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Critical Conservation Gaps for Microendemic Axolotls Reveal Inadequate Protection in Central Mexico

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ABSTRACT: Salamanders of the genus *Ambystoma* in the Trans-Mexican Volcanic Belt are experiencing severe population declines due to habitat loss and fragmentation. This study evaluated critical protection gaps for four Critically Endangered microendemic species: *A. amblycephalum*, *A. andersoni*, *A. dumerilii* and *A. mexicanum*. We compiled and cleaned 89 validated presence records from databases and the literature. Refined areas of occupancy were calculated using minimum convex polygons adjusted with elevation masks, hydrographic network filters, and species-specific buffer zones (50–100 m). Bioclimatic variables (temperature and precipitation-based) were derived from MexHiResClimDB, and overlap with protected areas, and the Ecosystem Integrity Index (EII) was quantified. The resulting areas of occupancy (0.38–108.19 km^2) were larger than previous IUCN estimates for *A. amblycephalum* and *A. dumerilii*, yet showed null or minimal overlap with protected areas for these two species (4.79% and 0%, respectively). Ecosystem integrity was low across all species (EII 0.05–0.43), indicating severe degradation. Climatic niches were narrow, differentiated, and associated with restricted altitudinal ranges. These results reveal a crisis of effective protection, where expanded distribution knowledge does not translate into improved conservation status, demanding urgent expansion of active conservation strategies to counteract severe habitat degradation caused by urbanization, intensive agriculture, pollution, and invasive species.

Keywords: Climatic niche; Ecosystem integrity; Geographic isolation; Habitat fragmentation; Paedomorphosis; Protected areas

1. Introduction

Global amphibian populations face unprecedented declines driven by habitat loss, climate change, invasive species, and emerging diseases such as chytridiomycosis [1,2]. Mexico, a biodiversity hotspot with more than 400 amphibian species [3], exhibits remarkable microendemic diversity, particularly in the Trans-Mexican Volcanic Belt (TMVB), a region shaped by volcanic activity and pronounced elevational gradients [4,5]. In this area, 92.3% of salamanders of the genus *Ambystoma* are classified as threatened according to the International Union for Conservation of Nature (IUCN) Red List, and 100% are protected under Mexico's NOM-059-SEMARNAT [6].

Four *Ambystoma* species from the TMVB are categorized as Critically Endangered (CR): *A. amblycephalum*, *A. andersoni*, *A. dumerilii*, and *A. mexicanum*. These microendemics are confined to isolated aquatic systems in central Mexico. *Ambystoma amblycephalum* inhabits high-elevation streams in Michoacán, with recent rediscoveries suggesting fragmented remnant populations [7]. *Ambystoma andersoni* and *A. dumerilii* are lacustrine endemics restricted to Lakes Zacapu and Pátzcuaro, respectively [8,9], while the iconic *A. mexicanum* persists only in the southern remnants of Lake Xochimilco [10].

Their restricted distributions, dependence on permanent water bodies, and sensitivity to disturbances render these species highly vulnerable. Habitat fragmentation caused by urbanization, intensive agriculture, pollution, and invasive species (such as predatory fish and the African clawed frog *Xenopus laevis*) has severely reduced population connectivity [10–12]. Critical areas such as Bosque de Agua and Xochimilco exhibit major protection gaps, with only limited coverage of suitable habitat within protected areas [10,11]. This highlights the urgent need to accurately assess areas of occupancy and ecosystem integrity to guide targeted conservation actions.

Despite their high ecological and cultural importance, conservation efforts in central Mexico remain inadequate. Existing protected areas (PA) often fail to encompass key breeding sites or dispersal corridors, increasing functional isolation and extinction risk [13]. Emerging threats, such as chytridiomycosis, have intensified declines in related *Ambystoma* species, demanding immediate action [14]. Understanding the extent of protection and ecosystem integrity for these four species is essential to prioritizing conservation strategies in the TMVB, where amphibians can serve as indicators of overall ecosystem health.

Our analysis reveals critical protection gaps, emphasizing the need for expansion of protected areas, connectivity restoration, and targeted interventions to mitigate ongoing declines and strengthen resilience against climatic and anthropogenic pressures.

2. Materials and Methods

2.1. Data Source

A total of 148 presence records for the four species were initially obtained from Global Biodiversity Information Facility (GBIF) and specialized literature. Following rigorous depuration to remove duplicates, records with metadata errors (e.g., imprecise coordinates or outside known geographic range), and cross-verification with Amphibian Species of the World [15], 89 valid records were retained: nine for *A. amblycephalum*, eight for *A. andersoni*, 36 for *A. dumerilii* and 36 for *A. mexicanum*. These depurated records served as the basis for all subsequent spatial and environmental analyses.

2.2. Determination of Distribution Areas

Initially, we calculated the extent of occurrence (EOO) using minimum convex polygons (MCP) in QGIS 3.40.15 (Bratislava) from the depurated records (compiled, cleaned, and validated), following IUCN recommendations for delineating the general accessible area [16]. These polygons provide a clear and straightforward of each species' approximate initial geographic distribution (Figure 1). However, since MCP tends to overestimate the actual distribution of species [17], and considering the strict microendemism of our study species with a strictly aquatic habitat and elevational dependence [18], we refined the areas using binary masks of optimal elevation extracted from the Mexican Elevation Continuum 4.0 (CEM 4.0) at 15 m resolution [19]. These masks were combined with specific hydrographic filters (hydrographic network) [20]: streams for *A. amblycephalum* and lakes for the remaining species, based on information from AmphibiaWeb, Amphibian Species of the World [15], and specialized literature. This procedure allowed us to obtain a more precise estimate of the occupied distribution (hereafter, refined areas of occupancy), thereby avoiding the overestimation inherent to MCP. Although we refer to our estimates as "refined areas of occupancy", these do not correspond to the formal Area of Occupancy (AOO) of the IUCN (which is calculated using 2 × 2 km grid cells), but rather to a refinement of the MCP with environmental filters.

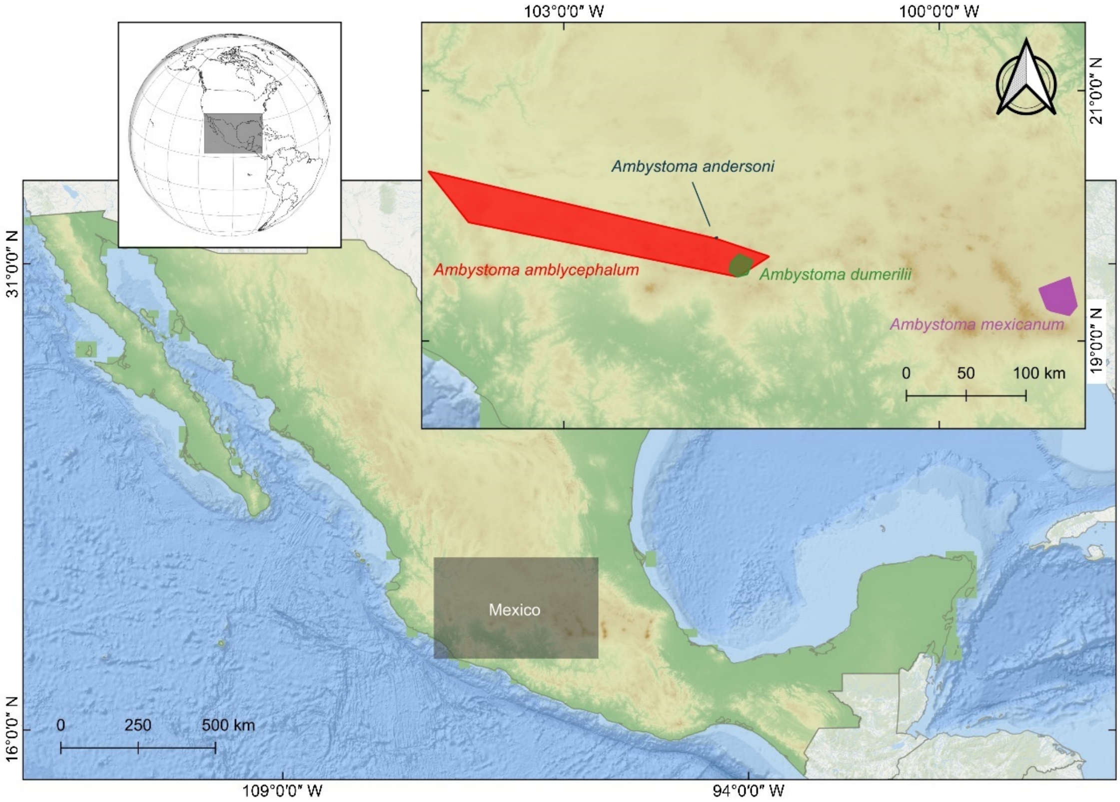


Figure 1. Minimum convex polygons (MCP) delineating the initial extent of occurrence (EOO) based on depurated records of the four *Ambystoma* species from central Mexico.

Additionally, we applied buffer zones to account for the limited mobility of these species: 100 m for *A. amblycephalum* [21], a stream species with occasional metamorphosis that enables limited terrestrial dispersal; and 50 m for the strictly paedomorphic lacustrine species *A. andersoni*, *A. dumerilii* and *A. mexicanum* [22], which remain permanently aquatic and exhibit minimal movement within their lake habitats. These buffers enabled the inclusion of transition zones and potential adjacent habitats.

2.3. Integration of Environmental Variables

From the depurated records, we extracted corresponding elevations using CEM 4.0 and calculated species-specific ranges through descriptive statistics (minimum and maximum elevation, median, and standard deviation), which helped delimit the refined areas of occupancy as described in the previous procedure. We incorporated land use and vegetation coverages from CONABIO [20] Series VII (30 m resolution), extracting the specific category for each record through spatial overlay in QGIS.

Climatic variables were derived from annual precipitation, maximum annual temperature, and minimum annual temperature data from MexHiResClimDB [23], a high-resolution (~500 m) database tailored to Mexico, ideal for species with restricted ranges. Monthly rasters of maximum temperature (tmax), minimum temperature (tmin), mean temperature (tmean), and precipitation (prec) for the period 1991–2020 were processed in R 4.3.1 [24] to generate the 19 standard bioclimatic variables (BIO1–BIO19; see Appendix A for complete variable definitions) by calculating monthly averages and applying moving-window functions for extreme quarters, following the procedure of Velasco and González-Salazar [25].

Bioclimatic values were extracted at presence points and analyzed through descriptive and exploratory statistical approaches in R (see Supplementary Material), including climatic space plots (BIO1 *vs*. BIO12) with 68% confidence ellipses, boxplots of key variables, principal component analysis (PCA), and bivariate density. For climatic space, we selected BIO1 (annual mean temperature) and BIO12 (annual precipitation) as the most relevant variables for delimiting thermal and hydrological niches in aquatic species dependent on permanent habitats [26,27]. Given their interpretative clarity and relevance for characterizing realized niches in this descriptive context, we prioritized the two-dimensional analysis with 68% ellipses (~1 SD), which focuses on the core of the realized niche and avoids the influence of outliers that would overestimate the potential niche [28]. Supplementary material includes a comparison with 95% ellipses (~2 SD) to explore peripheral variability.

2.4. Evaluation of Conservation Gaps and Effective Protection

To quantify the level of protection and vulnerability of the refined areas of occupancy for the four study species, we calculated two complementary indicators. As a positive conservation proxy, we determined the percentage of the distribution area overlapping with PA. Total areas of the refined polygons were calculated in km^2, and intersection with the official PA layer [20] was performed to obtain the overlap percentage. This indicator reflects the extent of legal protection and potential management measures in the species' distribution areas.

As a negative proxy, we used the Ecosystem Integrity Index (EII), a multifactorial indicator that assesses ecosystem health and functionality through the integration of metrics such as habitat fragmentation, biological connectivity, vegetation cover loss, and anthropogenic influence (e.g., roads, urbanization), based on satellite remote sensing data and spatial modeling [20,29]. The EII is normalized between 0 and 1 (base layer 2018 at 250 m), where values close to 0 indicate high degradation and low ecosystem integrity, and values close to 1 reflect ecosystems with high integrity and minimal alteration. This index provides a quantitative measure of environmental degradation across the distribution ranges, enabling identification of vulnerable areas beyond the mere presence of PA and highlighting cumulative impacts that could compromise the long-term viability of these threatened microendemic aquatic species. EII values were extracted directly from the national CONABIO [20] layer overlaid with the refined distribution polygons in QGIS, focusing on species-specific means and ranges to evaluate overall integrity.

3. Results

3.1. Spatial Distributions and Effective Protection

The refined areas of occupancy were small and varied widely in their level of effective protection (Table 1). *Ambystoma amblycephalum* exhibited the largest area (108.19 km^2), followed by *A. dumerilii* (74.32 km^2), *A. mexicanum* (15.14 km^2), and *A. andersoni* with the most reduced (0.38 km^2). Overlap with PA varied considerably: high in *A. mexicanum* (98.41%) and *A. andersoni* (86.84%), but null in *A. dumerilii* (0.00%) and very low in *A. amblycephalum* (4.79%). Mean EII values were generally low (0.05–0.43), with ranges indicating significant degradation in most occupied areas, particularly in *A. dumerilii* (mean 0.06 ± 0.07).

Table 1. Refined area of occupancy, overlap with Protected Areas (PA), and Ecosystem Integrity Index (EII, scale 0 = high degradation to 1 = high integrity) for four microendemic *Ambystoma* species in central Mexico.

Species	Refined Area of Occupancy (km^2)	Protection in PA (km^2/%)	EII (Mean ± SD; Range)
Ambystoma amblycephalum	108.19	5.19/4.79%	0.43 ± 0.23 (0.00–0.96)
Ambystoma andersoni	0.38	0.33/86.84%	0.39 ± 0.23 (0.00–0.97)
Ambystoma dumerilii	74.32	0.00/0.00%	0.06 ± 0.07 (0.00–0.26)
Ambystoma mexicanum	15.14	14.90/98.41%	0.05 ± 0.12 (0.00–0.97)

3.2. Altitudinal Characteristics and Land Use

Altitudinal ranges extracted from CEM 4.0 were remarkably narrow for all four species, reflecting their condition as strict microendemics dependent on specific aquatic habitats. *Ambystoma amblycephalum* presented the broadest range (1930–2014 m a.s.l.), while *A. andersoni* showed the most restricted (1984–1993 m a.s.l.). *A. dumerilii* had an intermediate range (2036–2359 m a.s.l.), and *A. mexicanum* recorded 2231–2343 m a.s.l. Central values (median) and variability (standard deviation), along with predominant land use and vegetation categories, are presented in Table 2.

This altitudinal narrowness is combined with a strong association to habitats altered by human activity: all species showed predominance of categories such as human settlements and rain-fed annual agriculture. *Ambystoma amblycephalum* was primarily linked to human settlements and rain-fed annual agriculture; *A. andersoni* to a mixture including tule and secondary vegetation; *A. dumerilii* exhibited the greatest diversity of uses (including irrigated agriculture, pine-oak forests, and induced grassland); and *A. mexicanum* to cultivated forest, halophytic grassland, and irrigated agriculture.

Table 2. Altitudinal ranges and main land use and vegetation categories (Series VII) associated with depurated presence records of four microendemic *Ambystoma* species in central Mexico.

Species	Altitudinal Range (m)	Median ± SD (m)	Land Use and Vegetation Categories (Series VII)
Ambystoma amblycephalum	1930–2014	1972 ± 105.5	Human settlements, Rain-fed annual agriculture
Ambystoma andersoni	1984–1993	1987 ± 2.77	Human settlements, Tule, Rain-fed annual agriculture, Shrub secondary vegetation, Pine-oak forest, Oak forest, Induced grassland
Ambystoma dumerilii	2036–2359	2137 ± 85.5	Human settlements, Rain-fed and irrigated annual agriculture, Tule, Shrub/tree secondary vegetation of low deciduous forest, Pine-oak and oak forests, Induced grassland, Irrigated annual agriculture
Ambystoma mexicanum	2231–2343	2260 ± 28.69	Cultivated forest, Halophytic grassland, Rain-fed annual agriculture, Human settlements, Irrigated annual agriculture

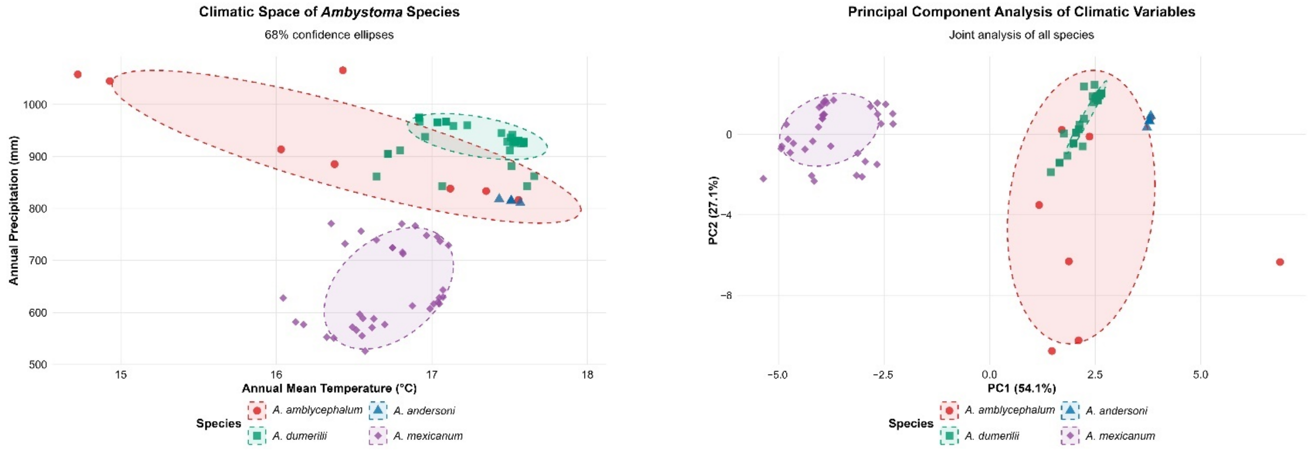
3.3. Climatic Niches

Values of annual mean temperature (BIO1) and annual precipitation (BIO12) revealed differentiated and notably restricted climatic niches among the four species (Table 3). *Ambystoma andersoni* presented the warmest and driest niche (BIO1: 17.5 °C, BIO12: 815 mm), with an extremely compact range. *A. mexicanum* showed the coldest conditions and lowest precipitation (BIO1: 16.7 °C, BIO12: 649 mm), while *A. amblycephalum* and *A. dumerilii* occupied intermediate positions, with greater precipitation variability. These patterns are represented in the two-dimensional climatic space (BIO1 *vs*. BIO12) with 68% confidence ellipses, where minimal overlap between species is observed, and a particularly compact cluster is evident in *A. andersoni* (Figure 2a).

Table 3. Mean values and ranges of annual mean temperature (BIO1, °C) and annual precipitation (BIO12, mm) extracted from MexHiResClimDB at the depurated presence records of the four *Ambystoma* species from central Mexico.

Species	BIO1: Annual Mean Temperature (°C)	BIO1 Range (°C)	BIO12: Annual Precipitation (mm)	BIO12 Range (mm)
Ambystoma amblycephalum	16.4	14.7–17.6	937	816–1066
Ambystoma andersoni	17.5	17.4–17.6	815	811–818
Ambystoma dumerilii	17.3	16.7–17.7	931	843–975
Ambystoma mexicanum	16.7	16.0–17.1	649	526–771

The joint principal component analysis (PCA) of the 19 bioclimatic variables confirmed a clear separation among species in multivariate space (Figure 2b). The first principal component (PC1, 54.1% explained variance) was primarily driven by temperature gradients (including BIO1, BIO5, BIO10, and BIO4), differentiating colder and more climatically seasonal niches (*A. amblycephalum* and *A. mexicanum*, negative and dispersed positions) from warmer and more stable ones (*A. dumerilii* and *A. andersoni*, positive and concentrated positions). The second component (PC2, 27.1% variance) was dominated by precipitation and seasonality-driven variables (mainly BIO12, BIO16, and BIO15), highlighting greater precipitation variability (both in total amount and seasonal distribution) for *A. amblycephalum* and *A. mexicanum*.



(**a**) (**b**)

Figure 2. Climatic analysis of the four *Ambystoma* species in central Mexico: (**a**) Two-dimensional climatic space based on annual mean temperature (BIO1, °C) and annual precipitation (BIO12, mm); (**b**) Joint principal component analysis (PCA) of the 19 bioclimatic variables. Scores of the records are shown on PC1 (54.1% explained variance) *vs*. PC2 (27.1% explained variance). PC1 is dominated by temperature gradients (primarily BIO1, BIO5, BIO10, and BIO4); PC2 by precipitation and seasonality variables (primarily BIO12, BIO16, and BIO15). See Appendix A for the names of the bioclimatic variables. Note 1: sample sizes vary among species (n = 6–36), therefore, confidence ellipses represent central tendency rather than robust niche boundaries. Note 2: The 68% confidence ellipse for *A. andersoni* is present but not visible at this scale due to extreme niche compactness (area = 0.14 units^2); see Table S1 for complete ellipse parameters.

Boxplots of key variables (BIO1, BIO12, BIO4, and BIO15) are presented in the Supplementary Material (Figure S1), highlighting greater thermal seasonality in *A. dumerilii* and *A. mexicanum*, and high precipitation seasonality (>95%) across all species, with greater dispersion in *A. amblycephalum* and *A. dumerilii*.

An exploratory comparison with 95% confidence ellipses is included in the Supplementary Material (Figure S2), which expands the climatic niche areas by an average factor of 2.63 relative to 68% (Table S1). This reveals marginal additional overlap in some species pairs, although central separation remains clear and the compactness of *A. andersoni* remains evident.

4. Discussion

4.1. Reduced Areas of Occupancy and Fragmentation as Defining Traits

The refined area of occupancy values obtained in this study differ notably from the official estimates reported by the IUCN for these species. While the IUCN reports an AOO of 18 km^2 for *A. amblycephalum* [30], our results reveal a refined area of 108.19 km^2, nearly six times larger. This discrepancy can be attributed primarily to the recent discovery of fragmented remnant populations in streams of the Michoacán region [7], which were not considered in the original 2016 assessment. Similarly, for *A. andersoni* we contrast our refined area of 0.38 km^2 against an EOO of 0.35 km^2 reported by the IUCN [31], showing consistency in the extreme restriction of its geographic range. For *A. dumerilii*, our area of 74.32 km^2 exceeds the IUCN AOO of 6 km^2 [32], likely due to the application of a 50 m buffer around Lake Pátzcuaro, which captures adjacent lacustrine habitats not included in the original assessment. Finally, *A. mexicanum* presents a refined area of 15.14 km^2, considerably smaller than the EOO of 467 km^2 reported by the IUCN [33], reflecting the drastic contraction of its distribution towards the southern remnants of Lake Xochimilco [10]. These comparisons underscore the importance of periodically updating threat assessments with recent field data and refined methodologies that incorporate specific environmental masks and filters for sensitive species such as these amphibians.

Our results reveal that the refined areas of occupancy for the four *Ambystoma* species are extremely small and markedly fragmented, with values ranging from 0.38 km^2 in *A. andersoni* to 108.19 km^2 in *A. amblycephalum* (Table 1). These extents are consistent with the strict microendemism pattern that characterizes salamanders in the TMVB [4,5], but highlight exceptional vulnerability when compared to other Neotropical amphibians. For example, the refined area of occupancy of *A. andersoni* (0.38 km^2) represents less than 5% of the area reported for *Pristimantis thymalopsoides* in the Ecuadorian Andes (~8 km^2) [34], a species already considered CR due to its extremely restricted distribution. It is even significantly smaller than that of miniaturized plethodontid salamanders of the genus *Thorius* in Mexico, such as *T. tlaxiacus* (~16 km^2) [35], or that of *Pseudoeurycea robertsi* (8 km^2) [36], another microendemic salamander from Nevado de Toluca categorized as CR. Even within *Ambystoma*, the range of *A. andersoni* is drastically smaller than that of *A. tigrinum* in North America (3,349,328 km^2) [37] or *A. gracile* in the northwestern United States (807,275 km^2) [38]. The reduced area of occupancy of *A. andersoni*, in particular, represents one of the most restricted geographic ranges documented for the genus, comparable only to isolated populations of *Ambystoma* in central Mexico [6].

4.2. Protection Gaps and Insufficient Nominal Protection

Unequal overlap with PA constitutes one of the most alarming findings. Although *A. mexicanum* and *A. andersoni* exhibit apparently high coverages (98.41% and 86.84%, respectively), these figures mask limited effective protection: the total area of *A. andersoni* is so small that even a high proportion represents less than 0.4 km^2 under legal protection. More critically, *A. amblycephalum* and *A. dumerilii* show overlaps below 5%, placing them in a condition of "nominal protection" (presence in risk lists) where most of their distribution remains outside any conservation management scheme. This pattern of protection gaps aligns with recent diagnoses for *A. altamirani* in Bosque de Agua, where less than 2% of suitable habitat lies within a PA [11], pointing to a systemic crisis of coverage for aquatic microendemics in the region.

4.3. Ecosystem Degradation and Conservation Paradox

Ecosystem Integrity Index values between 0.05 and 0.43 confirm that even within the identified occupied areas, ecosystems have suffered severe degradation. The average of 0.06 ± 0.07 for *A. dumerilii* is particularly concerning, indicating that virtually its entire distribution in Lake Pátzcuaro operates under conditions of high anthropogenic disturbance. These levels are comparable to those reported for *A.*

ordinarium in degraded pine-oak forests of Michoacán [39], and demonstrate that legal protection alone does not guarantee ecosystem functionality. The discrepancy between nominal protection (presence in risk lists) and actual habitat integrity represents a critical conservation paradox: the species are "protected on paper" but persist within functionally collapsed ecosystems [40], as quantified by the low EII values reported here.

4.4. Realized Climatic and Vulnerability to Global Change

The climatic conditions we identified (annual mean temperatures between 16–17.5 °C and precipitation of 650–930 mm) reflect climatic conditions associated with their restricted distributions that, while enabling the adaptive radiation of *Ambystoma* in the TMVB [4], currently constitute a factor of hypersensitivity. The narrow range of temperatures observed for *A. andersoni* (range 17.43–17.57 °C) and the low precipitation associated with *A. mexicanum* (526–771 mm) suggest limited tolerance margins in the face of increasing climatic variability. This specialization correlates with narrow altitudinal ranges (1930–2359 m a.s.l.), where modest upward shifts in distribution as projected for Mexican amphibians under climate change scenarios could result in the loss of thermally suitable habitats without upward migration alternatives due to the volcanic dome topography [5,12].

4.5. Persistence in Anthropized Matrices and "Refuge in Disturbance"

The dominance of anthropized land uses in presence records (human settlements, rain-fed and irrigated agriculture, secondary vegetation) indicates that remnant populations persist predominantly in highly anthropized landscapes. For *A. amblycephalum*, the association with rain-fed annual agriculture in high-mountain streams suggests forced dependence on modified hydrological systems, where hydrological regulation and agrochemical contamination represent constant threats. In *A. mexicanum*, the presence in cultivated forests and halophytic grasslands in southern Xochimilco documents historical contraction from natural lacustrine habitats to anthropogenic remnants [10], a "refuge in disturbance" pattern that, while enabling temporary persistence of the species, exposes populations to extreme fluctuations in water quality and competition with invasive species, and emerging diseases such as chytridiomycosis [10,14,41].

4.6. Syndrome of Risk Factors in TMVB Microendemics

Overall, these findings confirm that microendemism in *Ambystoma* of the TMVB constitutes a syndrome of risk factors: spatially restricted distributions, obligate dependence on permanent aquatic habitats, narrow realized climatic specialization, and location in landscapes dominated by human activity. Habitat fragmentation acts synergistically with these factors, reducing population connectivity at scales that compromise long-term demographic viability. The 50–100 m buffers zones applied, based on the limited mobility of these salamanders [21,22], likely underestimate real connectivity requirements for maintaining effective gene flow, particularly in *A. amblycephalum,* where metamorphosis in some individuals may temporarily increase terrestrial habitat needs. The convergence of these factors positions these four species on a trajectory of decline that, without urgent active management interventions, is at risk of extinction over the coming decades, replicating the pattern already observed in the extirpation of *A. mexicanum* from Lake Chalco and the severe contraction in Xochimilco [10,15].

4.7. Conservation Implications

Conservation strategies for aquatic microendemics in the TMVB must move beyond nominal protection toward active and integrated management. Based on our results and existing frameworks for threatened amphibians in Mexico [40], we recommend: (1) quantitative risk recategorization under revised IUCN criteria, accounting for actual occupancy areas and low ecosystem integrity values; (2) immediate creation of micro-reserves for species with ranges <1 km^2, such as *A. andersoni*; (3) connectivity expansion through restored aquatic corridors; (4) systematic population monitoring with emphasis on emerging threats such as chytridiomycosis [14]; and (5) community involvement through sustainable management of individual private landholdings, ejidos (communal lands), and environmental education. The convergence of microendemism, climatic conditions associated with their restricted distributions, and anthropized matrices places these species on a trajectory of decline that can only be reversed through the simultaneous and urgent implementation of these measures.

Additionally, given the virtually impossible natural dispersal among these geographically isolated populations, assisted migration and controlled individual exchange programs should be evaluated as complementary conservation tools, provided that prior genetic characterization and strict biosecurity protocols are in place to minimize risks of disease transmission and disruption of locally adapted genotypes [14].

5. Conclusions

The gap between legal protection and effective protection for four microendemic *Ambystoma* species in the TMVB refined areas of occupancy ranging from 0.38 to 108.19 km^2, null or minimal overlaps with protected areas in *A. amblycephalum* and *A. dumerilii*, and EII values revealing severe degradation across their entire distributions underscores the inadequacy of current conservation strategies for specialized aquatic amphibians in anthropized landscapes. Notably, our refined estimates for *A. amblycephalum* and *A. dumerilii* exceed previous IUCN assessments, reflecting the discovery of additional remnant populations and improved mapping resolution; however, these populations persist predominantly outside protected areas in functionally degraded ecosystems, meaning that the expanded distribution knowledge documented here does not translate into improved conservation or population viability. The combination of narrow climatic niches, restricted altitudinal ranges, and persistence in matrices dominated by agriculture and urbanization places these species on a trajectory of accelerated decline. This pattern can only be reversed through the immediate expansion of effective protected areas, restoration of connectivity between fragmented habitats, and integrated adaptive management with local communities. Our results demonstrate that microendemism in the TMVB is not merely a biogeographic attribute but a synergistic risk factor that urgently demands prioritizing this region as a critical hotspot for global amphibian conservation.

Supplementary Materials

The following supporting information can be found at: https://www.sciepublish.com/article/pii/1002, Figure S1: Boxplots of key bioclimatic variables for the four *Ambystoma* species: BIO1 (annual mean temperature, °C), BIO12 (annual precipitation, mm), BIO4 (temperature seasonality), and BIO15 (precipitation seasonality). The boxplots show the median (central line), interquartile range (box), and full range (whiskers); Figure S2: Exploratory comparison of climatic niches with 95% confidence ellipses. (a) Two-dimensional climatic space based on annual mean temperature (BIO1, °C) *vs*. annual precipitation (BIO12, mm), with points by species and 95% ellipses; (b) Joint principal component analysis (PCA) of the 19 bioclimatic variables with 95% ellipses; Table S1: Comparison of confidence ellipse areas at 68% and 95% in the climatic space (BIO1, annual mean temperature *vs*. BIO12, annual precipitation), along with the centers (mean coordinates) for each species.

Appendix A

Table A1. Description of the 19 standard bioclimatic variables (BIO1–BIO19) generated from MexHiResClimDB.

Code	Name	Description
BIO1	Annual Mean Temperature	Annual mean temperature (°C)
BIO2	Mean Diurnal Range	Mean of monthly (max temp − min temp) (°C)
BIO3	Isothermality (BIO2/BIO7 × 100)	Ratio of diurnal range to annual temperature range (×100)
BIO4	Temperature Seasonality (standard deviation × 100)	Standard deviation of monthly mean temperatures (×100)
BIO5	Max Temperature of Warmest Month	Highest temperature of any month (°C)
BIO6	Min Temperature of Coldest Month	Lowest temperature of any month (°C)
BIO7	Temperature Annual Range (BIO5–BIO6)	Difference between warmest and coldest month temperatures (°C)
BIO8	Mean Temperature of Wettest Quarter	Mean temperature during the three wettest months (°C)
BIO9	Mean Temperature of Driest Quarter	Mean temperature during the three driest months (°C)
BIO10	Mean Temperature of Warmest Quarter	Mean temperature during the three warmest months (°C)
BIO11	Mean Temperature of Coldest Quarter	Mean temperature during the three coldest months (°C)
BIO12	Annual Precipitation	Total annual precipitation (mm)
BIO13	Precipitation of Wettest Month	Precipitation of the wettest month (mm)
BIO14	Precipitation of the Driest Month	Precipitation of the driest month (mm)
BIO15	Precipitation Seasonality (Coefficient of Variation)	Coefficient of variation of monthly precipitation (×100)
BIO16	Precipitation of the Wettest Quarter	Precipitation during the three wettest months (mm)
BIO17	Precipitation of the Driest Quarter	Precipitation during the three driest months (mm)
BIO18	Precipitation of Warmest Quarter	Precipitation during the three warmest months (mm)
BIO19	Precipitation of the Coldest Quarter	Precipitation during the three coldest months (mm)

Note: These variables follow the standard definitions used in WorldClim [42] and were generated from monthly rasters (tmax, tmin, tmean, prec) of MexHiResClimDB [23] for the period 1991–2020.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

Claude AI (Claude Sonnet 4.6, Anthropic, San Francisco, CA, USA) was used to improve the writing style and clarity of the text; Kimi (Kimi K2.5, Moonshot AI, Beijing, China) was employed to eliminate redundancies and improve content cohesion; and Grok AI (Grok 4.1, xAI, Palo Alto, CA, USA) was used to verify English grammar and language accuracy. All AI-generated suggestions were carefully reviewed and validated by the authors, who take full responsibility for the manuscript content. These artificial intelligence tools were used solely for editorial assistance and did not contribute to the scientific content, data analysis, or intellectual conception of this work.

Author Contributions

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The raw data supporting the conclusions of this article will be made available by the authors on request.

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