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The long-term impact of the pathogenic fungus *Batrachochytrium dendrobatidis* on a neotropical salamander community in Mexico

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***Abstract.*—Mexico ranks first in salamander diversity in the Neotropical region, and most species are threatened by habitat loss and emerging diseases. The pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) is associated with severe amphibian population declines worldwide, particularly in the Neotropics, but the extent of the declines at the amphibian community level is not completely understood. In central Veracruz, Mexico, the fungal pathogen *Bd* has been detected in the salamander hotspot on the Cofre de Perote volcano. In this study, we evaluated the presence of *Bd* and its potential link to population declines and changes in the salamander community in this region. First, we characterized the current salamander community along the elevational gradient of Cofre de Perote through fieldwork. Then, we assessed shifts in the salamander community in historical and contemporary frameworks covering a time window of more than 40 years. In addition, we investigated the presence of *Bd* over time in this region in both formalin-preserved and recently collected specimens. Finally, we analyzed the associations between habitat and host species traits with current *Bd* prevalence and infection load. The results indicated that the salamander community of Cofre de Perote experienced significant reductions in abundance, species richness, and diversity over the past ~40 years. The declines in two species distributed at 2,400 m asl are potentially related to *Bd*. Meanwhile, population fluctuations recorded before the first appearance of *Bd* in the region or in recent sampling were probably related to habitat loss and stochastic factors. The prevalence of *Bd* was mainly influenced by host species, while infection load was associated with tree density. The results suggest that *Bd* is likely one of several factors affecting these species and that its presence in this area of high salamander diversity remains a threat.**

Keywords. Amphibian declines, chytridiomycosis, elevational gradient, historic comparison, Plethodontidae, Veracruz

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Introduction

Salamanders from the Neotropical region exhibit remarkable diversity, constituting over 40% of salamander species worldwide (AmphibiaWeb 2024; Frost 2023; Wake 1987). This diversity is particularly pronounced in mountainous areas, where the intricate topography and low vagility of these taxa contribute to high species richness and an elevated proportion of endemism within relatively small areas (Kozak 2017;

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Wake and Lynch 1976; Wake 1987). Mexico stands out as a global hotspot for salamander diversity within the Neotropics, harboring almost 20% of the world's salamander species (AmphibiaWeb 2024). With a staggering 159 salamander species documented in Mexico, over 80% of which are endemic to the country (Parra-Olea et al. 2014), it serves as a critical reservoir of amphibian biodiversity. However, this diversity is being severely threatened, with an alarming 70% of Mexican salamander species classified as threatened according to the International Union for Conservation of Nature (IUCN 2024).

In Mexico, pervasive habitat loss and degradation, coupled with pollution, invasive species, and potential climate change and emerging diseases, have profoundly impacted various salamander populations (Parra-Olea et al. 2014; Scheele et al. 2019; Young et al. 2001). Of particular concern is the rise of diseases, which can lead to amphibian extirpations even in pristine habitats (Adams et al. 2017; Lips et al. 2006; Scheele et al. 2019), particularly for species with restricted distributions, such as endemic Mexican salamander species. However, assessing the population trends of tropical salamanders over time presents significant challenges due to their secretive habits and varied activity patterns (Parra-Olea et al. 1999; Rovito et al. 2009; Wake 1987), which complicates studies on the association between emerging diseases and salamander population dynamics.

Among the emerging diseases affecting amphibians, chytridiomycosis stands out as the most significant in terms of the number of species affected and the geographic regions impacted (Scheele et al. 2019). This fungal disease, caused by *Batrachochytrium dendrobatidis* (*Bd*), targets the keratinized parts of amphibian skin and the tadpole mouth, disrupting respiration and ionic exchange across the epidermis, and ultimately leading to death by cardiac arrest (Voyles et al. 2007, 2009). However, chytridiomycosis does not affect amphibian species randomly, as specific environmental conditions are necessary for *Bd* survival and proliferation (Becker et al. 2015; Piotrowski et al. 2004). Optimal conditions for *Bd* growth, including ambient temperatures between 17 and 23 °C and high ambient humidity, are coincidentally found in habitats that host exceptional amphibian diversity, especially salamanders (Scheele et al. 2019; Vredenburg et al. 2010).

In Mexico, severe declines in amphibian populations were observed in the 1970s and 1980s, which were initially attributed to the introduction of *Bd* during that period (Cheng et al. 2011). However, recent studies have revealed the presence of *Bd* in northwestern Mexico since the late 19th century, with sporadic high prevalence peaks observed since the 1930s (Basanta et al. 2021). Alternative hypotheses for the prevalence peaks in the 1970s include the introduction of a more virulent lineage, an increase in the virulence of the native lineage, or immunological factors rendering salamanders more susceptible to pathogens (Adams et al. 2017; Basanta et al. 2021; Mendoza-Almeralla et al. 2015). Currently, over 100 Mexican amphibian species across the country have tested positive for *Bd* (Basanta et al. 2021; Bolom-Huet et al. 2019; Mendoza-Almeralla et al. 2015), potentially contributing to the population declines recorded predominantly in Oaxaca, Chiapas, and Veracruz during the 1970s and 1980s (Cheng et al. 2011; Lips et al. 2004; Rovito et al. 2009).

A salamander hotspot within Mexico occurs in the central Veracruz. On the Cofre de Perote volcano, 21 plethodontid salamander species inhabit a relatively small area (García-Castillo et al. 2018; Hanken and Wake 1998; Parra-Olea et al. 1999, 2001, 2010, 2020; Sandoval-Comte et al. 2017; Wake et al. 1992). This remarkable diversity faces threats such as habitat loss (up to 3.7% annually on Cofre de Perote), pollution, and climate change (INEGI 1991; Muñoz-Delfín 2012; Villers-Ruiz and Castañeda-Aguado 2013). Although salamander population declines in central Veracruz are poorly documented, *Bd* has been detected in 13 amphibian species in this region (Basanta et al. 2021; Cheng et al. 2011; Murrieta-Galindo et al. 2014). Furthermore, examples of salamander population declines were observed in *Thorius pennatulus* on the Pico de Orizaba and the Cofre de Perote volcano, which potentially occurred during the 1980s and 1990s, highlighting the vulnerability of these species (Sandoval-Comte et al. 2012). However, the factors contributing to salamander population declines, and their broader implications at the community level, remain poorly understood.

This study aims to assess the historical and current presence of *Bd* and its potential correlation with population declines and changes in the salamander community on the Cofre de Perote Volcano in central Veracruz, Mexico. By characterizing the salamander community over the past four decades and identifying species experiencing population declines, we aim to elucidate the relationship between declines and the historical presence of *Bd*. Furthermore, we seek to evaluate the association between current *Bd* prevalence and infection loads with habitat characteristics and host species attributes. The overall goal is to shed light on the factors driving salamander declines and the long-term effect of the presence of this fungal pathogen in a salamander community.

Materials and Methods

Study area

The study area encompasses the slopes of the Cofre de Perote volcano in central Veracruz, Mexico, situated between 19°31'48"–19°4'12" N and 96°55'12"–97°4'48" W, spanning elevations ranging from ca. 1,100 to ca. 3,500 meters above sea level (m asl) (Fig. 1). A total of 12 localities were sampled within this region, including: (a) Barranca de Teocelo (ca. 1,100 m asl), (b) Citlalapa (ca. 1,400 m asl), (c) Martinica (ca. 1,700 m asl), (d) Xico Viejo (ca. 1,600 m asl), (e) Coxmatla (ca. 2,000 m asl), (f) Cortadura (ca. 2,000 m asl), (g) La Joya (ca. 2,000 m asl),

(h) Ixhuacan (ca. 2,100 m asl), (i) Loma Alta (ca. 2,100 m asl), (j) Las Vigas (ca. 2,400 m asl), (k) Las Lajas (ca. 3,000 m asl), and (l) El Conejo (ca. 3,500 m asl).

Four of these localities (a, g, j, and k) are historical localities that were previously sampled between 1972 and 1997 by Dr. David B. Wake and collaborators from the University of California, Berkeley (Parra-Olea et al. 1999; Wake and Lynch 1976; Wake et al. 1992).

Salamander sampling and data collection

Systematic fieldwork was conducted in six localities (a, d, g, j, k, and l), including the four historical localities, between December 2015 and July 2017. These six locations were visited on five occasions, with extensive sampling efforts undertaken in December 2015, April, July, and October 2016, and July 2017, covering the dry (December and April) and rainy seasons (July and October).

A team of five herpetologists conducted diurnal and nocturnal surveys lasting four hours at each locality, employing the visual encounter survey method (Heyer et al. 1994). Salamanders encountered were captured using the inner part of a new plastic bag and held until processing. Identification of salamander species was performed using specialized literature (Darda 1994; García-Castillo et al. 2018; Hanken and Wake 1998; Parra-Olea et al. 2001, 2005, 2010, 2020; Sandoval-Comte et al. 2017). Voucher specimens, comprising one to three individuals of each species, were collected and deposited in the National Collection of Amphibians and Reptiles (CNAR) at the Instituto de Biologia, UNAM, under collecting permits SGPA/DGVS/00947/16, SGPA/DGVS/002176/18, and SGPA/DGVS/03368/19.

To obtain information on salamander community characteristics (abundance, species richness, structure, evenness, and diversity) in the past, we obtained data from the field notebooks of Dr. David B. Wake, which are stored at the Museum of Vertebrate Zoology at the University of California at Berkeley. We were able to obtain abundance data for salamander species starting in the 1970s at the four historical localities, and they were surveyed mainly during the rainy seasons of 1972, 1974, 1976, 1979, 1981, and 1997 (Hanken and Wake 1998; Parra-Olea et al. 1999; Wake and Lynch 1976; Wake et al. 1992) (Supplementary material I).

To evaluate changes in salamander community characteristics over time, we selected a historic period (from July 1976 to October 1981) and a recent period (from October 2016 to July 2017), which are comparable since in each period, the four historical localities were sampled in the same months and similar sampling efforts were applied. In addition, we accounted for a key constraint in the historical data, where salamander counting by species ceased at 100 individuals for the common species (Hanken and Wake 1998; Parra-Olea et al. 1999; Wake et al. 1992). Consequently, counting also ceased upon reaching 100 individuals for species

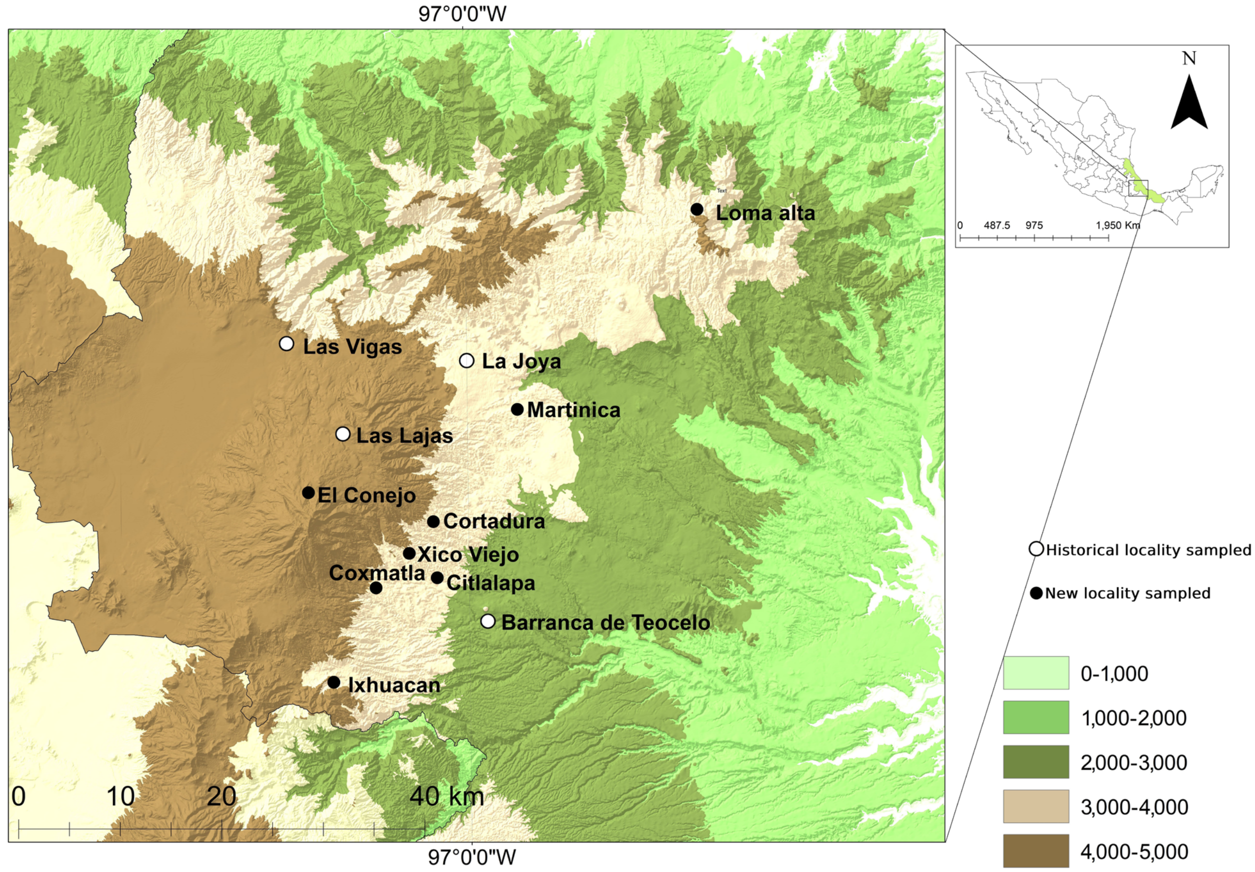

Fig. 1. Study area. Locations of the 12 sampling localities, including four historical localities, in the central Veracruz state region, Mexico.

that surpassed that abundance in the recent sampling.

Habitat characterization and host species attributes

Habitat characterization was conducted across all localities (a–l) by establishing three 25 x 25 m quadrants (625 m^2) at each site. The following habitat characteristics were measured: elevation, tree density, canopy cover, and leaf litter depth. Elevation data were recorded using a Garmin Etrex GPS unit. Tree density was determined by counting the number of trees with a diameter greater than 10 cm within each quadrant. Canopy cover density was assessed by capturing images at 20 random points within each quadrant during high-luminosity diurnal hours, using a Canon Power Shot SX420 camera set to an f11 diaphragm aperture and 1/124 shutter speed. The percentage of pixels occupied by vegetation in the images was calculated using Photoshop software. Leaf litter depth was estimated at 20 random points within each quadrant by inserting a graduated ruler into the leaf litter until it reached the soil. Climatic variables (minimum and maximum annual temperatures) for each locality were sourced from literature sources with field data spanning from 1973 to 1987 (Gómez 1991, 1993; Gómez and Angulo 1993; Gómez and Soto 1990; Soto and Gómez 1990). Habitat characteristics are summarized in Supplementary material III.

Host species attributes known to be associated with *Bd* infection patterns (Rebollar et al. 2014; Scheele et al. 2019) were investigated for all the salamander species recorded. The attributes evaluated were host species (taxonomic identity), maximum snout-vent length (SVL), elevational affinity, and microhabitat use. Maximum SVL data for each species were sourced from specialized literature (García-Castillo et al. 2018; Hanken and Wake 1998; Parra-Olea et al. 2001, 2005, 2010, 2020). Elevational affinity categories were assigned based on the predominant altitude range where individuals of each species were found, and categorized as follows: from low mid-elevation lands (1,100–1,499 m asl), from mid-elevation lands (1,500–1,999 m asl), from high mid-elevation lands (2,000–2,499 m asl), from high-elevation lands (> 2,500 m asl), and widely distributed (1,100–3,000 m asl). Microhabitat use information was extracted from specialized literature sources (García-Castillo et al. 2018; Hanken and Wake 1998; Parra-Olea et al. 2001, 2010, 2020; Ramírez-Bautista and Arizmendi 2004a–c) and categorized into three groups: terrestrial, facultative arboreal, and arboreal (Supplementary material III).

Recent and historical sampling of *Bd*

To investigate the presence of *Bd* in the study area over time, a noninvasive skin swab sampling method was used for both recent and historical samples. This approach minimizes specimen damage compared to histological detection methods (Boyle et al. 2004). During sample collection, sterile gloves were employed and changed between individuals to prevent cross-contamination.

For recent sampling, individuals were rinsed with approximately 50 ml of sterile water before swabbing to mitigate potential PCR inhibitors present in the soil (Watson and Blackwell 2000). In contrast, for historical sampling, museum specimens were rinsed with 70% ethanol before swabbing to reduce the risk of cross-contamination by other specimens in the same jar (Cheng et al. 2011). In both recent and historical sampling, each specimen was swabbed 30 times over the ventral surface from the neck to the vent, as well as along the limbs and tail, following a modification of Briggs' method (2009). The skin swab samples obtained were stored in 1.5 mL microcentrifuge tubes containing 96% ethanol at 4 °C until processing (Hyatt et al. 2007).

Additional skin swab samples were collected in October 2018 and October 2019 at select localities (b, c, e, f, h, and i) to enhance the dataset on current *Bd* presence in central Veracruz (Supplementary material I). The goal was to sample the same salamander species recorded during recent sampling as extensively as possible, thereby increasing the sample size per species. The collection and storage of skin swab samples followed the previously described methodology.

DNA extraction and *Bd* detection

DNA extraction from skin swab samples was conducted using two methods: (1) the PrepMan Ultra protocol (Applied Biosystems, Carlsbad, CA, USA) with 50 µl per sample for 95 historical samples and 71 recent samples, and (2) DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA) following the manufacturer's instructions, including pretreatment with lysozyme and a final elution volume of 100 µl. This was performed as part of a concurrent study on bacterial microbiome characterization in these samples. Method 2 was employed for an additional 174 recent samples. To minimize the presence of salts and proteins that could impact the reaction, both extractions from methods 1 and 2 were diluted 1:10 with water and run in duplicate using TaqMan real-time PCR (qPCR) following Boyle et al. (2004). Synthetic standards with 10, 10^2, 10^3, 10^4, and 10^5 copies of the internal transcribed spacer (ITS1) region of haplotype Bd_Hap001 were also included (Longo et al. 2013).

Each qPCR reaction comprised a final volume of 25 µl, containing 12.5 µl TaqMan Master Mix 2X, *Bd* primers at a final concentration of 900 nM, MGB probe at 250 mM, and 5 µl of DNA. Amplification was conducted using an Applied Biosystems Step One Real-Time System with initial denaturation at 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 sec and 60 °C for 1 min. One negative control was included per plate to detect false positives and one positive control with known zoospore numbers (100 zoospores of the MexMkt *Bd* strain).

To quantify the infection load, raw genomic outputs from qPCR for samples processed with extraction method 1 were multiplied by 80 to account for the extraction volume, dilution factor, and template reaction volume used (Boyle et al. 2004; Hyatt et al. 2007). For samples processed with extraction method 2, raw genomic outputs were converted to zoospore equivalents (ZEs) by multiplying by the ratio of zoospore numbers from the positive control divided by their raw genomic output. The resulting ZEs number was then multiplied by 160, to account for the extraction volume in method 2 (100 µl, or twice that of method 1), dilution factor, and template reaction volume used. Samples were considered *Bd* positive if both replicates yielded values above 0.1 ZE and exhibited exponential amplification curves before cycle 46.

Data analysis

To analyze the community characteristics over time, the species distributed across the four historical localities (a, g, j, and k) were consolidated into a single community. Data from years when all four locations were sampled in the same month were considered, and the information was pooled into two periods: a historic period (1976–1981) and a recent period (2016–2017) (Supplementary material I).

Community structure was assessed using abundance-range curves (Magurran 2004), where the number of individuals detected per species served as a measure of relative abundance, and species richness was measured by the total number of species recorded (Meza-Parral and Pineda 2015). Relative abundance by species against species rank (i.e., the order of species from most to least abundant) (Magurran 2004) was plotted for each period. To test for differences in structure between the curves, an analysis of covariance was conducted with the logarithm of relative abundances as the dependent variable, species rank as a regressor, and the period as a factor (Hernández-Ordóñez et al. 2015), and this analysis was conducted using the Stats package in R 4.3.0 (R Core Team 2020).

As a measure of diversity, the equivalent number of species (D1) or exponential of the Shannon index was calculated using the maximum likelihood method, along with its 95% confidence intervals, using the SpadeR package in R (Chao 2016). The D1 metric indicates the number of species in a virtual community where all species have the same abundance, weighting each by its observed relative abundance (Jost 2006).

To identify salamander population declines, the abundances per species by year in each historical locality were observed and the percentage of change (increase or decline) from one year to the following year were calculated. Significance tests for percentages were not conducted due to the extremely small sample sizes in most cases. For species exhibiting declining trends, the number of individuals of each species recorded by locality in each year were plotted. Species were diagnosed as showing population declines if their abundance decreased significantly by more than 85% without evidence of recovery in subsequent years, following the criteria outlined by Lips et al. (2003). Conversely, an increase in population abundance was assigned if it exceeded 85% from one year to the next.

The prevalence of *Bd* was determined for all salamanders recorded by locality per year, along with 95% confidence intervals, using the Clopper-Pearson test implemented in the GenBinomApps package in R. The infection loads obtained for recent samples could not be directly compared with those obtained for historical samples due to the degradation of fungal genetic material in the formalin-preserved specimens (Cheng et al. 2011). Consequently, only the presence or absence of *Bd* was analyzed in comparisons involving both recent and historical samples.

To test the hypothesis of an association between salamander species abundance variation and *Bd* prevalence over time, semiparametric regression models with an identity link function were employed, assuming a Gaussian distribution of salamander abundance by locality and year, which was normalized by log transformation. The models evaluated incorporated the nonmetric variables of *Bd* prevalence and year of sampling, as well as their additive effect, as predictors, and salamander abundances as response variables, using the regression splines method (Nurcahayani et al. 2021). The best model was determined via the maximum likelihood ratio test (MLRT) and the corrected Akaike information criterion for small sample sizes (AICc). Analyses were conducted with the Stats package in R.

To investigate the correlations between the current prevalence of *Bd* with habitat characteristics and host species attributes, Fisher's exact test followed by post hoc analysis with Benjamini–Hochberg correction was employed. These analyses were conducted to compare *Bd* prevalence across 12 localities and among 13 salamander species using the Stats package in R. Subsequently, logistic regression models were used to assess the associations between habitat characteristics and host species attributes with *Bd* prevalence. The models incorporated a logit link function, considering a binomial distribution of the presence-absence of *Bd* as the dependent variable. Predictive variables included locality, elevation, tree density, canopy cover percentage, leaf litter depth, maximum annual temperature, minimum annual temperature, host species, maximum snout-vent length (SVL), elevational affinity category, and microhabitat use.

To streamline interpretation and restrict the set of models considered, predictive variable combinations and interaction terms were omitted. The best logistic regression models were selected based on the lowest AICc, following Burnham and Anderson (2004). Furthermore, the relative importance of each predictor was calculated using Akaike weights and MLRT was conducted for the best model in each case. These analyses were performed using the qpcR, lmerTest, multcomp, and mgcv packages in R.

The infection load was compared across localities that had at least one individual positive for *Bd* using the non-parametric Kruskal-Wallis test, followed by pairwise Wilcoxon rank sum tests in the Stats package in R. Associations between *Bd* infection load, habitat characteristics, and host species attributes were assessed via general linear models using the identity link function, with *Bd* infection load normalized by log transformation and assumed to follow a Gaussian distribution. The best general linear models were selected based on the methods described above. All statistical analyses were performed in R version 4.3.0 (R Core Team 2023).

Results

Changes in salamander community characteristics over time

The salamander community exhibited notable differences between the historic period (1976–1981) and the recent period (2016–2017), with the historic period showing higher abundance, richness, and diversity. During the historic period, spanning from 1976 to 1981, a total of 587 salamanders representing 14 species from six genera, including two *Aquiloeurycea*, one *Bolitoglossa*, three *Chiropterotriton*, two *Isthmura*, four *Pseudoeurycea*, and two *Thorius* species. The dominant species included *Pseudoeurycea leprosa* and *Aquiloeurycea cephalica*, each with over 100 individuals, while the rarest species was *Bolitoglossa platydactyla*, with only one individual observed. In contrast, the recent period (2016–2017) survey data limited to the four historical localities yielded 372 salamanders comprising nine species from five genera: two *Aquiloeurycea*, one *Bolitoglossa*, three *Chiropterotriton*, two *Pseudoeurycea*, and one *Thorius* species. Like the historic period, *Pseudoeurycea leprosa* and *Aquiloeurycea cephalica* were the dominant species, each with over 100 individuals, while the rarest species observed were *Bolitoglossa platydactyla*, *Chiropterotriton totonacus*, and *Pseudoeurycea melanomolga*, each represented by a single individual. The structure of the abundance-range curve differed significantly between the historical and recent periods, as indicated by the covariance test (t = 20.647, $p < 0.001$) (see Fig. 2). Furthermore, there were notable differences in diversity between the two periods, with the historic period (1976–1981) exhibiting higher diversity with a D1 = 8.03 (95% CI: 7.45–8.60) than the recent period (2016–2017) with a D1 = 3.50 (95% CI: 3.15–4.02).

Throughout the entire time period analyzed (1972–2017), fluctuations in salamander abundances were observed across all species without exception (see Supplementary material IV). However, evidence of declines exceeding 85% was only apparent in four instances. At Barranca de Teocelo, while there was insufficient evidence to confirm declines in any species, the two species *Pseudoeurycea lineola* and *Thorius pennatulus* have not been recorded since 1979 (see Fig. 3A). At La Joya, *Isthmura gigantea* exhibited a relevant decline between 1974 and 1979, with a continuing decline in subsequent years (see Fig. 3B). Similarly, at La Joya, *Chiropterotriton lavae* showed declines since 1974. At Las Vigas, both *Aquiloeurycea cephalica* and *Chiropterotriton totonacus* experienced simultaneous declines between 1976 and 1997 (see Fig. 3C). Finally, at Las Lajas, *Pseudoeurycea melanomolga* showed a progressive decline without signs of recovery in subsequent years. Also at this site, *Isthmura naucampatepetl* was recorded only in 1979 and 1981 (each time with low abundance), and it has not been observed since.

Presence of *Bd* on the Cofre de Perote volcano over time

A total of 139 individuals collected from four localities near the Cofre de Perote volcano over a period spanning more than 40 years (1972–2016) were examined for the presence of *Bd* (Table 1). *Bd* was first detected in the salamander community in 1979, with *Aquiloeurycea cephalica* and *Thorius munificus* from Las Vigas showing prevalences of 20% and 25%, respectively, and *Pseudoeurycea melanomolga* from Las Lajas exhibiting a prevalence of 25%. Since these initial detections, the prevalence of *Bd* has varied significantly across the different years (X^2 = 30.99, p<0.001). Pairwise comparisons revealed a significant increase in prevalence between 1997 and 2016 (X^2 = 27.01, p = 0.044).

At the local level, ten individuals from five species collected in Barranca de Teocelo (Fig. 3A) between 1972 and 2016 tested negative for *Bd*. However, the absence of *Bd* should be interpreted cautiously due to the small sample size (Table 1). At La Joya (Fig. 3B), *Bd* was first detected in 1981 with a prevalence of 33%,

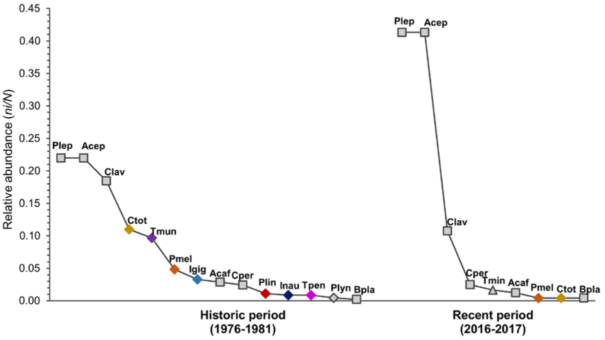

Fig. 2. Abundance-range curves of the salamander communities in the four historical localities in a historic period (1976–1981) and a recent period (2016–2017). Relative abundance is expressed as the quotient of the number of individuals of each species (*ni*) and the total number of individuals in each period (*N*). Squares represent salamander species without variation in the two periods, colored diamonds represent species with declines recorded in the recent period, and triangles represent species recorded only in the recent period. Acaf = *Aquiloeurycea cafetalera*, Acep = *Aquiloeurycea cephalica*, Bpla = *Bolitoglossa platydactyla*, Clav = *Chiropterotriton lavae*, Cper = *Chiropterotriton perotensis*, Ctot = *Chiropterotriton totonacus*, Igig = *Isthmura gigantea*, Inau = *Isthmura naucampatepetl*, Plep = *Pseudoeurycea leprosa*, Plin = *Pseudoeurycea lineola*, Plyn = *Pseudoeurycea lynchi*, Pmel = *Pseudoeurycea melanomolga*, Tmin = *Thorius minydemus*, Tmun = *Thorius munificus*, and Tpen = *Thorius pennatulus*.

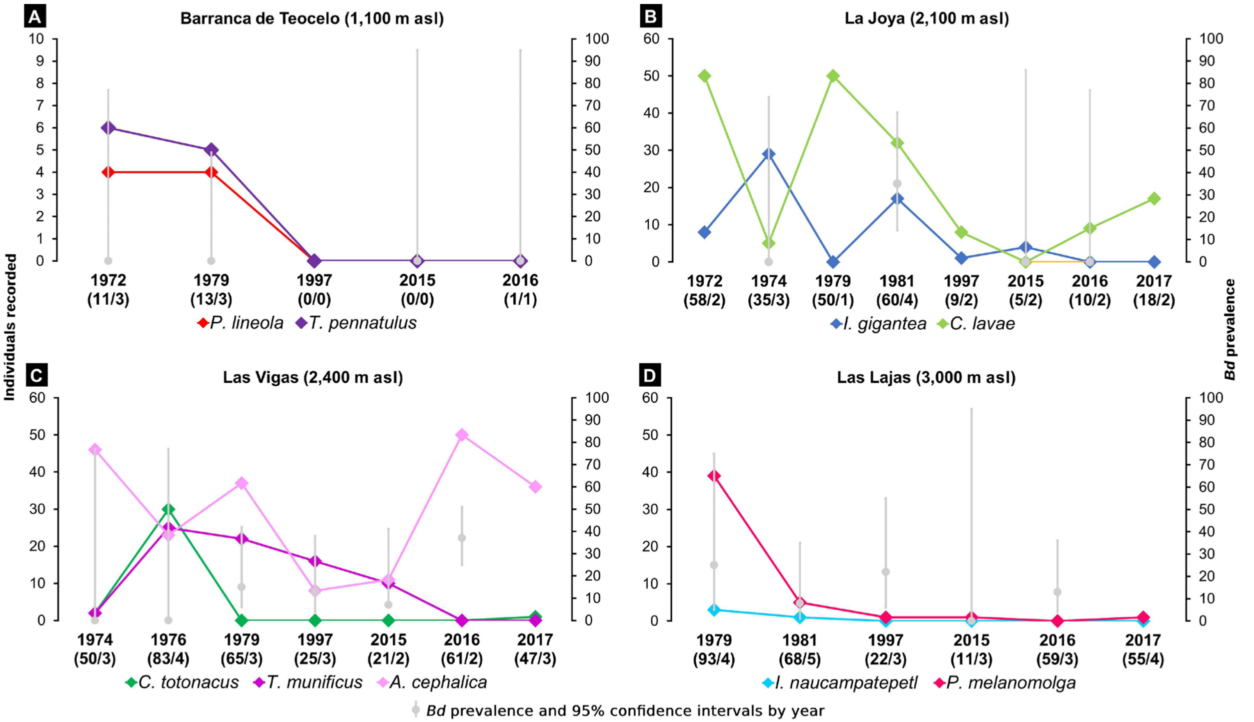

Fig. 3. Individuals of nine salamander species recorded (left y-axis) and *Batrachochytrium dendrobatidis* (*Bd*) prevalence (right y-axis) over time in the four historical localities on the Cofre de Perote volcano, Veracruz. **(A)** Species with population fluctuations in Barranca de Teocelo. **(B)** Species with population fluctuations in La Joya. **(C)** Species with population fluctuations in Las Vigas. **(D)** Species with population fluctuations in Las Lajas. In parentheses, the total number of all individuals in each year is shown before the slash, and the species richness recorded during each year is shown after the slash.

coinciding with the decline of *Isthmura gigantea* and the initial decline of *Chiropterotriton lavae*. At Las Vigas, *Bd* prevalence fluctuated between 20% and 37% from 1979 to 2016, with significant declines observed in *Aquiloeurycea cephalica* and *Chiropterotriton totonacus* between 1979 and 1981 (Fig. 3C). Finally, *Bd* was detected in 1979 at Las Lajas, corresponding with the decline observed in *Pseudoeurycea melanomolga* since that year (Fig. 3D).

Regarding the objective of evaluating the association between salamander population declines and *Bd* prevalence over time, the model that includes *Bd*

Table 1. Prevalence (%) of *Batrachochytrium dendrobatidis* (*Bd*) in salamander populations from the Cofre de Perote volcano, Central Veracruz, Mexico, in eight different years of sampling. Confidence intervals are shown in square brackets and sample size (*n*) is shown the column to the right of each year. Data in bold and red indicate the locality/year for samples that were positive for the pathogen.

Locality	Species	1972	*n*	1974	*n*	1976	*n*	1979	*n*	1981	*n*	1997	*n*	2015	*n*	2016	*n*
Teocelo	*A. cafetalera*							0% [0–86]	3							0% [0–95]	1
Teocelo	*T. pennatulus*	0% [0–77]	5					0% [0–95]	1					0% [0–95]	1	0% [0–95]	1
Joya	*A. cafetalera*			0% [0–95]	1					**25%** **[0.6–81]**	4					0% [0–95]	1
Joya	*I. gigantea*			0% [0–77]	5					**40%** **[5–85]**	5			0% [0–86]	3		
Joya	*T. minydemus*															0% [0–81]	4
Vigas	*A. cephalica*			0% [0–86]	3			**20%** **[0.5–72]**	5			**20%** **[0.5–72]**	5	0% [0–77]	5	**85%** **[55–98]**	13
Vigas	*P. leprosa*	0% [0–81]	4	0% [0–90]	2	0% [0–95]	1	0% [0–86]									
Vigas	*T. munificus*			0% [0–95]	1	0% [0–81]	4	**25%** **[0.6–81]**	4			**20%** **[0.5–72]**	5	11% [0–48]	9	**25%** **[0.6–81]**	4
Lajas	*A. cephalica*															**100%** **[16–100]**	2
Lajas	*C. perotensis*									**33%** **[1–91]**	3			0% [0–95]	1	0% [0–81]	3
Lajas	*P. leprosa*									0% [0–81]	4	**0.22** **[3–60]**	9	0% [0–77]	5		
Lajas	*P. melanomolga*							**25%** **[0.6–81]**	4	0% [0–77]	5			0% [0–95]	1	0% [0–90]	2
Total		0% [0–34]	9	0% [0–24]	12	0% [0–77]	5	**18%** **[4–43]**	17	**19%** **[5–42]**	21	**21%** **[6–46]**	19	0% [0–28]	25	45% [27–64]	31

prevalence and year of sampling was significant, such as the model of *Bd* prevalence and year of sampling individually, according to the MLRT (Supplementary material II A). The corrected Akaike information criterion showed that the best model was the one which included *Bd* prevalence and year of sampling as predictors (Supplementary material II B).

Contemporary *Bd* presence in relation to habitat characteristics and host species attributes

A total of 273 individuals from 12 localities were analyzed to investigate the current prevalence and infection load of *Bd* in salamanders from the Cofre de Perote volcano (Supplementary material V). Among all the samples analyzed, 50 tested positive for *Bd*, resulting in a prevalence of 18.3% (Clopper-Pearson CI: 13.9–23.4%). Fisher's exact test revealed significant variability in prevalence among localities ($p < 0.001$), and the only pairwise significant difference was between the Las Vigas and Cortadura localities (p-adjusted = 0.012) (Fig. 4). Moreover, prevalence varied significantly among host species ($p < 0.001$), with *Aquiloeurycea cephalica* exhibiting a higher prevalence than *Aquiloeurycea cafetalera*, *Parvimolge townsendi, Pseudoeurycea leprosa*, and *Pseudoeurycea lynchi* (p-adjusted < 0.010 in all cases).

Logistic regression models showed that host species was the best predictor for *Bd* prevalence (Table 2). Coefficient model analyses indicated that the species *Aquiloeurycea cephalica* exhibited the highest *Bd* prevalence in comparison to *Aquiloeurycea cafetalera* (Supplementary material VI A). On the other hand, the best predictor for infection load was the tree density (Table 3), which showed a positive relationship with the infection load (Supplementary material VI B).

Discussion

The salamander community examined in this study experienced the amphibian decline wave documented in the Neotropical region during the 1970s and 1980s, resulting in alterations in its abundance, species richness, structure, and diversity. The presence of the pathogen *Bd* may be associated with the population declines observed in species such as *Aquiloeurycea cephalica* and *Chiropterotriton totonacus* at Las Vigas. However, the fluctuations in the salamander populations and communities observed could also be influenced by habitat loss and degradation in the region, stochastic events, and the secretive behavior of plethodontids (Parra-Olea et al. 1999; Rovito et al. 2009; Sandoval-Comte et al. 2012; Stegen et al. 2013). Although the natural history of these species remains poorly understood, our findings underscore its relevance to *Bd* infection at the local level. Importantly, the effects of *Bd* infection on community dynamics could impact their resilience against new threats or exacerbate the existing stressors.

Changes in the salamander community over time

The timeframe of our analysis coincides with a period marked by amphibian extirpations and population

Table 2. Logistic regression models for *Batrachochytrium dendrobatidis* (*Bd*) prevalence performed for seven habitat characteristics and four host species attributes. The best model for prevalence in shown in bold and red text.

	Prevalence predictor	**AICc**	**ΔAICc**	**AICwt**	**Deviance**
Habitat characteristic	Locality	252.061	9.298	0.00625	12.736
	Elevation	263.024	21.416	0.00001	0.381
	Tree density	257.506	15.898	0.00023	2.504
	Canopy cover	263.728	22.121	0.00001	0.110
	Leaf litter	254.707	13.100	0.00093	3.580
	Minimum annual temperature	263.808	22.201	0.00001	0.079
	Maximum annual temperature	251.047	9.439	0.00583	4.988
Species attribute	**Host species**	**242.763**	**0.000**	**0.65340**	**16.312**
	Maximum SVL	263.781	22.174	0.00001	0.090
	Elevational affinity	243.134	1.347	0.33325	10.409
	Microhabitat use	260.505	18.853	0.00005	2.137

Table 3. General linear models for predicting *Batrachochytrium dendrobatidis* (*Bd*) infection load in positive samples performed for seven habitat characteristics and four host species attributes. The best individual model for infection load is shown in bold and red text.

	Infection load predictor	**AICc**	**ΔAICc**	**AICwt**	**Deviance**
Habitat characteristic	Locality	239.134	7.254	0.00016	23.321
	Elevation	229.358	2.864	0.10452	3.279
	Tree density	**226.494**	**0.000**	**0.43759**	**8.663**
	Canopy cover	229.427	2.933	0.10096	3.145
	Leaf litter	228.512	2.018	0.15952	4.401
	Min. annual temp.	230.929	4.434	0.04766	0.193
	Max. annual temp.	230.345	3.850	0.06382	1.351
Species attribute	Host species	241.137	11.386	0.00147	9.778
	Maximum SVL	230.892	4.397	0.04855	0.266
	Elevational affinity	236.307	8.704	0.00564	3.586
	Microhabitat use	233.075	6.314	0.01862	0.432

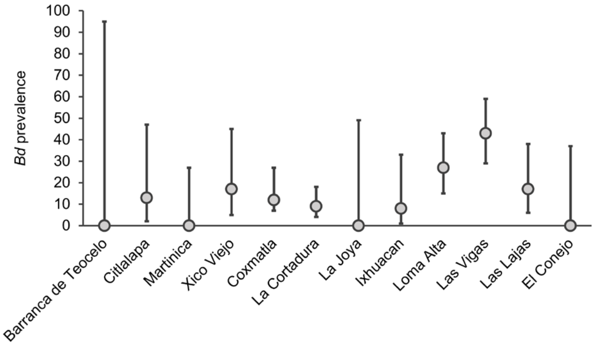

Fig. 4. Prevalence of *Batrachochytrium dendrobatidis* (*Bd*) in 12 localities sampled between 2015 and 2019 in central Veracruz, Mexico. Gray lines show 95% confidence intervals.

declines in Mexico that affected both frog and salamander species (Lips et al. 2006; Parra-Olea et al. 1999; Rovito et al. 2009). In this study, we demonstrated that the salamander community on the Cofre de Perote volcano experienced declines in abundance, species richness, and diversity over a period of approximately 40 years. The primary declines were observed at locations situated within the elevational range of 2,100 to 2,400 m asl, which is recognized for hosting the highest abundance and richness of salamander species in the mountainous regions of the Neotropics (Kozak 2017; Wake and Lynch 1976; Wake 1987; Wake et al. 1992). It is important to recognize that this could be influenced by variations in the activity patterns observed in plethodontid salamanders (Wake and Lynch 1976; Parra-Olea et al. 1999). We observed this variation in certain species that managed to regain their abundance in years after declines (e.g., *Aquiloeurycea cafetalera* and *Chiropterotriton lavae* at La Joya, or *Aquiloeurycea cephalica* and *Pseudoeurycea leprosa* at Las Vigas).

The potential factors contributing to declines may vary for the different species and locations. At Barranca de Teocelo (1,100 m asl), *Pseudoeurycea lineola* and *Thorius pennatulus* have not been recorded since 1979. Nonetheless, these species are known to be uncommon and difficult to find (IUCN 2020c,d; Ramírez-Bautista and Arizmendi 2004c); thus, we suspect that stochasticity influences their lack of detection. At La Joya (2,100 m asl), *Chiropterotriton lavae* and *Isthmura gigantea* experienced declines in 1974 and 1979, respectively, prior to the first record of *Bd* in the area. This region has been significantly impacted by extensive logging and mining activities since at least the 1970s (Parra-Olea et al. 1999; Wake et al. 1992). Therefore, despite differences in their natural history, such as *Chiropterotriton lavae* being a small species inhabiting bromeliads and *Isthmura gigantea* being one of the largest terrestrial salamanders in Mexico (IUCN 2016; IUCN 2020b), both species may have been impacted by habitat modification.

At Las Vigas (2,400 m asl), *Aquiloeurycea cephalica* and *Chiropterotriton totonacus* exhibited declines between 1979 and 1997, coinciding with the first appearance of *Bd* at this locality. Furthermore, logging activities are common at this locality (Muñoz-Delfín 2012). Thus, both *Bd* and habitat loss could have been related to the observed declines. A similar scenario occurs at Las Lajas, where *Bd* has been present since 1979 according to our results, and habitat loss has been ongoing due to forest fires (Muñoz-Delfín 2012), cattle grazing, and firewood collection (Rodríguez-Luna et al. 2011). Therefore, fluctuations in the *Pseudoeurycea melanomolga* population could be associated with the simultaneous effects of *Bd* and habitat loss.

Presence of *Bd* on the Cofre de Perote volcano

The oldest record of *Bd* presence in Mexico was in the 1890s, in amphibians from Baja California Sur (Basanta et al. 2021). Meanwhile, the oldest record of *Bd* presence in Veracruz dates from 1940 (Basanta et al. 2021). However, *Bd* was not recorded until 1979 at our historical localities, coinciding with the start of amphibian declines recorded in the 1980s (Cheng et al. 2011; Rovito et al. 2009). The lack of detection of *Bd* in retrospective studies could be due to a low *Bd* prevalence, reducing the probability of successful detection (Adams et al. 2017). On the other hand, our results are based on a reduced sample size before 1979 (26 individuals). Therefore, we cannot exclude the possible presence of *Bd* before 1979 on the Cofre de Perote Volcano; however, that year could delimit a period when the prevalence of *Bd* likely increased.

Research on the genetic variation of *Bd* in Mexico over time demonstrates that the *Bd* lineage present since at least 1981 in Veracruz was the global panzootic lineage (Bd-GPL-1) (Basanta et al. 2021). Because *Bd* was recorded on the Cofre de Perote volcano for the first time in 1979, establishing a statement about the *Bd* lineage in the region and its influence on declines was not possible. On the other hand, environmental stressors, such as habitat loss and deterioration, could be related to *Bd* prevalence fluctuations over time, as was hypothesized by Basanta et al. (2021). Our results suggest that *Bd* is not an isolated factor driving population declines on the Cofre de Perote volcano, but it is known to interact synergistically with other stressor factors such as habitat loss (Becker et al. 2015; Wake and Vredenburg 2008), which is widely prevalent on this mountain (Muñoz-Delfín 2012).

Current *Bd* presence in relation to habitat and salamander species attributes

This study found a differential *Bd* prevalence among the salamander species and elevational bands sampled in central Veracruz. The prevalence of *Bd* had its lowest values in species distributed in sites within the elevational band between 1,100 and 1,500 m asl. This elevational band presented temperatures that were non-optimal for *Bd* proliferation, with a maximum higher than 23 °C (Familiar-Lopez 2010; Piotrowski et al. 2004), which could drive this trend. On the other hand, we observed an influence of host species traits on *Bd* infection patterns.

For example, *Aquiloeurycea cephalica* is a species with a wide geographical and elevational distribution range (IUCN 2020a; Ramírez-Bautista and Arizmendi 2004a), and it was the species with the highest *Bd* prevalence. Nonetheless, the abundance of this species showed a recovery in this study, suggesting that amphibian species with a large distribution range show less severe declines and a higher probability of recovery (Bielby et al. 2008; Scheele et al. 2019).

Host species was the best predictor of *Bd* prevalence, emphasizing the role of host associated factors in *Bd* infection patterns. Interestingly, the two species that differed most in *Bd* prevalence were very close phylogenetically: *Aquiloeurycea cephalica* and *A. cafetalera* (Parra-Olea et al. 2010), thus ecological differences could be implicated. These species were collected in very different habitats of pine forest and cloud forest, respectively. The cloud forest has optimal environmental conditions for *Bd* growth (Familiar-López 2010; Piotrowski et al. 2004); conversely, *A. cafetalera* had the lowest *Bd* prevalence, and it was also rarely found. On the other hand, the pine forest has temperatures lower than the optimal for *Bd* (Familiar-López 2010; Piotrowski et al. 2004); however, *A. cephalica* had the higher prevalence and was the dominant species in the assemblage. Multiple factors could be driving the differences in *Bd* prevalence, including physiological and immunological differences across host species (Rollins-Smith et al. 2011; Woodhams et al. 2007). However, we suspect that host populational density is a major factor implicated in *Bd* transmission in these species.

Tree density had a positive relationship with *Bd* infection load. A high tree density can be associated with greater canopy cover and leaf litter depth, factors related to high levels of *Bd* infection (Becker et al. 2015; Scheele et al. 2019). The high tree density can also reduce water evaporation, promoting low temperatures and high humidity, conditions that benefit the salamanders physiologically, which may increase their capacity to tolerate *Bd* infection (Rollins-Smith et al. 2011). Finally, our tree count included trees with 10 cm diameter or greater, highlighting the relevance of middle-size arboreal elements in maintaining certain environmental conditions that influence the interaction between *Bd* and the host salamanders.

The inter- and intraspecific variations in the *Bd* prevalence and infection load observed in salamanders from the Cofre de Perote require further exploration of alternative explanatory factors. For example, neotropical salamander immune defense mechanisms and/or skin microbiome composition could play a role in differential responses to *Bd*, as has been observed in other systems (Bataille et al. 2015; Bird et al. 2018; Loudon et al. 2014; Rebollar et al. 2020; Rollins-Smith et al. 2011; Woodhams et al. 2007). Furthermore, the genetic structures of populations may affect their immune responses against *Bd* (Bataille et al. 2015; Woodhams et al. 2007). On the other hand, an evaluation of susceptibility to *Bd* in *Pseudoeurycea leprosa* (a dominant species in the Trans Mexican Volcanic Belt, including the Cofre de Perote) showed that this species can present the infection at levels undetectable by qPCR (Mendoza-Almeralla et al. 2016), which could introduce a bias in our results. Thus, future research on these factors and *Bd* infection dynamics in these species is still necessary.

Conclusions

This study assessed the trends in abundances and *Bd* infection patterns in a community composed of 13 plethodontid salamander species in central Veracruz, Mexico. Over a period of ~40 years, this salamander community experienced changes in properties that were probably related to their resilience in the face of new threats (Allison 2004). The fungal pathogen *Bd* likely played a role in the declines of some species; however, habitat loss and deterioration likely had major impacts on the declines of some species. Our findings indicate that the salamanders presented distinct *Bd* prevalences by locality, by species, and even at the intra-specific level. Factors that influence pathogen responses must be investigated in detail, such as population-based genetic variability, skin microbiome composition, and environmentally local stressors. Such efforts will provide a better understanding of the factors for preventing and mitigating the threats to these amphibian species in the future.

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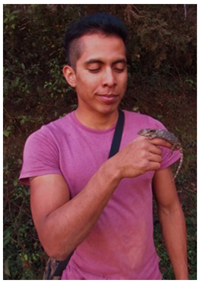

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Supplementary material I. Individuals recorded by historical, recent, and additional sampling carried out between December 1972 and October 2019. Species richness recorded by sampling session/locality is shown in brackets. Grey polygons show the periods compared in their abundance, species richness, evenness, and diversity, due to the similar sampling effort applied. (-) sampling session/localities without records. (*) counts of individuals influenced by truncation due to high species abundance (>100 individuals).

		Historical sampling							**Recent sampling**				**Additional sampling**	
		December 1972	**November 1974**	**July 1976**	**July 1979**	**October 1981**	**October 1997**	**December 2015**	**April 2016**	**July 2016**	**October 2016**	**July 2017**	**October 2018**	**October 2019**
Historic localities	**B. de Teocelo**	12 (3)	-	-	14 (4)	0 (0)	0 (0)	1 (1)	0 (0)	2 (1)	2 (2)	1 (1)	-	-
	La Joya	58 (2)	34 (3)	-	52 (1)	58 (4)	9 (2)	5 (2)	1 (1)	6 (2)	12 (3)	20 (3)	-	-
	Las Vigas	114* (4)	103* (3)	165* (4)	178* (4)	-	25 (3)	21 (2)	7 (2)	29 (3)	89 (2)	48 (3)	-	-
New localities	**Las Lajas**	-	-	-	89 (4)	23 (5)	22 (3)	11 (3)	6 (3)	22 (2)	59 (3)	77 (4)	-	-
	Xico Viejo	-	-	-	-	-	-	6 (4)	1 (1)	3 (3)	3 (3)	8 (3)	-	-
	El Conejo	-	-	-	-	-	-	2 (1)	0 (0)	1 (1)	17 (3)	36 (3)	-	-
	Loma Alta	-	-	-	-	-	-	-	-	-	-	-	32 (1)	8 (1)
	Cortadura	-	-	-	-	-	-	-	-	-	-	-	30 (2)	18 (3)
	Coxmatla	-	-	-	-	-	-	-	-	-	-	-	26 (3)	22 (3)
	Citlalapa	-	-	-	-	-	-	-	-	-	-	-	-	8 (2)
	Ixhuacan	-	-	-	-	-	-	-	-	-	-	-	-	13 (3)
	Martinica	-	-	-	-	-	-	-	-	-	-	-	-	17 (2)
	Total	184 (9)	147 (7)	165 (4)	333 (11)	81 (9)	56 (7)	46 (8)	15 (6)	63 (8)	182 (9)	190 (10)	88 (4)	86 (4)

Supplementary material II. A. Coefficients of semiparametric regression models for the nonmetric predictor variables of *Bd* prevalence and year of sampling and the response variable of salamander abundance by locality by year.

	Parametric coefficient			
	Estimate	**SE**	**t value**	***p***
Intercept	0.83885	0.08049	10.42	<0.001*
	Approximate significance of smooth term			
	edf	**Ref.df**	**F**	***p***
Prevalence	1	1	15.14	0.001*
Year	1	1	4.76	0.037*

B. Semiparametric regression models for the nonmetric predictor variables of *Bd* prevalence and year of sampling and the response variable of salamander abundance by locality by year. The best model is shown in bold and red text.

Model	**AICc**	**ΔAICc**	**AICwt**	**Deviance**
Prevalence+year	**49.912**	**0.000**	**0.8028**	**37.996**
Prevalence	52.756	2.844	0.1937	28.789
Year	60.835	10.923	0.0034	12.063

Supplementary material III. Habitat characteristics and host species attributes evaluated by logistic regression and general linear models for their relationships with the prevalence and infection load of *Batrachochytrium dendrobatidis* (*Bd*) in the salamander community from the Cofre de Perote volcano, central Veracruz, Mexico.

Locality	**Elevation**	**Tree density (trees/Ha)**	**Canopy cover (%)**	**Leaf litter (cm)**	**Maximum annual temperature**	**Minimum annual temperature**
B. Teocelo	1,100	510	65.1	3.6	34	7
Citlalapa	1,450	998	77.6	11.3	27	10
Martinica	1,800	794	76.5	19.8	33	4
Xico Viejo	1,600	818	65.1	4.1	29	11
Coxmatla	2,030	712	64.6	12.5	23	6
Cortadura	2,002	1,690	85.2	20.1	20	1
La Joya	2,012	870	68.2	9.2	23	6
Ixhuacan	2,100	1,395	89.1	13.7	27	2
Loma Alta	2,200	1,062	73.9	9.9	24	6
Las Vigas	2,400	698	72.5	6.6	24	0
Las Lajas	3,000	618	64.9	1.9	17	-1
El Conejo	3,500	986	37.6	0.9	14	-1

Host species	**Maximum SVL (mm)**	**Elevational affinity category**	**Microhabitat use**
Aquiloeurycea cafetalera	59.8	Mid-elevation lands	Facultative arboreal
Aquiloeurycea cephalica	62.7	Widely distributed	Terrestrial
Chiropterotriton perotensis	32.8	High-elevation lands	Terrestrial
Chiropterotriton nubilus	33.2	Mid-elevation lands	Arboreal
Isthmura gigantea	128	Widely distributed	Terrestrial
Parvimolge townsendi	24	Low mid-elevation lands	Facultative arboreal
Pseudoeurycea leprosa	57	High-elevation lands	Terrestrial
Pseudoeurycea lynchi	53.5	Mid-elevation lands	Facultative arboreal
Pseudoeurycea melanomolga	65.6	High-elevation lands	Terrestrial
Thorius minydemus	21.3	High mid-elevation lands	Terrestrial
Thorius munificus	25.9	High mid-elevation lands	Terrestrial
Thorius pennatulus	21.4	Low mid-elevation lands	Terrestrial

Supplementary material IV. Fluctuations of salamander populations over a period of 45 years (1972-2017) in four historical localities in Central Veracruz (Barranca de Teocelo, La Joya, Las Vigas, and Las Lajas). The percentage of change was calculated from one year of sampling to the subsequent year. Data in brackets show the species abundance in the years indicated in each column. If the previous abundance was zero, the change was calculated based on one virtual individual. Red = decline trend. Blue = recovery trend.

B. De Teocleo	1972–1979	1979–1997	1997–2015	2015–2016	2016–2017
Aquiloeurycea cephalica	0% (0–0)	0% (0–0)	100% (0–1)	0% (1–1)	0% (1–1)
Aquiloeurycea cafetalera	400% (1–4)	100% (4–0)	0% (0–0)	100% (0–1)	100% (1–0)
Pseudoeurycea lineola	0% (5–5)	100% (5–0)	0% (0–0)	0% (0–0)	0% (0–0)
Thorius pennatulus	67% (6–4)	100% (4–0)	0% (0–0)	0% (0–0)	0% (0–0)

La Joya	1972–1974	1974–1979	1979–1981	1981–1997	1997–2015	2015–2016	2016–2017
Aquiloeurycea cafetalera	100% (0–1)	100% (1–0)	900% (0–9)	100% (9–0)	0% (0–0)	100% (0–1)	0% (1–1)
Chiropterotriton lavae	90% (50–5)	940% (5–52)	38% (52–32)	75% (32–8)	100% (8–0)	900% (0–9)	89% (9–17)
Isthmura gigantea	250% (8–28)	100% (28–0) **	1,500% (0–15)	93% (15–1)	400% (1–4)	100% (4–0)	0% (0–0)
Pseudoeurycea lynchi	0% (0–0)	0% (0–0)	200% (0–2)	100% (2–0)	0% (0–0)	0% (0–0)	0% (0–0)
Thorius minydemus	0% (0–0)	0% (0–0)	0% (0–0)	0% (0–0)	100% (0–1)	100% (1–2)	0% (2–2)

Las Vigas	1972–1974	1974–1976	1976–1979	1979–1997	1997–2015	2015–2016	2016–2017
Aquiloeurycea cephalica	0% (100–100)	0% (100–100)	0% (100–100)	92% (100–8)	37% (8–11)	591% (11–76)	53% (76–36)
Chiropterotriton totonacus	200% (0–2)	1,350% (2–29)	72% (29–50)	100% (50–0)	0% (0–0)	0% (0–0)	100% (0–1)
Pseudoeurycea leprosa	50% (4–2)	500% (2–12)	50% (12–6)	83% (6–1)	100% (1–0)	1,000% (0–10)	9% (10–11)
Thorius munificus	92% (12–1)	2,300% (1–24)	8% (24–22)	17% (22–16)	54% (16–10)	100% (10–0)	0% (0–0)

Las Lajas	1979–1981	1981–1997	1997–2015	2015–2016	2016–2017
Aquiloeurycea cephalica	0% (1–1)	100% (1–0)	0% (0–0)	700% (0–7)	86% (7–1)
Chiropterotriton perotensis	1,100% (0–11)	55% (11–5)	80% (5–1)	200% (1–3)	0% (3–3)
Pseudoeurycea leprosa	89% (47–5)	220% (5–16)	44% (16–9)	444% (9–49)	6% (49–50)
Pseudoeurycea melanomolga	87% (38–5)	80% (5–1)	0% (1–1)	100% (1–0)	100% (0–1)

Supplementary material V. Salamander samples analyzed for *Batrachochytrium dendrobatidis* (*Bd*) in 12 localities from the Cofre de Perote volcano, central Veracruz, Mexico. Localities: (a) Barranca de Teocelo, (b) Citlalapa, (c) Martinica, (d) Xico Viejo, (e) Coxmatla, (f) Cortadura, (g) La Joya, (h) Ixhuacan, (i) Loma Alta, (j) Las Vigas, (k) Las Lajas, and (l) El Conejo. The number of individuals positive for *Bd* are shown in parentheses. The minimum and maximum zoospore equivalent numbers (infection load) are shown in square brackets. IUCN category codes: CR = Critically Endangered, EN = Endangered, VU = Vulnerable, and LC = Least Concern.

Species	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**Total**	**Prevalence**	**IUCN category**
Aquiloeurycea cafetalera	1 (0) [0]	4 (0) [0]	3 (0) [0]	–	20 (3) [59–1,247]	29 (4) [13–303,575]	–	12 (1) [48]	–	–	–	–	69 (8) [13–303,575]	11.80%	VU
Aquiloeurycea cephalica	–	–	–	–	–	–	–	–	–	18 (11) [2–11,181]	2 (2) [3–5]	–	20 (13) [2–11,181]	62.10%	LC
Chiropterotriton nubilus	–	–	–	–	11(2) [989–10,950]	3 (0) [0]	–	–	–	–	–	–	14 (2) [989–10,950]	14.30%	CR
Chiropterotriton perotensis	–	–	–	–	–	–	–	–	–	–	4 (0) [0]	4 (0) [0]	8 (0) [0]	0%	CR
Isthmura gigantea	–	–	–	–	–	–	3 (0) [0]	–	–	–	–	–	3 (0) [0]	0%	EN
Parvimolge townsendi	–	4 (1) [310]	8 (0) [0]	5 (2) [653–320,397]	–	18 (2) [2–9]	–	–	–	–	–	–	35 (5) [2–320,397]	10%	VU
Pseudoeurycea leprosa	–	–	–	–	–	–	–	–	–	–	5 (0) [0]	–	5 (0) [0]	0%	LC
Pseudoeurycea lynchi	–	–	–	–	–	27 (1) [131]	–	1 (0) [0]	37 (11) [13–4,413]	–	–	–	65 (12) [13–4,413]	28.90%	EN
Pseudoeurycea melanomolga	–	–	–	–	–	–	–	–	–	–	3 (0) [0]	4 (0) [0]	7 (0) [0]	0%	EN
Thorius munificus	–	–	–	–	–	–	–	–	–	13 (2) [4–780]	–	–	13 (2) [4–780]	15.38%	CR
Thorius minydemus	–	–	–	–	–	–	4 (0) [0]	–	–	–	–	–	4 (0) [0]	33.30%	EN
Thorius pennatulus	–	–	2 (0) [0]	–	–	–	–	–	–	–	–	–	2 (0) [0]	0%	EN
Prevalence	0%	12.50%	0%	40%	16.10%	9%	0%	7.70%	29.70%	41.93%	14.28%	0%			

Supplementary material VI. A. Coefficients of the best logistic regression model for *Batrachochytrium dendrobatidis* (*Bd*) prevalence evaluated by Akaike information criterion. (*) significant effect of level.

Variable	**Estimate**	**SE**	**Z value**	***p***
Intercept	-2.1547	0.3735	-5.769	<0.001*
Species: *A. cephalica*	2.6471	0.5347	4.950	<0.001*
Species: *C. nubilus*	0.2088	0.8432	0.248	0.804
Species: *C. perotensis*	0.2088	1.1324	0.184	0.854
Species: *I. gigantea*	-15.4114	1,978.0902	-0.008	0.994
Species: *P. leprosa*	-15.4114	1,097.2471	-0.014	0.989
Species: *P. lynchi*	0.6693	0.4916	1.361	0.173
Species: *P. melanomolga*	-15.4114	1,495.2957	-0.010	0.992
Species: *P. townsendi*	0.5124	0.5817	0.881	0.378
Species: *T. munificus*	0.5452	0.8599	0.634	0.526
Species: *T. mynidemus*	1.4615	1.2804	1.141	0.254
Species: *T. pennatulus*	-15.4114	2,797.442	-0.006	0.996

B. Coefficients of the best logistic regression model for *Batrachochytrium dendrobatidis* (*Bd*) infection load evaluated by Akaike information criterion. (*) significant effect of variable.

Variable	**Estimate**	**SE**	**Z value**	***p***
Intercept	0.6596	0.3389	1.946	0.0575
Tree density	0.8403	0.3938	2.134	0.0380*