alleles (--max-alleles 2 –min-alleles 2). We implemented an additional filter on minimum depth (--min-meanDP 14), as low coverage can increase genotyping error rates (Fountain et al., 2016) (Supplementary Table S1). We tested several filters for missing data setting max-missing in VCFtools from 0.5 to 1. Accordingly, we used a max-missing value of 1, thus SNPs with no missing data were retained. We also considered deviations from Hardy–Weinberg equilibrium (HWE = 0.00001) to eliminate possible sequencing errors and only biallelic SNPs were retained. Finally, we used a linkage disequilibrium filter ($r < 0.7$) to make sure that each SNP is segregating independently. We estimated a kinship coefficient (Manichaikul et al., 2010) with VCFtools to assess sibship between individuals and to remove potential siblings prior to population genetic analyses to avoid bias. Individuals showed values ≤ 0.3 (Supplementary Tables S2 and S3), therefore, we assume that sampled individuals are not closely related and we retained all individuals for further analyses.

Genetic variation, genetic differentiation and gene flow

We estimated genetic diversity (observed and expected heterozygosity) for each species based on the filtered SNP dataset with GenoDive 3.04 (Meirmans, 2020). To reduce sample size bias this software incorporates the correction factor ((2N/(2N-1))) proposed by Nei (Nei, 1987). We also estimated

standardized multilocus heterozygosity (sMHL) with library inbreedR (Stoffel et al., 2016) from R (R Core Team, 2011). We compared sMHL between species with a Wilcoxon signed ranked test and we estimated mean and standard deviation with R. Inbreeding coefficient (F_{IS}) for each species and its statistical significance through a Hardy-Weinberg equilibrium test, as well as pairwise F_{ST} and Nei's genetic distance were also estimated with GenoDive.

To determine the genetic differentiation patterns between species, we performed a principal component analysis (PCA) using the gl.pca function from adegenet library (Jombart, 2008; Jombart and Ahmed, 2011) in R (R Core Team, 2011). We performed a discriminant analysis of principal components (DAPC; Jombart et al., 2010), where we retained 20 principal components and one discriminant function. We also estimated the Euclidean distance between individuals and obtained a heatmap with library dartR 1.9.9.1 (Gruber et al., 2018). In addition, we run the admixture program (Alexander and Lange, 2011) with the admixed model to determine the number of genetic groups present in the sample. We tested K values from 1 to 5. This analysis estimates individual ancestries, and signals of admixture may relate to gene flow or retention of ancestral traits. The best value of K was evaluated according to the cross-validation test.

To estimate bidirectional migration rates, we ran BA3-SNPs algorithm (Mussmann et al., 2019). We converted our database using PGDspider 3.0.0 (Lischer et al., 2012). We performed preliminary runs to adjust parameters. Accordingly, we set –m0.15 and –a0.40. We ran two independent runs, setting different seed values. We ran 50,000,000 iterations and 10,000,000 burn-in. We assessed convergence for each independent run with Tracer 1.7 (Rambaut et al., 2018).

To depict the genealogical relationship between individuals, we estimated an identity by state (IBS) matrix with TASSEL 5 (Bradbury et al., 2007). We used this information to obtain a NeighborNet with SplitsTree4 (Huson and Bryant, 2006, 2024; Bryant and Huson, 2023), where edges represent lineages of descent and reticulation at the base of the net can be interpreted as admixture events.

Effective population size and historical demography

We estimated current effective population size (N_e) with the software currentNE (Santiago et al., 2023), which estimates N_e based on linkage disequilibrium (LD). We obtained one database for each species, without LD filter (20,646 SNPs), and we defined random mating (-k 0) and 15 chromosomes and 28 cM genome size in accordance with the assembly statistics provided for the genome of *A. mexicanum* (GCF_040938575.1).

To estimate the historical demography of each species we estimated the folded site frequency spectrum (SFS) with easySFS (Gutenkunst et al., 2009). Then, to explore the changes in the historical effective population sizes of *A. andersoni* and *A. dumerilli*, we used program Stairway Plot 2 (Liu and Fu, 2020). Stairway plot estimates changes in N_e through time based on the SFS (Liu and Fu, 2020). We set the generation time to three years of age, which is the reported age to sexual maturity and adult size for *A.*

macrodactylum based on skeletochronological methods (Russel et al., 1996). We masked singletons and used ½, ¼, ¾ and n-2 break points, where n is sample size, for each species, as suggested in the manual. We kept the rest of the parameters as default (Liu and Fu, 2020).

Results

From the 45 analysed individuals, we obtained 11,714 SNPs with no missing data. The observed (H_O) and expected heterozygosity (H_E) revealed similar levels of genetic diversity in the two species (Table 1). A higher number of unique SNPs were found in *A. andersoni* (Table 1; Supplementary Figure S1). Also, both species showed similar mean sMLH (Wilcoxon test, p = 0.324; Fig. 2). Nevertheless, *A. andersoni* showed higher standard deviation (mean = 0.9786 ± 0.141 s.d.) than *A. dumerilii* (mean = 1.043 ± 0.014 s.d.). Inbreeding coefficient (F_{IS}) showed significant negative values for both species (Table 1).

Table 1
Estimates of genetic diversity for *Ambystoma andersoni* and *A. dumerilii* endemic to Michoacán, Mexico estimated from 11,714 SNPs. N = sample size, Private SNPs based on variable sites, H_O = Observed heterozygosity, H_E = Expected heterozygosity, and F_{IS} = Inbreeding coefficient.

Species	**N**	**Private SNPs**	***H_O**	***H_E**	**F_{IS}**
B. *andersoni*	30	2588	0.369	0.286	-0.290***
B. *dumerilii*	15	1888	0.393	0.295	-0.331***
* Nei's sample size correction factor was applied.					
***p < 0.001					

The genetic differentiation coefficient between species (paired F_{ST}) was 0.322 (p < 0.001), and Nei's genetic distance was 0.211. Genetic distance among individuals showed that individuals within each species are more similar than those between species (Supplementary Figure S2). The PCA, DAPC and admixture analyses also suggest that each species constitute a well-differentiated genetic group (Fig. 3 and Supplementary Figure S3). Principal component analysis (PCA) revealed that the first two components explained 39.8% of the variance: the first component (PC1) explained 32.2% of the total variance, whereas the second component (PC2) explained 7.6% (Fig. 3a). The individuals of *A. dumerilii* formed a tight cluster, whereas for *A. andersoni* individual points were scattered on PC2, suggesting greater genetic variance within *A. andersoni* than within *A. dumerilii* (Fig. 3a).

In the admixture analysis, the best support was obtained for K = 2 (CV = 0.36337; Supplementary Figure S4), and the presence of admixed individuals was not observed (Fig. 3b). These results suggest lack of gene flow between the two species. Two independent runs conducted with BA3 showed similar results (LogL = -324339.03 and − 324341.09, respectively). Estimated migration rates between species were

close to zero (M_{12} = 0.0104 and M_{21} = 0.0196). NeighborNet based on IBS showed no reticulation at the base of the net further supporting lack of admixture between species (Supplementary Figure S5).

Estimated effective population size (with bias correction) for *A. andersoni* was 31.36 (24.8–39.52 90% CI) and for *A. dumerilii* analysis did not reach convergence. The historical demographic analyses suggested that both species have undergone population declines, with *A. andersoni*'s effective population size decreasing steadily for more than approximately 6000 years (Fig. 4a), while *A. dumerilii* has undergone a population decline for the past ~ 8000 years (Fig. 4b).

Discussion

In the present study, *A. andersoni* and *A. dumerilii* showed similar levels of genetic diversity. Both species showed high levels of genetic variation, which contrast with our expectation of low genetic variation given their restricted distributions and isolated populations. In addition, we found that, as expected given their allopatric distributions and dispersal limitations, each species constitutes a well-defined genetic group with no signs of interspecific gene flow. Regarding their historical demography, both species show signals of population declines possibly associated with environmental changes occurring during the early to mid-Holocene.

Even if studies based on different molecular markers are not directly comparable with those estimated from SNPs because of their characteristics (i.e. differences in selection pressure, in the rate of evolution and in the biological gene function), their general patterns on genetic diversity and genetic differentiation can be compared. In this context, previous analysis that included *A. andersoni* and *A. dumerilii* showed the same pattern of high genetic diversity based on the *DAB* gene of the Major Histocompatibility Complex (MHC) (Tracy et al., 2015). Moreover, the high levels of genetic diversity found in this study for *A. andersoni* and *A. dumerilii* are consistent with previous reports for other species of genus *Ambystoma*, that used distinct molecular markers (Storfer, 1999; Spear et al., 2005; Sunny et al., 2014; Nunziata et al., 2016; Heredia-Bobadilla et al., 2017). For example, studies involving species with restricted distributions, such as *A. leorae*, *A. altamirani* and *A. rivulare*, revealed high levels of genetic diversity for microsatellite data, with H_E values ranging from 0.591 to 0.613 (Sunny et al., 2014; Monroy-Vilchis et al., 2019). For SNP data, levels of genetic diversity in *A. dumerilii* and *A. andersoni* were similar to those estimated for a population of *A. opacum* (H_E = 0.24) and higher than those estimated for a population *A. talpoideum* (H_E = 0.17), which are species with wider distributions (Nunziata et al., 2016).

For sMLH we also found high values of genetic variation, but interestingly, *A. andersoni* showed a higher standard deviation, with a few individuals showing lower levels of genetic variation. This parameter is an estimate of the individual's heterozygosity standardized by the mean heterozygosity of the population (Coltman et al., 1999; Stoffel et al., 2016), and it may suggest that inbreeding is starting to show in this population, a process that could eventually lead to inbreeding depression (Miller et al., 2014). Accordingly, a program focused on monitoring the genetic variation of these populations should be implemented.

Also, contrary to our expectations, neither species show signals of inbreeding (F_{IS} = − 0.290 and − 0.331), despite their restricted distribution, isolation, demographic decline and small population sizes. This result agrees with reported data for populations of species with wider ranges (*A. opacum* and *A. talpoidieum*), based on SNP data; even when *A. talpoideum* population has declined since 1980's (Nunziata et al., 2016). On the other hand, our results contrast with significant levels of inbreeding that have been reported for species with restricted distributions such as *A. rivulare*, *A. altamirani* and *A. leorae* (Parra-Olea et al., 2012; Sunny et al., 2014; Tracy et al., 2015; Heredia-Bobadilla et al., 2016; Heredia-Bobadilla et al., 2017). Levels of inbreeding can relate to aspects of the mating system such as outcrossing, age to first reproduction, number of clutches and clutch size (Frankham 2005; Hedrick 2010). We should consider that F_{IS} could be biased by technical artifacts such as the presence of paralogous sequences and repetitive elements that are present at high frequency in the complex genome of *A. mexicanum* (Nowoshilow et al., 2018). In this sense, we applied strict filters for missing data, Hardy-Weinberg equilibrium and genotyping coverage to reduce possible technical artifacts that could bias our results (O'leary et al., 2018). Additionally, F_{IS} is sensitive to sample size (Aguirre-Liguori et al., 2020), so these results should be taken with caution. Future studies should consider larger sampling size; however, low population sizes may impede increasing sample sizes, even if sampling effort is increased.

As noted in previous research (Storfer, 1999; Spear et al., 2005; Everson et al., 2021), gene flow has been identified as an important determinant of the genetic structure for *Ambystoma* populations. In the present study, no signs of recent gene flow were detected between *A. andersoni* and *A. dumerilii*, with each species constituting well-differentiated genetic groups. In contrast, Everson et al. (2021) reported positive migration rates between *A. andersoni* and *A. dumerilii*; nonetheless, their analyses could not differentiate between recent and historical gene flow. In this context, there might have been historical gene flow between these sister taxa, but recent gene flow may be precluded by the isolation of these populations and their dispersal capacity. On the other hand, our results are consistent with those reported for *Ambystoma* species in Mexico in which low or null levels of gene flow were observed between species (Sunny et al., 2014; Percino et al., 2016; Heredia-Bobadilla et al., 2017; Monroy-Vilchis et al., 2019).

Effective population size (N_e) in *A. andersoni* was low, and the analysis did not converge in *A. dumerilii*. Occurrence of rare alleles and deviations from random mating will introduce bias in N_e estimation (Marendel et al., 2020; Gargiulo et al., 2024). In addition, ancient population events, such as Pleistocene population expansion or contraction, may affect inferences of current N_e, and signals of ancient demographic processes are easier to detect than recent population change (Reid and Pinsky, 2022). Even if there are potential biases affecting N_e estimates, our results suggest that N_e for *A. andersoni* is below the lower threshold recommended for the maintenance of evolutionary potential (Franklin and Frankham, 1998), as is the case for *A. opacum* and *A. talpoideum* (Nunziata et al., 2016). Moreover, it has been reported that *Ambystoma* species with restricted distributions tend to have small effective population sizes (Parra-Olea et al., 2012; Sunny et al., 2014; Monroy-Vilchis et al., 2019). In this context,

genetic drift would promote the loss of genetic diversity, which in turn may increase vulnerability to diseases, environmental changes and other stressors (Parra-Olea et al., 2012). Conducting additional genetic and ecological studies would provide a comprehensive view of temporal changes in genetic diversity and population dynamics.

Historical demographic analyses suggest that *A. andersoni* and *A. dumerilii* may have undergone population declines. However, as previously mentioned, these results should be interpreted with caution given the sample sizes (Marendel et al., 2020; Gargiulo et al., 2024). For *A. andersoni*, a constant population decline has been observed since the mid-Holocene (~ 6,000 years ago). The Zacapu Lagoon, inhabited by *A. andersoni*, is a small and isolated lagoon that originated in the Upper Pliocene-Pleistocene (3.6-0.012 MYA) and was impacted by drought and deforestation in AD 1,300 (Demant, 1992; Arnauld and Faugère-Kalfon, 1998). This lake was an endorheic basin in which the waters of other rivers, streams and springs converged but were separated by episodes of droughts that occurred since 1300 AD (Arnauld and Faugère-Kalfon, 1998). Currently, it is impacted by multiple anthropic activities such as intensive agriculture, urbanization, tourism, and industrial activities (Zubieta Rojas, 2005). Historical and current habitat degradation processes, coupled with the paedomorphic condition of this species, which limits its dispersal capacity (Arnauld and Faugère-Kalfon, 1998), could have important implications regarding the loss of genetic diversity and the long-term viability of these populations (Arnauld and Faugère-Kalfon, 1998; Parra-Olea et al., 2012).

Our results suggest that *A. dumerilii* has experienced a continuous population decline since the early Holocene (~ 8,000 years ago), a demographic trend mirrored by *A. andersoni* starting approximately 6,000 years ago. Lake Patzcuaro was formed during the Pleistocene as a result of volcanic activity that isolated preexisting drainages from the Lerma River. Then, this lake remained stable from the Last Glacial Maximum into the early Holocene. Throughout the Holocene there is evidence of changes in lake levels and environmental fluctuations possibly associated to volcanic impacts and human activities (Bradbury, 2000). Declines in effective population in both sites, likely correspond to shifts in precipitation regimes and the gradual desiccation of Lake Pátzcuaro and Zacapu Lagoon (Metcalfe et al., 2007).

Regarding current effective population sizes, the statistical power and sample size must be considered; thus, our estimates may be influenced by sample size or by a lack of recent signal to resolve contemporary fluctuations and should be taken with caution. Nonetheless, low current effective population size, alongside migration rates near zero ($m < 0.02$), increases the extinction risk of both species (Frankham 2005). Moreover, ongoing habitat fragmentation driven by eutrophication and wastewater discharge (Vargas and Guzmán Ramírez, 2009) poses an imminent threat; thus, even if we detected high genetic variation, it would be necessary to design specific conservation strategies for each species, considering their genetic diversity and the threats they face (Sunny et al., 2014). In both species habitat protection and restoration activities are needed to support their existence in the mid to long term. In addition, *ex situ* and *in situ* conservation actions could help prevent the loss of genetic variation in these species.

It is crucial to perform follow-up studies that include regular monitoring of the genetic diversity and demographics of *A. andersoni* and *A. dumerilii*. It is also essential to analyse data related to immune responses that could be associated with the susceptibility of these species to the presence of emerging diseases such as *B. dendrobatidis* and *B. salamandrivorans.* In addition, the impacts of environmental and anthropogenic factors should be explored in detail. In both cases, it is essential to promote public awareness about the conservation of these endemic species and their habitats and to involve local communities in conservation and monitoring activities.

Declarations

Competing Interests

The authors declare they have no financial interests to disclose

Genetic diversity in two endemic axolotl species from Michoacan, Mexico.

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Author Contribution

All authors contributed to project design and execution. E.D.d.-S. and G.C.-M. led the drafting of the manuscript. Y.M.-L. conducted sample collection. M.D.B. standardized and performed laboratory protocols. E.D.d.-S., G.C.-M., E.S.-C. and A.C.-T. analyzed the data. E.A.R., Y.M.-L. and G.C.-M. obtained funding. G.C.-M., E.A.R. and E.Z. supervised the project. All authors read and edited initial versions of the manuscript and approved the final version.

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Data Availability

Raw reads were deposited in NCBI SRA (Bioproject PRJNA1163358)

References

1. Aguirre-Liguori, J. A., Luna-Sánchez, J. A., Gasca-Pineda, J., & Eguiarte, L. E. (2020). Evaluation of the minimum sampling design for population genomic and microsatellite studies: an analysis based on wild maize. *Frontiers In Genetics*, *11*, 870. https://doi.org/10.3389/fgene.2020.00870
2. Ávila Akerberg, V. D., González Martínez, T. M., Hernández González, A., & Vázquez Trejo, M. (2021). El género *Ambystoma* en México ¿Qué son los ajolotes? Ciencia ergo sum 2(2):1. https://doi.org/ces.v28n.2a10.
3. Anderson, J. D., & Worthington, R. D. (1971). The life history of the Mexican salamander *Ambystoma ordinarium* Taylor. *Herpetologica*, *27*, 165–176. https://www.jstor.org/stable/3891075
4. Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinform*, *12*(1), 246. https://doi.org/10.1186/1471-2105-12-246
5. Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, *11*(10), 697–709. https://doi.org/10.1038/nrg2844
6. Allentoft, M. E., & O’Brien, J. (2010). Global amphibian declines, loss of genetic diversity and fitness: a review. *Diversity*, *2*(1), 47–71. https://doi.org/10.3390/d2010047
7. Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
8. Arnauld, M-C., & Faugère-Kalfon, B. (1998). Evolución de la ocupación humana en el Centro-Norte de Michoacán (Proyecto Michoacán, CEMCA). In: Darras V (coor) Génesis, culturas y espacios en Michoacán. CEMCA, pp 13–34.
9. Basanta, M. D., Ávila-Akerberg, V., Byrne, A. Q., Castellanos-Morales, G., González Martínez, T. M., Maldonado-López, Y., Rosenblum, E. B., Suazo-Ortuño, I., Parra Olea, G., & Rebollar, E. A. (2022). The fungal pathogen *Batrachochytrium salamandrovirans* is not detected in wild and captive amphibians from Mexico. *PeerJ*, *10*, e14117. https://doi.org/10.7717/peerj.14117
10. Bouzat, J. L. (2010). Conservation genetics of population bottlenecks: the role of chance, selection and history. 11:463–478. https://doi.org/10.1007/s10592-010-0049-0

11. Bradbury, J. P. (2000). Limnologic history of Lake Patzcuaro, Michoacán, México for the past 48,000 years: impacts of climate and man. *Phylogeog Palaeoclimatol Palaeoecol*, *163*, 69–95. https://doi.org/10.1016/S0031-0182(00)00146-2
12. Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308
13. Broad Institute (2021). Picard (Version 2.27.1) [Computer software]. Available at http://broadinstitute.github.io/picard/
14. Bryant, D., & Huson, D. H. (2023). NeighborNet: improved algorithms and implementation. *Front Bioinform*, *3*, 1178600. https://ddoi.org/10.3389/fbinf.2023.1178600
15. Cardillo, M., Mace, G. M., Gittleman, J. L., Jones, K. E., Bielby, J., & Purvis, A. (2008). The predictability of extinction: biological and external correlates of decline in mammals. *Proc R Soc B Biol Sci*, *275*(1641), 1441–1448. https://doi.org/10.1098/rspb.2008.0179
16. Castellanos-Morales, G., Ortega, J., Castillo-Gámez, R. A., Sackett, L. C., & Eguiarte, L. E. (2015). Genetic variation and structure in contrasting geographic distributions: widespread versus restricted black-tailed prairie dogs (Subgenus *Cynomys*). *Journal Of Heredity*, *S1*, 478–490. https://doi.org/10.1093/jhered/esv021
17. Catchen, J., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: building and genotyping loci *de novo* from short-read sequences. *G3*, *1*(3), 171–182. https://doi.org/10.1534/g3.111.000240
18. Catchen, J., Hohenlohe, P., Bassham, S., Amores, A., & Cresko, W. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. https://doi.org/10.1111/mec.12354
19. Ceballos, G., & Ehrlich, P. R. (2002). Mammal population losses and the extinction crisis. *Science*, *296*(5569), 904–907. https://doi.org/10.1126/science.1069349
20. Ceballos, G., Ehrlich, P. R., & Dirzo, R. (2017). Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proc Natl Acad Sci U S A*, *114*(30), E6089–E6096. https://doi.org/10.1073/pnas.1704949114
21. Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. (1999). Parasite-mediated selection against inbred Soay sheep in free-living island population. *Evolution*, *53*(4), 1259–1267. https://doi.org/10.1111/j.1558-5646.1999.tb04538.x
22. Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., et al. (2011). The variant call format and VCFtools. *Bioinform*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330
23. Demant, A. (1992). Marco geológico regional de la Laguna de Zacapu, Michoacán, México. In: Michelet D (coor) El proyecto Michoacán 1983–1987. Medio ambiente e introducción a los trabajos arqueológicos. Collection etudes mesoamericaines II-12/Cuadernos de Estudios Michoacanos 4, CWMCA pp 55–72.

24. Díez-del-Molino, D., Sánchez-Barreiro, F., Barnes, I., Gilbert, M. T. P., & Dalén, L. (2018). Quantifying temporal genomic erosion in endangered species. *Trends In Ecology & Evolution*, *33*(3), 176–185. https://doi.org/10.1016/j.tree.2017.12.002
25. Everson, K. M., Gray, L. N., Jones, A. G., Lawrence, N. M., Foley, M. E., Sovacool, K. L., Kratovil, J. D., Hotaling, S., Hime, P. M., Storfer, A., et al. (2021). Geography is more important than life history in the recent diversification of the tiger salamander complex. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, *118*(17), e2014719118. https://doi.org/10.1073/pnas.2014719118
26. Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Conservation Biology*, *10*, 1500–1508. https://doi.org/10.1046/j.1523-1739.1996.10061500.x
27. Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, *126*(2), 131–140. https://doi.org/10.1016/j.bio.con.2005.05.002
28. Franklin, R., & Frankham, R. (1998). How large must populations be to retain evolutionary potential? *Animal Conserv*, *1*, 69–70. https://doi.org/10.1111/j.1469-1795.1998.tb00228.x
29. Fisher, M., & Garner, T. W. J. (2020). Chytrid fungo and global amphibian declines. *Nature Rev Microbiol*, *18*(6), 1–12. https://doi.org/10.1038/s41579-020-0335-x
30. Fountain, E. D., Pauli, J. N., Reid, B. N., Palsboll, P. J., & Peery, M. Z. (2016). Finding the right coverage: the impact of coverage and sequence quality on single nucleotide polymorphism genotyping error rates. *Molecular Ecology*, *16*, 966–978. https://doi.org/10.1111/1755-0998.12519
31. Gargiulo, R., Decroocq, V., González-Martínez, S. C., Paz-Vinas, I., Aury, J-M., Kupin, I. L., Palomino, C., Schmitt, S., Scotti, I., & Heuertz, M. (2024). Estimation of contemporary effective population size in plant populations: limitations of genomic datasets. *Evol App*, *17*, e13691. https://doi.org/10.1111/eva.13691
32. Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. *arXiv preprint*. https://doi.org/10.48550/arXiv.1207.3907. arXiv:1207.3907.
33. Gruber, B., Unmack, P. J., Berry, O. F., & Georges, A. (2018). dartR: an R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Mol Ecol Resour*, *18*(3), 691–699. https://doi.org/10.1111/1755-0998.12745
34. Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *Plos Genet*, *5*(10), e1000695. https://doi.org/10.1371/journal.pgen.1000695
35. Hedrick, P. W. (2010). *Genetics of populations* (4th ed.). Jones and Bartlett.
36. Heredia-Bobadilla, R-L., Monroy-Vilchis, O., Zarco-González, M. M., Martínez-Gómez, D., Mendoza-Martínez, G. D., & Sunny, A. (2016). Genetic structure and diversity in an isolated population of an endemic mole salamander (*Ambystoma rivulare* Taylor, 1940) of central Mexico. *Genetica*, *144*(6), 689–698. https://doi.org/10.1007/s10709-016-9935-9
37. Heredia-Bobadilla, R-L., Monroy-Vilchis, O., Zarco-González, M. M., Martínez-Gómez, D., Mendoza-Martínez, G. D., & Sunny, A. (2017). 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. *Journal Of Genetics*, *96*(6), 873–883. https://doi.org/10.1007/s12041-017-0823-6

38. Huacúz Elías D del C. (2001). *Estado de conservación del género Ambystoma en Michoacán, México*. Universidad Michoacana de San Nicolás de Hidalgo, UNAM, Secretaría de Medio Ambiente y Recursos Naturales.
39. Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology And Evolution*, *23*(2), 254–267. https://doi.org/10.1093/molbev/msj030
40. Huson, D. H., & Byant, D. (2024). The SplitsTree App: inteactive analysis and visualization using phylogenetic trees and networks. *Nature Methods*, *21*, 1773–1774. https://doi.org/10.1038/s41592-024-02406-3
41. IUCN SSC Amphibian Specialist Group (2015). *Ambystoma andersoni* (errata version published in 2020). The IUCN Red List of Threatened Species 2015: e.T59051A176772780. https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T59051A176772780.en. Accessed 13 February 2025.
42. IUCN SSC Amphibian Specialist Group (2020). *Ambystoma dumerilii*. *The IUCN Red List of Threatened Species* 2020: e.T59055A53973725. https://dx.doi.org/10.2305/IUCN.UK.2020-1.RLTS.T59055A53973725.en. Accessed 13 February 2025.
43. Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinform*, *24*(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129
44. Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinform*, *27*(21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521
45. Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *Bmc Genetics*, *11*(1), 94. https://doi.org/10.1186/1471-2156-11-94
46. Kardos, M., Armstrong, E. E., Fitzpatrick, S. W., Hauser, S., Hedrick, P. W., Miller, J. M., Tallmon, D. A., & Funk, W. C. (2021). The crucial role of genome-wide genetic variation in conservation. *Proceedings Of National Academy Of Sciences*, *118*(48), e2104642118. https://doi.org/10.1073/pnas.2104642118
47. Keeler, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trend Ecol Evol*, *17*, 230–241. https://doi.org/10.1016/S0169-5347(02)02489-8
48. Lacy, R. C. (1997). Importance of genetic variation to the viability of mammalian populations. *Journal Of Mammalogy*, *78*(2), 320–335. https://doi.org/10.2307/1382885
49. Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinform*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324
50. Lischer, H. E., & Excoffier, L. (2012). PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics*, *28*(2), 298–299. https://doi.org/10.1093/bioinformatics/btr642

51. Liu, X., & Fu, Y-X. (2020). Stairway Plot 2: demographic history inference with folded SNP frequency spectra. *Genome Biology*, *21*, 280. https://doi.org/10.1186/s13059-020-02196-9
52. Luedtke, A. J., Chanson, J., Neam, K., et al. (2023). Ongoing declines for the world's amphibians in the face of emerging threats. *Nature*, *622*, 308–314. https://doi.org/10.1038/s41586-023-06578-4
53. Manichaikul, A., Mychaleckyj, J. C., Rich, S. S., Daly, K., Sale, M., & Chen, W-M. (2010). Robust relationship inference in genome-wide association studies. Bioinform 26(22):2867–2873. https://doi.org/101093/bioinformatics/btq559.
54. Marendel, F., Charrier, G., Lamy, J-B., Le Cam, S., Lone, P., & Trenkel, V. M. (2020). Estimating effective population size using RADseq: effects of SNP selection and sample size. *Econ Evil*, *10*, 1929–1937. https://doi.org/10.1002/ece3.6016
55. Meirmans, P. G. (2020). GENODIVE version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. *Mol Ecol Resour* 20:1126–1131. https://doi.org/10.1111/1755-0998.13145.
56. Mendoza, T. V. (2012). Importancia Ecológica y Cultural de una Especie Endémica de Ajolote (*Ambystoma dumerilii*) del Lago de Pátzcuaro, Michoacán. *Etnobiologia*, *10*(2), 40–49.
57. Mendoza-Almeralla, C., Burrowes, P., & Parra-Olea, G. (2015). La quitridiomicosis en los anfibios de México: una revision. *Rev Mex Biodiver*, *86*(1), 238–248. https://doi.org/10.7550/rmb.42588
58. Metcalfe, S. E., Davies, S. J., Braisby, J. D., Leng, M. J., Newton, A. J., Terrett, N., & O'Hara, S. L. (2007). Long and short-term change in the Pátzcuaro Basin, central Mexico. *Palaeogeog Palaeoclim Palaeoecol*, *247*(3–4), 272–295. https://doi.org/10.1016/j.palaeo.2006.10.018
59. Miller, J. M., Malenfant, R. M., David, P., Davis, C. S., Poissant, J., Hogg, J. T., Festa-Bianchet, M., & Coltman, D. W. (2014). Estimating genome-wide heterozygosity: effects of demographic history and marker type. *Heredity*, *112*, 240–247. https://doi.org/10.1038/hdy.2013.99
60. Monroy-Vilchis, O., Heredia-Bobadilla, R. L., Zarco-González, M. M., Ávila-Akerberg, V., & Sunny, A. (2019). Genetic diversity and structure of two endangered mole salamander species of the Trans-Mexican Volcanic Belt. *Herpetozoa*, *32*, 237–248. https://doi.org/10.3897/herpetozoa.32.e38023
61. Mussman, S. M., Douglas, M. R., Chafin, T. K., & Douglas, M. E. (2019). BA3-SNPs: contemporary migration reconfigures in BayesAss for next generation sequence data. *Met Ecol Evol*, *10*(10), 1808–1813. https://doi.org/10.1111/2041-210X.13252
62. Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University.
63. Nowoshilow, S., Schloissnig, S., Fei, J. F., et al. (2018). The axolotl genome and the evolution of key tissue formation regulators. *Nature*, *554*, 50–55. https://doi.org/10.1038/nature25458
64. Nunziata, S. O., Lance, S. T., Scott, D. E., Lemmon, E. M., & Weisrock, D. W. (2016). Genomic data detect corresponding signatures of population size change on an ecological time scale in two salamander species. *Molecular Ecology*, *26*, 1060–1074. https://doi.org/10.1111/mec.13988
65. Olazcuaga, L., Lincke, B., DeLacey, S., Durkee, L. F., Merbourne, B. A., & Hufbauer, R. A. (2023). Population demographic history and evolutionary rescue: influence of a bottleneck event. *Evolutionary Applications*, *16*, 1483–1495. https://doi.org/10.1111/eva.13581

66. O'Leary, S. J., Puritz, J. B., Willis, S. C., Hollenbeck, C. M., & Portnoy, D. S. (2018). These aren't the loci you're looking for: principles of effective SNP filtering for molecular ecologists. *Molecular Ecology*, *27*(16), 3193–3206. https://doi.org/10.1111/mec.14792

67. Parra-Olea, G., Zamudio, K. R., Recuero, E., Aguilar-Miguel, X., Huacuz, D., & Zambrano, L. (2012). Conservation genetics of threatened Mexican axolotls (*Ambystoma*). *Animal Conservation*, *15*(1), 61–72. https://doi.org/10.1111/j.1469-1795.2011.00488.x

68. Parra-Olea, G., Flores-Villela, O., & Mendoza-Almeralla, C. (2014). Biodiversidad de anfibios en México. *Rev Mex Biodivers*, *85*, S460–S466. https://doi.org/10.7550/rmb.32027

69. Percino-Daniel, R., Recuero, E., Vázquez-Domínguez, E., Zamudio, K. R., & Parra-Olea, G. (2016). All grown-up and nowhere to go: paedomorphosis and local adaptation in *Ambystoma* salamanders in the Cuenca Oriental of México. *Biological Journal Of The Linnean Society*, *118*(3), 582–597. https://doi.org/10.1111/bij.12750

70. Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

71. R Core Team (2021). R: a language and environment for statistical computing. R Foundation for Statistical computing. https://cran.r-project.org

72. Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

73. Rochette, N. C., & Catchen, J. M. (2017). Deriving genotypes form RAD-seq short-read data using Stacks. *Nature Protocols*, *12*, 2640–2659. https://doi.org/10.1038/nprot.2017.123

74. Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, *28*(21), 4737–4754. https://doi.org/10.1111/mec.15253

75. Russell, A., Powell, G. L., & Hall, D. R. (2011). Growth and age of Alberta long-toed salamanders (*Ambystoma macrodactylum krausei*): a comparison of two methos of estimation. *Canadian Journal Of Zoology*, *74*, 397–412. https://doi.org/10.1139/z96-047

76. Santiago, E., Caballero, A., Kôpke, C., & Novo, I. (2023). Estimation of the contemporary effective population size from SNP data while accounting for mating structure. *Mol Ecol Resour*, *24*(1), e13890. https://doi.org/10.1111/1755-0998.13890

77. SEMARNAT. (2018). *Programa de accion para la conservación de las especies: Ambystoma spp*. SEMARNAT/CONANP.

78. Smith, J. J., Timoshevskaya, N., Timoshevskiy, V. A., Keinath, M. C., Hardy, D., & Voss, S. R. (2019). A chromosome-scale assembly of the axolotl genome. *Genome Research*, *29*(2), 317–324. https://doi.org/10.1101/gr.241901.118

79. Spear, S. F., Peterson, C. R., Matocq, M. D., & Storfer, A. (2005). Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology*, *14*(8), 2553–2564.

https://doi.org/10.1111/j.1365-294X.2005.02573.x

80. Spielman, D., Brook, B. W., & Frankham (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, *101*(42), 15261–15264. https://doi.org/10.1073/pnas.0403809101
81. Stoffel, M. A., Esser, M., Kardos, M., Humble, E., Nichols, H., David, P., & Hoffman, J. I. (2016). inbreedR: an R package for the analysis of inbreeding based on genetic markers. *Methods In Ecology And Evolution*, *7*, 1331–1339. https://doi.org/10.1111/2041-210X.12588
82. Storfer, A., Cross, J., Rush, V., & Caruso, J. (1999). Adaptive coloration and gene flow as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri*. *Evolution*, *53*(3), 889–898. https://doi.org/10.1111/j.1558-5646.1999.tb05383.x
83. Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783–1786. https://doi.org/10.1126/science.1103538
84. Sunny, A., Monroy-Vilchis, O., Fajardo, V., & Aguilera-Reyes, U. (2014). Genetic diversity and structure of an endemic and critically endangered stream river salamander (Caudata: *Ambystoma leorae*) in Mexico. *Conservation Genetics*, *15*, 49–59. https://doi.org/10.1007/s10592-013-0520-9
85. Tracy, K. E., Kiemnec-Tyburczy, K. M., DeWoody, J. A., Parra-Olea, G., & Zamudio, K. R. (2015). Positive selection drives the evolution of a major histocompatibility complex gene in an endangered Mexican salamander species complex. *Immunogenetics*, *67*, 323–335. https://doi.org/10.1007/s00251-015-0835-4
86. Valencia-Vargas, R., & Escalera-Vázquez, L. H. (2021). Abundancia de la salamandra *Ambystoma andersoni* con relación a la dinámica estacional y heterogeneidad espacial en el lago de Zacapu, Michoacán, México. *Rev Mex Biodivers*, *92*, 1–12. https://doi.org/10.22201/ib.20078706e.2021.92.3283
87. Vargas, S., & Guzmán Ramírez, N. B. (2009). *Deterioro de la cuenca del lago de Pátzcuaro cambios en la identidad étnica p'urhépech*. Asociación Latinoamericana de Sociología.
88. Waples, R. S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conser Genet*, *7*, 167–184. https://doi.org/10.1007/s10592-005-9100-y
89. Zubieta Rojas, T. (2005). *Plan de Manejo del Área Natural Protegida Laguna de Zacapu y su Ribera*. Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Biología, y Secretaría de Urbanismo y Medio Ambiente.
90. Statements & Declarations

Figures

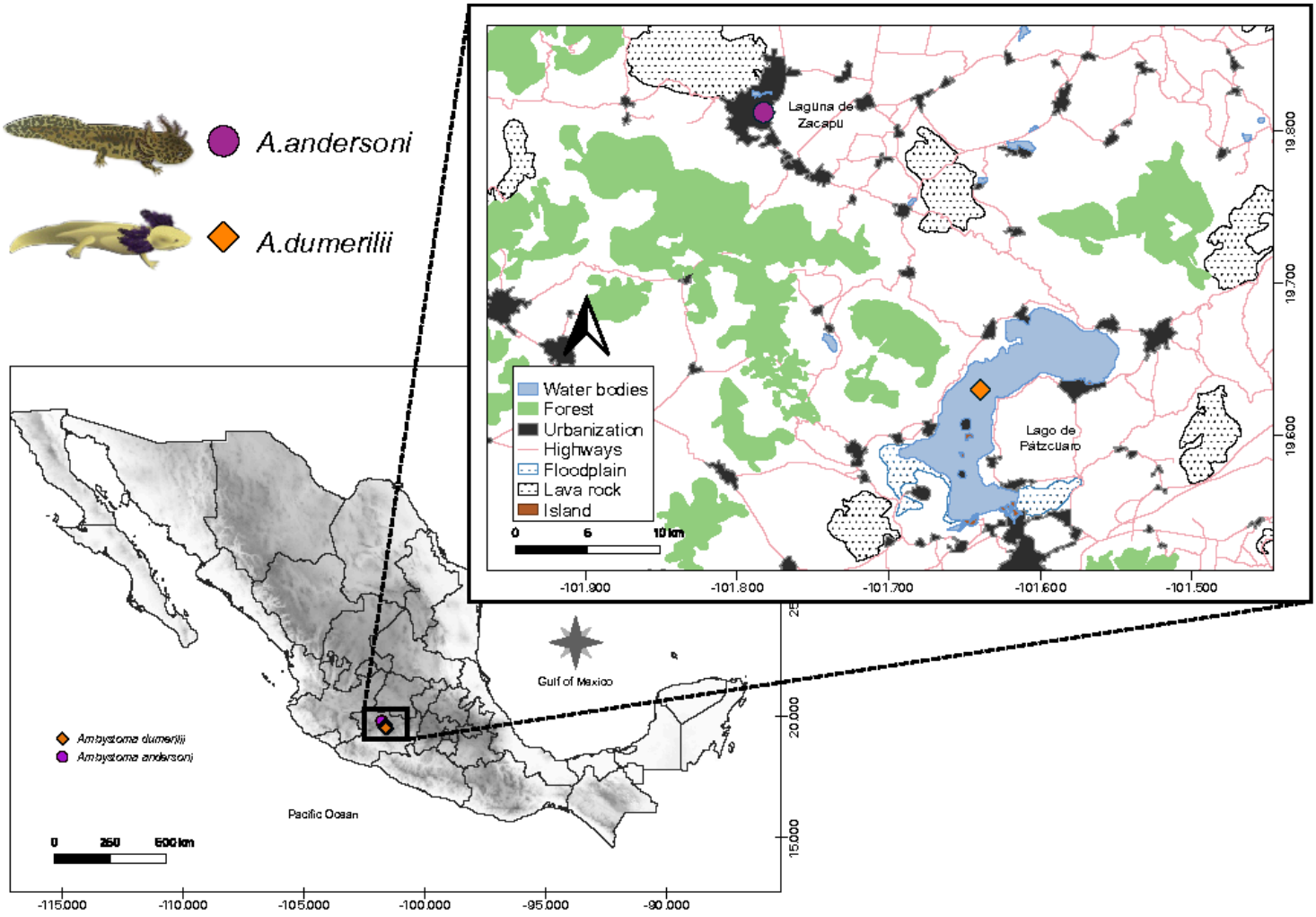


Figure 1

Location map of sampling areas in Zacapu Lagoon (pink circle) and Pátzcuaro Lake (blue diamond) in the state of Michoacán, México. The figure depicts in grayscale the topographic features of Mexico (INEGI, 2024). Occurrence points in accordance with data available at Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO, 2024).

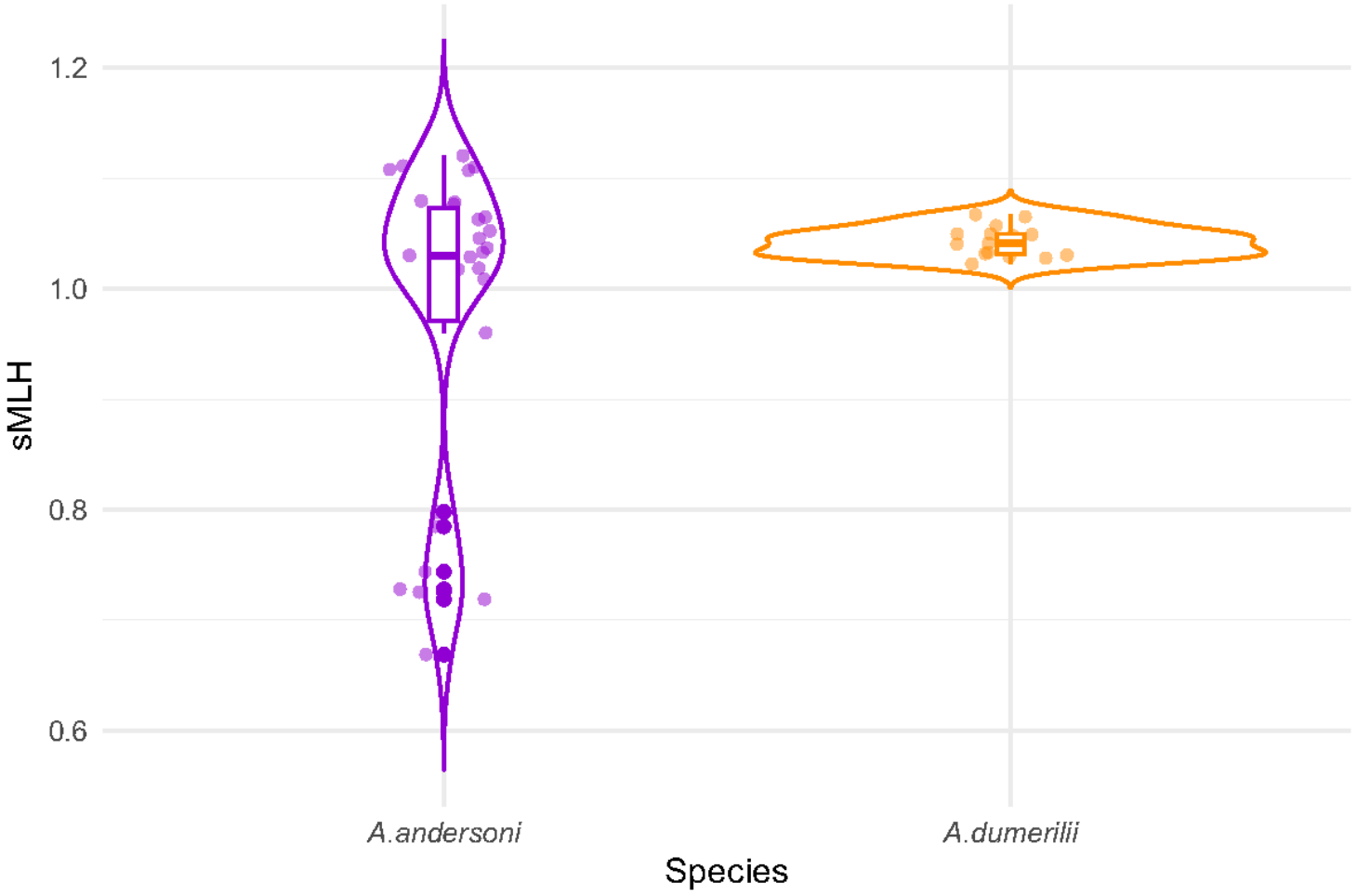


Figure 2

Violin plots depicting de distribution of standardized multilocus heterozygosity (sMLH) for *A. andersoni* (violet) and *A. dumerilii* (orange). Points correspond to individual's sMLH, box plots depict Q1, median and Q3, and the lines show data distribution.

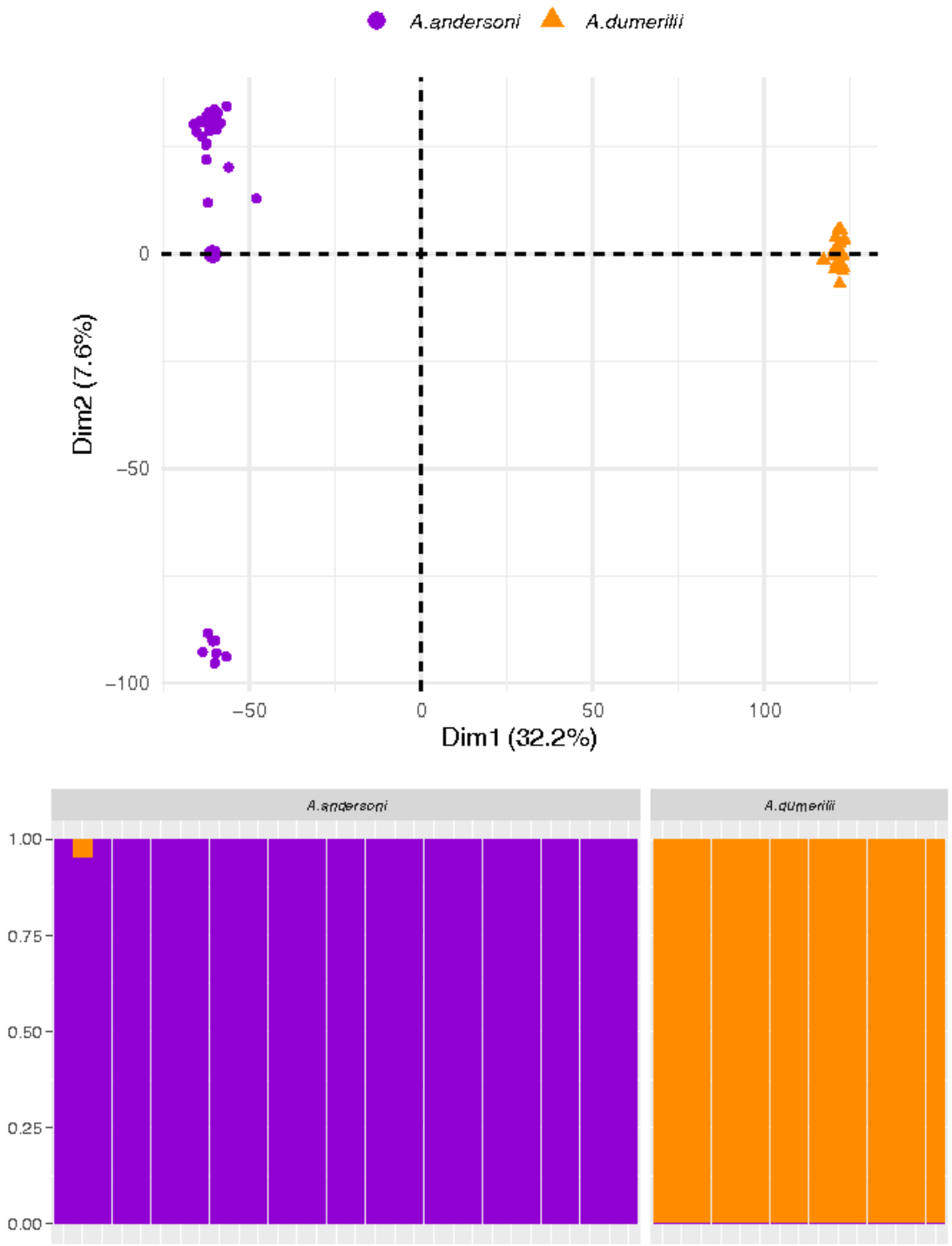


Figure 3

a) Principal Component Analysis (PCA); b) Admixture results for *A. andersoni* (violet) and *A. dumerilii* (orange).

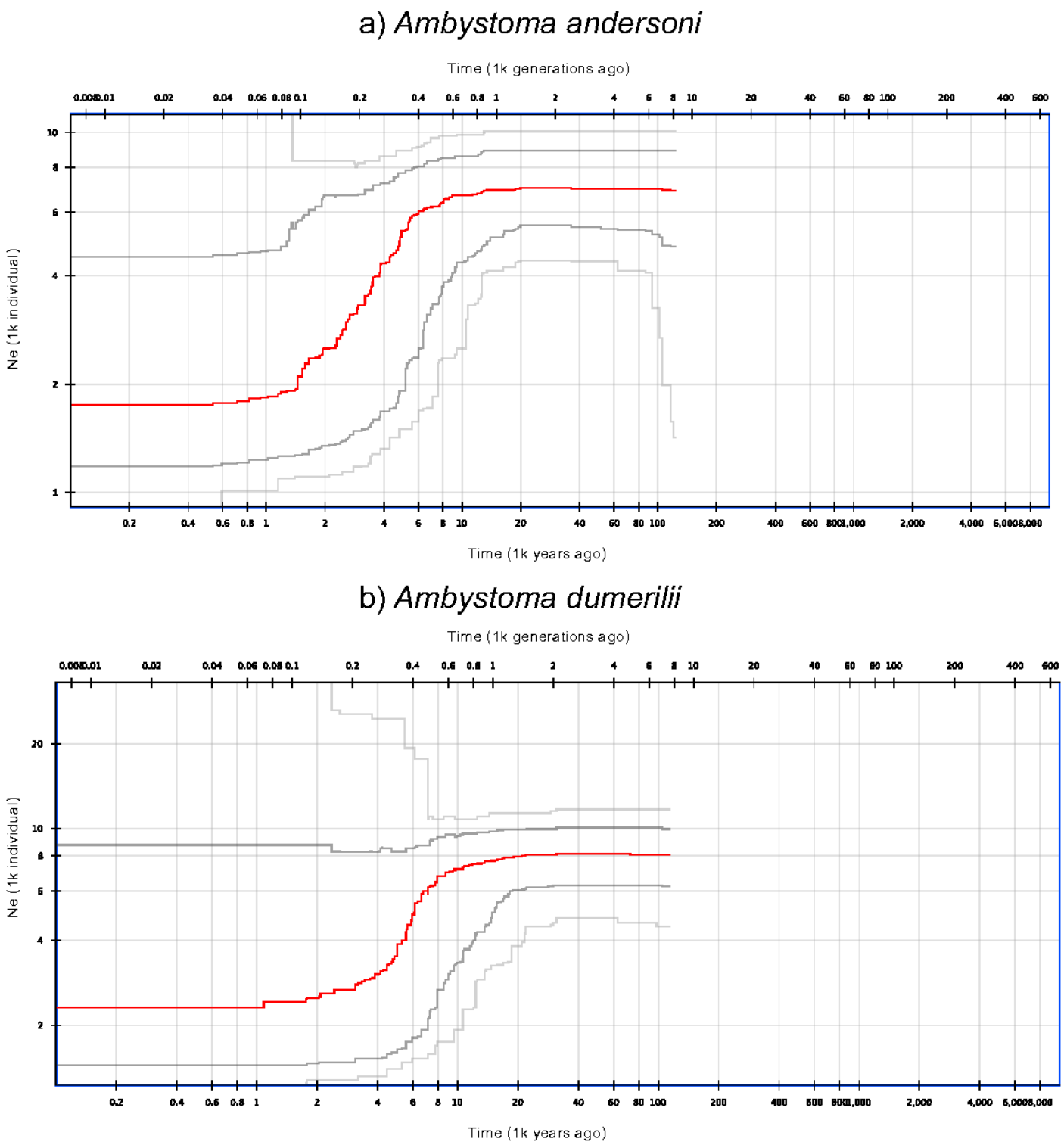


Figure 4

Historical demography estimation (in generations) for a) *A. andersoni* and b) *A. dumerilii* based on linkage disequilibrium obtained with Skyline plots. Red line represents median value, and grey lines represent confidence intervals.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials100426.docx