EVALUATING PATHOGEN OCCURRENCE AND COEXISTING THREATS ACROSS AMPHIBIAN SPECIES DISTRIBUTIONS

by

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A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Biological Sciences

West Lafayette, Indiana

May 2021

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ACKNOWLEDGMENTS

I am especially grateful to my advisor, Dr. Catherine L. Searle. She is the best advisor any graduate student can have. Dr. Searle always transmits enthusiasm and positiveness to her students. Her faith on my projects motivated me to become a better scientist, to improve my scientific writing, and to be resilient during difficult times. Thanks to my committee: Dr. Ximena E. Bernal for providing strong feedback during my examinations, which really helped me improve my critical thinking; Dr. Dennis J. Minchella, as chair of my examinations and annual meetings, always established a welcoming and friendly environment and provided valuable ideas to include in my chapters; Dr. Jeffrey D. Holland for expressing kind and encouraging words towards my improvement in Academia and providing statistical guidance.

I am very grateful to Gina Rupp for her guidance during all my time in the Graduate School and being concerned for my status during recent COVID-19 times. Thanks to all past and present members of the Searle Lab, who provided not only their help but friendship. I am especially grateful to Spencer Siddons and Kiersten Nelson who shared a lot of my field adventures in Costa Rica; to Kacie Jonasen, Jeannine Toth, and Jasmina Davis for their help with the laboratory work. I am also very grateful with all my office colleagues, there is no doubt that Lily G-365 is a room of high-quality scientific production but also a place to provide mutual support and have profound discussions about the fantasy football league and the eternal rivalry between fish and frog teams! Frogs always won by the way!

I am also thankful to the rest of the EEB community—graduate students, professors, technicians, and postdocs—for sharing nice times during our seminars, potlucks, ice cream parties, EEB coffee hour, and journal club. Thanks to the Costa Rican community at Purdue for continuous support since 2015, especially to Daniel Vasquez for statistical counseling. I am also grateful to all people who assisted me in the field in Costa Rica or help me with logistics. Thanks to all researchers that helped me with the design of my research, especially Adrian Garcia, Gerardo Chaves, Sofia Martinez, Kelsey Neam, and Federico Bolaños. Thanks also to Alessandro Catenazzi and Alex Shepack for laboratory training and standardization of methods. Thanks also to Grace Sanabria for the illustrations. Finally, thanks to my parents and siblings, for encouraging me to pursue a Ph.D. in the United States of America and providing continuous support despite the geographic distance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The incidence and frequency of emerging infectious diseases (EIDs) of wildlife have increased in the last 50 years. The spread of EIDs is a major concern because it can cause vulnerable species and even entire taxa to experience population decline and extinction. For example, numerous amphibian species drastically declined or went extinct in Mesoamerica between the 1980s and early 2000s, likely due to the introduction and spread of the pathogenic fungus *Batrachochytrium dendrobatidis* (hereafter '*Bd*'). While most of the initial declines were documented at high elevations, further studies conducted throughout the region after 2005 revealed that some species also declined in lowland undisturbed ecosystems and that prevalence and intensity of *Bd* infection vary with host species, geographic location, seasonality, and microhabitat conditions. **In Chapter 1**, I examined the dynamics of *Bd* in lowland amphibian communities in the tropic forests of Costa Rica. I found that *Bd* is widespread and exhibits enzootic dynamics (i.e., well adapted to local climate, exhibits low prevalence, and low to undetectable mortality rates). **In Chapter 2**, I described the current *Bd* enzootic dynamics across elevations and ecoregions in Costa Rica. I found that *Bd* exhibits seasonal dynamics, especially in lowlands. I also identified direct-developing, stream-dwelling amphibians as one of the groups most affected by the introduction and spread of *Bd*. **In Chapter 3**, I quantified the spread of *Bd* from pre-2005, when populations experienced epizootics (i.e., times of high infection prevalence and high disease-associated mortality) to post-2005, when populations experienced enzootics in Costa Rica. I found that 80% of the area of undisturbed ecosystems overlaps with the predicted distribution of enzootic *Bd* and identified several hotspots for disease. Finally, **in Chapter 4**, I conducted a threat assessment in different spatial scales for 46 direct-developing, stream-dwelling frog species endemic to Mesoamerica. At both regional and local levels, I found evidence that *Bd* was the main driver of the decline of most species. Together, my results add to the understanding of host-pathogen dynamics in the Tropics and address actions for regions and species that need immediate conservation management.

CHAPTER 1. INFECTION WITH *BATRACHOCHYTRIUM DENDROBATIDIS* IS COMMON IN TROPICAL LOWLAND HABITATS: IMPLICATIONS FOR AMPHIBIAN CONSERVATION

Reprinted by permission from John Wiley & Sons, Ltd. Ecology and Evolution. Infection with *Batrachochytrium dendrobatidis* is common in tropical lowland habitats: implications for amphibian conservation. Hector Zumbado-Ulate, Adrián García-Rodríguez, Vance T. Vredenburg, and Catherine L. Searle. 2019.

1.1 Abstract

Numerous species of amphibians declined in Central America during the 1980s and 1990s. These declines mostly affected highland stream amphibians and have been primarily linked to chytridiomycosis, a deadly disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). Since then, the majority of field studies on *Bd* in the Tropics have been conducted in midland and highland environments (>800 m) mainly because the environmental conditions of mountain ranges match the range of ideal abiotic conditions for *Bd* in the laboratory. This unbalanced sampling has led researchers to largely overlook host–pathogen dynamics in lowlands, where other amphibian species declined during the same period. We conducted a survey testing for *Bd* in 47 species (n = 348) in four lowland sites in Costa Rica to identify local host–pathogen dynamics and to describe the abiotic environment of these sites. We detected *Bd* in three sampling sites and 70% of the surveyed species. We found evidence that lowland study sites exhibit enzootic dynamics with low infection intensity and moderate to high prevalence (55% overall prevalence). Additionally, we found evidence that every study site represents an independent climatic zone, where local climatic differences may explain variations in *Bd* disease dynamics. We recommend more detection surveys across lowlands and other sites that have been historically considered unsuitable for *Bd* occurrence. These data can be used to identify sites for potential disease outbreaks and amphibian rediscoveries. pear in this acknowledgment.

1.2 Introduction

Globally, biodiversity is decreasing at an alarming rate even in seemingly pristine and protected environments (Barnosky et al., 2011; Novacek & Cleland, 2001). Species declines are

driven by numerous anthropogenic actions, acting alone or synergistically with natural threats (Hooper et al., 2012; Rödder et al., 2010; Sala et al., 2000). Previous studies suggest that immediate conservation efforts should prioritize actions on endangered taxa that are rapidly declining and the habitats that protect these species (Brooks et al., 2006; Foden et al., 2013; Giraudo & Arzamendia, 2018). However, there is often incomplete information on which populations are suffering the greatest declines and which locations provide them with the best chances of long-term persistence. For example, for several endangered species or clades, the majority of conservation actions have been designed based on opportunistic field studies conducted in sites where historic declines occurred (Kriger & Hero, 2007a). The potential bias caused by this unbalanced sampling might lead researchers to overestimate the rate of decline or to miss less dramatic declines and environmental threats across the range of the declining species. Therefore, extending the sampling to heterogeneous habitats across the entire geographic distribution of threatened species is crucial to detect and quantify potential threats as well as to establish suitable and more effective conservation actions (Hitchman et al., 2018; Miller et al., 2018; Olson et al., 2013).

Historic research on global amphibian population declines provides numerous examples of conservation actions in response to environmental threats in specific ecosystems. During the last four decades, at least 43% of described amphibian species declined or became extinct worldwide from multiple causes (Collins, 2010; Monastersky, 2014; Stuart et al., 2004; Wake & Vredenburg, 2008; Young et al., 2001). One widespread cause of amphibian population declines is the introduction of infectious pathogens. For example, *Batrachochytrium dendrobatidis* (Longcore et al., 1999) (hereafter *Bd*) is a fungus that causes chytridiomycosis, a deadly cutaneous disease that affects amphibians in all continents where amphibians occur (Berger et al., 1998; Fisher et al., 2009). Global assessments conservatively estimate that chytridiomycosis has caused the severe decline or extinction of over 200 species (Skerratt et al., 2007). Highland stream-dwelling amphibians have been hypothesized to be more prone to massive *Bd*-related die-offs than amphibians in other habitats (Hero et al., 2005; Hirschfeld et al., 2016; Lips, 1998; Lips et al., 2003). Evidence suggests that tropical highland stream environments match the range of ideal abiotic conditions where *Bd* reproduces best in the laboratory (Berger et al., 2004; Longcore et al., 1999; Piotrowski et al., 2004). However, the spatial dynamics of *Bd* are intricate and still poorly understood. It is known that the intensity and occurrence of epizootic outbreaks and length of negative effects upon amphibian communities have varied globally (Catenazzi, 2015). In addition,

numerous field studies show that prevalence and intensity of *Bd* infection vary with host species, microhabitat, temperature, humidity, seasonality, and geographic location (Kinney et al., 2011; Kriger & Hero, 2007b; Kriger et al., 2007; Phillott et al., 2013; Searle et al., 2011b). Thus, identifying conditions that constrain the geographic distribution of this pathogen will help elucidate why some species and populations suffer declines from *Bd* and identify locations that may be environmental refuges from infection (Murray et al., 2011; Rödder et al., 2008; Rosenblum et al., 2013).

The strong elevational gradients in the mountain ranges of Central America (Savage, 2002) create habitat heterogeneity and high endemism of amphibians in midlands and highlands (>800 m elevation). The cool and moist environments in tropical highlands provide suitable conditions for the *Bd* epizootic that occurred in Central America during the 1980s and 1990s, causing the extinction of an unknown number of amphibian species, especially highland stream-breeding species (Cheng et al., 2011; Lips et al., 2008; Pounds et al., 2006; Pounds & Crump, 1994; Rovito et al., 2009). Historical declines in montane amphibian species reflect why most studies on amphibian host-*Bd* dynamics in the tropics have been conducted in premontane and upper elevation localities (Lips, 1999,1998; Puschendorf et al., 2006; Ryan et al., 2008). For example, a considerable amount of *Bd* infection data has been opportunistically collected from montane ecosystems, increasing the focus of conservation actions on highlands while overlooking other potential environments where amphibians may also be impacted by *Bd* (Puschendorf et al., 2013). For example, the suitability of lowland ecosystems for the spread of *Bd* has been frequently disregarded (Puschendorf et al., 2009) even though is known that some amphibian species (Fig. 1.1) and clades have suffered dramatic unexplained declines in these zones (Chaves et al., 2014; La Marca et al., 2005; Puschendorf et al., 2009; Ryan et al., 2008; Whitfield et al., 2007; Zumbado-Ulate et al., 2014).

Despite the focus on highlands for most *Bd*-related studies, the few studies conducted in lowlands of Central America have found new locations where this pathogen occurs, suggesting that *Bd* is more widely distributed than previously thought (Flechas et al., 2015; Kilburn et al., 2010; von May et al., 2018; Whitfield et al., 2013; Whitfield et al., 2012; Woodhams et al., 2008; Zumbado-Ulate et al., 2014). Predictive models and abiotic suitability for *Bd* across heterogenous landscapes (Brannelly et al., 2018; Garcia-Rodríguez et al., 2012; Puschendorf et al., 2009; Rödder

Figure 1.1. Female individual of the Critically Endangered Golfito robber frog (*Craugastor taurus*). This species was very common in lowlands of Southern Costa Rica but catastrophically declined during the 1980s and 1990, presumably due to chytridiomycosis. Currently it is only present in Punta Banco (one of our study sites), and Puerto Armuelles (Panama).

et al., 2008) can be generated using available bioclimatic databases such as WorldClim. This dataset contains 19 bioclimatic variables generated by land area interpolations of climate point data from 1950 to 2000. These variables were derived from monthly precipitation and temperature data at weather stations around the world and describe annual means (e.g., annual precipitation and temperature) and average of extreme environmental values (e.g., maximum temperature of warmest month) (Hijmans et al., 2005). Thus, combining information on infection prevalence and abiotic conditions (e.g., from the WorldClim dataset) across the entire geographic distribution of a host can provide a more informative distribution of both the host and pathogen to identify potential hotspots of future disease outbreaks and potential environmental refuges from disease (Green, 2017; James et al., 2015; Rödder et al., 2010).

In this study, we sampled for *Bd* at four tropical lowland locations in Costa Rica and contrasted *Bd* prevalence and intensity of infection across study sites. We predicted that different host–pathogen dynamics occur across study sites because they exhibit latitudinal and altitudinal variation (Kriger & Hero, 2008; Kriger et al., 2007). We extracted all 19 bioclimatic variables of the WorldClim to describe the different ranges of temperature and precipitation across study sites, which are the main environmental variables that affect *Bd* growth and dispersal (Nowakowski et al., 2016; Savage et al., 2011). Additionally, we hypothesized that all study sites would exhibit low levels of *Bd* prevalence and intensity of infection suggesting stable enzootic infections of *Bd* (Retallick et al., 2004; Scheele et al., 2017; Woodhams et al., 2008). Finally, we also expected a higher prevalence of *Bd* in amphibian assemblages occurring in permanent streams than in ephemeral ponds and terrestrial assemblages, as has been found in previous studies (Kriger & Hero, 2007a; Lips et al., 2003).

1.3 Methods

1.3.1 Lowland sampling sites

We sampled four assemblages of amphibians between November and December 2011, at four tropical lowland locations in Costa Rica (Fig. 1.2). We defined tropical lowlands as all tropical locations within 0–800 m elevation according the Holdridge Life Zone System (Holdridge, 1967). Study sites consisted mostly of tropical moist forest and tropical wet forest with transitional ecosystems including semi-deciduous and evergreen forests, with temperature and precipitation ranges characteristic of these life zones. Our four sampling sites grouped into two main zones:

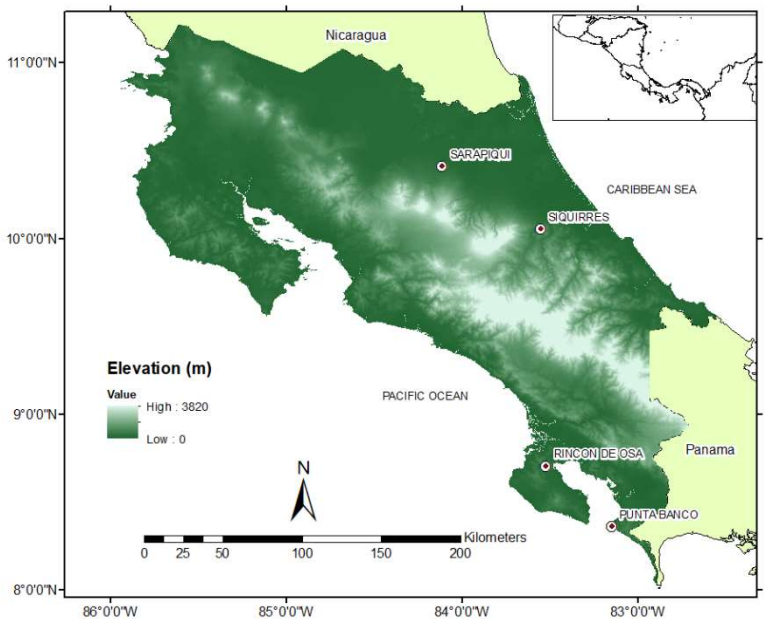

Figure 1.2. Map of Costa Rica showing elevational gradient and lowland sites surveyed for *Batrachochytrium dendrobatidis*.

1.3.1.1 Caribbean zone

Here, we sampled at Tirimbina Private National Wildlife Refuge at La Virgen, Sarapiquí, on the north Caribbean lowlands (10.41 N, –84.11 W, 0–200 m elevation), and at the Costa Rican Amphibian Research Center, at Guayacán, Siquirres (10.06 N, –83.55 W, 400–600 m elevation).

1.3.1.2 Pacific zone

Here, we focused on the areas surrounding the smalls towns of Rincon de Osa (8.71 N, –83.52 W, 0–50 m elevation) and Punta Banco (8.36 N, –83.15 W, 0–50 m elevation), where we sampled across patches of coastal forest. Our sampling in this zone was limited because we were only able to access private farms upon the authorization of landowners.

1.3.2 Pathogen detection

At each site, 4 people systematically searched for amphibians for 36–48 hours during the day and night (9–12 hours/person). Within each site, we conducted visual encounter surveys of amphibians (Heyer et al., 1994) and classified them by the habitat where they were captured: stream-dwellers (permanent flowing water), pond-dwellers (standing ephemeral waterbodies such as swamps, pools, and ditches), and forest-dwellers (leaf-litter, tree holes, or bromeliad plants in the understory, and canopy). Caught amphibians were stored individually in clean, unused plastic bags. Each individual was inspected for visible signs of chytridiomycosis, such as hyperplasia, hyperkeratosis, abnormal shedding, depigmentation, and lethargic behavior (Berger et al., 1998; Voyles et al., 2009) and swabbed to detect *Bd* with a cotton swab (Medical Wire and Equipment, MW–113) using nitrile gloves. To swab, we ran a total of 20 strokes on every individual as follows: five strokes on one hand, five strokes on the ventral patch, five strokes on one foot, and five strokes along inner thigh. Swabs were stored dry in 1.5 ml Eppendorf tubes and frozen at –20º C until DNA extraction. All amphibians were immediately released after sampling. During this study we followed field protocols (Kriger et al. 2006a; Skerratt el al. 2008) which were approved by the National System of Conservation Areas of Costa Rica (SINAC, research permit 001–2012–SINAC) which ensures that animals are being cared for in accordance with standard protocols and treated in an ethical manner.

We extracted DNA from swabs using PrepMan Ultra (Boyle et al., 2004). All extractions were diluted 1:10 in 0.25X TE buffer and run in singlicate (Kriger et al., 2006b) following diagnostic quantitative PCR (qPCR) standard protocols (Boyle et al., 2004) using an Applied BioSystems Prism 7300 Sequence Detection System to test for the presence and quantity of *Bd* genome equivalents. All *Bd*-positive samples were run again in singlicate confirmatory assay. Negative controls (DNase/RNase-free distilled water) were run in triplicate on every 96-well PCR plate. We used 100, 10, 1, and 0.1 zoospore quantification standards to produce a quantification curve. We multiplied the qPCR score by 80 to calculate the zoospore genomic equivalents in the original sample and calculated the average value from the two singlicate assays (Vredenburg et al., 2010; Warne et al., 2016).

1.3.3 Data analysis

We were interested in understanding how *Bd* prevalence and intensity varied among our study sites and habitats (predictor variables). For our analyses, we pooled all species together instead of using species as predictor or running independent tests for each species because the samples sizes per species were highly variable (from 1–44). This high variance in the sample size could produce significant models that may be an artifact of opportunistic sampling instead of a real pattern. Therefore, we analyzed habitat as a proxy of amphibian community composition because the species variable was 100% correlated to habitat. To contrast *Bd* prevalence, we used fix-effects generalized linear models (GLMs) to find the most suitable model using binomial response variables (infected or not infected). Candidate models were ranked according to the Akaike's information criterion (AIC) to determine the relative importance of predictor variables within each model set. The model with the lowest AIC was considered the most robust (Burnham & Anderson, 2004). To compare infection intensity among locations and habitats (predictors), we generated fix-effects general linear models (LMs) with data only from infected individuals. We built our models using the log-transformed *Bd* load (estimated number of genomic equivalents) as a response variable and included site and habitat as predictors. Candidate models were ranked according to the coefficient of regression (R^2), with the model with the highest R^2 considered the most robust (Zar, 2013). For the most robust GLM we tested the significance of the predictors using an ANOVA with a chi-square approximation to find the probabilities of predictor variables within the most suitable models, and for the most robust LM we used an ANOVA. Finally, we conducted post-hoc, pair-wise comparisons (Tukey test) to confirm where the differences occurred between significant predictors.

To describe the local abiotic environment for the sampled lowland sites we generated buffers (radius = 10 km) around each one of our four study sites. Because we wanted to achieve a full description of the abiotic environment, we extracted values for all the cells occurring within each buffer (mean = 355 cells/site, Table 1.1) from all 19 bioclimatic variables of WorldClim (version 1.4; www.worldclim.org) at a spatial resolution of 30 arc-s (Hijmans et al., 2005). We compared the abiotic environment among sites using a principal component analysis (PCA). To contrast climatic dissimilarities between lowland study sites we also generated a pairwise matrix of Euclidean distances between the centroids of climatic envelopes. All analyses were conducted in R v.3.5.1 (R Core Team, 2014).

Table 1.1. Mean values (standard deviation) of the 19 bioclimatic variables from the WorldClim dataset and loads (coordinates) for PCA axes 1 and 2 showing the specific contribution of each of the bioclimatic variables used in the environmental analysis of four lowland sites in Costa Rica.

Bioclimatic Variables	Punta Banco	Rincón de Osa	Sarapiquí	Siquirres	PC1	PC2
BIO_1 = Annual Mean Temperature	25.5 (0.7)	25.6 (0.6)	25.4 (0.7)	24.4 (1.1)	0.1	-0.1
BIO_2 = Mean Diurnal Range	10.1 (0.7)	11.0 (0.2)	9.0 (0.0)	9.0 (0.0)	0.0	-0.4
BIO_3 = Isothermality	75.4 (0.9)	76.6 (0.7)	77.3 (0.7)	79.4 (0.7)	-0.1	0.4
BIO_4 = Temperature Seasonality	77.9 (5.5)	78.0 (1.8)	73.3 (5.6)	76.1 (2.5)	0.0	-0.6
BIO_5 = Max Temperature of Warmest Month	32.8 (0.8)	33.2 (0.7)	31.6 (0.7)	30.4 (1.1)	0.1	-0.5
BIO_6 = Min Temperature of Coldest Month	19.2 (0.9)	18.9 (0.9)	19.8 (0.7)	19.0 (1.2)	0.1	0.1
BIO_7 = Temperature Annual Range	13.8 (1.0)	14.2 (0.4)	12.0 (0.2)	11.2 (0.4)	0.0	-0.6
BIO_8 = Mean Temperature of Wettest Quarter	25.0 (0.7)	25.1 (0.7)	25.3 (0.9)	24.2 (1.2)	0.1	-0.1
BIO_9 = Mean Temperature of Driest Quarter	25.8 (0.6)	25.8 (0.7)	25.9 (0.7)	25.1 (1.2)	0.1	-0.1
BIO_{10} = Mean Temperature of Warmest Quarter	26.6 (0.8)	26.7 (0.8)	26.4 (0.8)	25.5 (1.1)	0.1	-0.2
BIO_{11} = Mean Temperature of Coldest Quarter	24.7 (0.8)	24.8 (0.8)	24.6 (0.6)	23.6 (1.1)	0.1	-0.1
BIO_{12} = Annual Precipitation*	3112.0 (134.0)	3976.4 (430.3)	4085.4 (185.5)	3784.4 (245.8)	128.1	31.4
BIO_{13} = Precipitation of Wettest Month*	586.3 (47.2)	712.7 (51.4)	460.4 (19.1)	440.1 (23.5)	13.8	-49.9
BIO_{14} = Precipitation of Driest Month	54.0 (14.8)	60.7 (19.5)	163.6 (13.4)	182.1 (18.7)	3.6	24.8
BIO15 = Precipitation Seasonality	64.5 (6.4)	62.8 (4.9)	30.0 (1.5)	27.2 (2.1)	-0.7	-7.4
BIO_{16} = Precipitation of Wettest Quarter*	1351.0 (87.7)	1719.3 (130.8)	1277.3 (56.9)	1173.9 (65.2)	41.7	-88.5

Table 1.1 continued

BIO_{17} = Precipitation of Driest Quarter*	176.8 (49.1)	237.4 (65.2)	589.9 (40.9)	625.1 (53.4)	14.8	82.8
BIO_{18} = Precipitation of Warmest Quarter	528.5 (27.6)	707.5 (82.4)	724.5 (41.9)	772.7 (72.4)	21.7	19.6
BIO_{19} = Precipitation of Coldest Quarter*	1071.8 (132.0)	1348.7 (152.2)	1163.9 (71.9)	1089.1 (60.4)	38.4	-36.0

Notes: *Temperature variables are measured in Celsius (environmental variables 1–11) and precipitation variables in mm (environmental variables 12–19).*
[a]*Bioclimatic variables with higher contribution.*

1.4 Results

We screened a total of 348 adult amphibians from 47 species for *Bd* (346 frogs and two salamanders, Table 1.2). From this list, a total of 44 species are classified as least concern and three are categorized as threatened: *Oophaga granulifera* is classified as vulnerable (VU), *Agalychnis lemur*, and *Craugastor taurus* are classified as critically endangered (CR) according to the International Union for Conservation of Nature (IUCN) (Red List of Threatened Species, version 2017–1; http://www.iucnredlist.org/). Overall, 33 species (70.2% of sampled species) tested positive for *Bd* and total prevalence of *Bd* was 54.6%. We did not detect *Bd* on three of the amphibian families sampled, including Plethodontidae, the only family of Salamanders in the Neotropics, however the sample size for these families was very small.

Prevalence of infection showed high heterogeneity among sites with values ranging from 0.0% in Rincon de Osa to 68.6% in Punta Banco (Table 1.3). This variation in *Bd* prevalence was best explained by the interaction effects model (Table 1.4), which showed significant effects of locality ($P<0.01$), and that the variation of *Bd* prevalence by site depends on the habitat ($P<0.001$; Fig. 1.3A). Despite being close in proximity, amphibian assemblages from Sarapiquí showed significant higher prevalence of *Bd* than assemblages from Siquirres ($P<0.01$, Fig. 1.3A, Table 1.3, 1.5). We also found high prevalence of *Bd* across habitats (Table 1.3), but no significant differences between habitats in our model ($P=0.20$).

Table 1.2. List of species and number of individuals tested for *Batrachochytrium dendrobatidis* in amphibian assemblages from four lowland sites in Costa Rica.

Species	Habitat	N (*Bd* positive)	Prevalence % (95% CI)	Genomic equivalents (±SE)		
				Sarapiquí	Siquirres	Punta Banco
Agalychnis callidryas	Pond	11 (5)	45.5 (16.7–76.6)	X	(249.2±214.1)	X
Agalychnis lemur[a]	Pond	5 (2)	40.0 (5.3–85.3)	X	(12.3±4.9)	X
Agalychnis spurrelli	Pond	5 (1)	20.0 (5.0–71.6)	X	(10.3±0.0)	X
Anotheca spinosa	Forest	1 (1)	100.0 (0.2–100.0)	X	(112.3±0.0)	X
Boana rufitela	Pond	10 (8)	80.0 (44.4–97.5)	(8.4±3.9)	X	X
Bolitoglossa colonnea	Forest	1 (0)	0.0 (0.0–97.5)	X	X	X
Centrolenella ilex	Stream	1 (1)	100.0 (0.2–10.00)	X	(57.4±0.0)	X
Cochranella granulosa	Stream	1 (1)	100.0 (0.2–100.0)	(3.9±0.0)	X	X
Craugastor bransfordi	Forest	24 (19)	79.2 (57.8–92.9)	(31.6±13.8)	(74.9±112.2)	X
Craugastor crassidigitus	Forest	6 (2)	33.3 (4.3–77.7)	(3.0±0.0)	(18.5±0.0)	X
Craugastor fitzingeri	Forest	44 (26)	59.1 (43.2–73.7)	(448.8±321.2)	(14.1±5.6)	(65.4±22.5)
Craugastor megacephalus	Forest	2 (1)	50.0 (12.6–98.7)	(0.6±0.0)	X	X
Craugastor mimus	Forest	10 (8)	80.0 (44.4–97.5)	(107.5±76.9)	X	X
Craugastor stejnegerianus	Forest	6 (2)	33.3 (4.3–77.7)	X	X	(2.2±0.9)
Craugastor taurus[ab]	Stream	15 (12)	80.0 (51.9–95.7)	X	X	(11632.5±6285.2)
Cruziohyla calcarifer	Forest	1 (0)	0.0 (0.0–97.5)	X	X	X
Dendrobates auratus	Forest	7 (1)	14.3 (0.4–57.9)	X	(4.9±0.0)	X

Table 1.2 Continued

Dendropsophus ebraccatus	Pond	22 (15)	68.2 (45.1–86.1)	X	(130.3±59.1)	X
Dendropsophus phlebodes	Pond	1 (1)	100.0 (0.2–10.0)	X	(15.9±0.0)	X
Dendropsophus ebraccatus	Pond	22 (15)	68.2 (45.1–86.1)	X	(130.3±59.1)	X
Dendropsophus phlebodes	Pond	1 (1)	100.0 (0.2–10.0)	X	(15.9±0.0)	X
Diasporus diastema	Forest	9 (4)	44.4 (13.7–78.8)	X	(1994.3±1724.7)	X
Diasporus vocator	Forest	1 (0)	0.0 (0.0–97.5)	X	X	X
Duellmanohyla rufioculis	Stream	1 (0)	0.0 (0.0–97.5)	X	X	X
Engystomops pustulosus	Pond	10 (0)	0.0 (0.0–30.8)	X	X	X
Hyalinobatrachium fleischmanni	Stream	1 (0)	0.0 (0.0–97.5)	X	X	X
Hyalinobatrachium valerioi	Stream	1 (0)	0.0 (0.0–97.5)	X	X	X
Hyloscirtus palmeri	Stream	1 (1)	100.0 (0.2–100.0)	X	(231.2±0.0)	X
Incilius melanochlorus	Pond	8 (1)	12.5 (0.3–52.6)	(3.3±0.0)	X	X
Leptodactylus fragilis	Pond	1 (0)	0.0 (0.0–97.5)	X	X	X
Leptodactylus insularum	Pond	3 (0)	0.0 (0.0–70.7)	X	X	X
Leptodactylus poecilochilus	Pond	1 (0)	0.0 (0.0–97.5)	X	X	X
Leptodactylus savagei	Pond	3 (0)	0.0 (0.0–70.7)	X	X	X
Lithobates vaillanti	Pond	2 (0)	0.0 (0.0–84.2)	X	X	X
Lithobates warszewitschii	Stream	26 (14)	53.8 (33.4–73.3)	(51.8±39.1)	(1391.1±704.7)	X
Oedipina gracilis	Forest	1 (0)	0.0 (0.0–97.5)	X	X	X
Oophaga granulifera[a]	Forest	1 (1)	100.0 (0.2–100.0)	X	X	(114.0±0.0)
Oophaga pumilio	Forest	23 (18)	78.3 (56.3–92.5)	(625.2±479.5)	X	X
Pristimantis cerasinus	Forest	7 (4)	57.1 (18.4–90.1)	(3.6±0.5)	X	X

Table 1.2 Continued

Pristimantis ridens	Forest	6 (3)	50.0 (11.8–88.2)	(3.0±0.0)	(6.4±3.2)	X
Rhaebo haematiticus	Stream	27 (17)	63.0 (42.4–80.6)	(3.1±0.8)	X	X
Rhinella horribilis	Pond	4 (0)	0.0 (0.0–60.2)	X	X	X
Scinax boulengeri	Pond	4 (1)	25.0 (63.1–80.6)	(195.2±0.0)	X	x
Scinax elaeochroa	Pond	6 (3)	50.0 (11.8–88.2)	X	(2.3±0.4)	x
Smilisca phaeota	Pond	5 (2)	40.0 (5.3–85.3)	X	(9.8±2.3)	x
Smilisca sordida	Stream	1 (1)	100.0 (0.2–100.0)	(430.4±0.0)	X	X
Tlalocohyla loquax	Pond	15 (11)	73.3 (44.9–92.2)	X	(1566.8±1020.7)	X
Teratohyla spinosa	Stream	4 (2)	50.0 (6.8–93.2)	(4.8±1.2)	X	X
Teratohyla pulverata	Stream	3 (1)	33.3 (84.0–90.6)	X	(39.8±0.0)	X
Total		348 (190)	54.6 (49.2-59.9)			

Notes: *For every species, the table shows the habitat where the species was captured, the sample size, the overall prevalence (95% CI) and the average (SE) of genomic equivalents of Batrachochytrium dendrobatidis zoospores quantified per study site estimated from Bd-positive samples).*
[a]Endangered species according to the International Union for Conservation of Nature (IUCN). [b]Prevalence value previously reported in Chaves et al. (2014).

Table 1.3. Prevalence (95% CI) and infection intensity (SE) of *Batrachochytrium dendrobatidis* in amphibian assemblages from four lowland sites and three lowland habitats of Costa Rica.

Predictors		N	Prevalence (95% CI)	Infection intensity (SE)
Site	Rincon de Osa	25	0.0 (0.0–13.7)	0.0 (0.0)
	Punta Banco	35	68.6 (50.7–83.2)	2.0 (0.2)
	Sarapiquí	144	67.4 (51.1–75.5)	0.9 (0.1)
	Siquirres	144	47.9 (39.5–56.4)	1.5 (0.1)
Habitat	Forest	150	62.7 (54.4–70.4)	1.2 (0.1)
	Pond	116	39.7 (30.7–49.2)	1.3 (0.1)
	Stream	82	61.0 (49.6–71.6)	1.2 (0.2)

Table 1.4. Candidacy generalized linear models (GLMs) and linear models (LMs) used to determine the best predictors of prevalence of *Batrachochytrium dendrobatidis* and infection intensity in amphibian assemblages from four lowland sites and three lowland reproductive habitats in four lowland sites of Costa Rica.

Model	AIC (GLMs)	R^2 (LMs)
Site*habitat (interaction model)	422.03	0.19
Site+habitat (additive model)	431.40	0.14
Site	432.90	0.13
Habitat	469.70	0.00

Notes: *The most robust models were selected according to the highest values for the Akaike information criteria (AIC) for the generalized linear models (GLMs) and the coefficient of regression (R^2) for the linear models (LMs)*

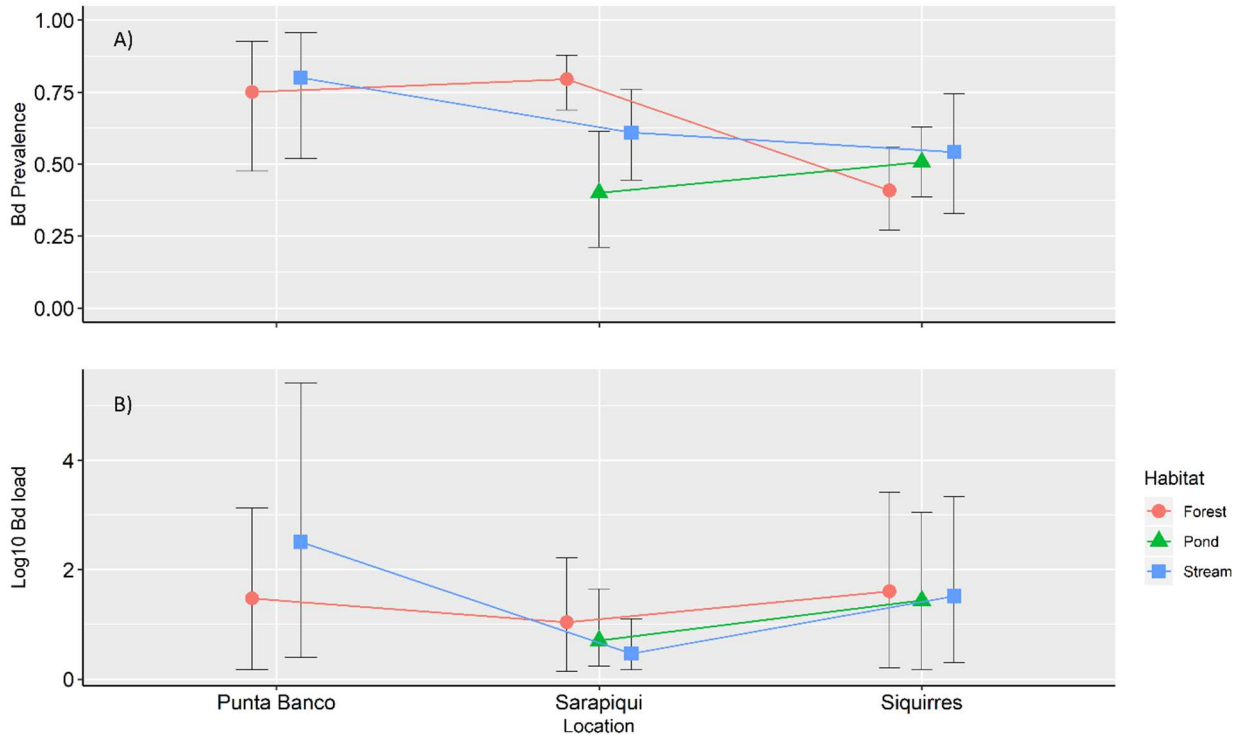

Figure 1.3. Prevalence and intensity of infection of *Batrachochytrium dendrobatidis* in amphibian assemblages from four surveyed lowland sites in Costa Rica. The line plots show A) prevalence of *B. dendrobatidis* among surveyed lowland sites per habitat (with 95% binomial confidence intervals) and B) average infection intensity (SE) of *B. dendrobatidis* in amphibian assemblages among surveyed lowland sites per habitat. The figure does not show results for Rincon de Osa because *Bd* prevalence at that site was 0%. Similarly, the plots do not display results for the category pond at Punta Banco because we did not collect any individuals from ponds at that location.

Table 1.5. Matrix of pair-wise comparisons showing P values obtained from a post-hoc analysis (Tukey test) to explain prevalence and infection intensity of *Batrachochytrium dendrobatidis* in amphibian assemblages from four lowland sites of Costa Rica.

Bd Prevalence			
	Punta Banco	Sarapiquí	Siquirres
Punta Banco			
Sarapiquí	0.98		
Siquirres	0.06	P<0.01*	
Bd Infection intensity			
	Punta Banco	Sarapiquí	Siquirres
Punta Banco			
Sarapiquí	P<0.001*		
Siquirres	0.12	P<0.001*	

Notes: *The table does not show results for Rincon de Osa because Bd prevalence at that site was 0%.*

Similarly, the differences in the infection intensity across study sites (Fig. 1.3B, Table 1.3) were best explained by the interaction model (R^2=0.19, Table 1.4), which also showed significant effects of location ($F_{2,166}$=15.5, P<0.001) and the interaction between habitat and location ($F_{3,166}$=3.6, P<0.01). Levels of infection intensity were significantly lower in Sarapiquí (Fig. 1.3B, Table 1.3, 1.5) when compared to Punta Banco (P<0.001) and Siquirres (P<0.01). Overall, the infection intensity ranged from 0.1–63 861 genome equivalents and four individuals had more than 10 000 zoospore genomic equivalents, a theoretical number that is considered a threshold that results in mass mortality and rapid population decline (Vredenburg et al., 2010). However, none of the sampled individuals including the four that were heavily infected, showed any evident signs of disease. Remarkably, three of these heavily infected individuals belong to the Critically Endangered species *Craugastor taurus*.

In our PCA analysis of the 19 bioclimatic variables, we retained the first two axes (Table 1.1) because they accounted for 98% of the total variance of our data. A tridimensional representation of PCA axes 1 and 2 (PCA 3 included as reference) shows four separated clusters of points, each one representing a study site (Fig. 1.4). As expected, we found the highest similarity in climatic conditions occurred among sites in each zone (Fig. A.1). We found that bioclimatic variables associated with precipitation (Annual Precipitation, Precipitation of Wettest Quarter, Precipitation of Driest Quarter) make a higher contribution in the variance of our climatic data than other variables (Table 1.1.

1.5 Discussion

We found *Bd* infections at three of the four lowland sites sampled and in 70.2% of the 47 sampled species for an overall *Bd* prevalence of 54.6% (Table 1.2, 1.3). Furthermore, we did not detect signs of disease in heavily infected individuals during the study and found low levels of infection in most of our samples. Similar community composition and population dynamics observed during our study and later visits (unpublished data) suggest that host-pathogen dynamics in surveyed lowlands are exhibiting enzootic dynamics, rather than epizootic dynamics (Brem & Lips, 2008; Woodhams et al., 2008; Briggs et al., 2010; Perez et al., 2014). Our findings also suggest that the distribution of *Bd* in Costa Rica is wider than historically considered (Puschendorf et al., 2009) and that the population declines during the 1980s and 1990s may not have been restricted to highlands. Comparable results were found in lowlands of Panama where *Bd* has been detected in multiple lowland sites (Woodhams et al., 2008; Kilburn et al., 2010; Perez et al., 2014). We suggest that future studies should include replicated sampling across seasons and sites that are outside the optimal environmental conditions for *Bd* growth, especially since most of these optimal conditions been estimated from lab studies. Additionally, under potential scenarios of climate change, sites that are currently considered unsuitable for *Bd* may experience future outbreaks of chytridiomycosis if environmental conditions become closer to ideal ranges for *Bd* growth (Endquist, 2002; AlMutairi et al., 2019). Furthermore, conducting more studies and replicated samplings in neglected sites or locations that are assumed to be pathogen-free may help to better describe spatial dynamics of both the host and pathogen. These proposed studies could reduce the effect of opportunistically collected data from montane ecosystems and help develop more effective conservation tools and actions for amphibians in a broader range of habitats (Grenyer et al., 2006; Woodhams et al., 2011; Scheele et al., 2014; Garner et al., 2016).

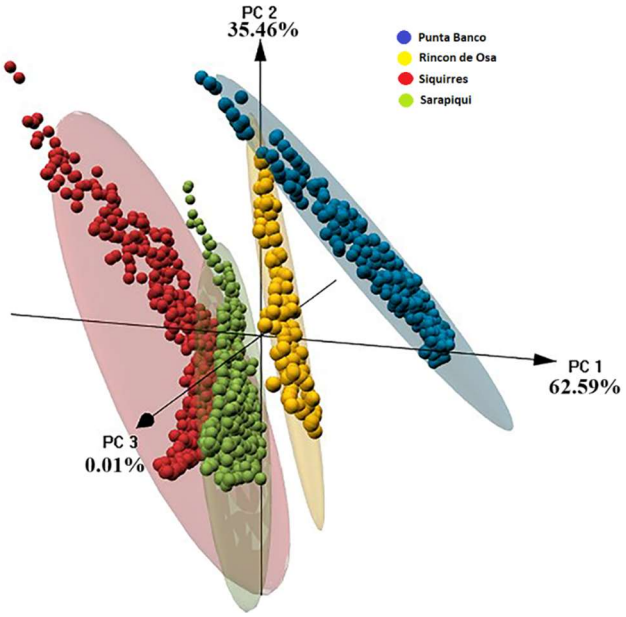

Figure 1.4. Abiotic environment of four surveyed lowland sites in Costa Rica. Tridimensional PCA biplot displays the extracted values within buffers (radius = 10 km) representing the four lowland sampling sites for the 19 bioclimatic variables from the WorldClim dataset.

The three endangered species sampled (*Craugastor taurus, Agalychnis lemur,* and *Oophaga granulifera*) tested positive for *Bd* (Table 1.2). The populations of *C. taurus* and *A. lemur* that we surveyed also tested positive in past surveys (Briggs et al., 2010; Whitfield et al., 2017). The continuous occurrence of these endangered species and the lack of clinical signs of chytridiomycosis in *Bd*-infected individuals (Berger et al., 1998; Voyles et al., 2009), suggest these populations are capable of surviving with enzootic *Bd* dynamics (Whitfield et al., 2017). Remarkably, infection levels in several individuals of the robber frog (*C. taurus*) were above 10000 *Bd* genomic equivalents, a theoretical threshold that has been linked to epizootic outbreaks, population die-offs, and local extinctions (Vredenburg et al., 2010). There are several explanations for these high infection loads without signs of population decline or disease. For example, it is possible that these populations can coexist with *Bd* because they carry cutaneous bacteria that release anti-*Bd* compounds, although none have been detected in individuals of the relict populations of the Golfito robber frog (Madison et al., 2017) or in a similar critically endangered species (*C. ranoides*) which also catastrophically declined in the 1980s (Puschendorf et al., 2009; Zumbado-Ulate et al., 2011). Additionally, antimicrobial peptides, and immune defenses (innate and adaptive) may play a role in this host-pathogen coexistence (e.g., Woodhams et al., 2016; Rollins-Smith, 2017). Alternatively, persistence of these populations could be associated with behavioral adaptations that rapidly clear infection or to local dry conditions that constrain *Bd* growth allowing susceptible frogs to coexist with low levels of *Bd* infection (Puschendorf et al., 2011; Chaves et al., 2014). Further studies on these endangered lowland populations can lead to management plans that protect and stabilize these relict populations.

The absence of *Bd* in fourteen surveyed species could be an artifact of the small sample sizes (1–10 individuals, Table 1.2) because some of these species have tested positive in other studies in Costa Rica and nearby Panama (e.g., *Engystomops pustulosus*, *Duellmanohyla rufioculis*, *Anotheca spinosa*, *Leptodactylus poecilochilus*) (Picco & Collins, 2007; Zumbado-Ulate et al., 2014; Rodríguez-Brenes et al., 2016). Low sample sizes were caused by low detectability during the survey period for some of the common species (e.g., *Rhinella horribilis, Smilisca sordida, Lithobates vaillanti, Leptodactylus savagei*) or due to the low year-round detectability for the more cryptic and rare species (e.g., fossorial and canopy dwellers like *Oedipina gracilis, Bolitoglossa colonnea, Cruziohyla calcarifer*). To increase species detectability and/or sample size, future studies in lowlands and neglected sites should conduct surveys restricting or focusing the sampling

on threatened species (e.g. Whitfield et al., 2017; Thorpe et al., 2018), to describe host-pathogen population dynamics, or preferably survey multiple species across seasons to obtain more accurate estimates of prevalence and infection intensity for all species within the amphibian community (Vredenburg et al., 2010; Kinney et al., 2011; Brannelly et al., 2015).

We found common lowland species with high prevalence of *Bd* (e.g., *Lithobates warszewitschii, Craugastor fitzingeri, Rhaebo haematiticus, Oophaga pumilio, Dendropsophus ebraccatus*). The species *L. warszewitschii, C. fitzingeri,* and *D. ebraccatus* also inhabit the montane ecosystems where historical enigmatic declines occurred. These species and others not sampled here (e.g. *Isthmohyla pseudopuma*) or with a small sample size (e.g. *Smilisca sordida*) seem to be highly tolerant to *Bd* and may function as competent reservoirs (Ostfeld & Keesing, 2000; Reeder et al., 2012; Scheele et al., 2017), amplifying *Bd* infection in the community (Searle et al., 2011a). Therefore, the high infection prevalence in these species that we found at lowland sites suggests that *Bd* is common and persists in these locations.

Our results showed that *Bd* was widespread across lowlands during the time of study, but *Bd* prevalence and intensity might exhibit seasonal dynamics. However, to detect a seasonality effect, multi-season studies collecting samples from a variety of amphibian assemblages must be conducted (e.g., Kinney et al., 2011; Savage et al., 2011; Phillott et al., 2013). Similar studies conducted in lowlands of Costa Rica also suggest seasonal dynamics. For example, remnant populations of the lowland robber frog *C. ranoides* in the tropical dry forest of Costa Rica exhibited infection prevalence values that varied from <1 to 60% across a dry season (December to May) (Zumbado-Ulate et al., 2014; Whitfield et al., 2017). Similarly, prevalence of *Bd* varied from <5% to around 35% in an amphibian assemblage in tropical lowland forest across 1-year period (Whitfield et al. 2012). Therefore, follow-up studies across lowlands in Costa Rica are needed to identify seasonal dynamics of *Bd* in Costa Rica, which may help design more suitable conservation strategies for lowlands endangered populations.

We did not find *Bd* in our samples from Rincon de Osa, and a similar study also reported a very low prevalence of *Bd* in the same study sites and nearby zones across the Osa Peninsula (Goldberg et al., 2009). Although our detected prevalence in Rincon de Osa was 0%, our binomial confidence interval (0–95%) overlaps with the prevalence value presented in this study. Therefore, our result for Rincon de Osa might be an artifact of our low sample size (n=24) which is not large enough to achieve 95% certainty of detecting 1 positive individual, based on the minimum disease

prevalence of ≥5% in infected amphibian assemblages (Skerratt et al., 2008). Climatic conditions at Rincon de Osa might constrain the dispersal and growth of *Bd* allowing coexistence between susceptible frogs and *Bd* (i.e., environmental refuge from chytridiomycosis, Puschendorf et al., 2011). However, the extirpation of the Golfito robber frog in this area, where it was abundant before the 1980s and 1990s (Chaves et al., 2014) suggests this may not be the case. We also found the highest levels of *Bd* prevalence in the Caribbean sites which coincide with studies conducted in the nearby locations within the same geographic zone (Whitfield et al., 2012, 2013, 2017). Thus, even within lowland zones, there is large variation in *Bd* prevalence across zones and sites.

Our statistical models showed no differences among habitats in relation to prevalence and infection intensity (Table 1.3), which coincides with similar studies (Lips et al., 2003; Kriger and Hero, 2007a; Brem & Lips, 2008). Some of the sampled species (e.g., *Craugastor fitzingeri, Oophaga pumilio, Rhaebo haematiticus, Rhinella horribilis*) may forage or move through different habitats that do not match their dwelling habitat, which may have affected our results. Previous studies have shown the highest infection prevalence and intensity in permanent streams suggesting that continuous streamflow provides more suitable conditions for the spread of *Bd* than other habitats (Lips et al., 2003; Kriger & Hero, 2007a). Lentic environments are more exposed to sunlight, resulting in temperatures >30 C (e.g., Adams et al., 2017), which in lab conditions is unsuitable for *Bd* (Piotrowski et al., 2004). Lentic environments also sustain invertebrates that feed on zoospores reducing the proportion of infected individuals (e.g., *Daphnia* spp., Searle et al., 2013). However, our findings suggest that the role of terrestrial lowland ecosystems in the dispersal of *Bd* might have been underestimated (but see Whitfield et al., 2012, 2013). Therefore, multi-season studies contrasting *Bd* dynamics across habitats are needed to elucidate the role of microhabitats in sustaining *Bd*.

We found significant evidence that every site of study represents an independent local abiotic environment according to the 19 environmental predictors that we used in our analysis (Fig. 1.4). This climatic independence was consistent with the heterogeneous prevalence of *Bd*, which suggests that every site exhibits a different host-pathogen dynamic in response to local environmental conditions. However, irregularity in elevation gradient across our study sites (e.g. Kriger & Hero, 2008), especially in the study site of Siquirres, where elevations varied from 400–600 m, could have influenced the differential prevalence we found across lowlands. We recommend controlling for elevational gradients (e.g., Kilburn et al., 2010) in follow-up studies.

Seasonality and particularly differences in precipitation (Table 1.1) may also play an important role in differential *Bd* prevalence between the Caribbean and South Pacific zones. The south Pacific zone, where Punta Banco and Rincon de Osa occur, presents a dry season extending from December to April, which coincided with our sampling. Conversely, the Caribbean zone does not have a well-established dry season, and the rainy season starts in December, when we conducted our surveys (Herrera, 1985). Other studies conducted at larger scale have also shown seasonal and latitudinal variation of *Bd* prevalence and infection (Kriger et al., 2007; Kinney et al., 2011; Phillott et al., 2013; Brannelly et al., 2015). Future studies should evaluate the effect of elevational gradients on the amphibian host-*Bd* dynamics.

Our results suggest that researchers should expand their sampling across the entire distribution of focal species and communities instead of only focusing on sites of historical declines. An adequate seasonal description of the suitable abiotic environment of pathogens across the host amphibian home range may help identify disease-free sites for effective repatriation or to determine instances where more technical strategies are needed to secure maintenance of declined populations (e.g., antifungal treatments to clear infection, bioaugmentation with commensal bacteria, habitat manipulation, ex-situ conservation, etc.) (Scheele et al., 2014; Garner et al., 2016). Furthermore, conducting more seasonal sampling in lowlands will increase the record of presence-absence datasets on *Bd* and can be used to generate more robust species distribution models (SDMs) from non-opportunistically collected data (Puschendorf et al., 2013). SDMs can help identify hotspots for future outbreaks of *Bd* and can be used to predict potential locations for amphibian rediscoveries (Puschendorf et al., 2009; García-Rodríguez et al., 2012). Recent validation surveys have led to the discovery of relict peripheral populations that occur in potential environmental refuges from disease (Puschendorf et al., 2011; Scheele et al., 2015; Raffel & Fox, 2018), validating increased surveys outside the boundaries of core geographic distributions (Nishida, 2006; Abarca et al., 2010; González-Maya et al., 2013; Chaves et al., 2014; Jiménez & Alvarado, 2017). A comprehensive assessment of a pathogen's distribution, prevalence and infection intensity can lead to more effective disease-management strategies based on specific locations, habitats and species.

1.6 References

Abarca, J., Chaves, G., García-Rodríguez, A., & Vargas, R. (2010). Reconsidering extinction: rediscovery of *Incilius holdridgei* (Anura: Bufonidae) in Costa Rica after 25 years. *Herpetological Review,* 41, 150.

Adams, A.J., Kupferberg, S.J., Wilber, M.Q., Pessier, A.P., Grefsrud, M., Bobzien, S., Vredenburg, V.T., & Briggs, C.J. (2017). Extreme drought, host density, sex, and bullfrogs influence fungal pathogen infection in a declining lotic amphibian. *Ecosphere,* 8, e01740.

AlMutairi, B.S., Grossmann, I., & Small, M.J. (2019). Climate model projections for future seasonal rainfall cycle statistics in Northwest Costa Rica. *International Journal of Climatology,* 2019, 1–114.

Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B., & Ferrer, E.A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature,* 471, 51–57.

Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonald, K.R., Skerratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G., Mahony, L.M., Sheppard, N., Williams, C., & Tyler, M.J. (2004). Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal,* 82, 434–439.

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., & Hines, H.B. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences,* 95, 9031–9036.

Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T., & Hyatt, A.D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms,* 60, 141–148.

Brannelly, L. A., Martin, G., Llewelyn, J., Skerratt, L. F., & Berger, L. (2018). Age-and size-dependent resistance to chytridiomycosis in the invasive cane toad *Rhinella marina. Diseases of Aquatic Organisms,* 131, 107–120.

Brannelly, L.A., Hunter, D.A., Lenger, D., Scheele, B.C., Skerratt, L.F., & Berger, L. (2015). Dynamics of chytridiomycosis during the breeding season in an Australian Alpine amphibian. *PLOS ONE,* 10, e0143629.

Brem, F., & Lips, K. (2008). *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Diseases of Aquatic Organisms,* 81, 189–202.

Briggs, C.J., Knapp, R.A., & Vredenburg, V.T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences,* 107, 9695–9700.

Brooks, T.M., Mittermeier, R.A., da Fonseca, G.A.B., Gerlach, J., Hoffmann, M., Lamoreux, J.F., Mittermeier, C.G., Pilgrim, J.D., & Rodrigues, A.S.L. (2006). Global biodiversity conservation priorities. *Science,* 313, 58–61.

Catenazzi, A. (2015). State of the world's amphibians. *Annual Review of Environment and Resources,* 40, 91–119.

Burnham, K.P., & Anderson, D.R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research,* 33, 61–304.

Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V.T., & Ryan, M.J. (2014). Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Tropical Conservation Science,* 7, 628–638.

Cheng, T.L., Rovito, S.M., Wake, D.B., & Vredenburg, V.T. (2011). Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences,* 108, 9502–9507.

Collins, J.P. (2010). Amphibian decline and extinction: what we know and what we need to learn. *Diseases of Aquatic Organisms,* 92, 93–99.

Enquist, C.A.F. (2002). Predicted regional impacts of climate change on the geographical distribution and diversity of tropical forests in Costa Rica. *Journal of Biogeography, 29*, 519–534.

Flechas, S., Vredenburg, V.T., & Amézquita, A. (2015). Infection prevalence in three lowland species of harlequin toads from the threatened genus *Atelopus. Herpetological Review,* 46, 528–532

Fisher, M.C., Garner, T.W.J., & Walker, S.F. (2009). Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology,* 63, 291–310.

Foden, W.B., Butchart, S.H.M., Stuart, S.N., Vié, J.-C., Akçakaya, H.R., Angulo, A., DeVantier, L.M., Gutsche, A., Turak, E., Cao, L., Donner, S.D., Katariya, V., Bernard, R., Holland, R.A., Hughes, A.F., O'Hanlon, S.E., Garnett, S.T., Şekercioğlu, Ç.H., & Mace, G.M. (2013). Identifying the world's most climate change vulnerable species: A systematic trait-based assessment of all birds, amphibians and corals. *PLOS ONE,* 8, e65427.

García-Rodríguez, A., Chaves, G., Benavides-Varela, C., & Puschendorf, R. (2012). Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions,* 18, 204–212.

Garner, T.W.J., Schmidt, B.R., Martel, A., Pasmans, F., Muths, E., Cunningham, A.A., Weldon, C., Fisher, M.C., & Bosch, J. (2016). Mitigating amphibian chytridiomycosis in nature. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 371, 2016.0207.

Giraudo, A.R., & Arzamendia, V. (2018). Descriptive bioregionalisation and conservation biogeography: what is the true bioregional representativeness of protected areas? *Australian Systematic Botany,* 30, 403–413.

Goldberg, C.S., Hawley, T.J., & Waits, L.P. (2009). Local and regional patterns of amphibian chytrid prevalence on the Osa Peninsula, Costa Rica. *Herpetological Review,* 40, 309–311.

González-Maya, J.F., Belant, J.L., Wyatt, S.A., Schipper, J., Cardenal, J., Corrales, D., Cruz-Lizano, I., Hoepker, A., Escobedo-Galván, A.H., Castañeda, F., & Fischer, A. (2013). Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica. *Amphibia-Reptilia,* 34, 573–578.

Green, D.M. (2017). Amphibian breeding phenology trends under climate change: predicting the past to forecast the future. *Global Change Biology,* 23, 646–656.

Grenyer, R., Orme, C.D.L., Jackson, S.F., Thomas, G.H., Davies, R.G., Davies, T.J., Jones, K.E., Olson, V.A., Ridgely, R.S., Rasmussen, P.C., Ding, T.-S., Bennett, P.M., Blackburn, T.M., Gaston, K.J., Gittleman, J.L., & Owens, I.P.F. (2006). Global distribution and conservation of rare and threatened vertebrates. *Nature,* 444, 93–96.

Hero, J.-M., Williams, S.E., & Magnusson, W.E. (2005). Ecological traits of declining amphibians in upland areas of eastern Australia. *Journal of Zoology,* 267, 221–232.

Herrera, W. (1985). Clima de Costa Rica (Vegetación y clima de Costa Rica, Vol. 2). Universidad Estatal a Distancia.

Heyer, W.R., Donnelly, M.A., McDiarmid, R.A., Hayek, L.C. & Foster, M.S. (eds). (1994). Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology,* 25, 1965–1978.

Hirschfeld, M., Blackburn, D.C., Doherty-Bone, T.M., Gonwouo, L.N., Ghose, S., & Rödel, M.-O. (2016). Dramatic declines of montane frogs in a Central African biodiversity hotspot. *PLOS ONE,* 11, e0155129.

Hitchman, S.M., Mather, M.E., Smith, J.M., & Fencl, J.S. (2018). Identifying keystone habitats with a mosaic approach can improve biodiversity conservation in disturbed ecosystems. *Global Change Biology,* 24, 308–321.

Holdridge, L.R. (1967). Life Zone Ecology. Tropical Science Center.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., & O'Connor, M.I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature,* 486, 105–108.

James, T.Y., Toledo, L.F., Rödder, D., da Silva Leite, D., Belasen, A.M., Betancourt-Román, C.M., Jenkinson, T.S., Soto-Azat, C., Lambertini, C., Longo, A.V., Ruggeri, J., Collins, J.P., Burrowes, P.A., Lips, K.R., Zamudio, K.R., & Longcore, J.E. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution,* 5, 4079–4097.

Jiménez, R., & Alvarado, G. (2017). *Craugastor escoces* (Anura: Craugastoridae) reappears after 30 years: rediscovery of an "extinct" Neotropical frog. *Amphibia-Reptilia,* 38, 257–259.

Kilburn, V.L., Ibáñez, R., Sanjur, O., Bermingham, E., Suraci, J.P., & Green, D.M. (2010). Ubiquity of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in anuran communities in Panamá. *EcoHealth, 7*, 537–548.

Kinney, V.C., Heemeyer, J.L., Pessier, A.P., & Lannoo, M.J. (2011). Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: affirmation of Vredenburg's "10,000 zoospore rule." *PLOS ONE,* 6, e16708.

Kriger, K.M., & Hero, J.-M. (2008). Altitudinal distribution of chytrid (*Batrachochytrium dendrobatidis*) infection in subtropical Australian frogs. *Austral Ecology,* 33, 1022–1032.

Kriger, K. M., & Hero, J.-M. (2007a). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions,* 13, 781–788.

Kriger, K. M., & Hero, J.-M. (2007b). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology,* 271, 352–359.

Kriger, K, Kriger, K. M., Hines, H. B., Hyatt, A. D., Boyle, D. G., & Hero, J.-M. (2006a). Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time TaqMan PCR. *Diseases of Aquatic Organisms,* 71, 141–148.

Kriger, K.M., Hero, J.-M., & Ashton, K.J. (2006b). Cost efficiency in the detection of chytridiomycosis using PCR assay. *Diseases of Aquatic Organisms,* 71, 149–154.

Kriger, K.M., Pereoglou, F., & Hero, J.-M. (2007). Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in Eastern Australia. *Conservation Biology,* 21, 1280–1290.

La Marca, E., Lips, K.R., Lotters, S., Puschendorf, R., Ibanez, R., Rueda-Almonacid, J.V., Schulte, R., Marty, C., Castro, F., Manzanilla-Puppo, J., Garcia-Perez, J.E., Bolanos, F., Chaves, G., Pounds, J.A., Toral, E., & Young, B.E. (2005). Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica,* 37, 190–201.

Lips, K.R. (1999). Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology,* 13, 117–125.

Lips, K.R. (1998). Decline of a tropical montane amphibian fauna. *Conservation Biology,* 12, 106–117.

Lips, K.R., Diffendorfer, J., Mendelson III, J.R., & Sears, M.W. (2008). Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLOS Biology,* 6, e72.

Lips, K.R., Reeve, J.D., & Witters, L.R. (2003). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology,* 17, 1078–1088.

Longcore, J.E., Pessier, A.P., & Nichols, D.K. (1999). *Batrachochytrium dendrobatidis* gen. et sp. nov. a chytrid pathogenic to amphibians. *Mycologia,* 91, 219–227.

Madison, J.D., Berg, E.A., Abarca, J.G., Whitfield, S.M., Gorbatenko, O., Pinto, A., & Kerby, J.L. (2017). Characterization of *Batrachochytrium dendrobatidis* inhibiting bacteria from amphibian populations in Costa Rica. *Frontiers in Microbiology,* 8, 290.

Miller, C.A., Tasse Taboue, G.C., Ekane, M.M.P., Robak, M., Sesink Clee, P.R., Richards-Zawacki, C., Fokam, E.B., Fuashi, N.A., & Anthony, N.M. (2018). Distribution modeling and lineage diversity of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in a central African amphibian hotspot. *PLOS ONE,* 13, e0199288.

Monastersky, R. (2014). Life-a status report. *Nature,* 516, 159–161.

Murray, K.A., Retallick, R.W., Puschendorf, R., Skerratt, L.F., Rosauer, D., McCallum, H.I., Berger, L., Speare, R., & VanDerWal, J. (2011). Assessing spatial patterns of disease risk to biodiversity: implications for the management of the amphibian pathogen, *Batrachochytrium dendrobatidis*. *Journal of Applied Ecology,* 48, 163–173.

Nishida, K. (2006). "Encounter with *Hyla angustilineata* Taylor, 1952 (Anura: Hylidae) in a cloud forest of Costa Rica. *Brenesia,* 66, 79–81.

Novacek, M.J., & Cleland, E.E. (2001). The current biodiversity extinction event: scenarios for mitigation and recovery. *Proceedings of the National Academy of Sciences,* 98, 5466–5470.

Nowakowski, A.J., Whitfield, S.M., Eskew, E.A., Thompson, M.E., Rose, J.P., Caraballo, B.L., Kerby, J.L., Donnelly, M.A., & Todd, B.D. (2016). Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances. *Ecology Letters,* 19, 1051–1061.

Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J., Garner, T.W.J., Weaver, G., & Fisher, M.C. (2013). Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PLOS ONE,* 8, e56802.

Ostfeld, R.S., & Keesing, F. (2000). Biodiversity and disease risk: the case of Lyme disease. *Conservation Biology,* 14, 722–728.

Perez, R., Richards-Zawacki, C.L., Krohn, A.R., Robak, M., Griffith, E.J., Ross, H., Gratwicke, B., Ibáñez, R., & Voyles, J. (2014). Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphibian & Reptile Conservation,* 8, 30–35.

Phillott, A.D., Grogan, L.F., Cashins, S.D., Mcdonald, K.R., Berger, L., & Skerratt, L.F. (2013). Chytridiomycosis and seasonal mortality of tropical stream-associated frogs 15 years after introduction of *Batrachochytrium dendrobatidis. Conservation Biology,* 27, 1058–1068.

Picco, A.M., & Collins, J.P. (2007). Fungal and viral pathogen occurrence in Costa Rican amphibians. *Journal of Herpetology,* 41, 746–749.

Piotrowski, J.S., Annis, S.L., & Longcore, J.E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia,* 96, 9–15.

Pounds, J.A, Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sánchez-Azofeifa, G.A., Still, C.J., & Young, B.E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature,* 439, 161–167.

Pounds, J.A., & Crump, M.L. (1994). Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. *Conservation Biology,* 8, 72–85.

Puschendorf, R., Hodgson, L., Alford, R.A., Skerratt, L.F., & VanDerWal, J. (2013). Underestimated ranges and overlooked refuges from amphibian chytridiomycosis. *Diversity and Distributions,* 19, 1313–1321.

Puschendorf, R., Hoskin, C.J., Cashins, S.D., McDonald, K., Skerratt, L.F., VanDerWal, J., & Alford, R.A. (2011). Environmental refuge from disease-driven amphibian extinction. *Conservation Biology,* 25, 956–964.

Puschendorf, R., Carnaval, A.C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., & Alford, R.A. (2009). Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions,* 15, 401–408.

Puschendorf, R., Bolaños, F., & Chaves, G. (2006). The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biological Conservation*, 132, 36–142.

R Core Team (2014). R: The R project for statistical computing. URL http://www.R-project.org/.

Raffel, T.R., & Fox, C. (2018). How do newts fight disease? They change their habitat. *Functional Ecology,* 32, 1142–1144.

Reeder, N. M., Pessier, A. P., & Vredenburg, V.T. (2012). A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. *PLOS ONE,* 7, e33567.

Retallick, R.W., McCallum, H., & Speare, R. (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLOS Biology,* 2, e351.

Rödder, D., Kielgast, J., & Lötters, S. (2010). Future potential distribution of the emerging amphibian chytrid fungus under anthropogenic climate change. *Diseases of Aquatic Organisms,* 92, 201–207.

Rödder, D., Veith, M., & Lötters, S. (2008). Environmental gradients explaining the prevalence and intensity of infection with the amphibian chytrid fungus: the host's perspective. *Animal Conservation,* 11, 513–517.

Rodríguez-Brenes, S., Rodríguez, D., Ibáñez, R., & Ryan, M.J. (2016). Spread of amphibian chytrid fungus across lowland populations of túngara frogs in Panamá. *PLOS ONE,* 11, e0155745.

Rollins-Smith, L. A. (2017). Amphibian immunity–stress, disease, and climate change. *Developmental & Comparative Immunology,* 66, 111–119.

Rosenblum, E.B., James, T.Y., Zamudio, K.R., Poorten, T.J., Ilut, D., Rodriguez, D., Eastman, J.M., Richards-Hrdlicka, K., Joneson, S., Jenkinson, T.S., Longcore, J.E., Parra Olea, G., Toledo, L.F., Arellano, M.L., Medina, E.M., Restrepo, S., Flechas, S.V., Berger, L., Briggs, C.J., & Stajich, J.E. (2013). Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proceedings of the National Academy of Sciences,* 110, 9385–9390.

Rovito, S.M., Parra-Olea, G., Vasquez-Almazan, C.R., Papenfuss, T.J., & Wake, D.B. (2009). Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of the National Academy of Sciences,* 106, 3231–3236.

Ryan, M.J., Lips, K.R., & Eichholz, M.W. (2008). Decline and extirpation of an endangered Panamanian stream frog population (*Craugastor punctariolus*) due to an outbreak of chytridiomycosis. *Biological Conservation,* 141, 1636–1647.

Sala, O.E., Chapin, F.S., Iii, Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., & Wall, D.H. (2000). Global biodiversity scenarios for the year 2100. *Science,* 287, 1770–1774.

Savage, A.E, Zamudio, K.R., & Sredl, M.J. (2011) Disease dynamics vary spatially and temporally in a North American amphibian. *Biological Conservation,* 144, 1910–1915.

Savage, J.M. (2002). The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas. University of Chicago Press.

Scheele, B.C., Hunter, D.A., Brannelly, L.A., Skerratt, L.F., & Driscoll, D.A. (2017). Reservoir-host amplification of disease impact in an endangered amphibian. *Conservation Biology,* 31, 592–600.

Scheele, B.C., Hunter, D.A., Skerratt, L.F., Brannelly, L.A., & Driscoll, D.A. (2015). Low impact of chytridiomycosis on frog recruitment enables persistence in refuges despite high adult mortality. *Biological Conservation,* 182, 36–43.

Scheele, B.C., Hunter, D.A., Grogan, L.F., Berger, L., Kolby, J.E., Mcfadden, M.S., Marantelli, G., Skerratt, L.F., & Driscoll, D.A. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology,* 28, 1195–1205.

Searle, C.L., Mendelson III, J.R., Green, L.E., & Duffy, M.A. (2013). *Daphnia* predation on the amphibian chytrid fungus and its impacts on disease risk in tadpoles. *Ecology and Evolution,* 3, 4129–4138.

Searle, C.L., Biga, L.M., Spatafora, J.W., & Blaustein, A.R. (2011a). A dilution effect in the emerging amphibian pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences,* 108, 16322–16326.

Searle, C.L., Gervasi, S.S., Hua, J., Hammond, J.I., Relyea, R.A., Olson, D.H., & Blaustein, A.R. (2011b). Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. *Conservation Biology,* 25, 965–974.

Skerratt, L., Berger, L., Hines, H., McDonald, K., Mendez, D., & Speare, R. (2008). Survey protocol for detecting chytridiomycosis in all Australian frog populations. *Diseases of Aquatic Organisms,* 80, 85–94.

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D., Hines, H.B., & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth,* 4, 125–134.

Stuart, S.N., Chanson, J., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D., & Waller, R. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science,* 306, 1783–1786.

Thorpe, C.J., Lewis, T.R., Fisher, M.C., Wierzbicki, C.J., Kulkarni, S., Pryce, D., Davies, L., Watve, A., & Knight, M.E. (2018). Climate structuring of *Batrachochytrium dendrobatidis* infection in the threatened amphibians of the northern Western Ghats, India. *Royal Society Open Science,* 5, 180211.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W.F., Dinudom, A., Cook, D., Webb, R., Alford, R.A., Skerratt, L.F., & Speare, R. (2009). Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science,* 326, 582–585.

von May, R., Catenazzi, A., Santa-Cruz, R., & Vredenburg, V.T. (2018) Microhabitat temperatures and prevalence of the pathogenic fungus *Batrachochytrium dendrobatidis* in lowland Amazonian frogs. *Tropical Conservation Science,* 11, 1–13.

Vredenburg, V.T., Knapp, R.A., Tunstall, T.S., & Briggs, C.J. (2010). Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences,* 107, 9689–9694.

Wake, D.B., & Vredenburg, V.T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences,* 105, 11466–11473.

Warne, R.W., LaBumbard, B., LaGrange, S., Vredenburg, V.T., & Catenazzi, A. (2016). Co-infection by chytrid fungus and ranaviruses in wild and harvested frogs in the Tropical Andes. *PLOS ONE,* 11, e0145864.

Whitfield, S., Alvarado, G., Abarca, J., Zumbado-Ulate, H., Zuñiga, I., Wainwright, M., & Kerby, J. (2017). Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms,* 126, 33–41.

Whitfield, S.M, Geerdes, E., Chacon, I., Ballestero Rodriguez, E., Jimenez, R., Donnelly, M., & Kerby, J. (2013). Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. *Diseases of Aquatic Organisms,* 104, 173–178.

Whitfield, S.M., Kerby, J., Gentry, L.R., & Donnelly, M.A. (2012). Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. *Biotropica,* 44, 779–784.

Whitfield, S.M., Bell, K.E., Philippi, T., Sasa, M., Bolaños, F., Chaves, G., Savage, J.M., & Donnelly, M.A. (2007). Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences,* 104, 8352–8356.

Woodhams, D.C., Bell, S.C., Bigler, L., Caprioli, R.M., Chaurand, P., Lam, B.A., Reinert, L.K., Stalder, U., Vazquez, V.M., Schliep, K., Hertz, A. & Rollins-Smith, L.A. (2016). Life history linked to immune investment in developing amphibians. *Conservation Physiology,* 4, cow025.

Woodhams, D.C., Bosch, J., Briggs, C.J., Cashins, S., Davis, L.R., Lauer, A., Muths, E., Puschendorf, R., Schmidt, B.R., Sheafor, B., & Voyles, J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology,* 8, 8.

Woodhams, D.C., Kilburn, V.L., Reinert, L.K., Voyles, J., Medina, D., Ibánez, R., Hyatt, A.D., Boyle, D.G., Pask, J.D., Green, D.M., & Rollins-Smith, L.A. (2008). Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. *EcoHealth, 5*, 268–274.

Young, B.E., Lips, K.R., Reaser, J.K., Ibanez, R., Salas, A.W., Cedeño, J.R., Coloma, L.A., Ron, S., La Marca, E., Meyer, J.R., Muñoz, A., Bolaños, F., Chaves, G., & Romo, D. (2001). Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology,* 15, 1213–1223.

Zar, J.H. (2013). Biostatistical analysis. Pearson Higher Ed.

Zumbado-Ulate, H., Bolaños, F., Gutiérrez-Espeleta, G., & Puschendorf, R. (2014). Extremely low prevalence of *Batrachochytrium dendrobatidis* in frog populations from Neotropical dry forest of Costa Rica supports the existence of a climatic refuge from disease. *EcoHealth,* 11, 593–602.

Zumbado-Ulate, H., Bolaños, F., Willink, B., & Soley-Guardia, F. (2011). Population status and natural history notes on the critically endangered stream-dwelling frog *Craugastor ranoides* (Craugastoridae) in a Costa Rican tropical dry forest. *Herpetological Conservation and Biology,* 6, 455–464.

CHAPTER 2. ENDEMIC INFECTION OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN COSTA RICA: IMPLICATIONS FOR AMPHIBIAN CONSERVATION AT REGIONAL AND SPECIES LEVEL

Reprinted by permission from MDPI. *Diversity*. Endemic infection of *Batrachochytrium dendrobatidis* in Costa Rica: Implications for amphibian conservation at regional and species level. Hector Zumbado-Ulate, Kiersten. N. Nelson, Adrián García-Rodríguez, Gerardo. Chaves, Erick Arias, Federico Bolaños, Steven M. Whitfield, and Catherine L. Searle. 2019.

2.1 Abstract

Batrachochytrium dendrobatidis (*Bd*) has been associated with the severe declines and extinctions of amphibians in Costa Rica that primarily occurred during the 1980s and 1990s. However, the current impact of *Bd* infection on amphibian species in Costa Rica is unknown. We aimed to update the list of amphibian species in Costa Rica and evaluate the prevalence and infection intensity of *Bd* infection across the country to aid in the development of effective conservation strategies for amphibians. We reviewed taxonomic lists and included new species descriptions and records for a total of 215 amphibian species in Costa Rica. We also sampled for *Bd* at nine localities between 2015–2018 and combined these data with additional *Bd* occurrence data from multiple studies conducted in amphibian communities across Costa Rica from 2005–2018. With this combined dataset, we found that *Bd* was common (overall infection rate of 23%) across regions and elevations, but infection intensity was below theoretical thresholds associated with mortality. *Bd* was also more prevalent in Caribbean lowlands and in terrestrial amphibians with an aquatic larval stage; meanwhile, infection load was the highest in direct-developing species (forest and stream-dwellers). Our findings can be used to prioritize regions and taxonomic groups for conservation strategies.

2.2 Introduction

Anthropogenic threats including habitat destruction, pollution, climate change, introduction of invasive species, and pathogens are causing a rapid and severe decline in global biodiversity (Novacek & Cleland, 2001). Scientific consensus states that we are in the midst of a sixth mass extinction event (Barnosky et al., 2011; Wake & Vredenburg, 2008). Within vertebrates,

amphibians are the most endangered taxonomic class with approximately 41% of described species classified as "globally threatened" (Monastersky, 2014; Stuart et al., 2004). The majority of the amphibian declines have occurred in the tropics of Australia, Central America, and South America (Catenazzi, 2015; Daszak et al., 1999), and have been observed even in seemingly pristine and protected environments (Collins, 2010; La Marca et al., 2005). However, information is still lacking regarding which species are suffering the greatest declines and which abiotic and biotic factors are contributing the most (Scheele et al., 2019). Identifying threatened species and factors contributing to global amphibian declines is vital for effective conservation and management efforts (Gerber et al., 2018; Meredith et al., 2016).

Costa Rica, with an area of only 51,100 km^2, is home to a great diversity of amphibians (Savage, 2002). More than 200 of the approximately 8000 described amphibian species are present in Costa Rica (Frost, 2019), and new species continue to be described. The vast species richness confined to a relatively small area is due to complex biogeographic events and climatic conditions throughout the country, and a long history of work has been done by in-country taxonomic specialists (Bagley & Johnson, 2014; Savage, 2002). Costa Rica is also an example of a country where numerous amphibian population declines have occurred in response to multiple environmental threats (Bolaños, 2009), highlighted by the enigmatic disappearance of the golden toad (*Incilius periglenes*) (Pounds & Crump, 1994). However, several species that catastrophically declined in the last thirty years, such as the harlequin frog, the Golfito robber frog, and the Holdridge's toad, have been recently rediscovered in viable populations (Abarca et al., 2010; Chaves et al., 2014; González-Maya et al., 2013). These findings suggest that highly susceptible species can recover from or at least persist when faced with deadly threats (Hero et al., 2005). Thus, Costa Rica is an excellent location to study not only how amphibian communities are affected by environmental threats but also their resistance and resilience from declines (Mendelson et al., 2019).

One widespread cause of amphibian declines is the introduction of the pathogen *Batrachochytrium dendrobatidis* (*Bd*) (Longcore et al., 1999). This fungus causes chytridiomycosis (Berger et al., 1998), a potentially deadly skin disease that has contributed to the decline of at least 500 amphibian species globally (Scheele et al., 2019). In Central America, amphibian declines peaked during the 1980s and 1990s and have been linked to the introduction of *Bd*, which caused deadly outbreaks of chytridiomycosis (i.e., epizootics) (Bolaños, 2009; Lips

et al., 2003; Puschendorf et al., 2006). It has been suggested that *Bd*-driven epizootic declines mostly affected species in highland lotic environments because moisture and temperature in these sites matches the optimal conditions for *Bd* growth in the lab (Piotrowski et al., 2004; Pounds et al., 2006). However, it is also well known that some amphibian species suffered unexpected and unexplained declines in lowland environments (<700 m above sea level) during the 1980s and 1990s, likely due to chytridiomycosis (Bolaños, 2009; Chaves et al., 2014; Puschendorf et al., 2009; Whitfield et al., 2007; Zumbado-Ulate et al., 2011). After the declines, the evolution of resistance and tolerance mechanisms in amphibian communities (Christie & Searle, 2018), and/or the evolution of less-pathogenic strains of *Bd* (Retallick & Miera, 2007), might have allowed susceptible amphibians to persist with endemic *Bd* infection (i.e., enzootics) (Briggs et al., 2005; Mendelson et al., 2019; Rachowicz et al., 2006; Retallick et al., 2004). However, susceptible species are still at a high risk of extinction under endemic infection if conditions shift in favor of the pathogen. For example, the introduction of an invasive species that is also a competent reservoir might amplify infection in the environment to epizootic levels (Briggs et al., 2010; O'Brien et al., 2011; Searle et al., 2011). Thus, examining the life history traits and conditions that may favor outbreaks of *Bd* is the key to understanding the underlying mechanisms behind why some infected species declined more severely than others and which species are most vulnerable to future outbreaks (Hitchman et al., 2018; Miller et al., 2018).

In this study, we present an updated list of all the amphibian species of Costa Rica, quantifying species diversity in each herpetological province and describing their conservation status. We also identified the effect of geography (herpetological province and altitudinal belt) and life-history traits associated with foraging and reproduction on current infection with *Bd*. For this, we sampled for *Bd* at nine tropical localities across Costa Rica from 2015–2018. In addition, we built a robust dataset by adding records from studies that detected *Bd* across Costa Rica from 2005–2018 in multi-species amphibian assemblages. We hypothesized that *Bd* is widespread across herpetological provinces and altitudinal belts in Costa Rica and would exhibit an infection intensity below theoretical thresholds associated with mass mortalities (Vredenburg et al., 2010). To compare across life-history traits, we developed an index that combines foraging habitat, reproductive habitat, and type of development. We hypothesized that *Bd* infection would vary across habitats, with the highest prevalence and infection intensity found in species with the greatest use of cool and humid environments (Brem & Lips, 2008; Kriger & Hero, 2007). The

knowledge from this work will aid policy-makers in identifying the most threatened regions and taxonomic clades to develop better conservation strategies in Costa Rica (Heard et al., 2018; Mendelson et al., 2019; Scheele et al., 2014).

2.3 Materials and methods

2.3.1 Species assessment

We updated the last official list of amphibian species in Costa Rica published in 2011 (Bolaños et al. 2011) by consulting the Herpetological Database ("Herp Database") of the Museo de Zoología at Universidad de Costa Rica (http://museo.biologia.ucr.ac.cr/) and taxonomists' lists (Savage & Bolaños 2009; Sasa et al. 2010). In addition, we georeferenced the distribution of all amphibian species within the five Costa Rican herpetological provinces (see Section 2.3.3.1). For this, we extracted all collection points for each species from the "Herp Database" (Datum WGS1984) and mapped them using a shapefile of the Costa Rican herpetological provinces and QGIS software 3.8.1 (QGIS Development Team, http://qgis.osgeo.org). For every species, we showed their status in Costa Rica [50] according to the International Union of Conservation of Nature (IUCN) [51] as follows: NA = "not applicable," DD = "data deficient," LC = "least concerned," NT = "near threatened," VU = "vulnerable," EN = "endangered," CR = "critically endangered," and EX = "extinct in the wild" (for additional details see http://www.iucnredlist.org/). We also included environmental vulnerability scores (EVS; Wilson & McCranie 2004), a regional vulnerability index that classifies amphibians and reptiles into four levels of risk: "no immediate risk" (EVS < 3), "low vulnerability" (EVS of 3–9), "medium vulnerability" (EVS of 10–13), and "high vulnerability" (EVS of 14–17). A high EVS indicates species that are restricted in distribution, occur in a single life zone, and have a highly derived reproductive mode. The EVS for Costa Rican amphibians reported here were extracted from Sasa et al. (2010). Finally, we compiled a list of all the species that have been screened for *Bd* and the methods used for detection: histology or polymerase chain reaction (PCR).

2.3.2 Field Dataset

To add to existing datasets of amphibian distribution and *Bd* infection, we surveyed nine amphibian assemblages across Costa Rica in both versants (Caribbean and Pacific) and at

elevations ranging from sea level to 1385 m (Fig. B.1). All surveys were conducted during the months of June and July between 2016–2018, except in the locality of Alto Lari, which was sampled in March 2015. At each site, we conducted visual and acoustic encounter surveys searching for amphibians in streams, ponds/puddles, and forest (leaflitter and canopy), and then caught individuals to screen them for *Bd* (see below). In total, we screened for *Bd* from 267 amphibians from 33 species (see Tables B.1 and B.2, Fig. B.2). Four of those species were classified in threatened categories: *Oophaga granulifera* (VU), *Ptychohyla legleri* (EN), *Craugastor ranoides* (CR), *and C. taurus* (CR).

All observed amphibians were collected using nitrile gloves and temporally placed individually in clean, unused plastic bags. Each individual was inspected for visible signs of chytridiomycosis, such as hyperplasia, hyperkeratosis, abnormal shedding, depigmentation, and lethargic behavior (Berger et al., 1998; Voyles et al., 2009). We swabbed (using MW–113 swabs) each individual's skin to detect *Bd* as follows: five strokes on one hand, five strokes on the ventral patch, five strokes on one foot, and five strokes along the inner thigh. The swabs were collected and placed into 1.5 mL screw-cap tubes and stored dry at -20°C until fungal DNA extraction. Once swabbed, all animals were released back in the site they were originally collected from. During this study we followed field protocols (Kriger et al., 2006b; Skerratt et al., 2008) approved by the National System of Conservation Areas of Costa Rica (SINAC), the Comisión Nacional para la Gestión de la Biodiversidad (CONAGEBIO), and animal care protocols from the Purdue Animal Care and Use Committee (PACUC 1604001392), ensuring that all animals are being cared for in accordance with standard protocols and treated in an ethical manner (Research permits 001–2012–SINAC, R-019-2016-OT-CONAGEBIO, R-023-2016-OT-CONAGEBIO, R-057-2016-OT-CONAGEBIO, R-060-2016-OT-CONAGEBIO).

We conducted diagnostic quantitative PCR (qPCR) on each swab to quantify *Bd* infection load following standard protocols (Boyle et al., 2004), with the following modifications: (1) the fungal DNA was extracted using 60 µL of PrepMan Ultra, and (2) an internal positive control (IPC) was used to detect inhibitors (Hyatt et al., 2007). Fungal DNA was diluted 1:10 in 0.25X TE buffer and run in singlicate (Kriger et al., 2006a) using a Step One Plus (Applied Biosystems). Negative controls (DNase/RNase-free water) were run in triplicate on every 96-well qPCR plate. We classified samples as positive when both dyes (*Bd* probe and IPC) amplified in each well. Samples absent of IPC amplification were considered inhibited. In order to eliminate inhibitors, we diluted

5 µL of a new dilution in 0.25X TE buffer in a proportion of 1:100. Ten samples were classified as inhibited and then determined to be negative after dilution. Quantification curves for genomic equivalents were constructed using 1000, 100, 10, and 1 zoospore quantification standards derived from a gBlock® Gene fragment (Integrated DNA Technologies). In order to calculate the zoospore genomic equivalents in the original sample, we multiplied the qPCR score by the dilution factor of 120 (dilution factor = 60 x 20 x 1/10). We estimated prevalence with 95% binomial confidence intervals (CIs) by locality.

2.3.3 Combined dataset

We generated a dataset from multiple studies that screened for *Bd* in multiple amphibian assemblages in Costa Rica after the year 2000 using conventional PCR and qPCR methods (Picco & Collins, 2007; Goldberg et al., 2009; Saenz et al., 2009; Whitfield et al., 2013; Zumbado-Ulate et al., 2014, 2019; Abarca, 2018) (Fig. 2.1, Table 2.1) (including the 267 individuals from 33 species we tested in the "field dataset" (see methods 2.3.2 and supporting data). In total, this "combined dataset" consisted of 1750 individual records from 79 species and 20 localities at elevations ranging from sea level to 2000 m. We identified the year 2000 as the starting of post-decline because most epizootic outbreaks of *Bd* occurred during the 1980's and early 1990's (Puschendorf et al., 2006, 2009; Lips et al., 2008). We also assumed that *Bd* expanded its range across Costa Rica by 2000 due to the rapid rate of spreading that this pathogen exhibits in tropical locations (Lips et al., 2005; Whitfield et al., 2016).

Although *Bd* was detected in 405 swabs in this dataset, quantification through qPCR was conducted only in 351 *Bd*-positive swabs (from the "field dataset" and three of the seven reviewed studies (Whitfield et al., 2013; Zumbado-Ulate et al. 2014, 2019). We did not consider studies that used histology as method of detection because most of these studies evaluated samples that were taken before 2000. We also excluded records of individuals that were identified only at the genus level, e.g., *Craugastor* spp. (Saenz et al., 2009) and *Agalychnis* spp. (Whitfield et al., 2013) and cases where only one species was screened for *Bd* (e.g., *Atelopus varius* in the locality of Uvita (Abarca, 2018). Finally, we classified all sampled amphibians according to herpetological province, altitudinal belt, and life history traits (foraging habitat, reproductive habitat, and type of development).

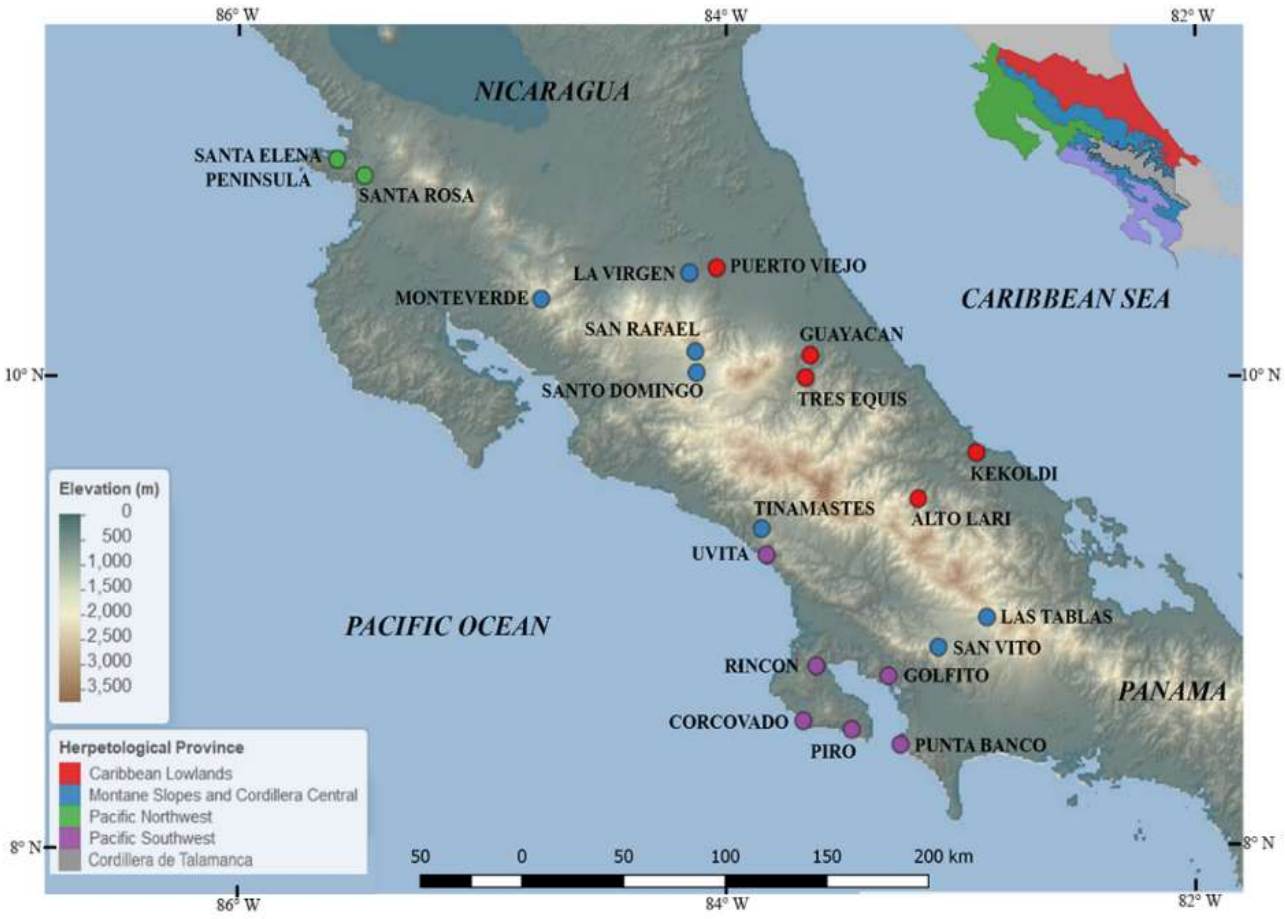

Figure 2.1. Map of 20 survey sites across Costa Rica. Sites are color-coded by herpetological province.

Table 2.1. Summary of studies where *Batrachochytrium dendrobatidis* (*Bd*) was detected in multi-species amphibian assemblages using conventional PCR and quantitative PCR (qPCR) in Costa Rica between 2005–2018. The table shows surveyed localities, herpetological province, sampling period, percentage of infection, and Holdridge's altitudinal belt. Symbology: CL—Caribbean Lowlands, MSCC—Montane Slopes and Cordillera Central, PN—Pacific Northwest, and PS—Pacific Southwest.

Study site (elevation m) and herpetological provinces	Sampling period	% of infection (n sampled)	Altitudinal belt	Reference
Monteverde (1400–2000), MSCC	Jul-05	12.2 (41)	Lower montane	(Picco & Collins, 2007)
San Vito de Coto Brus (1120–1385), MSCC		9.3 (43)	Premontane	
Rincón de Osa (0–100), PS	May-June 2006	0.1 (91)	Lowland	(Goldberg et al., 2009)
Piro (0–100), PS		0.0 (62)	Lowland	
Corcovado (0–100), PS		0.1 (25)	Lowland	
Kekoldi (0–100), CL	Jan-18	7.9 (126)	Lowland	
La Virgen de Sarapiquí (0–200), CL	January-March 2011	21.3 (253)	Lowland	
Santa Elena Peninsula (0–200), PN	January-March 2007-2008	0.0 (310)	Lowland	(Saenz et al., 2009)
Santa Rosa (0–200), PN		9.0 (100)	Lowland	
Punta Banco–Burica (0–100), PS	November-December 2011	68.6 (35)	Lowland	(Whitfield et al., 2013) (Zumbado-Ulate et al., 2014)
Rincón de Osa (0–100), PS		0.0 (25)	Lowland	
Puerto Viejo de Sarapiquí (0–200), CL		67.4 (144)	Lowland	
Guayacán de Siquirres (400–600), CL		47.9 (144)	Lowland	
San Vito de Coto Brus (1120–1385), MSCC	Unknown/ not indicated	10.5 (19)	Lowland	
Punta Banco–Burica (0–100), PS		0.0 (20)	Lowland	
Guayacán de Siquirres (400–600), CL		5.3 (19)	Lowland	
San Rafael de Heredia (1800), MSCC		66.7 (15)	Lower montane	
Santo Domingo de Heredia (1000-1200), MSCC		45.5 (11)	Premontane	
Las Tablas (1350), MSCC		28.6 (14)	Lower montane	

2.3.3.1 Herpetological Provinces

We classified all surveyed assemblages within the five herpetological provinces proposed by Savage (2002) and modified by Sasa and colleagues (2010).

Caribbean Lowlands: This faunal area represents 30% of Costa Rica and includes the lowlands of the Caribbean versant and the northern most region of the country, predominantly consisting of lowland wet forest. Sampling for *Bd* through PCR has been conducted in the localities of La Virgen de Sarapiquí, Puerto Viejo de Sarapiquí, Tres Equis de Turrialba, Guayacán de Siquirres, Kekoldi, and the remote Alto Lari. Alto Lari was surveyed as part of a recent expedition

following an enigmatic path that connects the Caribbean Lowlands with the highlands of Cordillera de Talamanca and is known as “the Gabb’s route” (Arias & Chaves, 2014).

Pacific Northwest: This herpetological province includes the lowlands of the Pacific Northwest and extends into the western side of the Central Valley, in the Meseta Central Occidental (Central Valley) up to the base of Cerros de Ochomogo. The Pacific Northwest consists of predominantly Lowland Dry Forest vegetation and constitutes 24% of Costa Rica’s area. This province contains a distinctive dry season that lasts five to six months. Within the Pacific Northwest, sampling for *Bd* has been conducted in the tropical dry forest at Guanacaste National Park (Santa Rosa and Santa Elena Peninsula stations).

Pacific Southwest: Encompassing the lowlands of the Pacific central and south, the herpetological provinces consist primarily of lowland wet forest and lowland moist forest and accounts for 15% of the country’s area. This herpetological province is biogeographically related to the Caribbean Lowlands and species have more recently been differentiated between these herpetological provinces due to isolation caused by the uplifting of the Cordillera de Talamanca. Within the Pacific Southwest, sampling for *Bd* through PCR has been conducted in the localities of Punta Banco–Burica, Golfito, Rincón de Osa, Piro, Corcovado, and Uvita.

Montane Slopes and Cordillera Central: This area represents 23% of Costa Rica and occurs along all of Costa Rica’s mountain ranges from 500–2100 m elevation in Cordillera de Guanacaste, 500–3400 in Cordillera Central, and 500–1600 in Cordillera de Talamanca (Lower Talamanca). The Montane Slopes and Cordillera Central province includes regions that receive the highest annual precipitation in the country. Sampling for *Bd* through PCR has been conducted in the localities of Monteverde, San Vito de Coto Brus, Las Tablas, Tinamastes de Pérez Zeledón, San Rafael de Heredia, and Santo Domingo de Heredia.

Cordillera de Talamanca: Found at elevations above 1600 m (upper Talamanca). This is the smallest faunal area (8% of Costa Rica) and consists primarily of montane rainforest and subalpine pluvial paramo. This faunal area is the least explored herpetological province of Costa Rica and there is no published data for *Bd* detection through PCR or qPCR in this faunal province.

2.3.3.2 Altitudinal Belt

We classified species according to the Holdridge's life zone system (Holdridge 1967), which divides Costa Rica into five altitudinal belts: lowland, premontane, lower montane, montane, and subalpine. Due to the elevational limits for altitudinal belts being slightly different among regions in Costa Rica, we established the limits of each belt as follows: lowland (0–700 m), premontane (700–1500 m), lower montane (1500–2700 m), montane (2700–3500 m), and subalpine (>3500 m).

2.3.3.3 Foraging-Reproduction Habitat Index

To compare *Bd* infection dynamics across taxonomic groups, we developed a foraging–reproduction habitat index (FRHI). The FRHI was created to classify species with a system of three letters that represented life history traits associated with foraging and reproduction (Table 2.2). First, we classified species according to their development into indirect- (I) or direct-developing amphibians (D). Second, we classified species according to their foraging habitat into terrestrial (T), arboreal (A), pond/puddle-dwellers (P), stream-dwellers (R), and phytotelma (F). Finally, species were classified according to their reproductive habitat into terrestrial (T), arboreal (A), pond/puddle-breeders (P), stream-breeders (R), and phytotelma (F).

Table 2.2. Categories and taxonomic examples of the foraging–reproduction habitat index (FRHI) that we developed for this study to analyze current prevalence of *Batrachochytrium dendrobatidis* across taxonomic groups. Symbology: First letter represents development: (I) indirect- or (D) direct-developing amphibians. Second letter represents foraging habitat: terrestrial (T), arboreal (A) pond/puddle-dwellers (P), stream-breeders (R), and phytotelma (F). Third letter represents reproductive habitat: terrestrial (T), arboreal (A), pond/puddle-dwellers (P), stream-breeders (R), and phytotelma (F).

FRHI	Species	Taxonomic Group (Examples)
DAA	2	*Diasporus* spp. (dink frogs, e.g., *Diasporus diastema*)
DAT	5	*Pristimantis* spp. (rain frogs, e.g., *Pristimantis cerasinus*)
DRT	3	*Craugastor punctariolus* clade (robber frogs, e.g., *Craugastor taurus*) *C. fitzingeri* (Pacific side)
DTT	13	*Craugastor* spp. (leaf-litter frogs. e.g., *Craugastor bransfordi*) *C. fitzingeri* (Caribbean side) Plethodontidae (e.g., *Oedipina gracilis)*
IAP	17	Hylidae (pond-breeding treefrogs, e.g., *Boana rufitela*)
IAR	15	Centrolenidae (glass frogs, e.g., *Teratohyla pulverata*) Hylidae (stream-breeding treefrogs, e.g., *Duellmanohyla rufioculis*)
ITF	4	Dendrobatidae (Poison-dart frogs, e.g., *Oophaga pumilio*)
ITP	12	Leptodactylidae (Leptodactylid frogs, e.g., *Leptodactylus melanonotus*) Microhylidae (sheep frogs, e.g., *Hypopachus variolosus*) Ranidae (Ranid frogs, e.g., *Lithobates forreri*) Bufonidae (toads, e.g., *Incilius coccifer*)
ITR	7	Bufonidae (river toads, e.g., *Rhaebo haematiticus*)

2.3.4 Statistical analysis

We reduced our "combined dataset" to 1741 samples from 74 species and 20 localities because there was insufficient information to accurately classify nine records of the species *Diasporus tigrillo, D. vocator, Hyloscirtus palmeri, Triprion spinosus,* and *Cruziohyla calcarifer* in the FRHI. For our analyses, we pooled all species together instead of using species as predictor because the samples sizes per species were highly variable (from 1–177), which could produce significant models that may be an artifact of opportunistic sampling instead of a real pattern. Instead, we used the FRHI, which is highly correlated with taxonomic group. We were unable to include time of sampling as predictor in our analyses because these data were missing in several of the amphibian assemblages sampled. All our analyses were performed with the R package "stats" (R Core Team 2018).

We analyzed *Bd* prevalence with fixed-effects generalized linear models (GLMs) using infected status as binomial response variable (uninfected or infected) and herpetological province,

altitudinal belt, and the FRHI as predictors. Ranking of the candidate GLMs followed the Akaike's information criterion (AIC) where the model with the lowest AIC was considered the most robust (Burnham & Anderson 2004). To analyze *Bd* infection intensity (estimated as the number of genomic equivalents), we analyzed the 351 *Bd*-positive swabs where *Bd* infection intensity was quantified through qPCR (see methods 2.3). We used linear models (LMs) to compare *Bd* infection intensity (response variable) across herpetological provinces, altitudinal belts, and FRHI (predictor variables). We log-transformed the *Bd* infection intensity to reduce skewness. Statistical significance of models was tested with ANOVA. For both, GLMs and LMs, we conducted post hoc, pairwise comparisons (Tukey's honestly significant difference; HSD-test), to confirm where the differences occurred between significant predictors. We were unable to run mix-effects models or fixed-effects interaction models because some combinations of predictors presented missing or low values, causing convergence difficulties.

2.4 Results

2.4.1 Species Assessment

The previous list of Costa Rican amphibians from 2011 included 196 species; however, we excluded the species *Pristimantis educatoris* (Ryan et al. 2010) due to taxonomic uncertainty (Batista et al. 2014) for a total of 195 species. Our new list of amphibians in Costa Rica included a total of 215 species grouped in three orders, 16 families, and 48 genera (Table B.3). This represented and addition of 20 species (10 anurans, 9 salamanders, and 1 caecilian; Table 2.3). The order Anura (frogs and toads) is the most diverse in Costa Rica, being 72% of the total species (13 families and 41 genera). Salamanders (order Caudata) are represented by only one family (Plethodontidae), three genera, and 53 species. Finally, caecilians (order Gymnophiona) are represented by two families, four genera, and eight species. A total of 63 species are endemic to Costa Rica (36 salamanders, 24 anurans, and 3 caecilians). We also included five anuran species that are not native to Costa Rica (*Eleutherodactylus coqui, E. johnstoni, E. planirostris, Lithobates catesbeianus,* and *Osteopilus septentrionalis*).

Table 2.3. List of new additions to the updated list of amphibians of Costa Rica.

	Family	Species	Source
	Centrolenidae	*Hyalinobatrachium dianae*	(Kubicki et al. 2015)
		Craugastor aenigmaticus	(Arias et al. 2018)
		Craugastor gabbi	(Arias et al. 2016)
	Craugastoridae	*Craugastor zunigai*	(Arias et al. 2019b)
		Diasporus amirae	(Arias et al. 2019a)
	Eleutherodactylidae	*Eleutherodactylus planirostris**	(Barquero & Araya 2016)
		Ecnomiohyla bailarina	(Kubicki & Salazar 2015)
		Ecnomiohyla veraguensis	Unpublished
	Hylidae	*Smilisca manisorum*	(McCranie 2017)
Anura	Phyllomedusidae	*Cruziohyla sylviae*	(Gray 2018)
		Bolitoglossa aurae	(Kubicki & Arias 2016)
		Bolitoglossa aureogularis	(Boza-Oviedo et al. 2012)
		Bolitoglossa kamuk	(Boza-Oviedo et al. 2012)
		Bolitoglossa pygmaea	Unpublished
		Bolitoglossa splendida	(Boza-Oviedo et al. 2012)
		Nototriton costaricense	(Arias & Kubicki 2018)
		Nototriton matama	(Boza-Oviedo et al. 2012)
		Oedipina berlini	(Kubicki 2016)
Caudata	Plethodontidae	*Oedipina nimaso*	(Boza-Oviedo et al. 2012)
Gymnophiona	Caeciliidae	*Caecilia volcani*	(Kubicki & Arias 2017)

Notes **Eleutherodactylus planirostris* is an invasive species that have been found in the Caribbean Lowlands of Costa Rica.

Regionally (Fig. 2.2a, Table B.3), the Cordillera de Talamanca is the most diverse province in terms of species per area (88 species, 2.2 species/100 km^2). It contains 23 species of amphibians that only occurred within this herpetological province (e.g., *Diasporus ventrimaculatus, Nototriton costaricense*). The Montane Slopes and Cordillera Central is the most diverse herpetological province (188 species, 1.3 species/100 km^2), with 27 species that are exclusively found within this herpetological province (e.g., *Cochranella euknemos, Nototriton picadoi*). The Caribbean Lowlands (101 species, 0.7 species/100 km^2) includes 20 species that are only found within this herpetological province (e.g., *Cruziohyla calcarifer, Caecilia volcani*). The Pacific Southwest (71 species, 0.9 species/100 km^2) has five species that only occur within this province (e.g., *Craugastor taurus, Oophaga granulifera*). Finally, the Pacific Northwest (66 species, 0.5 species/100 km^2) includes only two species that are found exclusively within this province (*Rhinophrynus dorsalis* and *Eleutherodactylus johnstonei*). A total of 20 species occur in all five

herpetological provinces (e.g., *Craugastor fitzingeri, Diasporus diastema, Dendropsophus ebraccatus, Hyalinobatrachium fleischmanni, Lithobates warszewitschii, Rhinella horribilis, Smilisca sordida*).

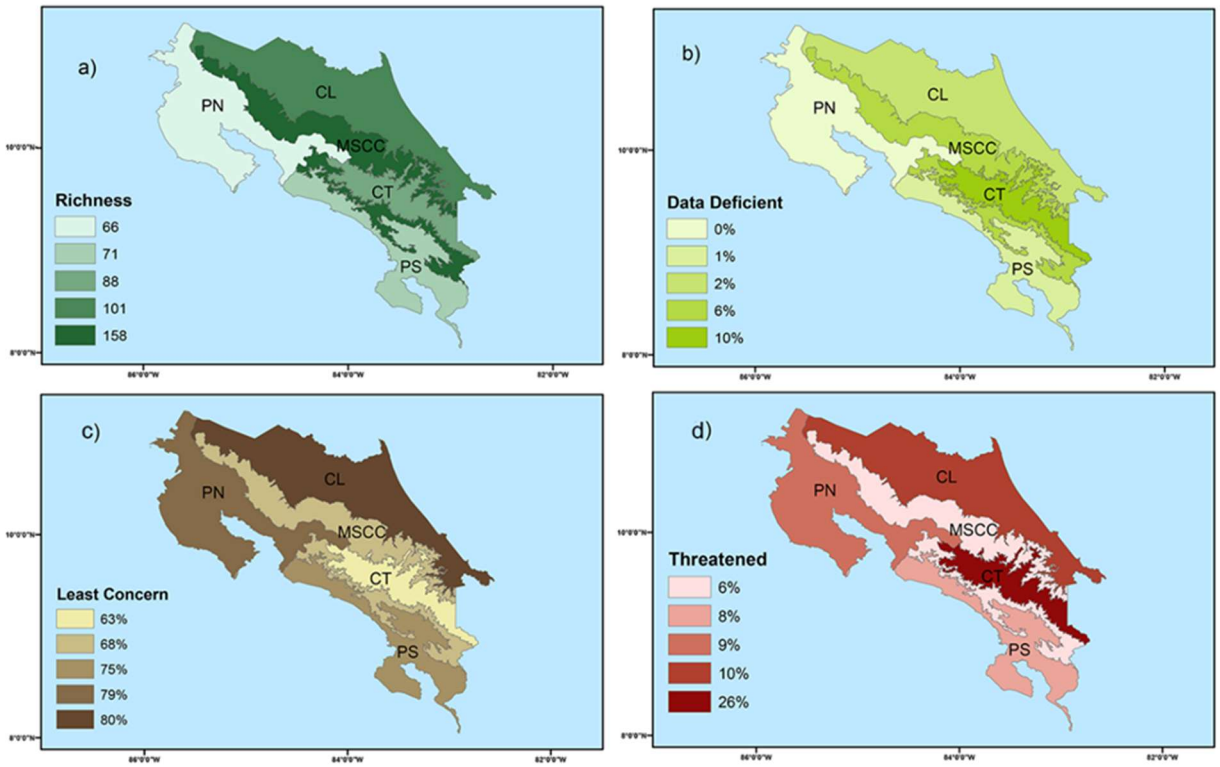

Figure 2.2. Map of Costa Rica showing (a) amphibian species richness for each herpetological province and percentage of amphibian species classified as (b) data deficient, (c) least concerned, and (d) threatened categories (near threatened, vulnerable, endangered, critically endangered, and extinct in the wild) for each herpetological province according to the Red List of Threatened Species from the International Union of Conservation of Nature (IUCN). Symbology: CL—Caribbean Lowlands, MSCC—Mountain Slopes and Cordillera Central, PN—Pacific Northwest, and PS—Pacific Southwest.

Overall, 200 species have been classified into IUCN categories at the country level and 15 species need future assessment. A total of 155 species do not fulfill the criteria to be considered in the threatened categories, including 136 species listed as LC, 18 as DD, and one as NA, a category for taxa that occur in the region but have been excluded from the regional Red List for a specific reason. Within threatened categories, two species are listed as EX, 24 as CR, ten as EN, seven as VU, and two as NT. Regionally, lowlands exhibited the lowest percentage (0–2%) of DD species (Figure 2.2b). Similarly, approximately 75–80% of species occurring in lowlands are listed as LC (Fig. 2.2c). In highlands, 6–10% of species are categorized as DD (Fig. 2.2b) and 26% of species

in Cordillera de Talamanca are classified within threatened categories (Fig. 2.2d). According to EVS, a total of 81 species were classified as "no immediate risk," four species at "low vulnerability," 50 species at "medium vulnerability," and 48 species at "high vulnerability" (Table B.3).

In our review, we found a total of 105 amphibian species (49%) that have been screened for *Bd* in Costa Rica (103 anurans and only 2 species of salamanders) (Table B.4). In the field, the most common method used to detect *Bd* was qPCR, especially after 2005. Conventional PCR was used only in one study in the Caribbean Lowlands (Saenz et al. 2009). Histology and qPCR have also been used in retrospective studies on preserved specimens from declined and extinct species.

2.4.2 Endemic dynamics

Overall, *Bd* prevalence in Costa Rica was estimated to be 0.23 (60% of species tested positive for *Bd*) (Table B.5). The most robust GLM found both herpetological province and the FRHI as significant predictors of *Bd* prevalence (AIC = 1700, $p < 0.001$, Table B.6). Among herpetological provinces (Fig. 2.3), the highest percentage of infected individuals was found in the Caribbean Lowlands (34%) and the lowest in the Pacific Northwest (4%). The Mountain Slopes, Cordillera Central, and Pacific Southwest had a similar percentage of infected individuals (≈23%). Furthermore, *Bd* was proportionally more prevalent in amphibians with terrestrial foraging and larval stage in phytotelma (ITF), pond-breeding treefrogs (IAP), and direct-developing species that breed in the forest (leaf-litter frogs DTT, rain frogs, DAT) (Fig. 2.4a). The species *Craugastor taurus* (the Golfito robber frog) was the species that had the highest average infection load (average *Bd* load of 11632.4 versus 571.6 genomic equivalents or 2.51 versus 1.18 after log transformation) (Table 2.4). We found an effect of the FRHI on infection load ($F_{8,342} = 7.91$, $p < 0.01$, Table B.6). Direct-developing frogs with terrestrial reproduction (robber frogs and leaf-litter frogs; DTR and DTT respectively) had the highest *Bd* loads (Fig. 2.4b, Table 2.4)

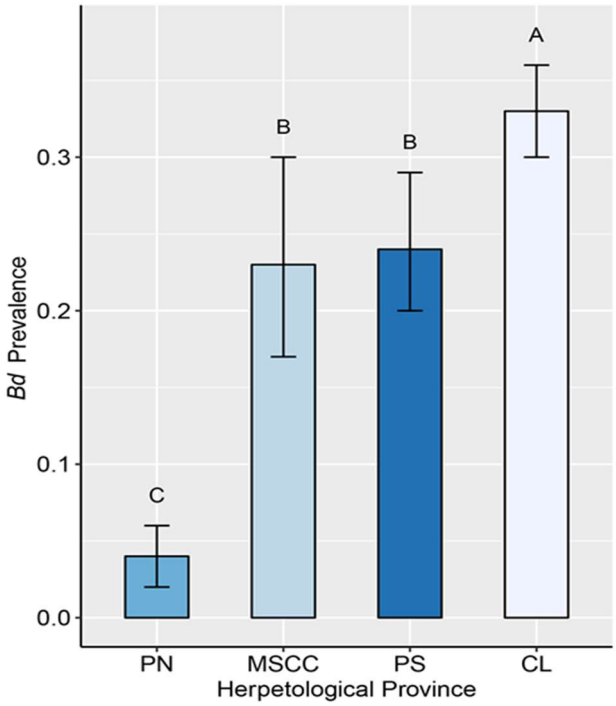

Figure 2.3. Mean prevalence of infection with *Batrachochytrium dendrobatidis* (*Bd*) in amphibian assemblages atfour herpetological provinces in Costa Rica (with 95% binomial CI). Means followed by a common letter are not significantly different according to the Tukey's honestly significant difference (HSD) test at the 5% level of significance. The plot does not display results for Cordillera de Talamanca because no sampling has been conducted for *Bd* in that province. Symbology: CL—Caribbean Lowlands, MSCC—Mountain Slopes and Cordillera Central, PN—Pacific Northwest, and PS—Pacific Southwest.

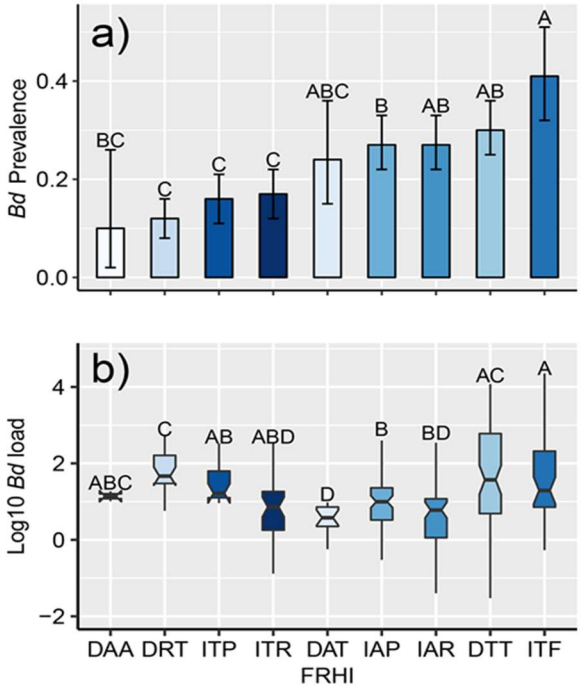

Figure 2.4. (a) Mean prevalence of infection with *Batrachochytrium dendrobatidis (Bd)* in amphibian assemblages (with 95% binomial CI) according to the foraging–reproduction habitat index (FRHI); (b) box plots with whiskers and notches that describe infection intensity for the 351 individuals where *Bd* was quantified using qPCR in Costa Rica between 2000–2018 according to the foraging–reproduction habitat index (FRHI). The box displays the inter-quantile range (25th–75th percentiles) with a center line representing the median (50th percentile). Notches show the median confidence region, and whiskers display the highest and lowest points. Means followed by a common letter are not significantly different according to the Tukey's honestly significant difference (HSD) test at the 5% level of significance. Symbology: First letter represents development: (I) indirect or (D) direct-developing amphibians. Second letter represents foraging habitat: terrestrial (T), arboreal (A), pond/puddle-dwellers (P), stream-breeders (R), and phytotelma (F). Third letter represents reproductive habitat: terrestrial (T), arboreal (A), pond/puddle-dwellers (P), stream-breeders (R), and phytotelma (F).

Table 2.4. Infection intensity in the 351 individuals where *Batrachochytrium dendrobatidis* (*Bd*) was quantified using qPCR in Costa Rica between 2000–2018. For every species, the table shows the foraging–reproduction habitat index (FRHI), the number of *Bd* positive swabs, the average (SE), and log10(SE) of genomic equivalents of *Bd* zoospores quantified per species estimated from the *Bd* + swabs. Symbology: First letter represents development: (I) indirect or (D) direct-developing amphibians. Second letter represents foraging habitat: terrestrial (T), arboreal (A), pond/puddle-dwellers (P), stream-breeders (R), and phytotelma (F). Third letter represents reproductive habitat: terrestrial (T), arboreal (A), pond/puddle-dwellers (P), stream-breeders (R), and phytotelma (F).

Species (FRHI)	*Bd*+ swabs	*Bd* load average (SE)	Log10 *Bd* load average (SE)
Agalychnis callidryas (IAP)	4	8.19 (3.81)	0.77 (0.21)
Agalychnis spurrelli (IAP)	5	39.83 (32.47)	1.10 (0.30)
Boana rufitela (IAP)	8	8.41 (4.12)	0.53 (0.23)
Bolitoglossa colonnea (DTT)	1	1.83 (0.00)	0.26 (0.00)
Cochranella granulosa (IAR)	1	3.95 (0.00)	0.60 (0.00)
Craugastor bransfordi (DTT)	23	1007.06 (483.50)	1.78 (0.25)
Craugastor crassidigitus (DTT)	5	1636.74 (1583.43)	1.69 (0.64)
Craugastor fitzingeri (DTT, DRT)	44	951.48 (310.27)	1.97 (0.17)
Craugastor megacephalus (DTT)	1	0.62 (0.00)	-0.21 (0.00)
Craugastor mimus (DTT)	9	125.48 (74.69)	1.01 (0.46)
Craugastor ranoides (DRT)	3	187.40 (174.13)	1.65 (0.55)
Craugastor stejnegerianus (DTT)	2	2.18 (0.95)	0.29 (0.20)
Craugastor taurus (DRT)	12	11632.50 (6564.67)	2.51 (0.41)
Dendropsophus ebraccatus (IAP)	34	315.85 (194.09)	1.00 (0.18)
Diasporus diastema (DAA)	2	14.44 (4.12)	1.14 (0.13)
Duellmanohyla rufioculis (IAR)	1	3.65 (0.00)	0.56 (0.00)
Engystomops pustulosus (ITP)	11	34.83 (13.26)	1.11 (0.3)
Espadarana prosoblepon (IAR)	3	3691.59 (3684.73)	1.06 (1.75)
Hyalinobatrachium colymbiphyllum (IAR)	1	0.01 (0.00)	-2.00 (0.00)
Hyalinobatrachium valerioi (IAR)	2	8.38 (2.04)	0.91 (0.11)
Incilius melanochlorus (ITR)	2	23.27 (19.93)	1.08 (0.56)
Leptodactylus melanonotus (ITP)	4	11.86 (0.66)	1.07 (0.02)
Leptodactylus poecilochilus (ITP)	1	1073.45 (0.00)	3.03 (0.00)
Leptodactylus savagei (ITP)	1	33.49 (0.00)	1.52 (0.00)
Lithobates forreri (ITP)	2	569.24 (241.10)	2.71 (0.20)
Lithobates warszewitschii (ITR)	14	978.92 (801.60)	1.47 (0.31)
Oophaga granulifera (ITF)	9	23.92 (11.31)	1.20 (0.11)
Oophaga pumilio (ITF)	34	1765.81 (778.67)	1.71 (0.25)
Pristimantis cerasinus (DAT)	9	14.82 (10.97)	0.47 (0.32)
Pristimantis ridens (DAT)	7	48.37 (32.34)	0.69 (0.50)
Rhaebo haematiticus (ITR)	22	239.20 (178.56)	0.70 (0.26)
Scinax boulengeri (IAP)	1	195.20 (0.00)	2.29 (0.00)
Scinax elaeochroa (IAP)	5	1384.15 (1350.51)	1.78 (0.58)
Smilisca phaeota (IAP)	4	37.25 (19.15)	1.44 (0.18)

Table 2.4. Continued

Smilisca sordida (IAP)	46	14.96 (9.27)	0.24 (0.16)
Teratohyla pulverata (IAR)	2	34.53 (22.92)	1.41 (0.35)
Teratohyla spinosa (IAR)	5	937.99 (825.54)	1.90 (0.57)
Tlalocohyla loquax (IAP)	11	144.66 (107.28)	1.22 (0.30)

2.5 Discussion

2.5.1 Species Assessment

We presented the first updated list of Costa Rican amphibians since 2011 (Bolaños et al. 2011). Compared to the last list, we added ten anurans, nine salamanders, and one caecilian (Table 2.3) for a total of 215 species (Table B.3). As is common throughout the world, anurans exhibit the highest amphibian species richness in Costa Rica, with 72% of listed species. However, the richness of salamander species is also high (25%). In Costa Rica, the diversity and endemism of amphibians (especially salamanders) increase with elevation and complex mountain topography (Savage 2002). Proportionally in terms of number of species per unit area (km2), the richest herpetological province is the Cordillera de Talamanca. In this herpetological province, the number of species continues to increase and most of the newly described species in our report came from this remote and almost inaccessible province (Arias & Chaves 2014). The Montane Slopes and Cordillera Central present the highest number of species (158 species). Within this province, numerous mountain ranges provide multiple microhabitats for niche differentiation and further speciation (Savage 2002; Sasa et al. 2010). In lowlands, the highest number of amphibian species occurs in the Caribbean Lowlands with 101 species. However, the Pacific Southwest presents more species per unit area. The Pacific Northwest only has 66 species, which is also the lowest number of species per unit area. This pattern may be attributed to the warm and dry conditions that occur in most part of this herpetological province (Savage 2002; Sasa et al. 2010).

According to IUCN, the species *Craugastor escoces* and *Incilius periglenes* are classified as EX in Costa Rica. However, *C. escoces* was recently rediscovered (Jiménez & Alvarado 2017). Similarly, several species that remained undetected after the 1980's ans1990' such as *Incilius holdridgei* (Abarca et al. 2010), *Craugastor taurus* (Chaves et al. 2014b), and *Atelopus varius* (González-Maya et al. 2013) have been rediscovered in peripheral populations during the last few years. However, the number of extinct species could be higher because multiple threatened species

still remain undetected in the field (e.g., *Craugastor andi*, *Incilius fastidiosus*, *Atelopus senex*). We recommend expedition surveys to find populations of declined and data deficient species (García-Rodríguez et al. 2012) and captive-breeding for species where ex-situ reproduction has been successful (e.g., harlequin frogs.) (Lewis et al. 2019). Although we acknowledge that there are limited funds available for these types of conservation efforts, knowledge from these sites is essential to be able to identify conditions that favor persistence of threatened species and identify species that should be targeted for future conservation efforts (Searle et al. 2011b).

Lowlands of Costa Rica exhibited the lowest proportion of DD species (0–2%; Fig. 2.2b) and the highest proportion of LC species (75–80%; Fig. 2.2c). On the other hand, highlands exhibited the highest percentage of DD species (6–10%; Fig. 2.2b). Similarly, Cordillera de Talamanca had the highest percentage of threatened species (26%; Fig. 2.2d). Based on these findings, we strongly recommend increasing sampling effort in the montane and subalpine altitudinal belts (>2800 m) that exclusively occur in Cordillera de Talamanca and Montane Slopes and Cordillera Central. These herpetological provinces present the highest rate of endemism (especially for salamanders) and contain several of the recently described species (Boza-Oviedo et al. 2012; Arias & Kubicki, 2018). Conducting expeditions and long-term studies in highlands will aid in monitoring threatened species and reducing information gaps allowing for more accurate assessments of amphibian species.

To better evaluate vulnerability of amphibian species, we utilized EVS (Table B.3). This index relies on ecological information for categorizing threat levels, which makes application easy for most species in a specific region (Wilson & McCranie 2004). Unlike the IUCN Red List of Threatened Species, previous evaluations of threats are not considered by this index (Sasa et al. 2010). For that reason, species that are classified as LC by IUCN can be classified as highly vulnerable in this index (e.g., *Duellmanohyla rufioculis*). We categorized 48 species (24 salamanders, 21 anurans, and three caecilians) in "high vulnerability" (e.g., *Atelopus senex, Craugastor andi, Bolitoglossa pesrubra, Nototriton guanacaste,* and *Oscaecilia osae*). These species exhibited the highest EVS values because their habitats are restricted and because they exhibit complex reproductive modes. Quantifying environmental threats and combining information from both indexes will help policy-makers to prioritize conservation actions for threatened species.

In Central America, habitat destruction is the most important threat impacting amphibian populations (Wilson & McCranie 2004; Sasa et al. 2010). Although approximately 30% of Costa Rica remains forested and protected, rapid urbanization, extensive agriculture, excessive pesticide use, illegal traffic, and inappropriate waste management negatively affect numerous amphibian populations. However, even in seemingly pristine locations, amphibian declines have occurred (Young et al. 2001). Additionally, climate change has been associated with the decline of several amphibian species in Costa Rica, by affecting their reproduction and likely increasing susceptibility to pathogens (Pounds et al. 2006). Although it has not been found in Central America, we recommend screening for the recently emerged fungus *Batrachochytrium salamandrivorans* (Martel et al. 2013), which causes chytridiomycosis in salamanders. Conveniently, swabbing methods and qPCR allow accurate detection of both fungi species in the same assay, which may facilitate rapid population assessments. For a fully detailed review of the environmental threats for amphibian communities in Costa Rica, we recommend the work of Sasa and collaborators (2010).

2.5.2 Post-Decline Dynamics

In this study, we found strong evidence that *Bd* is widespread in Costa Rica. Our results also suggests that post-decline *Bd* exhibits enzootic dynamics, characterized by high prevalence of infection across regions and pathogenic loads below thresholds associated with mass mortalities (Briggs et al. 2010; Vredenburg et al. 2010). We found *Bd* in all the herpetological provinces and altitudinal belts surveyed (Fig. 2.3 and 2.4, Table B.5 and B.6), for a total infection rate of 23%. We also found that *Bd* was more prevalent in terrestrial amphibians with an aquatic larval stage and direct-developing frogs exhibited the highest pathogenic loads.

We found the lowest infection rate (<5%) in the Pacific Northwest and the highest (33%) in the Caribbean Lowlands (Fig. 2.3). However, these values may be an effect of sampling periods. A study conducted at La Selva Biological Station in the Caribbean Lowlands (Whitfield et al. 2012) reported infection rates varying from <5% during the warmest months (May-early November) to 35% in the coolest months (Mid November to January) in three common amphibian species. In addition, the gradual population declines observed at La Selva over several decades (Whitfield et al. 2007) and opportunistic observations of small-scale mortality events during cold periods (S. Whitfield, unpublished data) suggest that *Bd* may be causing mortality in amphibians long after its initial invasion. Similar mortality events in response to seasonality could be occurring in

amphibian communities in "refuges from decline" in the Pacific Northwest (Zumbado-Ulate et al. 2014; Whitfield et al. 2017) and Pacific Southwest (Zumbado-Ulate et al. 2019) of Costa Rica; however, they have not been yet documented. There, seasonal changes in precipitation and temperature caused *Bd* prevalence to vary from >5% in the peak of the dry season (March and April) to 80% in the coldest months (November-December). Therefore we recommend follow-up studies at these sites to identify if seasonal disease dynamics are causing mortality events in regions that have been considered unsuitable for *Bd* (Puschendorf et al. 2011).

Bd was found across all altitudinal belts across Costa Rica. Similar results of high *Bd* prevalence across all elevations has been found in Panama (Woodhams et al. 2008; Kilburn et al. 2010; Perez et al. 2014). These findings suggests that current environmental conditions are suitable for *Bd* at most elevations in Central America (Zumbado-Ulate et al. 2019). It is also plausible that *Bd*-driven declines during the 1980's and 1990's were not exclusively restricted to highlands (Puschendorf et al. 2009) but were relatively undetected at lower elevations. Another hypothesis is that species with high susceptibility historically occupied high elevations sites, but severely declined or went extinct after *Bd* was introduced, leaving only species with mid- to low susceptibility across elevations (Acosta-Chaves et al. 2019). On the other hand, the absence of samples from montane and subalpine belts and uncontrolled variables (e.g., changes in species composition, climatic disturbances) could have reduced the statistical power to determine changes in *Bd* prevalence across altitudinal belts. We suggest that future studies increase sampling at high elevations (>2700 m) to better understand the local spatial dynamics of *Bd* across elevations.

Our results showed that infection with *Bd* was common in amphibians across all life-history traits evaluated in the FRHI (Table 2.2). However, *Bd* was significantly more prevalent in terrestrial amphibians with a larval stage (Fig. 2.4a), especially those that complete metamorphosis in phytotelma (ITF). All the species within the ITF category belonged to the family Dendrobatidae, which have previously been shown to easily acquire *Bd* infection (Whitfield et al. 2012; Zumbado-Ulate et al. 2014). The high susceptibly of the Dendrobatidae family is likely due to their preferred habitat (e.g. water-filled bromeliads for many species), as it offers suitable conditions for *Bd* infection (Garner et al. 2009). In addition, dendrobatid adults forage in the tropical forest floor and stream-associated low vegetation, which are environments that can sustain *Bd* (Kolby et al. 2015). Regarding infection intensity (Fig. 2.4b), the FRHI showed similar results to studies that have used the aquatic index (Brem & Lips 2008). We found that direct-developing species with terrestrial

reproduction had significant higher levels of infection load than other species with different life-history traits (Fig. 2.4b, Table 2.4). This life-history trait is exhibited by leaf-litter frogs and all the species within the *Craugastor punctariolus* clade (robber frogs), which is one of the most affected clades by chytridiomycosis in Central America (Ryan et al. 2008; Zumbado-Ulate et al. 2011). Robber frogs spend a majority of their life cycle along fast-flowing streams (Campbell & Savage 2000), an aquatic environment that seems highly suitable for *Bd* in Central America (Lips et al. 2003b). In addition, these frogs appear to be highly susceptible to *Bd*-driven mass mortalities outside warm and dry ecosystems (Puschendorf et al. 2009; Köhler et al. 2012; Chaves et al. 2014b). Our results suggest the FRHI is particularly useful to identify taxonomic units that are more susceptible to *Bd* (Searle et al. 2011b).

2.5.3 Conclusions

Our results demonstrated that the number of identified amphibian species in Costa Rica is still growing, and there may be potential future additions, e.g., *Bolitoglossa anthracina* (Hanken et al. 2005) and *B. indio* (Sunyer et al. 2012). A continuous assessment of species and regions is needed to identify continuing threats to amphibian biodiversity. We found that *Bd* was widespread across species, herpetological provinces, and altitudinal belts in samples collected since 2000. Conducting more studies in remote regions, such as Cordillera de Talamanca, may help to better describe spatial dynamics of both amphibian hosts and *Bd*. In addition, future studies should test whether seasonal disease dynamics are causing mortality events in regions that are considered unsuitable for *Bd*. Under potential scenarios of climate change, environmental conditions may shift to ideal ranges for *Bd* infection (Pounds et al. 2006) and seasonal regions that sustain critically endangered species (e.g. tropical dry forest) may experience future outbreaks of chytridiomycosis (AlMutairi et al. 2019). We also recommend continuous surveillance of invasive species, which might amplify *Bd* in the environment causing future epizootics (Searle et al. 2011a). This vital information will aid in the development of more effective conservation strategies for amphibians across a broader range of habitats (Grenyer et al. 2006; Woodhams et al. 2011; Scheele et al. 2014; Garner et al. 2016).

2.6 References

Abarca J, Chaves G, García-Rodríguez A, & Vargas R. (2010). Reconsidering extinction: rediscovery of *Incilius holdridgei* (Anura: Bufonidae) in Costa Rica after 25 years. *Herpetological Review* 41,150.

Abarca JG. (2018). Quitridiomicosis en Costa Rica: Aislamiento y descripción de cepas circulantes del patógeno y análisis de la microbiota del hospedero como posible factor en la incidencia de la enfermedad. Master's thesis. Universidad de Costa Rica, Costa Rica.

Acosta-Chaves VJ, Madrigal-Elizondo V, Chaves G, Morera-Chacón B, García-Rodríguez A, & Bolaños F. (2019). View of shifts in the diversity of an amphibian community from a premontane forest of San Ramón, Costa Rica. *Revista de Biología Tropical* 67, 259–273.

AlMutairi BS, Grossmann I, & Small MJ. (2019). Climate model projections for future seasonal rainfall cycle statistics in Northwest Costa Rica. International *Journal of Climatology* 39, 2933– 2946

Arias E, & Chaves G. (2014). 140 years after William M. Gabb's climb to Cerro Pico Blanco. *Mesoamerican Herpetology* 1, 176–180..

Arias E, Chaves G, Crawford AJ, & Parra-Olea G. (2016). A new species of the *Craugastor podiciferus* species group (Anura: Craugastoridae) from the premontane forest of southwestern Costa Rica. *Zootaxa* 4132, 347–363.

Arias E, Chaves G, & Parra-Olea G. (2018). A new species of *Craugastor* (Anura: Craugastoridae) from the montane rainforest of the Cordillera de Talamanca, Costa Rica. *Journal of Herpetology* 17, 211–232.

Arias E, Chaves G, Salazar S, Salazar-Zúñiga JA, & García-Rodríguez A. (2019a). A new species of dink frog, genus *Diasporus* (Anura: Eleutherodactylidae), from the Caribbean foothills of the Cordillera de Talamanca, Costa Rica. *Zootaxa* 4609, 269–288.

Arias E, Hertz A, & Parra-Olea G. (2019b). Taxonomic assessment of *Craugastor podiciferus* (Anura: Craugastoridae) in lower Central America with the description of two new species. *Amphibian & Reptile Conservation* 13, 173–197.

Arias E, & Kubicki B. (2018). A new moss salamander, genus *Nototriton* (Caudata: Plethodontidae), from the Cordillera de Talamanca, in the Costa Rica-Panama border region. *Zootaxa* 4369, 487–500.

Bagley JC, & Johnson JB. (2014). Phylogeography and biogeography of the lower Central American Neotropics: diversification between two continents and between two seas. *Biological Reviews* 89, 767–790.

Barnosky AD, Matzke N, Tomiya S, Wogan GO, Swartz B, Quental TB, Marshall C, McGuire JL, Lindsey EL, Maguire KC, & Mersey B. (2011). Has the Earth's sixth mass extinction already arrived? Nature 471, 51–57.

Barquero MD, & Araya MF. (2016). First record of the Greenhouse frog, *Eleutherodactylus planirostris* (Anura: Eleutherodactylidae), in Costa Rica. Herpetology Notes 9, 145–147.

Batista A, Hertz A, Köhler G, Mebert K, & Vesely M. (2014). Morphological variation and phylogeography of frogs related to *Pristimantis caryophyllaceus* (Anura: Terrarana: Craugastoridae) in Panama. *Salamandra* 50, 155–171.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, & Hines HB. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences* 95, 9031–9036.

Bolaños F. (2009). Situación de los anfibios de Costa Rica. *Biocenosis* 22:95–108.

Bolaños F, Savage JM, & Chaves G. (2011). Anfibios y reptiles de Costa Rica. Listas zoológicas actualizadas UCR. Available from http://museo.biologia.ucr.ac.cr/Listas/Anteriores/HerpCREsp.htm (accessed June 27, 2019).

Boyle DG, Boyle DB, Olsen V, Morgan JAT, & Hyatt AD. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60,141–148.

Boza-Oviedo E, Rovito SM, Chaves G, García-Rodríguez A, Artavia LG, Bolaños F, & Wake DB. (2012). Salamanders from the eastern Cordillera de Talamanca, Costa Rica, with descriptions of five new species (Plethodontidae: *Bolitoglossa*, *Nototriton*, and *Oedipina*) and natural history notes from recent expeditions. *Zootaxa* 3309, 36–61.

Brem F, & Lips K. (2008). *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Diseases of Aquatic Organisms* 81, 189–202.

Briggs CJ, Knapp RA, & Vredenburg VT. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences* 107, 9695–9700.

Briggs CJ, Vredenburg VT, Knapp RA, & Rachowicz LJ. (2005). Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. *Ecology* 86, 3149–3159.

Burnham KP, & Anderson DR. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research* 33, 261–304.

Campbell JA, & Savage JM. (2000). Taxonomic reconsideration of Middle American frogs of the *Eleutherodactylus rugulosus* group (Anura: Leptodactylidae): a reconnaissance of subtle nuances among frogs. *Herpetological Monographs* 14, 186–292.

Catenazzi A. (2015). State of the World's Amphibians. *Annual Review of Environment and Resources* 40, 91–119.

Chaves G, Bolaños F, Rodríguez JE, & Matamoros Y. (2014a). Actualización de las Listas Rojas Nacionales de Costa Rica. Anfibios y Reptiles. Conservation Breeding Specialist Group (SSC/IUCN)/CBSG Mesoamerica).

Chaves G, Zumbado-Ulate H, García-Rodríguez A, Gómez E, Vredenburg VT, & Ryan MJ. (2014b). Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Tropical Conservation Science* 7, 628–638.

Christie MR, & Searle CL. 2018. Evolutionary rescue in a host–pathogen system results in coexistence not clearance. *Evolutionary Applications* 11, 681–693.

Collins JP. (2010). Amphibian decline and extinction: What we know and what we need to learn. *Diseases of Aquatic Organisms* 92, 93–99.

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, & Speare R. (1999). Emerging infectious diseases and amphibian population declines. Emerging infectious diseases 5:735–748.

Frost DR. (2019). Amphibian Species of the World: an Online Reference. Version 6.0. Available from http://research.amnh.org/vz/herpetology/amphibia/ (accessed June 27, 2019).

García-Rodríguez A, Chaves G, Benavides-Varela C, & Puschendorf R. (2012). Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions* 18, 204–212.

Garner TWJ, Schmidt BR, Martel A, Pasmans Frank, Muths E, Cunningham AA, Weldon C, Fisher MC, & Bosch J. (2016). Mitigating amphibian chytridiomycosis in nature. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371, 20160207.

Garner TWJ, Walker S, Bosch J, Leech S, Marcus Rowcliffe J, Cunningham AA, & Fisher MC. (2009). Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos* 118, 783–791.

Gerber BD, Converse SJ, Muths E, Crockett HJ, Mosher BA, & Bailey LL. (2018). Identifying species conservation strategies to reduce disease-associated declines. *Conservation Letters* 11, e12393.

Goldberg CS, Hawley TJ, & Waits LP. (2009). Local and regional patterns of amphibian chytrid prevalence on the Osa Peninsula, Costa Rica. *Herpetological Review* 40, 309–311.

González-Maya JF et al. (2013). Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica. *Amphibia-Reptilia* 34, 573–578.

Gray AR. (2018). Review of the genus *Cruziohyla* (Anura: Phyllomedusidae), with description of a new species. *Zootaxa* 4450, 401–426.

Grenyer R., Orme CDL, Jackson SF, Thomas GH, Davies RG, Davies TJ, Jones KE, Olson VA, Ridgely RS, Rasmussen PC & Ding TS. (2006). Global distribution and conservation of rare and threatened vertebrates. *Nature* 444, 93–96.

Hanken J, Wake DB, & Savage JM. (2005). A solution to the large black salamander problem (genus *Bolitoglossa*) in Costa Rica and Panamá. *Copeia* 2005, 227–245

Heard GW, Scroggie MP, Ramsey DSL, Clemann N, Hodgson JA, & Thomas CD. (2018). Can habitat management mitigate disease impacts on threatened amphibians? *Conservation Letters* 11, e12375.

Hero J-M, Williams SE, & Magnusson WE. (2005). Ecological traits of declining amphibians in upland areas of eastern Australia. *Journal of Zoology* 267, 221–232.

Hitchman SM, Mather ME, Smith JM, & Fencl JS. (2018). Identifying keystone habitats with a mosaic approach can improve biodiversity conservation in disturbed ecosystems. *Global Change Biology* 24, 308–321.

Holdridge LR. 1967. Life Zone Ecology. Tropical Science Center, Costa Rica. Available from https://www.ca*Bd*irect.org/ca*Bd*irect/abstract/19670604180.

Hyatt, AD, Boyle AH, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, Phillott R, & Campbell R. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73, 175–192.

IUCN. (2019). The IUCN Red List of Threatened Species. Version 2019-1. Available from https://www.iucnredlist.org/en (accessed June 27, 2019).

Jiménez R, & Alvarado G. (2017). *Craugastor escoces* (Anura: Craugastoridae) reappears after 30 years: rediscovery of an "extinct" Neotropical frog. *Amphibia-Reptilia* 38, 257–259.

Kilburn VL, Ibáñez R, Sanjur O, Bermingham E, Suraci JP, & Green DM. (2010). Ubiquity of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in anuran communities in Panamá. *EcoHealth* 7, 537–548.

Köhler G, Batista A, Carrizo A, & Hertz A. (2012). Field notes on *Craugastor azueroensis* (Savage, 1975) (Amphibia: Anura: Craugastoridae). *Herpetology Notes* 5, 157–162.

Kolby JE, Ramirez SD, Berger L, Richards-Hrdlicka KL, Jocque M, & Skerratt LF. (2015). Terrestrial dispersal and potential environmental transmission of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). *PLOS ONE* 10, e0125386.

Kriger KM, & Hero J-M. (2007). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions* 13, 781–788.

Kriger KM, Hero J-M, & Ashton KJ. (2006a). Cost efficiency in the detection of chytridiomycosis using PCR assay. *Diseases of Aquatic Organisms* 71, 149–154.

Kriger KM, Hines HB, Hyatt AD, Boyle DG, & Hero J-M. (2006b). Techniques for detecting chytridiomycosis in wild frogs: Comparing histology with real-time Taqman PCR. *Diseases of Aquatic Organisms* 71, 141–148.

Kubicki B. (2016). A new species of salamander (Caudata: Plethodontidae: *Oedipina*) from the central Caribbean foothills of Costa Rica. *Mesoamerican Herpetology* 3, 819–840.

Kubicki B, & Arias E. (2016). A beautiful new yellow salamander, genus *Bolitoglossa* (Caudata: Plethodontidae), from the northeastern slopes of the Cordillera de Talamanca, Costa Rica. *Zootaxa* 4184, 329–346.

Kubicki B, & Arias E. (2017). Vulcan's Slender Caecilian, *Caecilia volcani*, in Costa Rica. *Mesoamerican Herpetology* 4, 488–492.

Kubicki B, & Salazar S. (2015). Discovery of the golden-eyed fringe-limbed treefrog, *Ecnomiohyla bailarina* (Anura: Hylidae), in the Caribbean foothills of southeastern Costa Rica. *Mesoamerican Herpetology* 2, 76–86.

Kubicki B, Salazar S, & Puschendorf R. (2015). A new species of glassfrog, genus *Hyalinobatrachium* (Anura: Centrolenidae), from the Caribbean foothills of Costa Rica. *Zootaxa* 3920, 069–084.

La Marca E, Lips KR, Lötters S, Puschendorf R, Ibáñez R, Rueda-Almonacid JV, Schulte R, Marty C, Castro F, Manzanilla-Puppo J, & García-Pérez JE. (2005). Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37, 190–201.

Lewis CHR, Richards-Zawacki CL, Ibáñez R, Luedtke J, Voyles J, Houser P, & Gratwicke B. (2019). Conserving Panamanian harlequin frogs by integrating captive-breeding and research programs. *Biological Conservation* 236, 180–187.

Lips KR, Burrowes PA, Mendelson JR, & Parra-Olea G. (2005). Amphibian declines in Latin America: Widespread population declines, extinctions, and impacts. *Biotropica* 37, 163–165.

Lips KR, Diffendorfer J, Mendelson JR, & Sears MW. (2008). Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLOS Biology* 6, e72.

Lips KR, Green DE, & Papendick R. (2003a). Chytridiomycosis in wild frogs from southern Costa Rica. *Journal of Herpetology* 37, 215–218.

Lips KR, Reeve JD, & Witters LR. (2003b). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17, 1078–1088.

Longcore JE, Pessier AP, & Nichols DK. (1999). *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91, 219–227.

Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F & Pasmans, F (2013). *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences* 110, 15325–15329.

McCranie JR. (2017). Morphological and systematic comments on the Caribbean lowland population of *Smilisca baudinii* (Anura: Hylidae: Hylinae) in northeastern Honduras, with the resurrection of *Hyla manisorum*. *Mesoamerican Herpetology* 4, 513–526.

Mendelson JR, Whitfield SM, & Sredl MJ. (2019). A recovery engine strategy for amphibian conservation in the context of disease. *Biological Conservation* 236, 188–191.

Meredith H, Van Buren C, Antwis RE. (2016). Making amphibian conservation more effective. *Conservation Evidence* 13,1–6.

Miller CA, Tasse Taboue GC, Ekane MMP, Robak M, Sesink Clee PR, Richards-Zawacki C, Fokam EB, Fuashi NA, & Anthony NM. (2018). Distribution modeling and lineage diversity of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in a central African amphibian hotspot. *PLOS ONE* 13, e0199288.

Monastersky R. (2014). Life-a status report. *Nature* 516, 158.

Novacek MJ, & Cleland EE. (2001). The current biodiversity extinction event: Scenarios for mitigation and recovery. *Proceedings of the National Academy of Sciences* 98, 5466.

O'Brien Valerie A., Moore Amy T., Young Ginger R., Komar Nicholas, Reisen William K., & Brown CR. (2011). An enzootic vector-borne virus is amplified at epizootic levels by an invasive avian host. *Proceedings of the Royal Society B: Biological Sciences* 278, 239–246.

Perez R, Richards-Zawacki CL, Krohn AR, Robak M, Griffith EJ, Ross H, Gratwicke B, Ibanez R, & Voyles J. (2014). Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphibian & Reptile Conservation* 8, 30–35.

Picco AM, & Collins JP. (2007). Fungal and viral pathogen occurrence in Costa Rican amphibians. *Journal of Herpetology* 41, 746–749.

Piotrowski JS, Annis SL, & Longcore JE. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96, 9–15.

Pounds AJ, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, Ron SR, Sánchez-Azofeifa GA, Still CJ, & Young BE (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439, 161–167.

Pounds JA, & Crump ML. (1994). Amphibian declines and climate disturbance: The case of the golden toad and the harlequin frog. *Conservation Biology* 8, 72–85.

Puschendorf R, Bolaños F, & Chaves G. (2006). The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biological Conservation* 132, 136–142.

Puschendorf R, Carnaval AC, VanDerWal J, Zumbado-Ulate H, Chaves G, Bolaños F, & Alford RA. (2009). Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: Proposing climatic refuges as a conservation tool. *Diversity and Distributions* 15, 401–408.

Puschendorf R, Hoskin CJ, Cashins SD, McDonald K, Skerratt LF, Vanderwal J, & Alford RA. (2011). Environmental refuge from disease-driven amphibian extinction. *Conservation Biology* 25, 956–964.

R Core Team. (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Viena. Available from https://www.r-project.org/ (accessed February 22, 2019).

Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, & Briggs CJ. (2006). Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87, 1671–1683.

Retallick RWR, McCallum H, & Speare R. (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLOS Biology* 2, e351.

Retallick RWR, & Miera V. (2007). Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. *Diseases of Aquatic Organisms* 75, 201–207.

Ryan M, Lips K, & Giermakowski JT. (2010). New species of *Pristimantis* (Anura: Terrarana: Strabomantinae) from lower Central America. *Journal of Herpetology* 44, 193–200.

Ryan MJ, Lips KR, & Eichholz MW. (2008). Decline and extirpation of an endangered Panamanian stream frog population (*Craugastor punctariolus*) due to an outbreak of chytridiomycosis. *Biological Conservation* 141, 1636–1647.

Saenz D, Adams CK, Pierce JB, & Laurencio D. (2009). Occurrence of *Batrachochytrium dendrobatidis* in an anuran community in the southeastern Talamanca region of Costa Rica. *Herpetological Review* 40, 311–313.

Sasa M, Chaves G, & Porras LW. (2010). The Costa Rican herpetofauna: conservation status and future perspectives. Pages 510–603 in J. H. Townsend and J. D. Johnson, editors. Conservation of Mesoamerican amphibians and reptiles. Eagle Mountain Press, Utah.

Savage JM. (2002). The Amphibians and Reptiles of Costa Rica: a Herpetofauna Between Two Continents, Between Two Seas. University of Chicago press.

Savage JM, & Bolaños F. (2009). A checklist of the amphibians and reptiles of Costa Rica: Additions and nomenclatural revisions. *Zootaxa* 2005, 1–23.

Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel AN, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A, & De la Riva I. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363, 1459–1463.

Scheele BC, Hunter DA, Grogan LF, Berger L, Kolby JE, Mcfadden MS, Marantelli G, Skerratt LF, & Driscoll DA. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology* 28, 1195–1205.

Searle CL, Biga LM, Spatafora JW, & Blaustein AR. (2011a). A dilution effect in the emerging amphibian pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences* 108, 16322–16326.

Searle CL, Gervasi SS, Hua J, Hammond JI, Relyea RA, Olson DH, & Blaustein AR. (2011b). Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. *Conservation Biology* 25, 965–974.

Skerratt L, Berger L, Hines H, McDonald K, Mendez D, & Speare R. (2008). Survey protocol for detecting chytridiomycosis in all Australian frog populations. *Diseases of Aquatic Organisms* 80, 85–94.

Stuart SN, Chanson J, Cox NA, Young BE, Rodrigues ASL, Fischman D, & Waller R. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.

Sunyer J, Wake DB, & Obando L. (2012). Distributional data for *Bolitoglossa* (Amphibia, Caudata, Plethodontidae) from Nicaragua and Costa Rica. *Herpetological Review* 43, 564–568.

Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, Skerratt LF, & Speare R. (2009). Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* 326, 582–585.

Vredenburg VT, Knapp RA, Tunstall TS, & Briggs CJ. (2010). Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences* 107, 9689–9694.

Wake DB, & Vredenburg VT. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences* 105, 11466–11473.

Whitfield SM, Alvarado G, Abarca J, Zumbado-Ulate H, Zuñiga I, Wainwright M, & Kerby J. (2017). Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms* 126, 33–41.

Whitfield SM, Bell KE, Philippi T, Sasa M, Bolaños F, Chaves G, Savage JM, & Donnelly MA. (2007). Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences* 104, 8352–8356.

Whitfield SM, Geerdes E, Chacon I, Ballestero Rodriguez E, Jimenez R, Donnelly M, & Kerby J. (2013). Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. *Diseases of Aquatic Organisms* 104, 173–178.

Whitfield SM, Kerby J, Gentry LR, & Donnelly MA. (2012). Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. *Biotropica* 44, 779–784.

Whitfield SM, Lips KR, & Donnelly MA. (2016). Amphibian decline and conservation in Central America. *Copeia* 104, 351–379.

Wilson LD, & McCranie JR. (2004). The conservation status of the herpetofauna of Honduras. *Amphibian & reptile conservation* 3, 6–33.

Woodhams DC, Kilburn VL, Reinert LK, Voyles J, Medina D, Ibáñez R., … Rollins-Smith LA (2008). Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. *EcoHealth* 5,268–274.

Woodhams DC, Bosch J, Briggs CJ, Cashins S, Davis LR, Lauer A, Muths E, Puschendorf R, Schmidt BR, Sheafor B, & Voyles J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology* 8, 8.

Young BE. Lips KR, Reaser JK, Ibanez R, Salas AW, Cedeño JR, Coloma LA, Ron S, La Marca E, Meyer JR, Muñoz A, Bolaños F, Chaves G, & Romo D. (2001). Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15, 1213–1223.

Zumbado-Ulate H, Bolaños F, Gutiérrez-Espeleta G, & Puschendorf R. (2014). Extremely low prevalence of *Batrachochytrium dendrobatidis* in frog populations from Neotropical dry forest of Costa Rica supports the existence of a climatic refuge from disease. *EcoHealth* 11, 593–602.

Zumbado-Ulate H, Bolaños F, Willink B, & Soley-Guardia F. (2011). Population status and natural history notes on the critically endangered stream dwelling frog *Craugastor ranoides* (Craugastoridae) in a Costa Rican tropical dry forest. *Herpetological Conservation and Biology* 6, 455–464.

Zumbado-Ulate H, García-Rodríguez A, Vredenburg VT, & Searle CL. (2019). Infection with *Batrachochytrium dendrobatidis* is common in tropical lowland habitats: Implications for amphibian conservation. *Ecology and Evolution* 9, 4917–4930.

CHAPTER 3. SPECIES DISTRIBUTION MODELS PREDICT THE GEOGRAPHIC EXPANSION OF AN ENZOOTIC AMPHIBIAN PATHOGEN

Reprinted by permission from John Wiley and Sons, Ltd. Biotropica. Species distribution models predict the geographic expansion of an enzootic amphibian pathogen. Hector Zumbado-Ulate, Adrián García-Rodríguez, and Catherine L. Searle. 2021.

3.1 Abstract

Globally, numerous amphibian species have declined due to the introduction of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). However, the understanding of the spatiotemporal dynamics remains incomplete. Therefore, estimating the current geographic distribution of *Bd* is urgently needed, especially in countries like Costa Rica, where susceptible species are still recovering from *Bd*-driven declines. We conducted model tuning and spatial analysis to compare the habitat suitability for epizootic and enzootic *Bd* in Costa Rica and to identify data-deficient regions, opportunistic sampling, and *Bd* hotspots. Our dataset combined two methods of detection (histology and PCR methods) for a total of 451 *Bd*-positive records from 34 localities. We found that the distribution of enzootic *Bd* in Costa Rica increased 60% since previous estimates in the early 2000s and extended to highlands and dry lowlands that were considered unsuitable for *Bd*. We also found that *Bd* is common across protected lands (80%) and within the herpetological provinces containing the highest amphibian richness and endemism in Costa Rica. Opportunistic sampling of *Bd* has focused on sites where epizootics occurred with the strongest intensity, leading to deficient or absent sampling across the Talamanca Range, the Nicoya Peninsula, and the northern lowlands. Our results showed that PCR increased the power of *Bd* detection in lowlands and favored the identification of *Bd* hotspots across the Caribbean side of Costa Rica. Our results add to the understanding of disease spread during enzootics and can be used to identify new hotspots for disease to mitigate future outbreaks of this pathogen.

3.2 Introduction

Global biodiversity has steadily declined over the last 50 years, and extinction risk at the species level has increased (Hooper et al., 2012). Current extinction rates suggest we are

approaching a sixth mass extinction (Barnosky et al., 2011) driven by a variety of anthropogenic actions such as habitat destruction, spread of pathogens, introduction of non-native species, illegal harvest, and climate change (Dirzo & Raven, 2003). Within vertebrates, amphibians are at a higher risk of extinction than any other taxon (Monastersky, 2014; Wake & Vredenburg, 2008). More than 40% of amphibian species have declined or gone extinct (Stuart et al., 2004) due to multiple environmental threats (Collins, 2010; Daszak et al., 2003). Therefore, the identification and quantification of these threats are needed to reconstruct the history of species declines and establish effective future management (Adams, et al., 2017; Meredith, et al., 2016).

With only 51,100 km2, Costa Rica is home of 215 species of amphibians, which represents nearly 2.7% of globally described amphibian species (Zumbado-Ulate et al., 2019a). The accurate description of amphibian taxa in Costa Rica, as well as its effective monitoring and conservation has been facilitated with the creation of conservation areas. Costa Rica possess a robust system of protected areas (public and private), which safeguards 26% of the country and favors the mitigation of anthropogenic threats (Andam et al., 2008; Lambin & Meyfroidt, 2011; Langholz & Lassoie, 2001). Similarly, the establishment of Savage's herpetological provinces in Costa Rica facilitated the study and conservation of amphibians (Sasa et al., 2010; Savage, 2002). The Savage's herpetological provinces system divides Costa Rica into five units that represent a unique combination of life zones and herpetological taxa, therefore facilitating specific conservation actions (Zumbado-Ulate et al., 2019a). Despite the application of these conservation efforts, numerous amphibian population declines (including enigmatic extinctions) have occurred in pristine and protected sites of Costa Rica (e.g., Bolaños, 2009; Lips et al., 2003; Pounds & Crump, 1994). Thus, identifying environmental threats across protected lands and herpetological provinces and quantifying their impacts on amphibian communities is crucial to propose specific conservation actions at the regional level (Nori et al., 2015).

A common threat to global amphibian populations is the introduction of the chytrid fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*; Longcore et al., 1999), which causes chytridiomycosis, a deadly skin disease in some species (Berger et al., 1998; Voyles et al., 2009). A recent assessment estimated that the global decline of at least 500 amphibian species is linked to *Bd* (Scheele et al., 2019). However, this number could be higher because the status of many species remains unknown (e.g., species that inhabit remote localities or exhibit a secretive biology). Similarly, the distribution of *Bd* in several countries remains insufficiently described due to the

lack of continuous studies on regional dynamics of *Bd*; therefore, the predicted distribution often relies in largescale continental models (James et al., 2015; Ron, 2005). Because every country uses different environmental policies and conservation strategies, updated distributions of *Bd* at the country level are needed to accurately identify potential areas for future outbreaks and amphibian population recovery (Rohr et al., 2011).

Understanding of the spatiotemporal dynamics of *Bd* remains incomplete, even in well-studied countries like Costa Rica (De León et al., 2019; Whitfield et al., 2016). Although *Bd* has existed in Costa Rica since at least the 1960s, optimal environmental conditions during the middle 1980s may have favored the emergence of a highly virulent strain of *Bd* in Costa Rica (De León et al., 2019). The rapid spread of this strain during the 1980s and 1990s throughout the country caused deadly outbreaks of chytridiomycosis (i.e., epizootics, Briggs et al., 2010), especially in stream-dwelling amphibian species from mid- and high-elevation ecosystems (1,000–2,500 m elevation; Bolaños, 2009; Lips et al., 2003; Pounds et al., 2006). However, recent studies have found low mortality and endemic *Bd* infection (i.e., enzootics, Briggs et al., 2010) across amphibians assemblages in all elevations (Whitfield et al., 2017; Zumbado-Ulate et al., 2019a, 2019b). Additionally, several declined species have been rediscovered since 2005 and may be recovering from epizootics (Abarca et al., 2010; Chaves et al., 2014; Gómez-Hoyos et al., 2018; Jiménez & Alvarado, 2017). Therefore, information on the current distribution of *Bd* is urgently needed to prevent future outbreaks of disease, especially in regions where *Bd* is endemic and coexists with highly susceptible species (García-Rodríguez et al., 2012; Zumbado-Ulate et al., 2019a).

Central America has a combination of complex topography and climatic variability (Lieberman et al., 1996; Powers et al., 2009) causing variation in expected suitable localities for *Bd* occurrence even at similar elevations (Puschendorf et al., 2009). Therefore, sensitive methods of detection are needed to accurately identify current hotspots for disease and unsuitable regions for infection. Detection of *Bd* has been conducted through histology, standard polymerase chain reaction (PCR), and quantitative-PCR (qPCR; Annis et al., 2004; Berger et al., 2000; Boyle et al., 2004). The choice of method of detection has relied mainly on the type of study and sample. For example, histology is mostly used to assess the severity of damage on amphibian skin caused by chytridiomycosis or to detect *Bd* on samples where DNA degradation has occurred (Skerratt et al., 2011; Wandeler et al., 2007). PCR detection is preferred when processing a large number of

samples (Kriger et al., 2006), or detecting *Bd* in regions where prevalence is low and destructive sampling of amphibian hosts is not possible or desirable (Skerratt et al., 2011). Among PCR techniques, qPCR (Boyle et al., 2004) has become a popular sampling method to detect *Bd* from live animals in the field (e.g., Whitfield et al., 2017), museum specimens (Cheng et al., 2011), and the environment (Kirshtein et al., 2007). Therefore, building occurrence datasets based on the combination of different methods of detection provides a solid framework for accurately estimating a pathogen's distribution across heterogeneous (Chestnut et al., 2014; Skerratt et al., 2011).

Species distribution models (SDMs) are powerful tools in conservation. One common application of SDMs is to predict suitable areas for disease (Peterson et al., 2004; Pinkard et al., 2010). At the continental level, two suitability maps have been often used to predict the distribution of *Bd* across the Americas (James et al., 2015; Ron, 2005); however, the large scale of these SDMs makes predictions of *Bd* distribution difficult at the country level. To address this issue in Costa Rica, Puschendorf et al. (2009) built a *Bd* suitability map conditional on host presence with the maximum entropy (MaxEnt) algorithm (Phillips et al., 2006), a presence-background approach that uses information from climatic conditions on detection-only datasets. This SDM predicted suitable areas for *Bd* during epizootics (e.g., mountain ranges between 1,000 and 2,500 m) and identified low suitability regions in several mid- to low-elevations. However, this model may not accurately reflect the enzootic distribution of *Bd* in Costa Rica because most detection trials were conducted on museum specimens collected during the 1980s and 1990s, when *Bd* was epizootic, and histology was the only method used for *Bd* detection. Additionally, the suitability map lacked tuning methods, which generate more accurate modeling of geographic distributions (Phillips & Dudík, 2008; Radosavljevic & Anderson, 2014). Therefore, improving upon existing SDMs with the use of updated and robust detection datasets along with increased rigor for model building and evaluation will improve the accuracy of predicting a pathogen's occurrence in a given region (Merow et al., 2013; Rödder et al., 2008; Warren & Seifert, 2011).

We generated an updated habitat suitability map for *Bd* in Costa Rica using a comprehensive dataset that combined two methods of detection: histology and PCR. Our main goal was to describe the current distribution of *Bd* and generate accurate predictions of *Bd* occurrence in latitudinal and altitudinal regions. We also aimed to estimate the occurrence of *Bd* a cross protected areas and herpetological provinces in Costa Rica, and to identify data-deficient regions, opportunistic

sampling, and *Bd* hotspots. We hypothesize that (a) *Bd* increased its spatial distribution after epizootics, even reaching regions previously considered as unsuitable; (b) *Bd* is highly common in protected areas and hotspots occur within the herpetological provinces with the highest amphibian richness; (c) and opportunistic sampling is linked to regions where *Bd* caused declines during epizootics.

3.3 Methods

3.3.1 Climatic data and analyses

To describe the abiotic conditions across Costa Rica, we downloaded the 19 bioclimatic layers from WorldClim v1.4 (Table C.1) at a spatial resolution of 30 arc-s (https://www.worldclim.org; Hijmans et al., 2005). For data extraction and model construction, we calibrated all models to the extent of Costa Rica by cropping the 19 bioclimatic layers with a defined bounding box (7.750–11.500 N, 82.150–86.250 W). Our maps and geographic analyses were created with ArcGIS 10.7 (ESRI®) and shape files from the Atlas Digital Costa Rica 2014 (Instituto Tecnológico de Costa Rica, 2014). We used the World Geodetic System datum (WGS84) as the coordinate reference system (CRS).

3.3.2 Detection dataset

We build a detection-only dataset (hereafter "combined dataset") based exclusively on detection on amphibian hosts using two methods of detection: histology and PCR. First, we reviewed literature (Goldberg et al. 2009; Picco & Collins, 2007; Saenz et al., 2009; Whitfield et al., 2017; Zumbado-Ulate et al., 2014; Zumbado-Ulate, et al., 2019a, 2019b; Table C.2) and found 401 records of *Bd* in 13 localities of Costa Rica detected through standard PCR or qPCR. Then, we added these *Bd*-positive records to the histology-only dataset (50 *Bd*-positive samples from 21 localities), which was used to produce the first suitability map for *Bd* in Costa Rica (Puschendorf et al., 2009) and includes all the histology-detection data available for this country. Overall, the combined dataset consisted of 451 *Bd*-positive records from 34 localities (Fig. C.1) and evaluated 13 amphibian families and 90 species, plus five individuals that were identified at the genus level. Given that PCR methods are more sensitive to detect *Bd* than histology (Kriger et al., 2006), we compared the abiotic range of detection of both methods in Costa Rica and found that histology

could potentially lead to underestimation of the epizootic distribution, but only in the tropical dry forest (Fig. C.2).

3.3.3 Species distribution models

We aimed to generated suitability maps to compare the endemic geographic distribution of *Bd* in Costa Rica (i.e., enzootic distribution, Zumbado-Ulate, Nelson, et al., 2019) with the distribution of the epizootic strain of *Bd* that emerged in the 1980s (De León et al., 2019). To generate the enzootic distribution, we used the combined dataset described above. To build the epizootic distribution, we used the histology dataset and rebuilt the first suitability maps for *Bd* in Costa Rica (Puschendorf et al., 2009) with tuned settings instead of default settings (see below). Since these two suitability maps only differed in the inputted *Bd*-presence data, we were able to directly determine the effects of timing (epizootic vs. enzootic) and detection method (histology only vs. histology + PCR) on *Bd* distribution. Suitability maps were calibrated with the MaxEnt algorithm (Phillips et al., 2006) in the R package "dismo" (Hijmans et al., 2013). Since MaxEnt accounts for collinearity and correlation between variables (Elith & Leathwick, 2009), we included all the 19 bioclimatic layers from WorldClim v1.4 (see Section 2.1) as predictors for model building. We conducted model tuning (Phillips & Dudík, 2008; Radosavljevic & Anderson, 2014) with the R package "ENMeval" (Muscarella et al., 2014). For data partition, we used the block method (Radosavljevic & Anderson, 2014). The block method traces latitude and longitude lines to split the occurrence localities into four bins of equal number of occurrences and then iteratively uses $k - 1$ bins ($k = 4$) for model training and the withheld bin for testing, resulting in four runs for each combination of settings (Muscarella et al., 2014). Because the block method leads to extrapolation of data beyond the model during each iteration, the model could predict unreliable distributions in insufficiently surveyed areas and remote regions (Elith and Graham, 2009). In total, we generated 72 candidate models by varying regularization multiplier values (ranging from 1 to 5 with increments of 0.5) and using different combinations of feature classes (L, Q, H, LQ, LH, LQH, LQHP, LQHPT, where L = linear, Q = quadratic, H = hinge, P = product, and T = threshold). For model selection, we considered three metrics in the following priority order: (a) mean area under the curve (mean AUC), (b) AUC difference, and (c) minimum training presence omission rate (mtpOR). High values of mean AUC reflect a better ability for a model to discriminate between conditions at withheld (testing) occurrence localities and those of background localities

(Radosavljevic & Anderson, 2014). If two models showed an identical mean AUC, we reviewed the AUC difference between training and test set, where best fitted models show lower values than over-fitted models (Warren & Seifert, 2011). If that did not differentiate models, we checked the mtpOR, that indicates the proportion of test localities with suitability values lower than that associated with the lowest-ranking training locality. Values greater than the expectation of zero typically indicate model overfitting (Table C.3 and C.4).

3.3.4 Geographic distribution of *Bd*

With the output of both suitability maps, we generated binary maps (polygons representing the epizootic and enzootic predicted distribution of *Bd*) using the 10th percentile presence threshold (Radosavljevic & Anderson, 2014). This rigorous criterion discards the localities with the lowest 10% of suitability values and considers the rest to be "suitable habitat." To estimate the relative distribution of suitable and unsuitable conditions across Costa Rica's altitudinal gradient, we extracted the value of elevation (altitude layer from the WorldClim dataset at the same resolution of our models ~1 km2) and the presence or absence value from our binary maps for each cell of the country. Then, we grouped all elevation values into six altitudinal belts of 500 m (from 0 to 3,000 m elevation) and a seventh belt including all elevations above 3,000 m. Finally, we estimated the number of cells predicted as suitable or unsuitable for each belt based on results from each model. We quantified the expansion of *Bd* by comparing the geographic area of both epizootic and enzootic binary maps. We also estimated the predicted occurrence of *Bd* across protected areas and herpetological regions in Costa Rica by quantifying the overlap between the enzootic distribution of *Bd* and polygons representing the protected areas of Costa Rica and Savage's herpetological provinces.

3.3.5 Data-deficient regions, opportunistic sampling, and *Bd* hotspots

We quantified and mapped the sampling of *Bd* in Costa Rica to identify data-deficient regions (i.e., regions where sampling is missing or scarce) and opportunistic sampling. We used kriging interpolation (Oliver & Webster, 1990) to identify *Bd* hotspots (i.e., regions where high prevalence of *Bd* is independent of opportunistic sampling). For this, we split the map of Costa Rica in nine equal regions using a fishnet map (cell size width = 122,500, cell size height = 117,500)

and calculated nine regional values of *Bd* prevalence in Costa Rica. Then, we created a kriging predictive surface using a spherical semi-variogram. Our predictions were tested with a zero-inflated linear model using region, sample size, and method of detection as predictors for the Poisson regression component, and sample size and method of detection as predictors for the logistic regression component. To evaluate the performance of our zero-inflated model, we ran a Poisson regression using the same predictors and compared both models using a corrected Vuong test (Desmarais & Harden, 2013) with the R package "pscl" (Jackman et al., 2017).

3.4 Results

3.4.1 Geographic distribution of *Bd*

When comparing both SDMs, we found that habitat suitability for enzootic *Bd* increased compared to the epizootic period in highlands above 2,500 m and lowlands (<500 m), especially on the Caribbean side (Fig. 3.1a). The enzootic distribution also increased across the Pacific side of Costa Rica, even predicting the occurrence of *Bd* in the tropical dry and semi-dry forests that were predicted as unsuitable during epizootics (Fig. 3.1b).

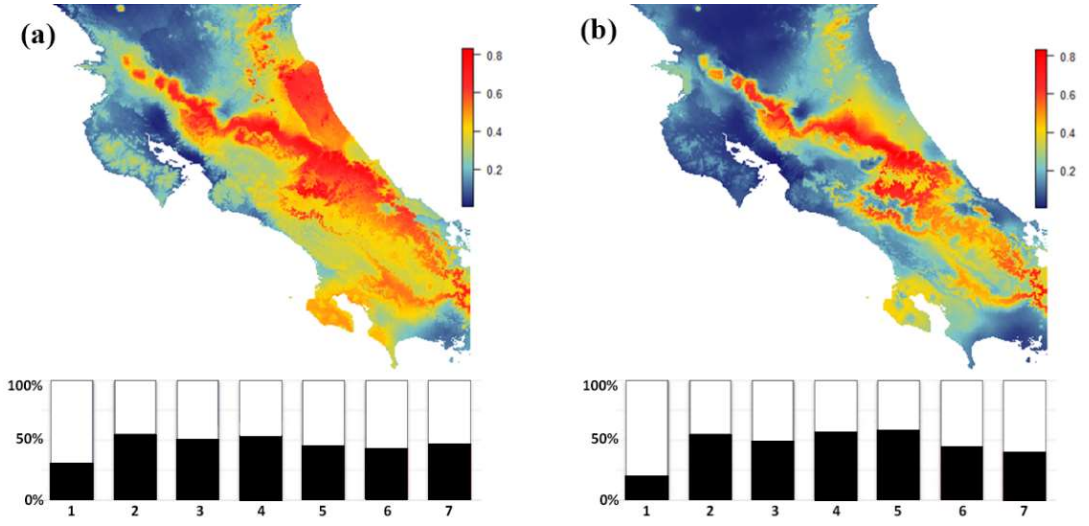

Figure 3.1. (a) Species distribution models for *Batrachochytrium dendrobatidis* (*Bd*) in Costa Rica. (a) The enzootic distribution shows high suitability across all elevations. (b) The epizootic distribution shows high suitability in mid-elevations throughout the country. The scale at the right of each map shows the average probability of occurrence. The bars under each map show suitability by elevation with black indicating the predicted percentage of climatic suitability for *Bd*. Altitudinal bars display belts of 500 m except the seventh bar that shows all elevations above 3,000 m (1 = 0–500 m; 2 = 500–1,000 m; 3 = 1,000–1,500 m, 4 = 1,500–2,000 m; 5 = 2,000–2,500 m; 6 = 2,500–3,000 m; 7 = 3,000–3,820 m).

We estimated the geographic distribution of enzootic *Bd* in 28,390 km^2 (55.6% of Costa Rica), which represents an increase of 61% in the total area of occurrence from epizootics (Fig. 3.2a). We also found that *Bd* is predicted to occur in 80% (10,500 km^2; Fig. 3.2b) of the geographic area classified as protected areas (26% of continental Costa Rica). When comparing the occurrence of *Bd* among herpetological provinces (Fig. 3.2 c,d), we found that *Bd* occurred in all five herpetological provinces, with the highest overlap in the provinces with the highest amphibian species richness: the Talamanca Range (89 species, 99.2% overlap), the Central Volcanic Range (159 species, 91.2% overlap), and the Caribbean lowlands (102 species, 58.3% overlap). Conversely, the occurrence of *Bd* in the North Pacific province was estimated in only 5.6%, which is also the driest province and holds the lowest amphibian richness (66 species).

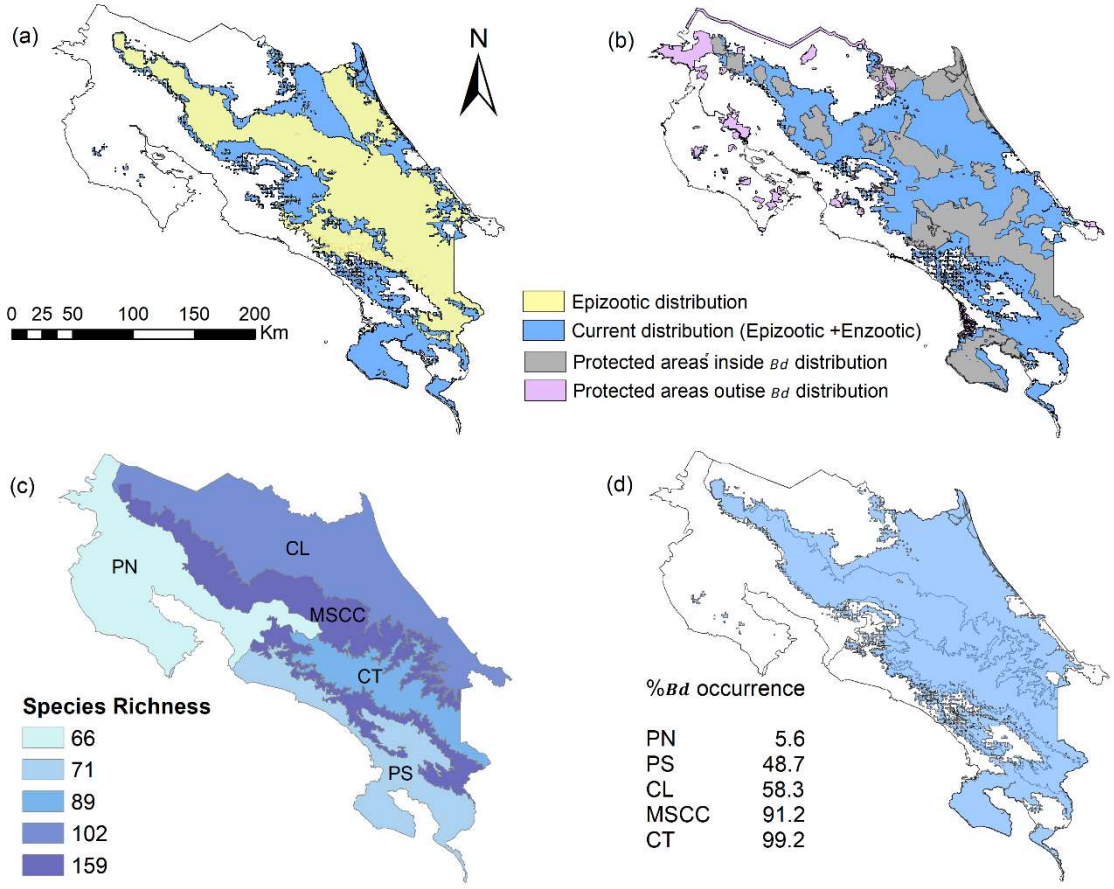

Figure 3.2. (a) Predictive binary map showing the expansion of enzootic *Batrachochytrium dendrobatidis* (*Bd*) in Costa Rica. Predicted distribution of enzootic *Bd* includes both blue and yellow polygons; (b) Predicted occurrence of enzootic *Bd* across protected lands in Costa Rica; (c) Amphibian species richness within each herpetological province (from Zumbado-Ulate et al., 2019a); and (d) predicted occurrence of enzootic *Bd* within each herpetological province. Symbology: PN—Pacific Northwest, and PS—Pacific Southwest, CL— Caribbean Lowlands, MSCC—Central Volcanic Range, CT—Talamanca Range.

Climatic variables showed different contributions to *Bd* suitability, ranging from 0.0% to 41.5% (Table C.1). The climate predictors that most contributed to enzootic distribution were mean precipitation of the driest month (BIO14 = 41.5%), minimum temperature of coldest month (BIO6 = 13.9%), precipitation of warmest quarter (BIO18 = 13.8%), mean temperature of wettest quarter (BIO8 = 12.8%), and precipitation of the coldest quarter (BIO19 = 10.1%). The contribution of these predictors in our updated suitability map suggests that habitat suitability for enzootic *Bd* in Costa Rica is strongly associated with seasonal climatic interactions across the year that favor or constrain *Bd* occurrence.

3.4.2 Data-deficient regions, opportunistic sampling, and *Bd* hotspots

Our results show that numerous localities in Costa Rica remain unsampled for *Bd*, or with a very low sample size. The more extensive data-deficient regions include most part of the Talamanca Range, the Nicoya Peninsula, and the Northwestern lowlands (Fig. 3.3a). We found that most *Bd* sampling efforts have been conducted in midlands and highlands of the Central Volcanic Range, the northern side of the Caribbean lowlands, Santa Elena Peninsula in the Northwest Pacific, and Southern Pacific lowlands (Fig. 3.3a). We found that enzootic *Bd* hotspots occur on the Caribbean side of Costa Rica (Fig. 3.3b and S3), specifically on the foothills of the Central Volcanic Range and the Talamanca Range ($Z = 5.15$, $p < .001$) and the

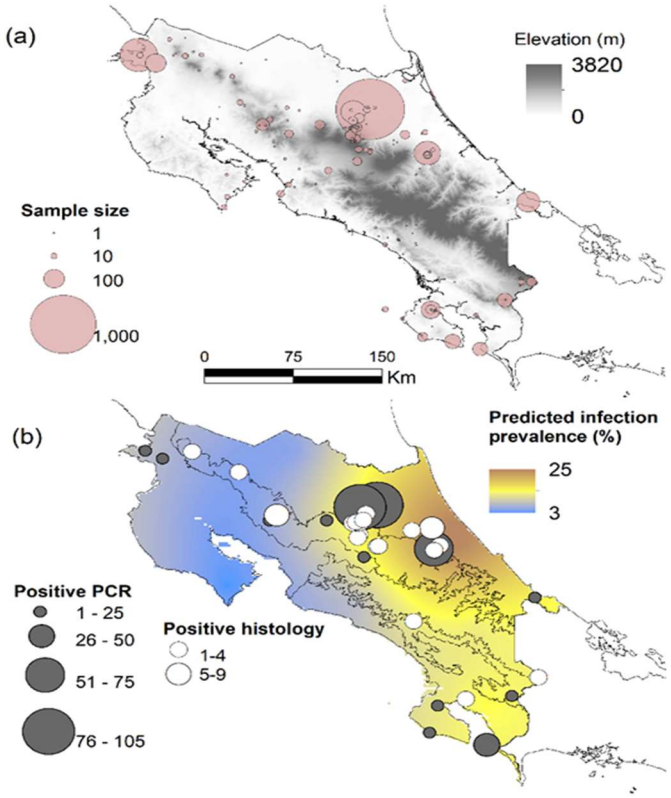

Figure 3.3 Historical sampling of *Batrachochytrium dendrobatidis* (*Bd*) in Costa Rica. (a) Dots show the 172 localities where *Bd* has been surveyed in Costa Rica. The dot size is proportional to the sample size per locality. Small dots and regions lacking dots represent data-deficient regions where *Bd* sampling is poor or missing. (b) Kriging surface predicts the prevalence of enzootic *Bd* in Costa Rica based on 451 positive records detected through histology or polymerase chain reaction (PCR). The map scale shows that the predicted percentage of infection has the highest values on the Caribbean lowlands and intermediate values on the Central Volcanic Range, the Talamanca Range, and Southern Costa Rica. The color-code dots show the number of *Bd* positives in each of the 34 localities for each method of detection.

Caribbean lowlands ($Z = 7.95$, $p < .001$). We identified that *Bd* detection in hotspots increased with sample size ($Z = 11.23$, $p < .001$) and PCR methods ($Z = 14.46$, $p < .001$). Our more robust zero-inflated linear model performed better than a Poisson regression built with the same predictors ($Z = -2.28$, $p = 0.01$).

3.5 Discussion

3.5.1 Geographic distribution of *Bd*

In this study, our main goal was to describe the enzootic distribution of *Bd* and generate accurate predictions of *Bd* occurrence in Costa Rica. Our findings suggest that the distribution of *Bd* has shifted as an enzootic, expanding its range to approximately 60% of the country and across all altitudinal belts of Costa Rica, from sea level to 3,800 m elevation, including the dry and semi-dry forest lowlands on the Pacific side that have been historically considered unsuitable for *Bd* (Puschendorf et al., 2009). Alternatively, the observed increase in *Bd* distribution from epizootic to enzootic dynamics could be related to increased detection of *Bd* through time. Thus, the epizootic distribution might be underestimated because histology is less sensitive than PCR in detecting *Bd* in areas with low prevalence/intensity (Kriger et al., 2006). One potential application that can be applied to validate changes in the distribution during enzootics consists of using host–pathogen integral projection models (IPMs; Ellner & Rees, 2006). IPMs have already been used on the amphibian-*Bd* system and may be used to predict changes in the distribution of pathogen loads over the course of enzootics based on environmental predictors (Wilber et al., 2016). Complementarily, the use of spatial analyses to compare the abiotic range of detection of methods of detection could help identify areas where an approach may fail to detect a pathogen, potentially leading to underestimates of the geographic distribution (Fig. C.2).

The increased suitability for *Bd* in lowlands of Costa Rica may explain the enigmatic declines experienced by several amphibian species in regions that were thought to be unsuitable for *Bd* (below 1,000 m elevation; Bolaños, 2009; Chaves et al., 2014; Zumbado-Ulate et al., 2011). The suitable elevational range predicted for enzootic *Bd* in Costa Rica matched with the elevational range reported for enzootic *Bd* in Panama, Mexico, Colombia, and Chile (Bacigalupe et al., 2019; Bolom-Huet et al. 2019; Flechas et al., 2017; Kilburn et al., 2010; Woodhams et al., 2008). The climatic variable contributions in our model (Table C.1) also suggested that habitat suitability for

enzootic *Bd* in Costa Rica is associated with seasonal climatic interactions across the year that favor or constrain *Bd* occurrence. For example, low precipitation of the driest month may reduce the ability of *Bd* to spread during the dry season. On the other hand, predictors associated with suitable temperatures and precipitation for amphibian aggregation would favor *Bd* transmission.

Our results predicted that enzootic *Bd* occurs in 80% of the area covered by protected areas and all five herpetological provinces (Fig. 3.2b–d). *Bd* distribution was predicted to be high in the herpetological provinces with the highest rate of amphibian endemism in Costa Rica: the Talamanca Range (99.2% overlap) and the Central Volcanic Range (91.2% overlap). A recent assessment (Zumbado-Ulate et al., 2019a) found that 67 of 105 amphibian species screened for *Bd* in Costa Rica tested positive and confirmed that enzootic *Bd* is currently infecting species that exhibit multiple life-history traits across all elevations. Fortunately, the lack of *Bd*-driven die-offs detected since epizootics (i.e., before 2,000) suggests that current amphibian communities in Central America might be exhibiting shifts in host responses to combat enzootic *Bd* and recover from epizootic declines (Christie & Searle, 2018; Voyles et al., 2018).

We found that numerous localities in Costa Rica remain unsampled for *Bd*, especially across the remote highlands of the Talamanca Range, the Nicoya Peninsula, and the Northwestern lowlands (Fig. 3.3a). In many other localities, the sample size analyzed is well below the threshold of 60, the minimum recommended size (Skerratt et al., 2008). We also found evidence of accumulation of *Bd* occurrences through many individual samples that were taken opportunistically on the midlands and highlands of the Central Volcanic Range and on the western side of the Caribbean lowlands, causing the potential misidentification of these regions as *Bd* hotspots. Opportunistic sampling occurred in these regions because they were highly affected by epizootics (Puschendorf et al., 2009), or due to the existence in these regions of experimental biological stations that have conducted long-term studies on *Bd* dynamics (e.g., Whitfield et al., 2013; Whitfield et al., 2012). We identified *Bd* hotspots in the areas of highest amphibian richness in Costa Rica: the Caribbean side and the eastern foothills on the Central Volcanic Range and the Talamanca Range (Fig. 3.3b and C.3). Our findings are supported by recent studies that found that environmental conditions for *Bd* on the Caribbean side of Costa Rica favor an average endemic percent infection of about 25% on local amphibian communities (Whitfield et al., 2012; Zumbado-Ulate et al, 2019b). We recommend the use of interpolation methods (e.g., kriging interpolation)

to map the occurrence of hotspots, and refuges (e.g., Hernandez-Stefanoni & Ponce-Hernandez, 2006).

3.5.2 Implications for conservation

Our study demonstrates that the use of comprehensive datasets that combine methods of detection generate robust predictions of the geographic distribution of pathogens. In our study, the use of the combined dataset increased the power to detect hotspots and cold spots (e.g., Puschendorf et al., 2011). Our findings suggest that other suitability maps for *Bd* in the Americas (at the continental and regional level) that were generated datasets derived from only one method of detection (e.g., Bacigalupe et al., 2019; Bolom-Huet et al., 2019; James et al., 2015; Ron, 2005) could be reanalyzed in follow-up studies with combined datasets and interpolation methods to revalidate high-risk areas, *Bd* hotspots and cold spots.

Our study also suggests that pathogens may expand their ranges after epizootics. Similar studies have modeled the distribution of pathogens during the enzootic stage, for example, West Nile virus vector mosquitoes (Larson et al., 2010), tick vectors of Lyme disease (Illoldi-Rangel et al., 2012), and sarcoptic mange in red foxes (Carricondo-Sanchez et al., 2017). However, shifts in the geographic distribution from epizootics to enzootics are not yet documented. For *Bd*, several studies have addressed the transition between epizootics to enzootics but mostly in terms of prevalence and susceptibility (Briggs et al., 2010; Catenazzi et al., 2017; Kilburn et al., 2010). Given that enzootic *Bd* seems to maintain high levels of virulence in Central America (Voyles et al., 2018), we recommend the use of backcast modeling to identify changes in the distribution of pathogens from epizootics to enzootics. In a different context, our datasets can be used to predict how climate change and other anthropogenic threats may influence suitable habitat for *Bd* in Costa Rica under current and future scenarios (Rohr et al., 2011; Rohr & Raffel, 2010; Xie et al., 2016). A comprehensive assessment of a pathogen's distribution can lead to more effective conservation strategies based on specific regions and species (Heard et al., 2018; Langwig et al., 2015).

3.6 References

Abarca, J., Chaves, G., García-Rodríguez, A., & Vargas, R. (2010). Reconsidering extinction: Rediscovery of *Incilius holdridgei* (Anura: Bufonidae) in Costa Rica after 25 years. *Herpetological Review*, *41*(2), 150.

Adams, A. J., Pessier, A. P., & Briggs, C. J. (2017). Rapid extirpation of a North American frog coincides with an increase in fungal pathogen prevalence: Historical analysis and implications for reintroduction. *Ecology and Evolution*, *7*(23), 10216–10232.

Andam, K. S., Ferraro, P. J., Pfaff, A., Sanchez-Azofeifa, G. A., & Robalino, J. A. (2008). Measuring the effectiveness of protected area networks in reducing deforestation. *Proceedings of the National Academy of Sciences*, *105*(42), 16089–16094.

Annis, S. L., Dastoor, F. P., Ziel, H., Daszak, P., & Longcore, J. E. (2004). A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. *Journal of Wildlife Diseases*, *40*(3), 420–428.

Bacigalupe, L. D., Vásquez, I. A., Estay, S. A., Valenzuela-Sánchez, A., Alvarado-Rybak, M., Peñafiel-Ricaurte, A., … Soto-Azat, C. (2019). The amphibian-killing fungus in a biodiversity hotspot: Identifying and validating high-risk areas and refugia. *Ecosphere*, *10*(5), e02724.

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., … Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature*, *471*(7336), 51–57.

Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., … Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences*, *95*(15), 9031–9036.

Berger, L., Speare, R., & Kent, A. (2000). Diagnosis of chytridiomycosis in amphibians by histologic examination. *Zoos' Print Journal*, *15*, 184–190.

Bolaños, F. (2009). Situación de los anfibios de Costa Rica. *Biocenosis*, *22*(1–2), 95–108.

Bolom-Huet, R., Pineda, E., Díaz-Fleischer, F., Muñoz-Alonso, A. L., & Galindo-González, J. (2019). Known and estimated distribution in Mexico of *Batrachochytrium dendrobatidis*, a pathogenic fungus of amphibians. *Biotropica*, *51*(5), 731–746.

Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T., & Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, *60*(2), 141–148.

Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences*, *107*(21), 9695–9700.

Carricondo-Sanchez, D., Odden, M., Linnell, J. D. C., & Odden, J. (2017). The range of the mange: Spatiotemporal patterns of sarcoptic mange in red foxes (*Vulpes vulpes*) as revealed by camera trapping. *PLOS ONE*, *12*(4), e0176200.

Catenazzi, A., Swei, A., Finkle, J., Foreyt, E., Wyman, L., & Vredenburg, V. T. (2017). Epizootic to enzootic transition of a fungal disease in tropical Andean frogs: Are surviving species still susceptible? *PLOS ONE*, *12*(10), e0186478.

Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V. T., & Ryan, M. J. (2014). Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae). Costa Rica. *Tropical Conservation Science*, *7*(4), 628–638.

Cheng, T. L., Rovito, S. M., Wake, D. B., & Vredenburg, V. T. (2011). Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences*, *108*(23), 9502–9507.

Chestnut, T., Anderson, C., Popa, R., Blaustein, A. R., Voytek, M., Olson, D. H., & Kirshtein, J. (2014). Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. *PLOS ONE*, *9*(9), e106790.

Christie, M. R., & Searle, C. L. (2018). Evolutionary rescue in a host–pathogen system results in coexistence not clearance. *Evolutionary Applications*, *11*(5), 681–693.

Collins, J. P. (2010). Amphibian decline and extinction: What we know and what we need to learn. *Diseases of Aquatic Organisms*, *92*(2–3), 93–99.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2003). Infectious disease and amphibian population declines. Diversity and Distributions, *9*(2), 141–150.

De León, M. E., Zumbado-Ulate, H., García-Rodríguez, A., Alvarado, G., Sulaeman, H., Bolaños, F., & Vredenburg, V. T. (2019). *Batrachochytrium dendrobatidis* infection in amphibians predates first known epizootic in Costa Rica. *PLOS ONE*, *14*(12), e0208969.

Desmarais, B. A., & Harden, J. J. (2013). Testing for zero inflation in count models: Bias correction for the Vuong test. *The Stata Journal*, *13*(4), 810–835.

Dirzo, R., & Raven, P. H. (2003). Global state of biodiversity and loss. *Annual Review of Environment and Resources*, 28(1), 137–167.

Elith, J., & Leathwick, J. R. (2009). Species distribution models: Ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics, 40*(1), 677–697.

Elith, J., & Graham, J. R. (2009). Do They? How Do They? Why Do They Differ? On finding reasons for differing performances of species distribution models. *Ecography*, *32*(1), 66–77.

Ellner, S. P., & Rees, M. (2006). Integral projection models for species with complex memography. *The American Naturalist, 167*(3), 410–428.

Flechas, S. V., Paz, A., Crawford, A. J., Sarmiento, C., Acevedo, A. A., Arboleda, A., … Amézquita, A. (2017). Current and predicted distribution of the pathogenic fungus *Batrachochytrium dendrobatidis* in Colombia, a hotspot of amphibian biodiversity. *Biotropica, 49*(5), 685–694.

García-Rodríguez, A., Chaves, G., Benavides-Varela, C., & Puschendorf, R. (2012). Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions, 18*(2), 204–212.

Goldberg, C. S., Hawley, T. J., & Waits, L. P. (2009). Local and regional patterns of amphibian chytrid prevalence on the Osa Peninsula, Costa Rica. *Herpetological Review, 40*(3), 309–311.

Gómez-Hoyos, D., Méndez-Arrieta, R., Méndez-Arrieta, A., Seisdedosde-Vergara, R., Abarca, J., Barrio-Amorós, C., & González-Maya, J. (2018). Anuran inventory in a locality of the buffer area of La Amistad International Park, Costa Rica: Pilot study for Citizen Science application. *Anales De Biología, 40*, 57–64.

Heard, G. W., Scroggie, M. P., Ramsey, D. S. L., Clemann, N., Hodgson, J. A., & Thomas, C. D. (2018). Can habitat management mitigate disease impacts on threatened amphibians? *Conservation Letters, 11*(2), e12375.

Hernandez-Stefanoni, J. L., & Ponce-Hernandez, R. (2006). Mapping the spatial variability of plant diversity in a Tropical forest: Comparison of spatial interpolation methods. *Environmental Monitoring and Assessment, 117*(1), 307–334.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology, 25*(15), 1965–1978.

Hijmans, R. J., Phillips, S., Leathwick, J., & Elith, J. (2013). Dismo: Species distribution modeling. R Package Version 0.8-17. http://Cran.r-Project. Org. https://cran.r-proje ct.org/web/packa ges/dismo/ index.html

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E. K., Hungate, B. A., Matulich, K. L., … O'Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature, 486,* 105–108.

Illoldi-Rangel, P., Rivaldi, C.-L., Sissel, B., Trout Fryxell, R., Gordillo-Pérez, G., Rodríguez-Moreno, A., … Sarkar, S. (2012). Species distribution models and ecological suitability analysis for potential tick vectors of Lyme Disease in Mexico. *Journal of Tropical Medicine, 2012*, 959101.

Instituto Tecnológico de Costa Rica. (2014). *Atlas de Costa Rica 2014. In RepositorioTEC*. https://repositoriotec.tec.ac.crhtt ps://repositoriotec.tec.ac.cr/handl e/2238/6749.

Jackman, S., Tahk, A., Zeileis, A., Maimone, C., Fearon, J., & Meers, Z. (2017). *The pscl package.* R Package Version 1.17-4. http://Cran.r-Project.Org

James, T. Y., Toledo, L. F., Rödder, D., da Silva Leite, D., Belasen, A. M., Betancourt-Román, C. M., … Longcore, J. E. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution*, *5*(18), 4079–4097.

Jiménez, R., & Alvarado, G. (2017). *Craugastor escoces* (Anura: Craugastoridae) reappears after 30 years: Rediscovery of an "extinct" Neotropical frog. *Amphibia-Reptilia*, *38*(2), 257–259.

Kilburn, V. L., Ibáñez, R., Sanjur, O., Bermingham, E., Suraci, J. P., & Green, D. M. (2010). Ubiquity of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in anuran communities in Panamá. *EcoHealth*, *7*(4), 537–548.

Kirshtein, J., Anderson, C., Wood, J., Longcore, J., & Voytek, M. (2007). Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. *Diseases of Aquatic Organisms*, *77*, 11–15.

Kriger, K. M., Hines, H. B., Hyatt, A. D., Boyle, D. G., & Hero, J.-M. (2006). Techniques for detecting chytridiomycosis in wild frogs: Comparing histology with real-time Taqman PCR. *Diseases of Aquatic Organisms*, *71*(2), 141–148.

Lambin, E. F., & Meyfroidt, P. (2011). Global land use change, economic globalization, and the looming land scarcity. *Proceedings of the National Academy of Sciences*, *108*(9), 3465–3472.

Langholz, J., & Lassoie, J. (2001). Combining conservation and development on private lands: Lessons from Costa Rica. *Environment, Development and Sustainability*, *3*(4), 309–322.

Langwig, K. E., Voyles, J., Wilber, M. Q., Frick, W. F., Murray, K. A., Bolker, B. M., … Kilpatrick, A. M. (2015). Context-dependent conservation responses to emerging wildlife diseases. *Frontiers in Ecology and the Environment*, *13*(4), 195–202.

Larson, S. R., Degroot, J. P., Bartholomay, L. C., & Sugumaran, R. (2010). Ecological niche modeling of potential West Nile virus vector mosquito species in Iowa. *Journal of Insect Science*, *10*(110), 1–17.

Lieberman, D., Lieberman, M., Peralta, R., & Hartshorn, G. S. (1996). Tropical forest structure and composition on a large-scale altitudinal gradient in Costa Rica. *The Journal of Ecology*, *84*(2), 137–152.

Lips, K. R., Green, D. E., & Papendick, R. (2003). Chytridiomycosis in wild frogs from southern Costa Rica. *Journal of Herpetology*, *37*(1), 215–218.

Lips, K. R., Reeve, J. D., & Witters, L. R. (2003). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology*, *17*(4), 1078–1088.

Longcore, J. E., Pessier, A. P., & Nichols, D. K. (1999). *Batrachochytrium dendrobatidis* gen. Et sp. Nov., a chytrid pathogenic to amphibians. *Mycologia*, *91*, 219–227.

Meredith, H., Van Buren, C., & Antwis, R. E. (2016). Making amphibian conservation more effective. *Conservation Evidence*, *13*, 1–6.

Merow, C., Smith, M. J., & Silander, J. A. Jr (2013). A practical guide to MaxEnt for modeling species' distributions: What it does, and why inputs and settings matter. *Ecography*, *36*(10), 1058–1069.

Monastersky, R. (2014). Life-a status report. *Nature*, *516*(7530), 158.

Muscarella, R., Galante, P. J., Soley-Guardia, M., Boria, R. A., Kass, J. M., Uriarte, M., & Anderson, R. P. (2014). ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. *Methods in Ecology and Evolution*, *5*(11), 1198–1205.

Nori, J., Lemes, P., Urbina-Cardona, N., Baldo, D., Lescano, J., & Loyola, R. (2015). Amphibian conservation, land-use changes and protected areas: A global overview. *Biological Conservation*, *191*, 367–374.

Oliver, M. A., & Webster, R. (1990). Kriging: A method of interpolation for geographical information systems. *International Journal of Geographical Information Systems*, *4*(3), 313–332.

Peterson, A. T., Bauer, J. T., & Mills, J. N. (2004). Ecologic and geographic distribution of filovirus disease. *Emerging Infectious Diseases*, *10*(1), 40–47.

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3), 231–259.

Phillips, S. J., & Dudík, M. (2008). Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. *Ecography*, *31*(2), 161–175.

Picco, A. M., & Collins, J. P. (2007). Fungal and viral pathogen occurrence in Costa Rican amphibians. *Journal of Herpetology*, *41*(4), 746–749.

Pinkard, E. A., Kriticos, D. J., Wardlaw, T. J., Carnegie, A. J., & Leriche, A. (2010). Estimating the spatio-temporal risk of disease epidemics using a bioclimatic niche model. *Ecological Modelling*, *221*(23), 2828–2838.

Pounds, A. J., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., … Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, *439*(7073), 161–167.

Pounds, J. A., & Crump, M. L. (1994). Amphibian declines and climate disturbance: The case of the golden toad and the harlequin frog. *Conservation Biology*, *8*(1), 72–85.

Powers, J. S., Becknell, J. M., Irving, J., & Pèrez-Aviles, D. (2009). Diversity and structure of regenerating tropical dry forests in Costa Rica: Geographic patterns and environmental drivers. *Forest Ecology and Management*, *258*(6), 959–970.

Puschendorf, R., Carnaval, A. C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., & Alford, R. A. (2009). Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: Proposing climatic refuges as a conservation tool. *Diversity and Distributions*, *15*(3), 401–408.

Puschendorf, R., Hoskin, C. J., Cashins, S. D., McDonald, K., Skerratt, L. F., Vanderwal, J., & Alford, R. A. (2011). Environmental refuge from disease-driven amphibian extinction. *Conservation Biology*, *25*(5), 956–964.

Radosavljevic, A., & Anderson, R. P. (2014). Making better MAXENT models of species distributions: Complexity, overfitting and evaluation. *Journal of Biogeography*, *41*(4), 629–643.

Rödder, D., Veith, M., & Lötters, S. (2008). Environmental gradients explaining the prevalence and intensity of infection with the amphibian chytrid fungus: The host's perspective. *Animal Conservation, 11*(6), 513–517.

Rohr, J. R., Halstead, N. T., & Raffel, T. R. (2011). Modelling the future distribution of the amphibian chytrid fungus: The influence of climate and human-associated factors. *Journal of Applied Ecology, 48*(1), 174–176.

Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences, 107*(18), 8269–8274.

Ron, S. R. (2005). Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. *Biotropica, 37*(2), 209–221.

Sáenz, D., Adams, C. K., Pierce, J. B., & Laurencio, D. (2009). Occurrence of *Batrachochytrium dendrobatidis* in an anuran community in the southeastern Talamanca region of Costa Rica. *Herpetological Review, 40*(3), 311–313.

Sasa, M., Chaves, G., & Porras, L. W. (2010). The Costa Rican herpetofauna: Conservation status and future perspectives. In J. H. Townsend, & J. D. Johnson (Eds.), Conservation of Mesoamerican amphibians and reptiles (pp. 510–603). Eagle Mountain, UT: Eagle Mountain Press.

Savage, J. M. (2002). The amphibians and reptiles of Costa Rica: A herpetofauna between two continents, between two seas, Chicago, IL: The University of Chicago Press.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A. N., Beukema, W., … Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science, 363*(6434), 1459–1463.

Skerratt, L., Berger, L., Hines, H., McDonald, K., Mendez, D., & Speare, R. (2008). Survey protocol for detecting chytridiomycosis in all Australian frog populations. *Diseases of Aquatic Organisms, 80*(2), 85–94.

Skerratt, L. F., Mendez, D., McDonald, K. R., Garland, S., Livingstone, J., Berger, L., & Speare, R. (2011). Validation of diagnostic tests in wildlife: The case of chytridiomycosis in wild amphibians. *Journal of Herpetology, 45*(4), 444–450.

Stuart, S. N., Chanson, J., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D., & Waller, R. (2004). Status and trends of amphibian declines and extinctions worldwide. Science, 306(5702), 1783–1786.

Voyles, J., Woodhams, D. C., Saenz, V., Byrne, A. Q., Perez, R., Rios-Sotelo, G., … Richards-Zawacki, C. L. (2018). Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science, 359*(6383), 1517–1519.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W. F., Dinudom, A., … Speare, R. (2009). Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science, 326*(5952), 582–585.

Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences, 105*(1), 11466–11473.

Wandeler, P., Hoeck, P. E. A., & Keller, L. F. (2007). Back to the future: Museum specimens in population genetics. *Trends in Ecology & Evolution, 22*(12), 634–642.

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in Maxent: The importance of model complexity and the performance of model selection criteria. *Ecological Applications, 21*(2), 335–342.

Whitfield, S. M., Alvarado, G., Abarca, J., Zumbado-Ulate, H., Zuñiga, I., Wainwright, M., & Kerby, J. (2017). Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms*, *126*(1), 33–41.

Whitfield, S. M., Geerdes, E., Chacon, I., Ballestero Rodriguez, E., Jimenez, R., Donnelly, M., & Kerby, J. (2013). Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. *Diseases of Aquatic Organisms*, *104*(2), 173–178.

Whitfield, S. M., Kerby, J., Gentry, L. R., & Donnelly, M. A. (2012). Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. *Biotropica*, *44*(6), 779–784.

Whitfield, S. M., Lips, K. R., & Donnelly, M. A. (2016). Amphibian decline and conservation in Central America. *Copeia*, *104*(2), 351–379.

Wilber, M. Q., Langwig, K. E., Kilpatrick, A. M., McCallum, H. I., & Briggs, C. J. (2016). Integral Projection Models for host–parasite systems with an application to amphibian chytrid fungus. *Methods in Ecology and Evolution*, *7*(10), 1182–1194.

Woodhams, D. C., Kilburn, V. L., Reinert, L. K., Voyles, J., Medina, D., Ibáñez, R., … Rollins-Smith, L. A. (2008). Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. *EcoHealth*, *5*(3), 268–274.

Xie, G. Y., Olson, D. H., & Blaustein, A. R. (2016). Projecting the global distribution of the emerging amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, based on IPCC climate futures. *PLOS ONE*, *11*(8), e0160746.

Zumbado-Ulate, H., Bolaños, F., Gutiérrez-Espeleta, G., & Puschendorf, R. (2014). Extremely low prevalence of *Batrachochytrium dendrobatidis* in frog populations from Neotropical dry forest of Costa Rica supports the existence of a climatic refuge from disease. *EcoHealth*,*11*(4), 593–602.

Zumbado-Ulate, H., Bolaños, F., Willink, B., & Soley-Guardia, F. (2011). Population status and natural history notes on the critically endangered stream dwelling frog *Craugastor ranoides* (Craugastoridae) in a Costa Rican tropical dry forest. *Herpetological Conservation and Biology*, *6*(3), 455–464.

Zumbado-Ulate, H., García-Rodríguez, A., Vredenburg, V. T., & Searle, C.L. (2019). Infection with *Batrachochytrium dendrobatidis* is common in tropical lowland habitats: Implications for amphibian conservation. *Ecology and Evolution*, *9*(8), 4917–4930.

Zumbado-Ulate, H., Nelson, K. N., García-Rodríguez, A., Chaves, G., Arias, E., Bolaños, F., … Searle, C. L. (2019). Endemic infection of *Batrachochytrium dendrobatidis* in Costa Rica: Implications for amphibian conservation at regional and species level. *Diversity*, *11*(8),129.

CHAPTER 4. ASSESSING THREATS FOR DIRECT-DEVELOPING STREAM-DWELLING FROGS IN MESOAMERICA

4.1 Abstract

Direct-developing, stream-dwelling frogs (commonly known as robber frogs) were among those that suffered from widespread amphibian declines in Mesoamerica between the 1980s and early 2000s, especially in seemingly pristine forested environments. Currently, 33% of robber frog species remain undetected since 2005 or before. However, the rediscovery of several vanished species during the last decade raises the hope that additional remnant populations may be found. We used species distribution modeling, range quantification, and climatic niche comparisons to assess threat risk (habitat deterioration and pathogen occurrence). At the regional level (i.e., Mesoamerica), we found that the percent of old-growth and secondary forest in each species range was at least 50% for 83% of species between the 1980s and early 2000s. We also found that 80% of the average range of each species currently overlaps with the predicted occurrence of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which suggests that *Bd* was the main driver of extinction before 2005. At the local level (i.e., focusing on two species ranges), we found that the present range of *Bd* corresponds with the reduction in geographic range and climatic niche of two species of robber frogs. Additionally, we found that climatic and microhabitat heterogeneity (biotic and abiotic) can predict the location of remnant populations with *Bd*. We use these results to propose specific actions for all species to improve their management and identify regions where additional remnant populations may occur. This study highlights the importance of considering multiple threats and spatial scales when assessing the status of declined and threatened species

4.2 Introduction

The values assigned to ecological processes and variables that determine species ranges can vary greatly across the space and are scale dependent (Isbell et al., 2017). For example, disease transmission changes across spatial scales as a function of species diversity (Keesing et al., 2010; Chase et al., 2019) and abiotic conditions (Johnson et al., 2019). Additionally, the range of pathogens and host species can be driven by processes that occur at different scales (e.g., biotic

processes at small scales, climate, and population density at larger scales; Cohen et al., 2016). Thus, multiple scales must be used when examining threats for threatened and endangered taxa.

Among vertebrates, amphibians represent the most endangered class (Monastersky, 2014). Over 56% of the approximately 8310 described species (Frost, 2021) are likely threatened by extinction (Cardillo, 2021; IUCN, 2019). Multiple anthropogenic threats have driven global amphibian declines including introduced pathogens, habitat destruction and fragmentation, climate change, and invasive species (Hof et al., 2011; Catenazzi, 2015). Thus, scientists must quantify the loss of amphibian biodiversity and identify the various threats to amphibians across landscapes, so that appropriate conservation actions can be implemented in regions where threats will have the largest impact (Isaac et al., 2012; Mendelson et al., 2019).

Although threats to amphibians are often considered individually, many amphibian species are facing multiple threats at the same time, which can often act synergistically to increase extinction risk above that expected from their additive contributions (e.g., Brook et al. 2008; Hof et al. 2011; but see Greenville et al., 2021). For example, the effects of introduced pathogens (Kilpatrick et al., 2010; Scheele et al., 2017a) can be exacerbated by climate change, pollutants, and habitat deterioration (Kiesecker et al., 2001; Becker et al., 2007; Rollins-Smith, 2017). Amphibians represent an ideal group on which to conduct multiscale analyses for conservation purposes because species tend to exhibit discontinuous ranges across the landscape (Semlitsch & Bodie, 2003), and the threats to amphibians vary widely across space and scale (Bosch et al., 2004; Grant et al., 2016; Cohen et al., 2016).

4.2.1 Amphibian declines in Mesoamerica

Mesoamerica is a global hotspot of amphibian biodiversity and endemism (Savage, 2002; Wilson & Johnson, 2010) and is home to approximately 850 described amphibian species (AmphibiaWeb, 2020). However, the destruction and alteration of habitat for mining, agriculture, and establishment of human settlements have jeopardized the majority of amphibian species in the region (e.g., Frías-Alvarez et al., 2010; Mayani-Parás et al., 2019). Furthermore, numerous amphibian species experienced drastic declines in seemingly undisturbed environments (Lips et al., 2005; Pounds et al., 2006; Whitfield et al., 2016) due to the spread of epizootic lineages of the pathogenic fungus *Batrachochytrium dendrobatidis* (hereafter '*Bd*;' Longcore et al., 1999) between the 1980s and early 2000s (Cheng et al., 2011; Lips et al., 2008; Puschendorf et al., 2006). During

Bd epizootic times (here, defined as the period before 2005), *Bd* rapidly spreads causing massive die-offs of multiple species and can even cause an entire amphibian community to collapse (e.g., Briggs et al., 2010; Catenazzi et al., 2011). Currently, several studies at the local level (e.g., Woodhams et al., 2008; Kilburn et al., 2010; Bolom-Huet et al., 2019; Zumbado-Ulate et al., 2019a; 2020) suggest that *Bd* is widespread and has not caused high rates of mortality after the early 2000s (hereafter '*Bd* enzootic times'). In this study, we set the start of *Bd* enzootic times to the year 2005 following spatiotemporal trends that suggest that *Bd* was enzootic across Mesoamerica by 2005 (Lips et al., 2008; Cheng et al., 2011).

Studies on *Bd* dynamics (e.g., Lips et al., 2003; Brem & Lips, 2008; Zumbado-Ulate et al., 2019b) have identified direct-developing, stream-dwelling frogs (commonly known as robber frogs) as one of the groups most affected by population declines in Mesoamerica. However, recent signs of partial recovery (e.g., Whitfield et al., 2017) and species rediscovery (Kolby & McCranie 2009; Chaves et al., 2014) suggest that remnant populations of robber frog species might occur under biotic and abiotic conditions that exclude pathogenic outbreaks (i.e., environmental refugia from disease; Puschendorf et al., 2011) or have rapidly evolved reduced susceptibility to *Bd*, allowing host-pathogen coexistence (Christie & Searle, 2018). Here, our main objective was to examine geographic patterns, and threat risk across for all known robber frog species in Mesoamerica. We were particularly interested in determining 1) how threats to robber frogs (habitat degradation and *Bd* occurrence) vary across space, 2) how *Bd* has affected the geographic range and climatic niche of robber frogs, 3) how threat risk assessment varies with spatial scale, and 4) which species and regions are most likely to experience future declines and extinctions. We did so by using species distribution modeling (SDM), range quantification, and climatic niche comparisons. Results from this work will provide a greater understanding of the role that environmental heterogeneity and habitat degradation play in driving the range and niche dynamics of pathogens and host species.

4.3 Methods

We measured the effect of threats at the regional level (Mesoamerica) for a total of 46 robber frog species and at the local level (i.e., species-range) for two Critically Endangered species. We did so by controlling the scope in both analyses (i.e., the ratio of the extent to resolution; Schneider, 2001), by holding the resolution of our raster layers constant (30 arc-s) and modifying the extent.

4.3.1 Regional-level analyses

4.3.1.1 Focal species

In our study, we included all species contained within the *Craugastor punctariolus* species series (34 spp.) and the subgenus *Campbellius* (12 spp.; former *C. milesi* group; Hedges et al., 2008) (Fig. D.1; Table D.1). Hereafter we will refer to our focal species as 'robber frogs' although other frog species from different clades are also known by this common name. Robber frogs are direct-developing, stream-dwelling species, endemic to Mesoamerica. Although most species inhabit old-growth and secondary forest ecosystems, a few species occur in regions with moderate levels of disturbance (e.g., *C. amniscola*; Ochoa-Ochoa & Whittaker 2014). Most species of robber frogs declined during *Bd* epizootic times (Lips et al., 2005; Whitfield et al., 2016) and 15 species (33%) have remained undetected since 2005 or earlier. A total of 90% of all robber frog species are considered threatened according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (hereafter 'IUCN Red List'; IUCN 2019). Similarly, the regional index of environmental vulnerability scores (EVS; Wilson & McCranie, 2004) shows that 45 species (98%) have the highest vulnerability to extinction (Wilson et al., 2013; Johnson et al., 2015).

4.3.1.2 Abiotic data

We downloaded the 19 BIOCLIM variables (Booth et al., 2014) from WorldClim v2.1 (Fick & Hijmans 2017) and the 16 environmental raster layers for ecological modeling 'ENVIREM' (Title & Bemmels, 2018), both at a spatial resolution of 30 arc-s. The ENVIREM dataset has been shown to potentially improve the performance of SDMs that use BIOCLIM variables. Following Title and Bemmels (2018), we excluded the ENVIREM layers 'aridityIndexThornthwaite' (which is redundant with the 'climaticMoistureIndex'), and 'monthCountByTemp10' (which is categorical). For data extraction, we cropped the 33 environmental layers with the R package 'Raster' (Hijmans et al., 2015) using a bounding box for Mesoamerica (N 5–40º, W 70–118º). We used the R package 'usdm' (Naimi, 2015) to detect collinearity among predictors by quantifying the Variance Inflation Factor (VIF) and selecting the predictors which had VIF < 3 and pair-wise correlations < 0.6. We retained eight environmental predictors in our abiotic dataset (hereafter 'regional abiotic dataset;' Table D.2). Our maps and geographic analyses were created with

ArcGIS 10.7.1 (ESRI® Redlands, CA, USA) using the World Geodetic System datum (WGS84). We used shapefiles from the Atlas Digital Costa Rica 2014 (Ortiz-Malavasi 2014) and the Database of Global Administrative Areas (GADM; https://gadm.org/data.html).

4.3.1.3 *Bd* occurrence records

We constructed a presence-only dataset from 181 *Bd*-positive localities in Mesoamerica (Table D.3). We also included 41 occurrence points from northern Colombia, southern United States, and Cuba as calibration points. To reduce the effect of spatial autocorrelation, we excluded the occurrence points that were separated by a distance < 15 km with the R package 'spThin' using 100 random repetitions (Aiello-Lammens et al., 2015). Our final dataset (hereafter '*Bd* dataset') consisted of 133 records (Fig. D.2).

4.3.1.4 Threat quantification

We quantified land use during *Bd* epizootic and enzootic times and predicted the range of *Bd* during enzootic times. We were not able to predict the past range of *Bd* during epizootic times due to the lack of sampling in most of Mesoamerica and global methodical limitations to detect *Bd* before the 2000s (but see Puschendorf et al., 2009; Zumbado-Ulate et al., 2020).

4.3.1.4.1 Land use

We downloaded 'The Human Footprint (2018 Release)' (Sanderson et al., 2002; Venter et al., 2016) at a spatial resolution of 30 arc-s. This dataset consists of two raster layers that quantify the cumulative human pressure on terrestrial ecosystems in 1993 and 2009, which match temporally with the *Bd* epizootic and *Bd* enzootic times in Mesoamerica. In each layer, scores ranging from 0 to 50 are assigned to each cell, with the highest values representing cells with the highest human footprint. To build an accurate categorical representation of the human footprint in Mesoamerica, we superposed land-use layers of Central America from the Atlas Digital Costa Rica 2014 (Ortiz-Malavasi, 2014) on both human footprint layers and reclassified the raster values into four categories according to each cell score: suitable habitat (old-growth and secondary forest ecosystems, 0-10), intermediate disturbance (small forest patches, croplands, and pastures; 11-20), rural development (21-30), and urban development (31-50). We transformed both reclassified (i.e.,

categorized) 1993 and 2009 human footprint layers into vector data (i.e., shapefiles). Then, we overlaid both shapefiles to quantify changes in land use during these 16 years.

4.3.1.4.2 Range of *Bd* in Mesoamerica

We modeled the range of *Bd* in Mesoamerica during enzootic times using the MaxEnt algorithm (Phillips et al., 2006). With the regional abiotic dataset (section 4.3.1.2), we generated 36 candidate models with the R package 'ENMeval' (Muscarella et al., 2014) using the following settings: Partition Method = 'block'; random points = 1000; algorithm = maxent.jar; regularization of multiplier values = 0.5–3 with increments of 0.5; feature classes = L, LQ, H, LQH, LQHP, LQHPT. We selected the model with the highest value of average test of the area under the receiver characteristic operator curve (AUC mean) and the lowest omission rate at minimum training presence (orMTP; Radosavljevic & Anderson, 2014). We used the selected settings to build a habitat suitability map with the R package 'dismo' (Hijmans et al., 2013). With the logistic output of our suitability map, we generated a binary map (absence-presence maps of the potential range of *Bd* in Mesoamerica) using the equal training sensitivity and specificity threshold (ETSS), which is less restrictive than other thresholds and recommended for conservation planning programs (Liu et al., 2005). We calculated the area of occurrence (AOO) of *Bd* in Mesoamerica by transforming our predictive binary map of *Bd* into a shapefile.

4.3.1.4.3 Assessing threat risk

We used updated range polygons for each focal species provided by the IUCN Amphibian Specialist Group. The IUCN range maps show the historical, present, and projected range of a species within its native range (Bland et al., 2015). The IUCN range polygons are generated by 1) plotting point data, 2) drawing a minimum convex polygon (MCP) around the points, 3) expanding the range considering the knowledge of habitat preferences, 4) removing areas known to be unsuitable (e.g., unsuitable habitat, elevation limits, or climate/temperature restrictions), and 5) smoothing polygons. Because IUCN has different classifications assigned to range polygons, we only considered the polygons corresponding to the extant and extinct range of our focal species and excluded those that represent regions of uncertain presence. We calculated the area of each range polygon (hereafter 'A_{MCP}') as a proxy of the species occurrence area. For species with

several range polygons, the total inhabited range area consisted of the sum of all individual polygon areas. For each species range, we also estimated the percent coverage of old-growth and secondary forest during *Bd* epizootic and enzootic times and the percent overlap with the predicted occurrence of *Bd*.

We conducted logistic regression using stepwise, backward selection (Hosmer et al., 2013) to examine the current detectability of robber frog species using binomial response variables (detected or undetected). To deal with quasi-perfect separation we followed a Bayesian analysis with non-informative prior assumptions (Gelman et al., 2008) using the function 'bayesglm' in the R package 'arm' (Gelman et al., 2020). Output models were ranked according to Akaike's information criterion (AIC; Burnham & Anderson, 2004). All species without records since 2005 or before were classified as 'undetected'. As predictors, we used 1) the A_{MCP} (quantitative, in km^2), 2) the elevational range inhabited by a species (quantitative, the difference between the upper and lower elevation limit, using values from the IUCN Red List), 3) the percent coverage of old-growth and secondary forest within the range of each species of robber frog during *Bd* epizootic times (quantitative), 4) the percent of overlap of *Bd* with the range of each species of robber frog (quantitative), and 5) clade (categorical, *C. punctariolus* species series or subgenus *Campbellius*). We ran a correlation test between continuous variables to identify highly correlated predictors. All correlations were below 0.6, therefore no predictors were excluded.

4.3.2 Local, species-range level analyses

4.3.2.1 Focal species

We selected two species to examine at the local, species-range level (Fig. 4.1): the 'dry forest robber frog', a subspecies of *C. ranoides* (Puschendorf et al., 2019) which remnant populations exclusively occur in the Santa Elena Peninsula, Costa Rica and the 'Golfito robber frog' (C. taurus), which only survives in a small portion of its historical range, in the areas of Pavones in Costa Rica and Puerto Armuelles, in Panamá (Chaves et al., 2014). We selected these species because they have been exhaustively monitored in Costa Rica since the early 2000s (Zumbado-Ulate & Willink, 2011; Chaves et al., 2014), allowing us to generate accurate SDMs and climatic niche modeling for *Bd* enzootic times.

4.3.2.2 Abiotic data and study extent

We followed the same methods described in section 4.3.1.2 but we used an extent (N 7–14º, W 80–88º) that included the potential range of study species in Costa Rica, Nicaragua, and Panamá. For the dry forest robber frog, our area of calibration included the province of Guanacaste and the counties of Upala and Guatuso in the province of Alajuela in Costa Rica, following the range proposed by Puschendorf et al. (2019). We also included the Department of Rivas in Nicaragua due to its proximity and climatic similarity with the Santa Elena Peninsula. For the Golfito robber frog, our area of calibration included the Central and South Pacific versant of Costa Rica, and the province of Chiriquí in Panamá (Savage, 2002). Our abiotic dataset consisted of six environmental predictors with pair-wise correlations < 0.6 (hereafter 'local abiotic dataset;' Table D.4).

4.3.2.3 Robber frog datasets

We obtained occurrence data from two sources: the herpetological database of the Museo de Zoología at Universidad de Costa Rica (http://museo.biologia.ucr.ac.cr/) and the Global Biodiversity Information Facility (GBIF) (Flemons et al., 2007). We also included new occurrence points generated from expeditions conducted in Costa Rica during the extent of this study. We cleaned data by checking typos, cross-checking geographic coordinates, and removing unreferenced records and duplicates from the dataset. To control spatial autocorrelation, we used the same methods described in section 4.3.1.3 but instead used a spatial filter of 1 km (matching the resolution of the abiotic dataset layers). Our final datasets consisted of 102 historic localities: 23 for the dry forest robber frog (eight of which are from *Bd* enzootic times) and 79 for the Golfito robber frog (14 of which are from *Bd* enzootic times).

4.3.2.4 Range quantification

We generated SDMs for the historic and present range of the dry forest robber frog and the Golfito robber frog to quantify the reduction in their ranges during *Bd* enzootic times using the local abiotic dataset (section 4.3.2.2). For the present range, we considered only the known remnant populations. We followed the same methods described in section 4.3.1.4.2 but we used the 'n-1 Jackknife' method instead of the block method to model the historic and present range of the dry forest robber frog and the present range of the Golfito robber frog because the number of

occurrences was < 25 (Muscarella et al., 2014). We used the output binary maps to estimate the AOO for both species as shown in section 4.3.1.4.2.

4.3.2.5 Niche dynamics

We compared the historic and present climatic niche of remnant populations of the dry forest robber frog and the Golfito robber frog with the climatic niche of *Bd* during enzootic times to quantify niche contraction after the spread of *Bd*. All *Bd* records for Costa Rica were extracted from the *Bd* dataset (section 4.3.1.3). We were not able to model the climatic niche of *Bd* during epizootic times because the pathogen was either undetected or the number of samples was < 5 in the study species' ranges before 2005. First, we estimated species' density of occurrences as probability distributions defined over the multivariate climatic niche (Z scores) using ordination (PCA-env sensu; Broennimann et al., 2012), smoothed kernel density (Broennimann et al., 2007; 2012; Petitpierre et al., 2012) and the local abiotic dataset. Second, we used the Z scores to estimate the niche overlap using the Schoener's *D* overlap index (Schoener, 1968). Finally, we estimated 'unfilling' (i.e., climatic niche of the historical populations that was not occupied by the present remnant populations; Warren et al., 2008) and 'expansion' (i.e., new climatic space occupied by *Bd* during enzootic times; Warren et al., 2008). We also ran a niche similarity test to assess if we could predict the climatic niche of one species better than expected by chance by modeling another based on the null hypothesis of niche equivalency (Warren et al., 2008). All tests were performed with the R package 'Ecospat' (Di Cola et al., 2017).

4.3.2.6 Habitat

We selected six stream networks in Costa Rica where robber frogs were common before the *Bd* epizootic times (Fig. 4.1). Remnant populations of robber frogs still survive and coexist with *Bd* in two of the stream networks (Santa Elena Peninsula, and Punta Banco stream networks; Zumbado-Ulate et al., 2014, 2019a). This approach allowed us to compare biotic and abiotic conditions in sites with and without remnant populations to identify environmental predictors that constrain or allow coexistence with *Bd.*

4.3.2.6.1 Abiotic conditions

To achieve a full description of the abiotic environment we generated buffers (radius = 10 km) around each of our six study stream networks. Within each buffer, we generated 500 random points. Our final dataset consisted of 2278 random points (722 random points were projected outside continental Costa Rica). For each random point, we extracted values for the six environmental predictors of the local abiotic dataset (Table D.5). The extracted values were scaled and compared using a principal component analysis (PCA) with the R package 'FactoMineR' (Lê et al., 2008). The results of the PCA were interpreted and visualized with the R package 'FactoExtra' (Kassambara & Mundt 2017). To show climatic dissimilarities between study sites we generated a hierarchical cluster analysis using Euclidean distances between the centroids of climatic envelopes and complete linkage as the agglomeration method. We visualized our cluster with the R package 'ggdendro' (de Vries & Ripley 2013).

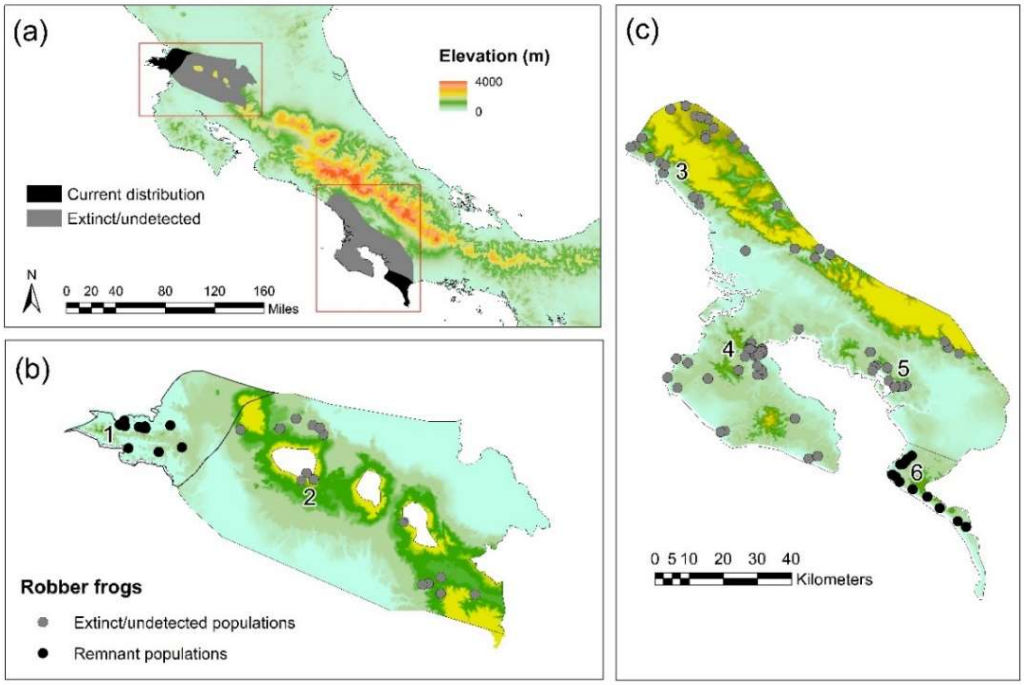

Figure 4.1. Study stream networks. a) range polygons showing the range of extinct and remnant populations of the dry forest robber frog (subspecies of *Craugastor ranoides* in the Santa Elena Peninsula, upper left rectangle) and the Golfito robber frog (*C. taurus,* bottom right rectangle). The white regions represent the top of the main volcanoes at Guanacaste Mountain Range, where the species is not predicted to occur; b) sampling sites for the lowland robber frog: 1) the Santa Elena Peninsula and 2) Rincón de la Vieja Volcano stream networks; c) sampling sites for the Golfito robber frog: 3) Uvita, 4) Rincón de Osa, 5) Golfito, and 6) Punta Banco stream networks. Black dots and polygons represent remnant populations; grey dots and polygons represent extinct/undetected populations.

4.3.2.6.2 Microhabitat conditions

We conducted six comparative field surveys to the six stream networks in Costa Rica during the dry seasons of 2017 and 2018. We only surveyed during the dry season because the study stream networks become inaccessible during most of the rainy season and amphibian detectability is highly reduced. In each site, we surveyed 25 m linear transects (the total number of transects varied according to the accessibility of each study stream; Table D.6). We characterized each transect by measuring the following variables in its midpoint: stream width and depth (as a proxy of water volume in m^3 by multiplying width x depth x 25), flow speed (m/s), percent of canopy cover, air and water temperature (°C), percent of relative humidity, water pH, and elevation (m). We also characterized the amphibian community by counting all individuals that were visually or acoustically detected in all the transects (Table D.7). With data from our counts, we estimated 1) the beta diversity (H) based on the numbers equivalent of Shannon's diversity index using the diversity order q = 1, which considers the proportional abundance of each species in a community, without favoring either rare or abundant species (Norris & Pollock 1998; Chao et al. 2014) and 2) the community heterogeneity (J) using the Shannon evenness index (Krebs, 1989). We quantified collinearity among stream predictors as shown in section 4.3.1.2 and retained six stream predictors (elevation, water pH, volume, flow speed, percent of canopy cover, and J, Table D.6). Finally, we compared our study streams using a PCA as described in 4.3.2.6.1.

4.4 Results

4.4.1 Regional-level analyses

4.4.1.1 Land use and suitability for *Bd*

We found that old-growth and secondary forests comprised 77% of Mesoamerica during *Bd* epizootic times but decreased 23 000 km^2 (to cover 76% of Mesoamerica) during *Bd* enzootic times (Fig. 4.2). Land classified as urban development experienced the highest increase in area in proportional terms, with a total gain of 65% (approximately 20 000 km^2).

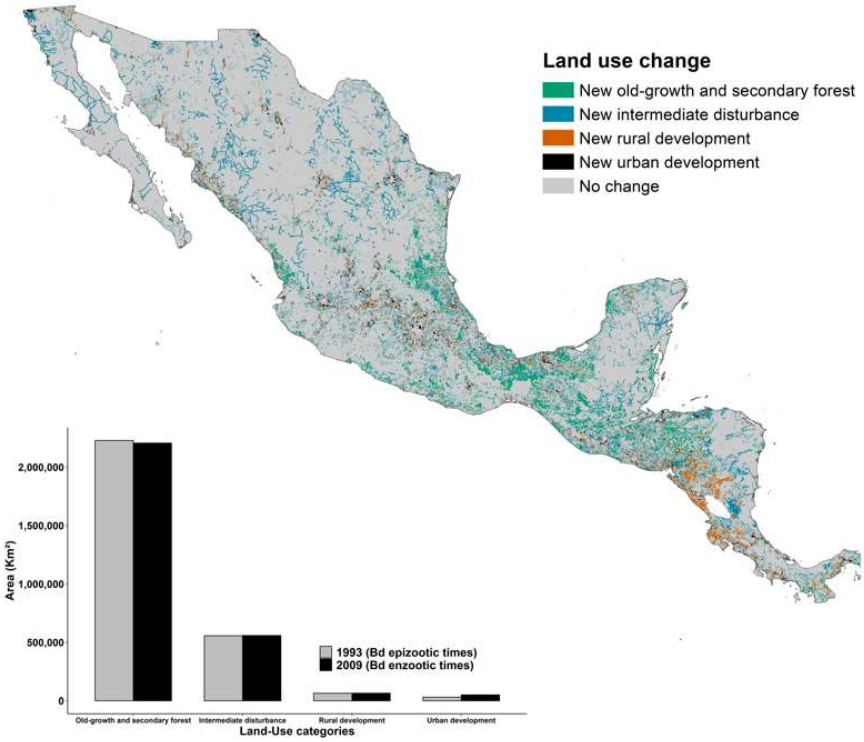

Figure 4.2. Map of Mesoamerica showing the land-use change between 1993 (during *Bd* epizootic times) and 2009 (during *Bd* enzootic times). The graph below the map shows that all disturbance categories increased their area to the detriment of old-growth and secondary forests, which decreased by 1% (23 000 km^2)

The regional suitability map for *Bd* in Mesoamerica derived from our most robust model (Table D.8) showed high suitability for *Bd* across the Sierra Madre Oriental and the Sierra Madre Occidental range in México, the Sierra de las Minas in Guatemala, and Talamanca range and Pacific and Caribbean lowlands of Costa Rica and Panamá (Fig. 4.3a). The model also predicted intermediate suitability across the lowlands of Nicaragua, Honduras, and southwestern México. The environmental predictors with the highest permutation were 'max temperature of warmest month' (74.6%), and 'precipitation of warmest quarter' (14.4%). The AOO of *Bd* in Mesoamerica during enzootic times was approximately 850 000 km^2 (41.6% of Mesoamerica; Fig. 4.3b). Furthermore, 72% of suitable area for *Bd* enzootic times occurs in old-growth and secondary forests and 23% in low disturbance habitats.

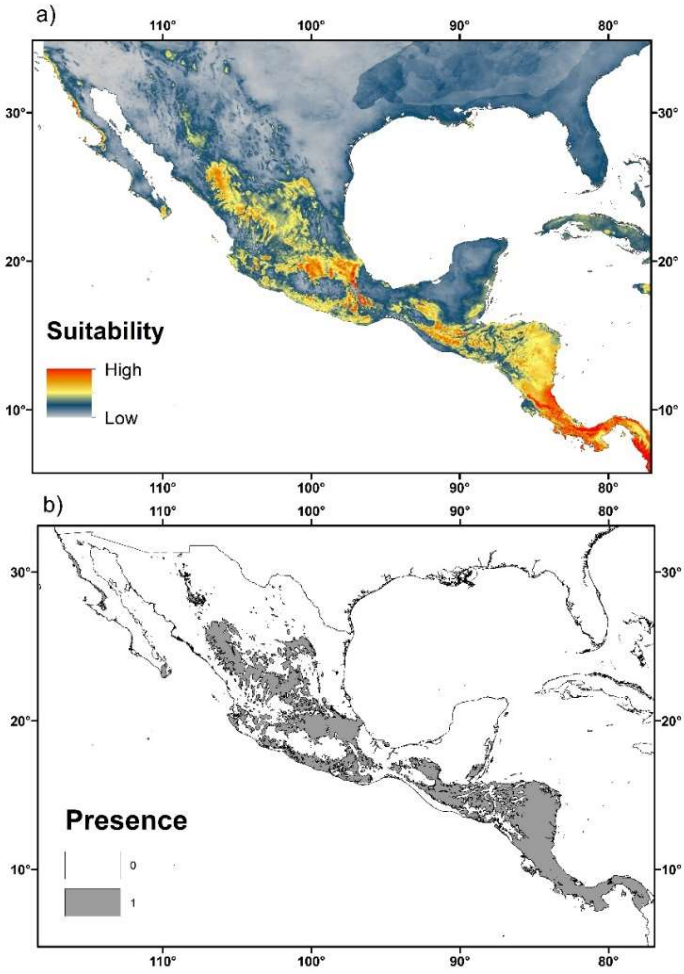

Figure 4.3. Predicted occurrence of *Batrachochytrium dendrobatidis* (*Bd*) in Mesoamerica; a) Suitability map showing gradients of suitability for *Bd* across Mesoamerica; b) the range polygon for *Bd* comprises 41.6% of the total area of Mesoamerica.

4.4.1.2 Threat risk

Overall, 46% of the species of robber frogs have ranges smaller than 1000 km^2, with 21% between 1000 and 5000 km^2, and 33% larger than 5000 km^2 (max. *C. vocalis* 170 000 km^2). The elevational range inhabited by a species is greater than 1000 m in only 41% of robber frog species, with only seven of those species exceeding 1500 m (max. *C. vocalis* 2200 m). Similar to the trend observed throughout Mesoamerica (Fig. 4.2), the percent of old-growth and secondary forest decreased for 26 species during *Bd* enzootic times. However, the percent of old-growth and secondary forest in each species range was at least 50% for 83% of species during *Bd* epizootic times. We found that environments with intermediate disturbance, which sometimes sustain populations of robber frogs that are more tolerant to habitat fragmentation (e.g., *C. amniscola, C. taurus*) experienced a decrease of about 2% during *Bd* enzootic times. On average, 80% of the

range of each species overlaps with the predicted occurrence of *Bd*. This overlap is greater than 90% in 31 species, 15 of which have been undetected since 2005 or before (Table 4.1).

In our first logistic model, we excluded the non-significant predictor with the highest p-value (habitat suitability for robber frogs; $p = 0.82$). With the same approach, we excluded A_{MCP} in a second model ($p = 0.48$), and the elevational range inhabited by a species in a third model ($p = 0.09$. Our most robust model (Table 4.2) fits significantly better than an empty model ($X^2 = 29.9$, df = 3, $p < 0.001$) and showed that undetectability significantly increases with *Bd* overlap and clade (subgenus *Campbellius* is more vulnerable).

Table 4.1. Range area and elevational distribution of 46 species of robber frogs in Mesoamerica. For each species, we present the area of minimum polygon convex (A_{MCP}) as a proxy of the total home range, the lower and upper elevation limits, and the percent of a species range that overlaps with 1) enzootic *Batrachochytrium dendrobatidis* (*Bd*), 2) old-growth and secondary forests (SH), 3) intermediate disturbance (ID), 4) rural development (RD), and 5) urban development (UD) during *Bd* epizootic times (Ep) and *Bd* enzootic times (En).

Species	A_{MCP} (km^2)	Elevation limits (m)	% of overlap								
			SH		ID		RD		UD		*Bd*
			Ep	En	Ep	En	Ep	En	Ep	En	En
Craugastor punctariolus species series											
Craugastor amniscola	6271.8	600-1000	60	62	37	32	2	3	1	4	28
*Craugastor anciano**	102	1400-1840	90	85	10	15	0	0	0	0	100
Craugastor angelicus	1517.6	656-1680	63	49	34	53	2	7	1	1	100
Craugastor aurilegulus	1801.6	50-1550	88	86	10	10	1	3	0	1	92
Craugastor azueroensis	1017.5	61-940	88	88	12	10	0	0	0	0	100
Craugastor berkenbuschii	66867.2	80-1900	54	67	41	30	4	3	1	2	40
Craugastor brocchi	8172.1	1200-2000	71	78	29	21	1	1	0	0	95
*Craugastor catalinae**	560.5	1219-1800	86	80	10	40	2	1	2	0	100
Craugastor charadra	3339.5	30-1370	79	69	19	26	2	2	0	0	83
Craugastor emleni	416.3	800-2000	65	65	34	32	1	2	0	1	100
Craugastor escoces	605.6	1100-2100	68	57	27	44	3	7	2	0	100
Craugastor evanesco	5692.7	80-709	89	85	11	15	0	0	0	0	100
Craugastor fleischmanni	2706.1	1050-2286	31	24	33	34	9	14	27	29	100
Craugastor inachus	794.4	500-1400	41	27	43	49	15	20	1	4	3
Craugastor laevissimus	38730.8	100-1700	64	71	33	25	2	2	1	2	74
*Craugastor merendonensis**	1.5	150-200	0	0	0	0	100	0	0	100	100
*Craugastor obesus**	3534	400-1450	94	91	6	8	0	0	0	0	100
*Craugastor olanchano**	641.4	1180-1350	94	95	6	5	0	0	0	0	100
Craugastor palenque	8664.7	300-350	77	93	23	7	0	0	0	0	3
Craugastor pechorum	5049.4	150-680	100	99	0	1	0	0	0	0	100
Craugastor pelorus	240.2	48-1700	80	84	15	11	5	3	0	2	79

Table 4.1. Continued

Craugastor pozo	13.8	760-1100	0	0	13	41	87	23	0	36	0
Craugastor psephosypharus	6368.6	150-1170	93	94	7	6	0	0	0	0	66
Craugastor punctariolus	3916.8	500-1000	88	81	12	19	0	0	0	0	100
Craugastor ranoides	54691.9	0-1300	70	59	25	32	2	5	2	3	97
*Craugastor rhyacobatrachus**	585.4	950-1800	77	61	21	37	1	2	1	0	100
Craugastor rivulus	4954.1	770-1250	74	70	25	29	1	1	0	0	93
Craugastor rugulosus	62351.1	200-2000	77	77	21	20	1	2	1	1	43
Craugastor rupinius	19504.8	400-1760	32	30	56	56	9	8	4	6	54
Craugastor sabrinus	7621.7	0-900	93	91	7	8	1	0	0	0	72
Craugastor sandersoni	7781.8	0-1160	89	88	10	12	1	0	0	0	48
Craugastor taurus	5958.1	25-525	64	54	31	37	5	8	0	1	100
Craugastor vocalis	167352.9	60-2150	86	84	13	14	1	1	1	1	26
Craugastor vulcani	1769.8	0-1200	42	89	58	11	0	0	0	0	41
Subgenus *Campbellius* (former *Craugastor milesi* group)											
*Craugastor adamastus**	5.8	600-650	100	100	0	0	0	0	0	0	100
*Craugastor chrysozetetes**	16.2	880-1130	100	100	0	0	0	0	0	0	100
*Craugastor cruzi**	14.72	1520	100	100	0	0	0	0	0	0	100
*Craugastor daryi**	499.2	1475-2290	18	52	79	48	3	1	0	0	100
*Craugastor epochthidius**	2250.3	150-1460	97	98	3	2	0	0	0	0	100
*Craugastor fecundus**	138.2	200-1260	99	93	1	8	0	0	0	0	100
Craugastor matudai	63.9	1500-2100	85	50	14	31	1	4	0	15	73
Craugastor milesi	94.3	1050-1841	87	74	14	24	0	2	0	0	100
*Craugastor myllomyllon**	20.4	875	26	78	75	22	0	0	0	0	100
*Craugastor omoaensis**	34.7	760-1150	74	41	26	58	0	1	0	0	99
*Craugastor saltuarius**	648.1	1550-1800	100	99	0	1	0	0	0	0	100
Craugastor stadelmani	818.8	1125-1900	100	97	1	3	0	0	0	0	93

**not seen in ≥ 15 years*

Table 4.2. Candidacy logistic regression models were used to identify the best predictors of the probability of extinction for 46 species of robber frog in Mesoamerica. The most robust model is shown in bold. Predictors: 1) the area of the minimum convex polygon (A_{MCP}, km^2), 2) the elevational range inhabited by a species (Elev; the difference in m between the upper elevation limit and the lower elevation limit), 3) the percent of old-growth and secondary forests during *Bd* epizootic times (SH), 4) the percent of overlap of *Bd* (*Bd*O), and 5) clade (*C. punctariolus* species series or subgenus *Campbellius*).

Model	AIC	Significant predictors <0.05	Excluded predictors
(Detection~ A_{MCP} + Elev+ SH + *Bd*O + Clade)	39.8	Clade	SH (p=0.84)
(Detection ~ A_{MCP} + Elev + *Bd*O + Clade)	37.8	Clade	A_{MCP} (p=0.56)
(Detection ~ Elev + *Bd*O + Clade)	**36.1**	**Clade, *Bd***	**Elev (p=0.09)**
(Detection ~ *Bd*O + Clade)	36.2	Clade	*Bd*O (p=0.06)

4.4.2 Local-level analyses

4.4.2.1 Change in the range and climatic niche

The historical and present suitability maps derived from our most robust models (Fig. D.3, Table D.8) showed that the historical AOO for the dry forest robber frog decreased from 2597.5 km^2 to 397.4 km^2 during *Bd* enzootic times (85% reduction of the historical range; Fig. D.3a and b), with small suitable patches throughout the tropical dry forest of the Santa Elena Peninsula (Fig. D.3c and d). For the Golfito robber frog, the AOO decreased from of 6199 km^2 to 328.2 km^2 during *Bd* enzootic times (95% reduction of the historical range; Fig. D.3a and b), with suitable habitat restricted to a semidry region that extends from Pavones in Costa Rica to Puerto Armuelles in Panamá (Fig. D.3c and d). The predictors with the highest permutation importance were 'precipitation of driest month' for the lowland robber frogs and 'mean monthly potential evapotranspiration of driest quarter' for the Golfito robber frog (Table D.4).

We also found a strong contraction in the climatic niche of both species of robber frogs after the spread of *Bd* during enzootic times. The climatic niche of the dry forest robber frog exhibited a reduction of 91% when compared with the historical climatic niche (Fig. 4.4a and b). *Bd* also experienced a niche expansion in the climatic range of the dry forest robber frog, increasing from 0 to 91% during *Bd* enzootic times. Moreover, the centroid of the climatic niche of the lowland robber frog changed its orientation towards environmental dry conditions, showing an overlap of 8.5% with *Bd* (Fig. 4.4c). This change in niche dynamics was different than expected by chance ($p < 0.001$). Similarly, the Golfito robber frog experienced a reduction of 83% in the climatic niche after the spread of *Bd* during enzootic times (Fig. 4.4d and 4e). This reduction coincided with an expansion of *Bd* from 0% to 92%. As observed for the dry forest robber frog, the centroid changed towards dry conditions, exhibiting a niche overlap of 7.5% with *Bd* (Fig. 4.4f). However, this change was not different than expected by chance ($p = 0.40$).

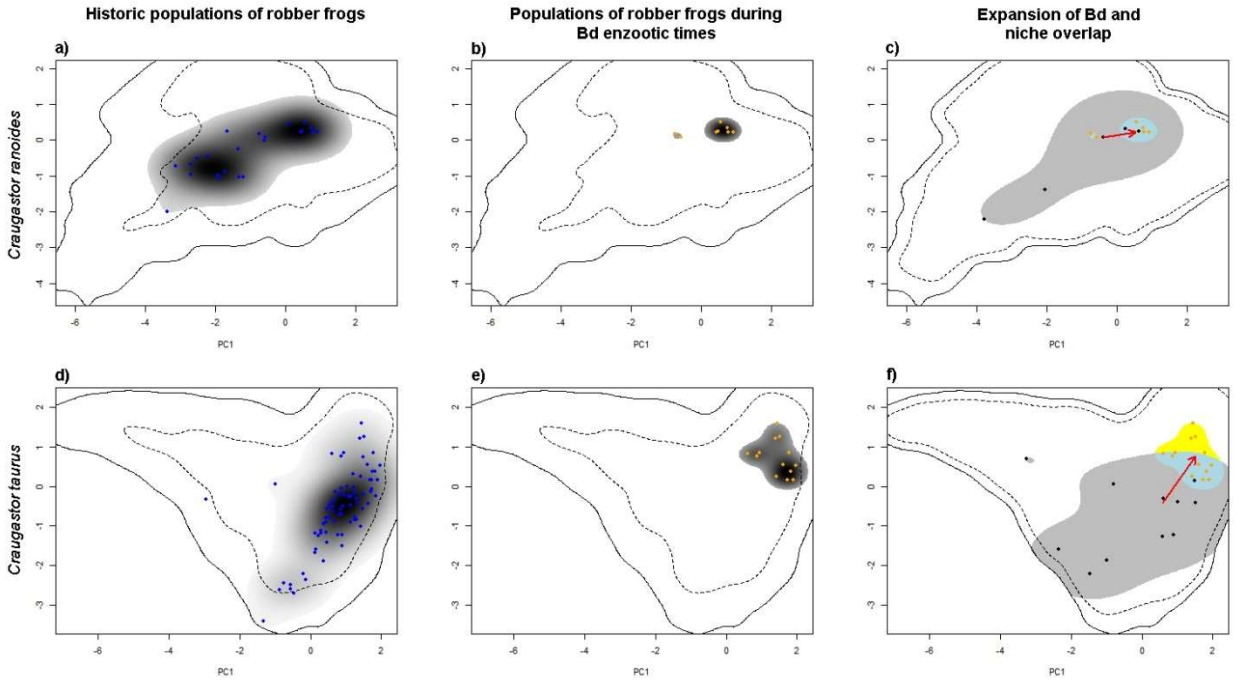

Figure 4.4. The climatic niche contraction of two species of robber frog matches the expansion of the pathogen *Batrachochytrium dendrobatidis* (*Bd*); a) climatic envelope generated from historic populations of the dry forest robber frog *Craugastor ranoides* across the Santa Elena Peninsula and Guanacaste Volcanic Range b) shows a climatic niche contraction of 91% during *Bd* enzootic times, which c) coincides with the expansion of *Bd*; Similarly, d) the climatic envelope generated from historic populations of the Golfito robber frog *C. taurus* e) shows a climatic niche contraction of 83% during *Bd* enzootic times and f) matches the expansion of *Bd*. For both species, the red arrows show the shift in position and direction of the centroid towards dry environmental conditions. Climatic niches of robber frogs are represented by black areas. Blue dots show historic populations and orange dots show remnant populations. Light blue represents niche overlap with *Bd* while yellow represents the climatic space where *Bd* does not occur.

4.4.2.2 Abiotic conditions

We retained the first three axes in our PCA for the local abiotic dataset in our study stream networks, which explained 85% of the total variance of our data (Fig. D.4). Our tridimensional representation of the PCA (Fig. 4.5a) showed six well-defined clusters of points, each one representing a study stream network. As expected, we found that the highest similarity in climatic conditions occurred among networks located in the same geographic region (Fig. D.5). The highest loading in the first PC was 'temperature annual range' (0.82), which correlated positively with all other predictors (Table D.9). In the second PC, the 'minimum temperature of the warmest month' and the 'mean monthly potential evapotranspiration of the driest quarter' had the highest loadings, 0.86 and 0.75 respectively and correlated negatively with the other predictors (Table D.9). These

results suggests that remnant populations in Santa Elena Peninsula and Punta Banco are associated with dry and hot climatic conditions (Fig. 4.5a).

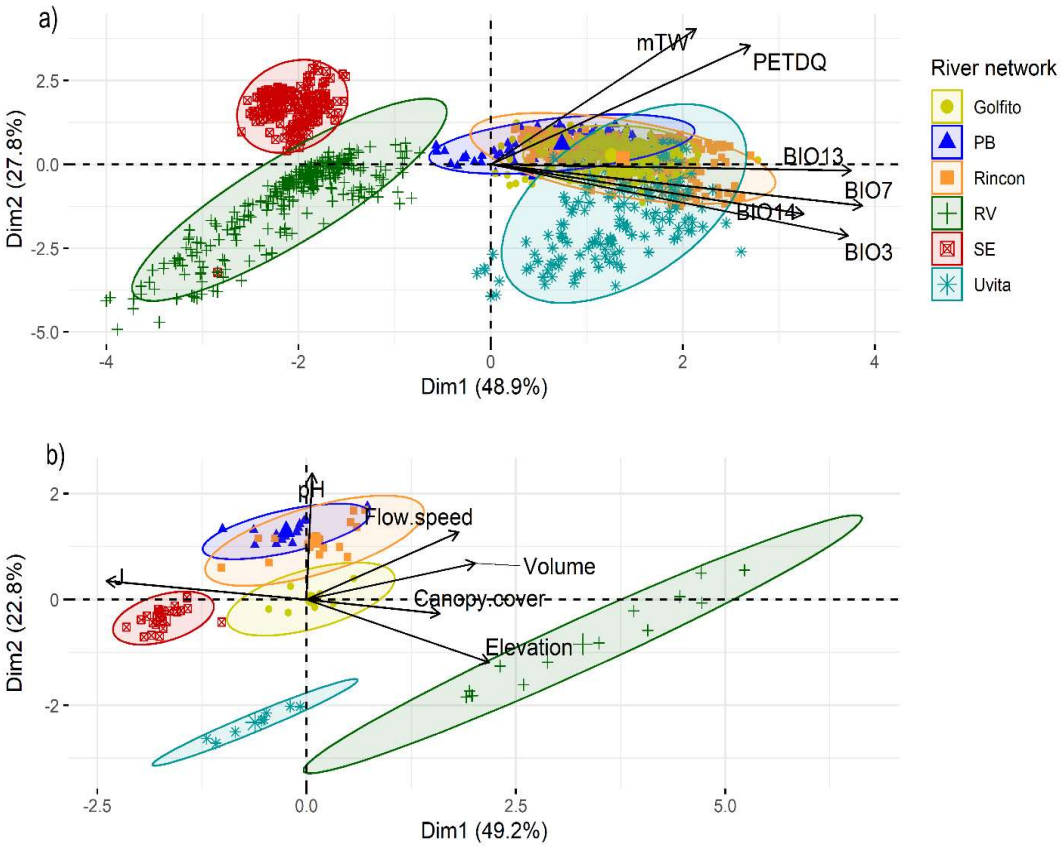

Figure 4.5. The Study stream networks: a) Climatic characterization of study locations using six predictors: 'isothermality' (BIO3), 'temperature annual range' (BIO7), 'precipitation of the wettest month' (BIO13), 'precipitation of driest month' (BIO14), 'minimum temperature of the warmest month' (mTW), and 'mean monthly potential evapotranspiration of driest quarter' (PETDQ); b) microhabitat characterization of study transects using six predictors: elevation, pH, community heterogeneity (J), volume, canopy cover, and flow speed. Localities: Golfito, Punta Banco (PB), Rincón de Osa (Rincon), Rincón de la Vieja Volcano (RV), Santa Elena Peninsula (SE) and Uvita.

4.4.2.3 Microhabitat

The highest beta diversity and community heterogeneity were observed in Santa Elena, Uvita, and Punta Banco (Table 4.3, D.6, and D.8). Two of these sites (Santa Elena and Punta Banco stream networks) sustain remnant populations of robber frogs. In our PCA of the six stream microhabitat predictors, we retained the first three axes, which accounted for 85% of the total variance of our data (Fig. D.6). The highest loading in the first PC was scored by community heterogeneity (-0.91), which correlated negatively with all other predictors (Table D.9). In the

second PC the highest loading was scored by water pH (0.91) and correlated negatively with elevation and canopy cover (Table D.9). These results suggests that remnant populations of robber frogs are associated with high community heterogeneity in low elevation, slow-flowing, low volume streams in Santa Elena Peninsula and water pH > 8 in Punta Banco (Fig. 4.5b; Fig. D.7).

Table 4.3. Amphibian abundance (N) and richness (S) found in linear transects in our six study stream networks: Santa Elena Peninsula (SE), Rincón de la Vieja Volcano (RV), Punta Banco (PB), Rincón de Osa, Golfito, and Uvita. For each site we estimated the community beta diversity (H; including standard error -SE-, lower -LCL- and upper confidence limits -UCL-), and community heterogeneity (J).

Estimator	Stream network					
	SE	RV	PB	Rincón de Osa	Golfito	Uvita
N	337	182	696	302	371	207
S	9	8	9	13	11	11
H (SE)	4.5 (0.2)	1.6 (0.2)	3.7 (0.2)	4.0 (0.3)	2.87 (0.2)	4.8 (.4)
LCL	4.5	1.6	3.7	4.0	2.9	4.8
UCL	4.9	2.0	4.0	4.5	3.3	5.7
% (J)	67.3	22.6	60.1	54.6	43.8	64.6

4.5 Discussion

Amphibian conservation is a global priority, especially after the accelerated global decline of amphibian populations during the last 40 years (Mendelson et al., 2019). Therefore, it is fundamental to measure the impact of anthropogenic threats across the landscape (Bosch et al., 2004; Cohen et al., 2016) to develop specific management plans for threatened species. We were especially interested in evaluating the threat risk and current detectability of 46 robber frog species. At the regional level (Mesoamerica), we found evidence that suggests that *Bd* was a primary cause of the decline of most robber frogs during *Bd* epizootic times. Our evidence also suggests that the species within the subgenus *Campbellius* are more vulnerable to extinction, which might be linked to their narrow geographic and elevational ranges (Lawton, 1993; Gaston, 1994; Gaston & Fuller, 2009; Scheele et al., 2019). At the local level, our results suggest that the spread of *Bd* during enzootic times caused contraction of the climatic niche of two species of robber frogs, and that variation in biotic and abiotic conditions within their original ranges have allowed remnant populations to coexist with this pathogen. Our findings suggest than using different spatial scales when conducting threat assessments could allow to obtain a more accurate picture of the threats and status of endangered taxa.

We found that the ranges of 81% of the species of the *C. punctariolus* species series and 91%

in the subgenus *Campbellius* contained at least 50% of old-growth and secondary forest cover during *Bd* epizootic times. In addition, we found that *Bd* is predicted to be widespread in the historical and present ranges of most robber frog species. These findings suggest that *Bd* was a primary driver of declines of robber frog populations rather than habitat deterioration. However, the increase of 65% of urban development in Mesoamerica between *Bd* epizootic and *Bd* enzootic times (approximately 20 000 km^2, Fig. 4.2) might also have strongly affected populations that historically inhabited areas close to human settlements (Campbell & Savage, 2000; McCranie & Wilson, 2002). Our findings also suggest that interactions between temperature and precipitation, especially during the warmest periods explain the present range of *Bd* in Mesoamerica, such that not all species and populations are equally likely to be affected by this pathogen.

Our analyses at the local level allowed us to assess the persistence of two species of robber frogs using methods that are less accurate at a larger scale. We found that the spread of *Bd* during enzootic times coincides spatially and temporarily with the climatic niche contraction the extinction of most populations of the dry forest robber frog and the Golfito robber frog across their historical ranges (see Scheele et al., 2017a). Our SDMs and niche climatic comparisons suggest that seasonal dry and semidry conditions have more impact on the present range of both species of robber frogs. This finding coincides with a similar study that found a strong niche contraction in the species *C. ranoides* across all its historic range as a consequence of chytridiomycosis (Granados-Martínez et al., 2021). Our findings also suggest that high community heterogeneity (e.g., Schmidt & Ostfeld 2001; Searle et al., 2011) and abiotic conditions outside of the optimal range for *Bd* transmission (e.g., pH 6–8; Piotrowski et al., 2004) could be suppressing pathogen transmission in locations where remnant populations exist in Santa Elena and Punta Banco stream networks. Therefore, follow-up field and laboratory studies are needed to assess if these environmental factors alone or in combination with other factors are constraining the spread of *Bd* in environmental refugia from decline.

4.5.1 Conservation implications

Here, we report the rediscovery of a remnant population of *C. amniscola* at the state park of La Pera, in Chiapas, México (Fig. D.8). The recent rediscoveries of several robber frogs (Appendix E) are a beacon of hope that other species are surviving, perhaps in key environmental refugia (Puschendorf et al., 2011). Results of this work can be used to make field inventories more

effective by identifying priority areas for locating remnant, or even undiscovered, populations of robber frogs (Appendix E). However, because a remnant population cannot be assumed to represent a recovering population (Mendelson et al., 2019), a long-term commitment to monitoring and conservation interventions may be required to ensure persistence. For example, management actions that create or preserve habitat refugia from chytridiomycosis and target other threatening processes such as habitat loss, have great potential for supporting species' recovery (Skerratt et al., 2016). This will become increasingly important as future shifts in environmental conditions or other emerging threats (Scheele et al., 2017a) may trigger a re-emergence of *Bd*, resulting in further declines or extinctions (Scheele et al., 2017b). Furthermore, this study can be used as a reference for the evaluation of other direct-developing, stream-dwelling species, or even other Neotropical amphibian groups that were also decimated by *Bd* (e.g., *Atelopus* spp., *Isthmohyla* spp.).

Studies that assess threat risk across different spatial scales can be used to develop more effective conservation strategies and targets. Our fine-resolution analyses proved to be effective for assessing threats within a species' range, especially those species that have relatively small ranges (Schwartz, 1999; Wiens, 1989). The results of this work can be integrated with important conservation tools, such as the IUCN Red List, which serve as valuable inputs into conservation priority setting and decision-making. Considering the key role that the IUCN Red List plays at the global and regional levels, it is imperative that the extinction risk assessments and their underlying data are updated regularly. The expanded understanding of disease-related declines in robber frogs presented in this study can be used to improve the quality of data in IUCN Red List assessments for these species, many of which have been undetected for long periods of time and for which data are scarce.

4.6 References

Aiello-Lammens, M. E., Boria, R. A., Radosavljevic, A., Vilela, B., & Anderson, R. P. (2015). spThin: An R package for spatial thinning of species occurrence records for use in ecological niche models. *Ecography*, *38*(5), 541–545.

AmphibiaWeb. (2020). *AmphibiaWeb*. https://amphibiaweb.org

Becker, C. G., Fonseca, C. R., Haddad, C. F. B., Batista, R. F., & Prado, P. I. (2007). Habitat split and the global decline of amphibians. *Science*, *318*(5857), 1775–1777.

Bland, L. M., Keith, D. A., Miller, R. M., Murray, N. J., & Rodríguez, J. P. (Eds.). (2017). *Guidelines for the application of IUCN Red List of ecosystems categories and criteria, version 1.1*. IUCN International Union for Conservation of Nature.

Bolom-Huet, R., Pineda, E., Díaz-Fleischer, F., Muñoz-Alonso, A. L., & Galindo-González, J. (2019). Known and estimated distribution in Mexico of *Batrachochytrium dendrobatidis*, a pathogenic fungus of amphibians. *Biotropica*, *51*(5), 731–746.

Booth, T. H., Nix, H. A., Busby, J. R., & Hutchinson, M. F. (2014). BIOCLIM: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. *Diversity and Distributions*, *20*(1), 1–9.

Bosch, J., Boyero, L., & Martínez-Solano, Í. (2004). Spatial scales for the management of amphibian populations. *Biodiversity & Conservation*, *13*(2), 409–420.

Brem, F., & Lips, K. (2008). *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Diseases of Aquatic Organisms*, *81*, 189–202.

Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences*, *107*(21), 9695–9700.

Broennimann, O., Treier, U. A., Müller-Schärer, H., Thuiller, W., Peterson, A. T., & Guisan, A. (2007). Evidence of climatic niche shift during biological invasion. *Ecology Letters*, *10*(8), 701–709.

Broennimann, O, Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M.-J., Randin, C., Zimmermann, N. E., Graham, C. H., & Guisan, A. (2012). Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography*, *21*(4), 481–497.

Brook, B. W., Sodhi, N. S., & Bradshaw, C. J. A. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, *23*(8), 453–460.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods & Research*, *33*(2), 261–304.

Campbell, J. A., & Savage, J. M. (2000). Taxonomic reconsideration of Middle American frogs of the *Eleutherodactylus rugulosus* group (Anura: Leptodactylidae): A reconnaissance of subtle nuances among frogs. *Herpetological Monographs*, *14*, 186–292.

Cardillo, M. (2021). Clarifying the relationship between body size and extinction risk in amphibians by complete mapping of model space. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1944), 20203011.

Catenazzi, A., Lehr, E., Rodriguez, L. O., & Vredenburg, V. T. (2011). *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. *Conservation Biology*, *25*(2), 382–391.

Catenazzi, A., (2015). State of the World's Amphibians. *Annual Review of Environment and Resources*, *40*(1), 91–119.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecological Monographs*, *84*(1), 45–67.

Chase, J. M., McGill, B. J., Thompson, P. L., Antão, L. H., Bates, A. E., Blowes, S. A., Dornelas, M., Gonzalez, A., Magurran, A. E., Supp, S. R., Winter, M., Bjorkman, A. D., Bruelheide, H., Byrnes, J. E. K., Cabral, J. S., Elahi, R., Gomez, C., Guzman, H. M., Isbell, F., … O'Connor, M. (2019). Species richness change across spatial scales. *Oikos*, *128*(8), 1079–1091.

Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V. T., & Ryan, M. J. (2014). Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Tropical Conservation Science*, *7*(4), 628–638.

Cheng, T. L., Rovito, S. M., Wake, D. B., & Vredenburg, V. T. (2011). Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences*, *108*(23), 9502–9507.

Christie, M. R., & Searle, C. L. (2018). Evolutionary rescue in a host–pathogen system results in coexistence not clearance. *Evolutionary Applications*, *11*(5), 681–693.

Cohen, J. M., Civitello, D. J., Brace, A. J., Feichtinger, E. M., Ortega, C. N., Richardson, J. C., Sauer, E. L., Liu, X., & Rohr, J. R. (2016). Spatial scale modulates the strength of ecological processes driving disease distributions. *Proceedings of the National Academy of Sciences*, *113*(24), E3359.

de Vries, A., & Ripley, B. D. (2013). *Ggdendro: Tools for extracting dendrogram and tree diagram plot data for use with ggplot. R package version 0.1–14*. http://CRAN. R-project. org/package= ggdendro

Di Cola, V., Broennimann, O., Petitpierre, B., Breiner, F. T., D'Amen, M., Randin, C., Engler, R., Pottier, J., Pio, D., Dubuis, A., Pellissier, L., Mateo, R. G., Hordijk, W., Salamin, N., & Guisan, A. (2017). Ecospat: An R package to support spatial analyses and modeling of species niches and distributions. *Ecography*, *40*(6), 774–787.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315.

Flemons, P., Guralnick, R., Krieger, J., Ranipeta, A., & Neufeld, D. (2007). A web-based GIS tool for exploring the world's biodiversity: The Global Biodiversity Information Facility Mapping and Analysis Portal Application (GBIF-MAPA). *Ecological Informatics*, *2*(1), 49–60.

Frías-Alvarez, P., Zúñiga-Vega, J. J., & Flores-Villela, O. (2010). A general assessment of the conservation status and decline trends of Mexican amphibians. *Biodiversity and Conservation*, *19*(13), 3699–3742.

Frost, D. R. (2021). *Amphibian Species of the World: An Online Reference. Version 6.1* [Data set]. American Museum of Natural History. https://amphibiansoftheworld.amnh.org/index.php

Gaston, K. J. (1994). Geographic range sizes and trajectories to extinction. *Biodiversity Letters*, *2*(6), 163–170.

Gaston, K. J., & Fuller, R. A. (2009). The sizes of species' geographic ranges. *Journal of Applied Ecology*, *46*(1), 1–9.

Gelman, A., Jakulin, A., Pittau, M. G., & Su, Y.-S. (2008). A weakly informative default prior distribution for logistic and other regression models. *Ann. Appl. Stat.*, *2*(4), 1360–1383.

Gelman, A., Su, Y.-S., Yajima, M., Hill, J., Pittau, M. G., Kerman, J., Zheng, T., & Dorie, V. (2016). *"Package 'arm'." data analysis using regression and multilevel/hierarchical models. R package, version 9.* (1.11-2) [Computer software]. https://CRAN.R-project.org/package=arm

Granados-Martínez, S., Zumbado-Ulate, H., Searle, C. L., Oliveira, B. F., & García-Rodríguez, A. (2021). Niche contraction of an endangered frog species driven by the amphibian chytrid fungus. *EcoHealth*.

Grant, E. H. C., Miller, D. A. W., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., Cruickshank, S. S., Fisher, R. N., Green, D. M., Hossack, B. R., Johnson, P. T. J., Joseph, M. B., Rittenhouse, T. A. G., Ryan, M. E., Waddle, J. H., Walls, S. C., Bailey, L. L., Fellers, G. M., Gorman, T. A., … Muths, E. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports*, *6*, 25625.

Greenville, A. C., Newsome, T. M., Wardle, G. M., Dickman, C. R., Ripple, W. J., & Murray, B. R. (2021). Simultaneously operating threats cannot predict extinction risk. *Conservation Letters*, *14*(1), e12758.

Hedges, S. B., Duellman, W. E., & Heinicke, M. P. (2008). New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa*, *1737*(1), 1–182.

Hijmans, R. J., Phillips, S., Leathwick, J., & Elith, J. (2013). Dismo: Species distribution modeling. *R Package Version 0.8-17. Http://Cran. r-Project. Org>*. https://cran.r-project.org/web/packages/dismo/index.html

Hijmans, R. J., Van Etten, J., Cheng, J., Mattiuzzi, M., Sumner, M., Greenberg, J. A., Lamigueiro, O. P., Bevan, A., Racine, E. B., & Shortridge, A. (2015). Package 'raster.' *R Package*.

Hof, C., Araújo, M. B., Jetz, W., & Rahbek, C. (2011). Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature*, *480*(7378), 516–519.

Hosmer, D. W., Lemeshow, S., & Sturdivant, R. X. (2013). *Applied logistic regression* (3rd ed., Vol. 398). John Wiley & Sons.

Isaac, N. J. B., Redding, D. W., Meredith, H. M., & Safi, K. (2012). Phylogenetically-informed priorities for amphibian conservation. *PLOS ONE*, *7*(8), e43912.

Isbell, F., Gonzalez, A., Loreau, M., Cowles, J., Díaz, S., Hector, A., Mace, G. M., Wardle, D. A., O'Connor, M. I., Duffy, J. E., Turnbull, L. A., Thompson, P. L., & Larigauderie, A. (2017). Linking the influence and dependence of people on biodiversity across scales. *Nature*, *546*(7656), 65–72.

IUCN. (2019). *The IUCN Red List of Threatened Species. Version 2019-1.* IUCN Red List of Threatened Species. https://www.iucnredlist.org/en

Johnson, E. E., Escobar, L. E., & Zambrana-Torrelio, C. (2019). An ecological framework for modeling the geography of disease transmission. *Trends in Ecology & Evolution*, *34*(7), 655–668.

Johnson, J. D., Mata-Silva, V., & Wilson, L. D. (2015). A conservation reassessment of the Central American herpetofauna based on the EVS measure. *Amphibian & Reptile Conservation*, *9*(2), 1–94.

Kassambara, A., & Mundt, F. (2017). Package 'factoextra'. Extract and visualize the results of multivariate data analyses, 76.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., Mitchell, C. E., Myers, S. S., Bogich, T., & Ostfeld, R. S. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, *468*(7324), 647–652.

Kiesecker, J. M., Blaustein, A. R., & Belden, L. K. (2001). Complex causes of amphibian population declines. *Nature*, *410*(6829), 681–684.

Kilburn, V. L., Ibáñez, R., Sanjur, O., Bermingham, E., Suraci, J. P., & Green, D. M. (2010). Ubiquity of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in anuran communities in Panamá. *EcoHealth*, *7*(4), 537–548.

Kilpatrick, A. M., Briggs, C. J., & Daszak, P. (2010). The ecology and impact of chytridiomycosis: An emerging disease of amphibians. *Trends in Ecology & Evolution*, *25*(2), 109–118.

Kolby, J. E., & McCranie, J. R. (2009). Discovery of a surviving population of the montane streamside frog *Craugastor milesi* (Schmidt). *Herpetological Review*, *40*(3), 282.

Krebs, C. J. (1989). *Ecological methodology*. Harper & Row.

Lawton, J. H. (1993). Range, population abundance and conservation. *Trends in Ecology & Evolution*, *8*(11), 409–413.

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25*(1), 1–18.

Lips, K. R., Burrowes, P. A., Mendelson, J. R., & Parra-Olea, G. (2005). Amphibian declines in Latin America: Widespread population declines, extinctions, and impacts. *Biotropica*, *37*(2), 163–165.

Lips, K. R., Diffendorfer, J., Mendelson, J. R., & Sears, M. W. (2008). Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *PLOS Biology*, *6*(3), e72.

Lips, K. R., Reeve, J. D., & Witters, L. R. (2003). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology*, *17*(4), 1078–1088.

Liu, C., Berry, P. M., Dawson, T. P., & Pearson, R. G. (2005). Selecting thresholds of occurrence in the prediction of species distributions. *Ecography*, *28*(3), 385–393.

Longcore, J. E., Pessier, A. P., & Nichols, D. K. (1999). *Batrachochytrium dendrobatidis* gen. Et sp. Nov., a chytrid pathogenic to amphibians. *Mycologia*, *91*, 219–227.

Mayani-Parás, F., Botello, F., Castañeda, S., & Sánchez-Cordero, V. (2019). Impact of habitat loss and mining on the distribution of endemic species of amphibians and reptiles in Mexico. *Diversity*, *11*(11), 210.

McCranie, J. R., & Wilson, L. D. (2002). *The Amphibians of Honduras*. Society for the Study of Amphibians and Reptiles.

Mendelson, J. R., Whitfield, S. M., & Sredl, M. J. (2019). A recovery engine strategy for amphibian conservation in the context of disease. *Biological Conservation*, *236*, 188–191.

Monastersky, R. (2014). Life-a status report. *Nature*, *516*(7530), 158.

Muscarella, R., Galante, P. J., Soley-Guardia, M., Boria, R. A., Kass, J. M., Uriarte, M., & Anderson, R. P. (2014). ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. *Methods in Ecology and Evolution*, *5*(11), 1198–1205.

Naimi, B. (2015). Usdm: Uncertainty analysis for species distribution models. R package version *1.1-15*.

Norris, J. L., & Pollock, K. H. (1998). Non-parametric MLE for Poisson species abundance models allowing for heterogeneity between species. *Environmental and Ecological Statistics*, *5*(4), 391–402.

Ochoa-Ochoa, L. M., & Whittaker, R. J. (2014). Spatial and temporal variation in amphibian metacommunity structure in Chiapas, Mexico. *Journal of Tropical Ecology*, *30*(6), 537–549.

Ortiz-Malavasi, E. (2014). *Atlas Digital Costa Rica*. https://repositoriotec.tec.ac.cr/handle/2238/6749

Petitpierre, B., Kueffer, C., Broennimann, O., Randin, C., Daehler, C., & Guisan, A. (2012). Climatic niche shifts are rare among terrestrial plant invaders. *Science*, *335*(6074), 1344.

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3), 231–259.

Piotrowski, J. S., Annis, S. L., & Longcore, J. E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, *96*(1), 9–15.

Pounds, A. J., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sánchez-Azofeifa, G. A., Still, C. J., & Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, *439*(7073), 161–167.

Puschendorf, R., Bolaños, F., & Chaves, G. (2006). The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biological Conservation*, *132*(1), 136–142.

Puschendorf, R., Carnaval, A. C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., & Alford, R. A. (2009). Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: Proposing climatic refuges as a conservation tool. *Diversity and Distributions*, *15*(3), 401–408.

Puschendorf, R., Hoskin, C. J., Cashins, S. D., McDonald, K., Skerratt, L. F., Vanderwal, J., & Alford, R. A. (2011). Environmental refuge from disease-driven amphibian extinction. *Conservation Biology*, *25*(5), 956–964.

Puschendorf, R., Wallace, M., Chavarría, M. M., Crawford, A. J., Wynne, F., Knight, M., Janzen, D. H., Hallwachs, W., Palmer, C. V., & Price, S. J. (2019). Cryptic diversity and ranavirus infection of a critically endangered Neotropical frog before and after population collapse. *Animal Conservation*, *22*(5), 515–524.

Radosavljevic, A., & Anderson, R. P. (2014). Making better MAXENT models of species distributions: Complexity, overfitting and evaluation. *Journal of Biogeography*, *41*(4), 629–643.

Rollins-Smith, L. A. (2017). Amphibian immunity–stress, disease, and climate change. *Developmental and Comparative Immunology*, *66*, 111–119.

Sanderson, E. W., Jaiteh, M., Levy, M. A., Redford, K. H., Wannebo, A. V., & Woolmer, G. (2002). The Human Footprint and the Last of the Wild: The human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. *BioScience*, *52*(10), 891–904.

Savage, J. M. (2002). *The Amphibians and Reptiles of Costa Rica: A Herpetofauna Between Two Continents, Between Two Seas*. The University of Chicago Press.

Scheele, B. C., Foster, C. N., Banks, S. C., & Lindenmayer, D. B. (2017a). Niche contractions in declining species: Mechanisms and consequences. *Trends in Ecology & Evolution*, *32*(5), 346–355.

Scheele, B. C., Skerratt, L. F., Grogan, L. F., Hunter, D. A., Clemann, N., McFadden, M., Newell, D., Hoskin, C. J., Gillespie, G. R., Heard, G. W., Brannelly, L., Roberts, A. A., & Berger, L. (2017b). After the epidemic: Ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis. *Biological Conservation*, *206*, 37–46.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., Catenazzi, A., De la Riva, I., Fisher, M. C., Flechas, S. V., Foster, C. N., Frías-Álvarez, P., Garner, T. W. J., Gratwicke, B., Guayasamin, J. M.,

Hirschfeld, M., … Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, *363*(6434), 1459–1463.

Schmidt, K. A., & Ostfeld, R. S. (2001). Biodiversity and the dilution effect in disease ecology. *Ecology*, *82*(3), 609–619.

Schneider, D. C. (2001). The rise of the concept of scale in ecology: The concept of scale is evolving from verbal expression to quantitative expression. *BioScience*, *51*(7), 545–553.

Schoener, T. W. (1968). The *Anolis* lizards of Bimini: Resource partitioning in a complex fauna. *Ecology*, *49*(4), 704–726.

Schwartz, M. W. (1999). Choosing the appropriate scale of reserves for conservation. *Annual Review of Ecology and Systematics*, *30*(1), 83–108.

Searle, C. L., Biga, L. M., Spatafora, J. W., & Blaustein, A. R. (2011). A dilution effect in the emerging amphibian pathogen *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences*, *108*(39), 16322–16326.

Semlitsch, R. D., & Bodie, J. R. (2003). Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology*, *17*(5), 1219–1228.

Skerratt, L. F., Berger, L., Clemann, N., Hunter, D. A., Marantelli, G., Newell, D. A., Philips, A., McFadden, M., Hines, H. B., Scheele, B. C., Brannelly, L. A., Speare, R., Versteegen, S., Cashins, S. D., & West, M. (2016). Priorities for management of chytridiomycosis in Australia: Saving frogs from extinction. *Wildlife Research*, *43*(2), 105–120.

Title, P. O., & Bemmels, J. B. (2018). ENVIREM: an expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography*, *41*(2), 291–307.

Venter, O., Sanderson, E. W., Magrach, A., Allan, J. R., Beher, J., Jones, K. R., Possingham, H. P., Laurance, W. F., Wood, P., Fekete, B. M., Levy, M. A., & Watson, J. E. (2016). Global terrestrial human footprint maps for 1993 and 2009. *Scientific Data*, 160067.

Warren, D. L., Glor, R. E., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution*, *62*(11), 2868–2883.

Whitfield, S. M., Alvarado, G., Abarca, J., Zumbado-Ulate, H., Zuñiga, I., Wainwright, M., & Kerby, J. (2017). Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms*, *126*(1), 33–41.

Whitfield, S. M., Lips, K. R., & Donnelly, M. A. (2016). Amphibian decline and conservation in Central America. *Copeia*, *104*(2), 351–379.

Wiens, J. A. (1989). Spatial scaling in ecology. *Functional Ecology*, *3*(4), 385–397. JSTOR.

Wilson, L. D., & Johnson, J. D. (2010). Distributional patterns of the herpetofauna of Mesoamerica, a biodiversity hotspot. *Conservation of Mesoamerican Amphibians and Reptiles*, *30*, 235.

Wilson, L. D., Johnson, J. D., & Mata-Silva, V. (2013). A conservation reassessment of the amphibians of Mexico based on the EVS measure. *Amphibian & Reptile Conservation*, *7*(1), 97–127.

Wilson, L. David, & McCranie, J. R. (2004). The conservation status of the herpetofauna of Honduras. *Amphibian & Reptile Conservation*, *3*(1), 6–33. PubMed.

Woodhams, D. C., Kilburn, V. L., Reinert, L. K., Voyles, J., Medina, D., Ibáñez, R., Hyatt, A. D., Boyle, D. G., Pask, J. D., Green, D. M., & Rollins-Smith, L. A. (2008). Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. *EcoHealth*, *5*(3), 268–274.

Zumbado-Ulate, H, Bolaños, F., Gutiérrez-Espeleta, G., & Puschendorf, R. (2014). Extremely low prevalence of *Batrachochytrium dendrobatidis* in frog populations from Neotropical dry forest of Costa Rica supports the existence of a climatic refuge from disease. *EcoHealth*, *11*(4), 593–602.

Zumbado-Ulate, H., García-Rodríguez, A., & Searle, C. L. (2020). Species distribution models predict the geographic expansion of an enzootic amphibian pathogen. *Biotropica*, *53*(1), 221–321.

Zumbado-Ulate, H, García-Rodríguez, A., Vredenburg, V. T., & Searle, C. L. (2019). Infection with *Batrachochytrium dendrobatidis* is common in tropical lowland habitats: Implications for amphibian conservation. *Ecology and Evolution*, *9*(8), 4917–4930.

Zumbado-Ulate, H, Nelson, K. N., García-Rodríguez, A., Chaves, G., Arias, E., Bolaños, F., Whitfield, S. M., & Searle, C. L. (2019). Endemic infection of *Batrachochytrium dendrobatidis* in Costa Rica: Implications for amphibian conservation at regional and species level. *Diversity*, *11*(8), 129.

Zumbado-Ulate, H., & Willink, B. (2011). *Craugastor ranoides* (NCN) geographic distribution. *Herpetological Review*, *42*, 236.

APPENDIX A. CHAPTER 1 SUPPLEMENTARY MATERIALS

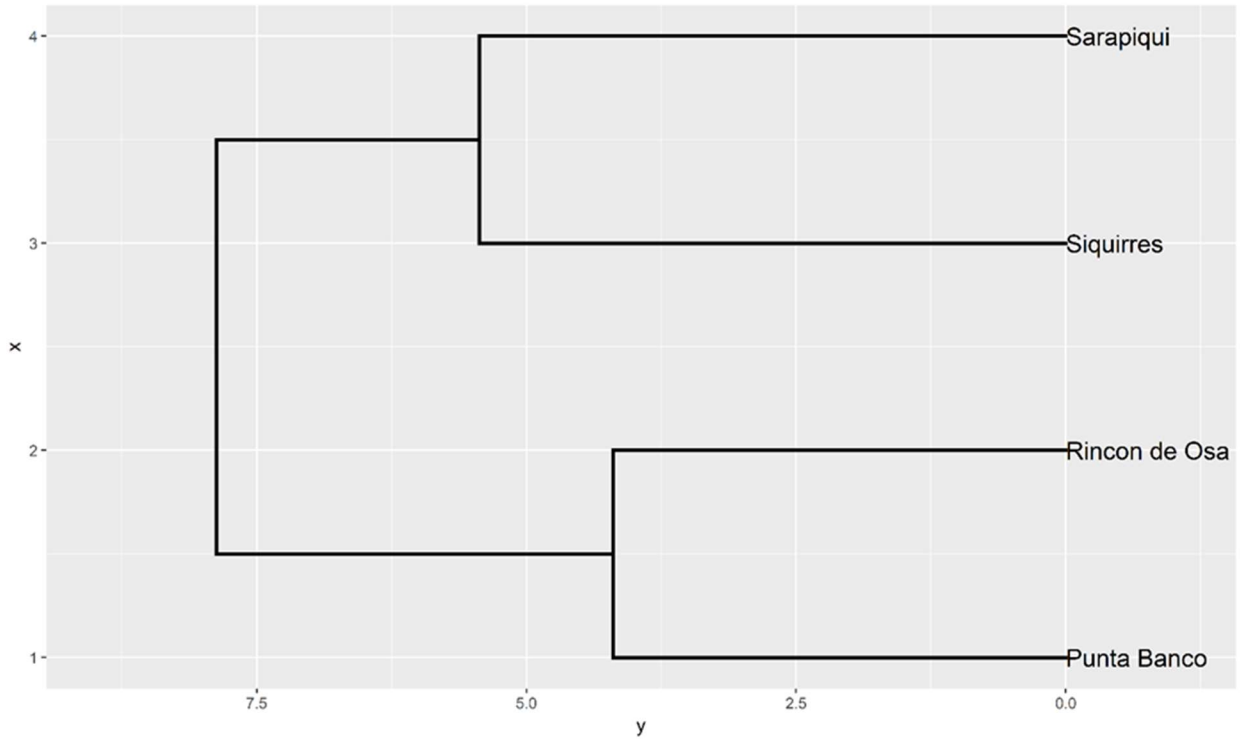

Figure A.1. Cluster analysis of four lowland sites in Costa Rica generated from a matrix of Euclidean distances between the centroids of climatic envelopes. Environmental values were extracted from the 19 bioclimatic variables of the WorldClim dataset. The cluster shows higher similarities between Sarapiquí and Siquirres (Caribbean side) and Rincón de Osa-Punta Banco (Pacific side).

APPENDIX B. CHAPTER 2 SUPPLEMENTARY MATERIALS

Field dataset (Fig. B.1-B.2, Table B.1-B.2): We surveyed nine amphibian assemblages across Costa Rica in both versants (Caribbean and Pacific) and at elevations ranging from sea level to 1385 m (Fig. B.1). All surveys were conducted during the months of June and July between 2016-2018, except the locality of Alto Lari, which was sampled in March 2015. In total, we screened for *Bd* from 267 amphibians from 33 species. A total of 19 (58%) of the sampled species tested positive for *Bd* (Table B.1 and B.2, Fig. B.2). The overall infection rate in the field dataset was 39%, however this value showed high heterogeneity among sites, with values ranging from 0% in Punta Bunco-Burica to 60% in Santa Elena Peninsula (Table B.2, Fig. B.2). In addition, we did not detect signs of disease in any infected individuals during our study and quantified low levels of infection in most of our samples (Table B.1).

Species assessment (Table B.3-B.4): We updated the last official list of amphibian species in Costa Rica published in 2011 [1] by consulting the the Museo de Zoología at Universidad de Costa Rica (http://museo.biologia.ucr.ac.cr/) and taxonomists' lists [2,3]. We also compiled a list of all the species that have been screened for *Bd* and the methods used for detection (histology or PCR). Our new list of amphibians in Costa Rica includes a total of 215 species grouped in three orders, 16 families, and 48 genera (Table B.1). This represents and addition of 20 species (ten anurans, nine salamanders, and one caecilian). In our review, we listed a total of 105 amphibian species (49%) that have been screened for *Bd* in Costa Rica (103 anurans and only two species of salamanders) (Table B.2). In the field, the most common method used to detect *Bd* was qPCR, especially after 2005. Overall, *Bd* prevalence in Costa Rica was estimated to be 0.23 (60% of species tested positive for *Bd*) (Table B.3). The most robust GLM found both herpetological province and the FRHI as significant predictors of *Bd* prevalence (AIC=1700, $P<0.001$, Table B.4).

Combined dataset (Table B.5-B.6): We generated a dataset of amphibian assemblages from multiple studies that screened for *Bd* in Costa Rica after 2000 using conventional PCR and qPCR methods [4–10] (including the 267 individuals from 33 species we tested in the "field dataset". In total, this "combined dataset" consisted of 1750 individual records from 79 species and 20 localities at elevations ranging from sea level to 2000. In our analyses, we found an effect of the FRHI ($F_{8,342}=7.91$, $P<0.01$, Table B.6).

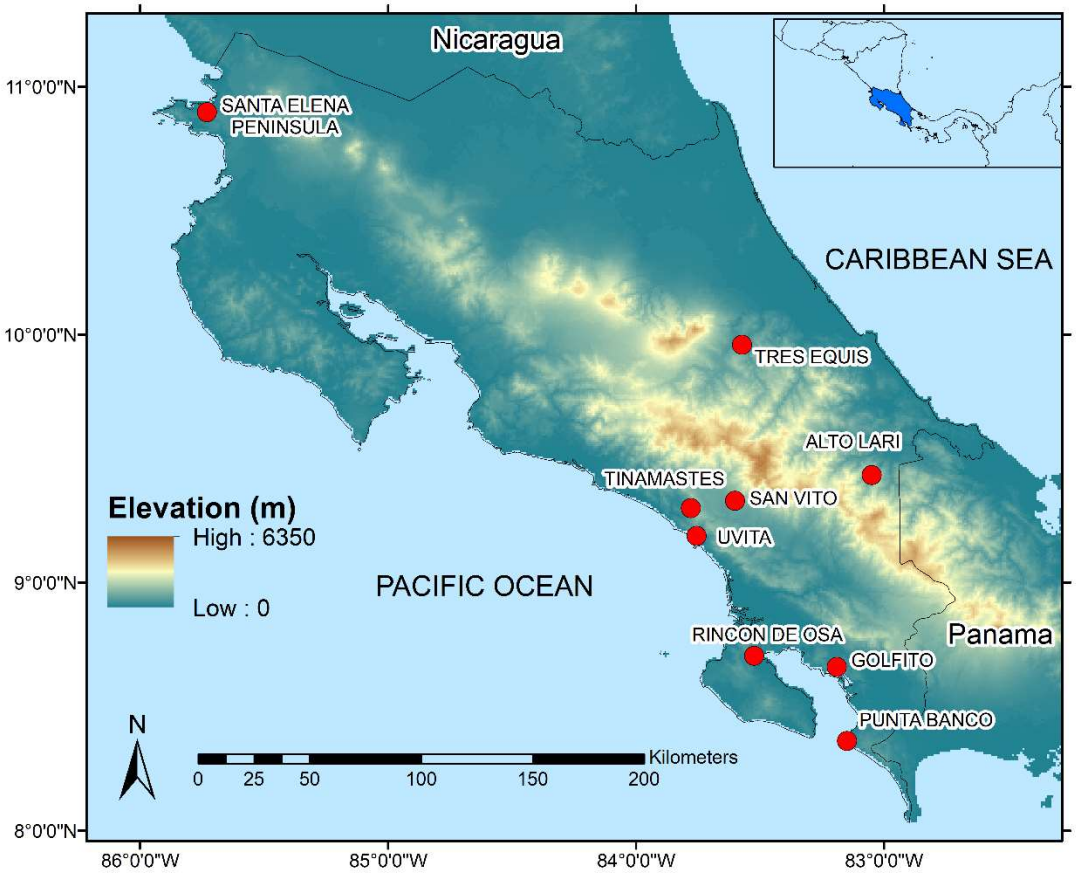

Figure B.1. A) Map of Costa Rica showing elevational gradient and nine study sites surveyed for *Batrachochytrium dendrobatidis* in our field dataset.

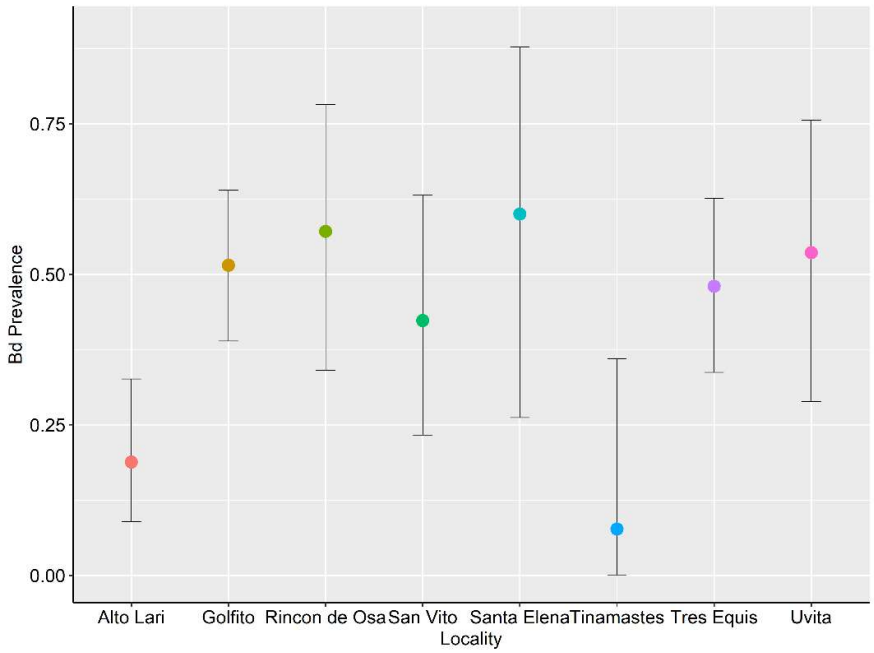

Figure B.2. Mean prevalence of *Batrachochytrium dendrobatidis* (*Bd*) in amphibian assemblages from the nine surveyed sites in Costa Rica in our field dataset (with 95% binomial CI). The plot does not display results for the study site at Punta Banco because no sampled individuals tested positive for *Bd*.

Table B.1. List of species where *Batrachochytrium dendrobatidis* (*Bd*) was surveyed in Costa Rica in our field dataset. For every species, the table shows the positive number of samples (total sample size), the overall prevalence (with 95% binomial CI), and the average (SE) of genomic equivalents of *Bd* zoospores quantified per species estimated from the *Bd*-positive samples.

Species	Positive (n)	Percentage of infection (95% CI)	Average of *Bd* Load (SE)
Agalychnis callidryas	1 (3)	33 (1-91)	0.6 (0.6)
Agalychnis spurrelli	3 (7)	43 (10-82)	3.8 (1.9)
Cochranella granulosa	0 (2)	0 (0-84)	0 (0)
Cochranella spinosa	0 (2)	0 (0-84)	0 (0)
Craugastor crassidigitus	2 (10)	20 (3-56)	2.3 (1.4)
Craugastor fitzingeri	0 (11)	0 (0-29)	0 (0)
Craugastor ranoides	2 (2)	1 (16-100)	13.3 (3.6)
Craugastor taurus	0 (9)	0 (0-34)	0 (0)
Dendropsophus ebraccatus	20 (35)	57 (39-74)	4.1 (0.9)
Diasporus tigrillo	0 (5)	0 (0-52)	0 (0)
Engystomops pustulosus	5 (6)	83 (36-100)	8.6 (2.9)
Espadarana prosoblepon	2 (5)	40 (5-85)	2.7 (2.7)
Hyalinobatrachium colymbiphyllum	1 (4)	25 (1-91)	0 (0)
Hyalinobatrachium valerioi	2 (4)	50 (7-93)	4.2 (2.6)
Boana rosenbergi	0 (1)	0 (0-98)	0 (0)
Incilius coniferus	0 (7)	0 (0-41)	0 (0)
Incilius luetkenii	0 (1)	0 (0-98)	0 (0)
Leptodactylus insularum	0 (1)	0 (0-98)	0 (0)
Leptodactylus melanonotus	4 (7)	57 (18-90)	6.8 (2.4)
Leptodactylus savage	1 (1)	100 (25-100)	33.5 (0)
Lithobates vaillanti	0 (3)	0 (0-70)	0 (0)
Oophaga granulifera	8 (10)	80 (44-98)	5.1 (1.0)
Oophaga pumilio	0 (1)	0 (0-98)	0 (0)
Pristimantis cerasinus	4 (16)	25 (7-52)	6.8 (6.4)
Pristimantis cruentus	0 (3)	0 (0-70)	0 (0)
Pristimantis ridens	2 (8)	25 (3-65)	12.3 (12.3)
Ptychohyla legleri	0 (1)	0 (0-98)	0 (0)
Rhaebo haematiticus	1 (11)	9 (1.-41)	0.7 (0.7)
Rhinella marina	0 (5)	0 (0-52)	0 (0)
Sachatamia ilex	0 (1)	0 (0-98)	0 (0)
Smilisca phaeota	2 (11)	18 (2-53)	10.1 (8.6)
Smilisca sordida	44 (71)	62 (50-73)	2.7 (0.5)
Teratohyla pulverate	1 (2)	50 (1-99)	2.9 (2.9)
Tlalocohyla loquax	1 (1)	100 (25-100)	13.1 (0)
Total	106 (267)	40 (34-46)	3.6 (0.68)

Notes: All the species are classified as Least Concern (LC) according to the International Union for Conservation of Nature (IUCN; https://www.iucnredlist.org) with the following exceptions: *Craugastor ranoides* (Critically Endangered), *C. taurus* (Critically Endangered); *Oophaga granulifera* (Vulnerable); *Ptychohyla legleri* (Endangered).

Table B.2. Percentage of individuals infected and infection intensity of *Batrachochytrium dendrobatidis* (*Bd*) at nine sites in Costa Rica in our field dataset. For each study site the table shows number of samples (number of *Bd*-positive samples) and prevalence (with 95% binomial CI) of *Bd* in amphibian assemblages.

Locality	Elevation (m)	Altitudinal Belt	n (*Bd* positive)	Percentage of individuals Infected (95% CI)
San Vito, Coto Brus	1120–1385	Premontane	26 (11)	42.3 (23.3-63.1)
Tinamastes, Pérez Zeledón	700–800	Premontane	13 (1)	7.7 (0.1-36.0)
Uvita, Osa	0–300	Tropical	19 (10)	53.6 (28.9-75.6)
Rincón, Osa	0–50	Tropical	21 (12)	57.1 (34.0-78.2)
Golfito	0–50	Tropical	66 (34)	51.5 (38.9-64.0)
Punta Banco-Burica	0–50	Tropical	14 (0)	0.0 (0.0-23.2)
Santa Elena Peninsula	0–100	Tropical	10 (6)	60.0 (26.2-87.8)
Tres Equis, Turrialba	400–600	Tropical	50 (24)	48.0 (33.7-62.6)
Alto Lari	300–600	Tropical	48 (9)	18.8 (8.9-32.6)

Table B.3. List of 215 amphibian species in Costa Rica distributed by herpetological province and elevational range. For every taxon, numbers in square brackets indicate the number of genera and numbers displayed in parenthesis indicates the number of species. For each of the three amphibian orders, the number preceding the genera represents the number of families. The table also specifies endemic species, IUCN status, and environmental vulnerability scores (EVS). Symbology for herpetological provinces: CL= Caribbean Lowlands; CT= Cordillera de Talamanca; MSCC=Mountain Slopes and Cordillera Central; PN= Pacific Northwest; PS=Pacific Southwest. IUCN Red List categories: DD = Data Deficient; LC = Lear Concerned; NT = Near Threatened; VU = Vulnerable; EN = Endangered; CR = Critically Endangered; EX = Extinct in the wild. EVS categories: 0 = now immediate risk (EVS<3); 1 = low vulnerability species (EVS of 3–9); 2 = medium vulnerability species (EVS of 10–13); 3 = high vulnerability species (EVS of 14–17).

Amphibian taxa	Endemic	EVS	IUCN status	CL	CT	MSCC	PN	PS	Elevation (m)
Anura 13 [41] (154)									
Aromobatidae [1] (1)									
Allobates talamancae		0	LC	X		X		X	0–800
Bufonidae [4] (18)									
Atelopus chiriquiensis	X	2	CR		X	X			1089–2500
Atelopus chirripoensis	X	3	CR		X				3400–3500
Atelopus senex		3	CR		X	X			1250–2200
Atelopus varius		2	CR	X	X	X	X	X	16–2110
Incilius aucoinae		3	LC			X	X	X	5–760
Incilius chompipe	X	NE	VU			X			1400–2250
Incilius coccifer		0	LC	X		X	X	X	1–1435
Incilius coniferus		0	LC	X	X	X	X	X	2–1720
Incilius epioticus		2	LC		X	X			1051–2060
Incilius fastidiosus		2	CR		X	X		X	760–2400
Incilius guanacaste	X	NE	DD			X			1700–2000
Incilius holdridgei	X	3	CR			X			1800–2200
Incilius luetkenii		0	LC			X	X		6–1140
Incilius melanochlorus		3	LC	X		X	X		5–1400
Incilius periglenes	X	3	EX			X			1500–1650
Incilius valliceps		0	LC	X					30–495
Rhaebo haematiticus		0	LC	X	X	X	X	X	20–1300
Rhinella horribilis		0	LC	X	X	X	X	X	1–1600
Centrolenidae [5] (14)									
Cochranella euknemos		0	LC			X			840–1500
Cochranella granulosa		0	LC	X		X	X	X	40–1500
Espadarana prosoblepon		0	LC	X	X	X	X	X	20–1900
Hyalinobatrachium chirripoi		0	LC	X					50–250
Hyalinobatrachium colymbiphyllum		0	LC	X	X	X		X	10–1800
*Hyalinobatrachium dianae**	X	NE	NE	X		X			400–900
Hyalinobatrachium fleischmanni		0	LC	X	X	X	X	X	0–1900
Hyalinobatrachium talamancae	X	3	LC	X		X			400–1500
Hyalinobatrachium valerioi		0	LC	X		X	X	X	6–1100
Hyalinobatrachium vireovittatum		2	LC			X		X	170–1000
Sachatamia albomaculata		0	LC	X		X	X	X	20–1500
Sachatamia ilex		0	LC	X		X			250–1000
Teratohyla pulverata		0	LC	X		X	X	X	0–1000
Teratohyla spinosa		0	LC	X		X			0–900
Craugastoridae [3] (41)									
*Craugastor aenigmaticus**	X	NE	NE		X				2300–2700
Craugastor andi		3	CR			X			900–1400
Craugastor angelicus	*X*	3	CR	X		X			650–1600

Table B.3. Continued

Craugastor bransfordii		2	LC	X		X	X		6–900
Craugastor catalinae		2	CR		X	X			1219–1800
Craugastor crassidigitus		0	LC	X	X	X	X	X	2–2000
Craugastor cuaquero	X	3	DD			X			1500–1650
Craugastor escoces	X	3	EX		X	X			1000–2110
Craugastor fitzingeri		0	LC	X	X	X	X	X	1–1500
Craugastor fleischmanni		3	CR		X	X			1050–2500
*Craugastor gabbi**	X	NE	NE			X		X	1100–1280
Craugastor gollmeri		1	LC	X	X	X			10–1520
Craugastor gulosus		2	DD		X	X			1000–1900
Craugastor megacephalus		0	LC	X		X			1–1200
Craugastor melanostictus		2	LC		X	X			1150–2700
Craugastor mimus		0	LC	X		X			15–1260
Craugastor noblei		0	LC	X		X		X	4–1200
Craugastor obesus		3	CR			X			400–1700
Craugastor persimilis	X	3	LC	X		X			0–1400
Craugastor phasma	X	3	DD		X				1850
Craugastor podiciferus		2	NT		X	X			1089–2650
Craugastor polyptychus		0	LC	X		X			2–900
Craugastor ranoides		1	CR	X		X	X	X	0–1300
Craugastor rayo	X	3	DD		X	X			1480–1820
Craugastor rhyacobatrachus		2	CR		X	X			400–1800
Craugastor rugosus		2	LC			X	X	X	10–1200
Craugastor stejnegerianus		2	LC			X	X	X	3–1400
Craugastor talamancae		1	LC	X		X			15–710
Craugastor taurus		2	CR					X	5–550
Craugastor underwoodi		2	LC		X	X		X	920–1760
*Craugastor zunigai**	X	NE	NE		X	X			1500–2100
Pristimantis altae		2	LC	X		X			50–1500
Pristimantis caryophyllaceus		0	NT	X	X	X			0–1900
Pristimantis cerasinus		0	LC	X	X	X	X		10–1400
Pristimantis cruentus		0	LC	X	X	X	X	X	40–2400
Pristimantis gaigei		0	LC	X					0–200
Pristimantis moro		0	LC			X			900–1250
Pristimantis pardalis		0	LC		X	X			300–1450
Pristimantis ridens		0	LC	X	X	X	X	X	0–1600
Pristimantis taeniatus		NE	LC			X			1000–1200
Strabomantis bufoniformis		0	LC	X					0–50
Dendrobatidae [4] (7)									
Dendrobates auratus		0	LC	X		X	X	X	2–819
Oophaga granulifera		2	VU					X	0–600
Oophaga pumilio		0	LC	X		X	X		0–980
Phyllobates lugubris		0	LC	X					0–750
Phyllobates vittatus	X	2	EN					X	0–600
Silverstoneia flotator		0	LC	X		X		X	0–900
Silverstoneia nubicola		0	CR			X		X	1050–1600
Eleutherodactylidae [2] (9)									
*Diasporus amirae**	X	NE	NE			X			1000–1100
Diasporus diastema		0	LC	X	X	X	X	X	0–1600
Diasporus hylaeformis	X	2	LC		X	X		X	1500–2500
Diasporus tigrillo	X	3	DD	X					400–450
Diasporus ventrimaculatus	X	NE	LC		X				2500–2700

Table B.3. Continued

Diasporus vocator		0	LC		X	X		X	0–1650
Eleutherodactylus coqui		NE	LC			X			650
Eleutherodactylus johnstonei		NE	LC				X		1200
*Eleutherodactylus planirostris**		NE	NE						Unknown
Hemiphractidae [1] (1)									
Gastrotheca cornuta		0	EN	X		X			300–800
Hylidae [13] (40)									
Boana rosenbergi		0	LC				X	X	0–900
Boana rufitela		0	LC	X		X			0–750
Dendropsophus ebraccatus		0	LC	X	X	X	X	X	0–1600
Dendropsophus microcephalus		0	LC	X	X	X	X	X	0–1200
Dendropsophus phlebodes		0	LC	X		X			0–750
Duellmanohyla lythrodes		2	DD	X					150–450
Duellmanohyla rufioculis	X	3	LC	X	X	X	X	X	650–1600
Duellmanohyla uranochroa		2	LC	X	X	X			300–1750
*Ecnomiohyla bailarina**		NE	NE	X					300–750
Ecnomiohyla fimbrimembra		2	EN			X			750–1900
Ecnomiohyla miliaria		0	VU	X		X		X	0–1350
Ecnomiohyla sukia	X	NE	LC	X		X			400–1000
*Ecnomiohyla veraguensis**		NE	NE	X					NE
Hyloscirtus colymba		0	CR			X			600–1200
Hyloscirtus palmeri		0	LC	X		X			400–1000
Isthmohyla angustilineata		2	CR		X	X	X		1500–2350
Isthmohyla calypsa		2	CR		X				1700–2300
Isthmohyla debilis		2	CR		X	X			900–1450
Isthmohyla lancasteri		2	LC	X		X			350–1400
Isthmohyla picadoi		2	LC		X	X			1700–2900
Isthmohyla pictipes		3	EN		X	X			1900–2800
Isthmohyla pseudopuma		2	LC		X	X			1100–2350
Isthmohyla rivularis		2	CR		X	X	X		1200–2450
Isthmohyla tica		2	CR		X	X	X		720–1750
Isthmohyla xanthosticta	X	3	DD			X			2150
Isthmohyla zeteki		2	LC		X	X			1200–1800
Osteopilus septentrionalis		0	LC	X					0-10
Ptychohyla legleri		2	EN			X		X	600–1500
Scinax boulengeri		0	LC	X			X	X	1–700
Scinax elaeochroa		0	LC	X	X	X	X	X	0–1200
Scinax staufferi		0	LC	X			X		0–700
Smilisca baudinii		0	LC			X	X	X	0–1600
*Smilisca manisorum**		NE	NE	X					0–750
Smilisca phaeota		0	LC	X		X	X	X	0–1100
Smilisca puma		2	LC	X					0–550
Smilisca sila		0	LC		X	X	X	X	0–1000
Smilisca sordida		0	LC	X	X	X	X	X	0–1550
Tlalocohyla loquax		0	LC	X		X			50–1100
Trachycephalus typhonius		0	LC	X		X	X	X	0–1100
Triprion spinosus		0	LC	X		X		X	350–1400
Leptodactylidae [2] (6)									
Engystomops pustulosus		0	LC	X		X	X	X	0–1550
Leptodactylus fragilis		0	LC	X			X	X	1–650
Leptodactylus insularum		0	LC				X	X	0–450
Leptodactylus melanonotus		0	LC	X	X	X	X	X	0–1450

Table B.3. Continued

Leptodactylus poecilochilus		0	LC	X		X	X	X	0–1150
Leptodactylus savagei		0	LC	X	X	X	X	X	0–1200
Microhylidae [2] (3)									
Ctenophryne aterrima		0	LC	X		X	X	X	0–1600
Hypopachus pictiventris		2	LC	X		X	X		1–800
Hypopachus variolosus		0	LC			X	X		0–1600
Phyllomedusidae [2] (7)									
Agalychnis annae		3	LC		X	X	X		780–1650
Agalychnis callidryas		0	LC	X		X	X	X	0–1250
Agalychnis lemur		0	CR	X		X			450–1600
Agalychnis saltator		0	LC	X		X			0–1000
Agalychnis spurrelli		0	LC	X		X		X	0–900
Cruziohyla calcarifer		0	LC	X					0–800
*Cruziohyla sylviae**		NE	NE	X					0-800
Ranidae [1] (6)									
Lithobates catesbeianus		NE	LC			X			1200
Lithobates forreri		0	LC	X	X	X	X	X	0–1550
Lithobates taylori		2	LC	X	X	X	X		0–3200
Lithobates vaillanti		0	LC	X		X	X		0–900
Lithobates vibicarius		2	LC		X	X			1400–2700
Lithobates warszewitschii		0	LC	X	X	X	X	X	0–1750
Rhinophrynidae [1] (1)									
Rhinophrynus dorsalis		0	LC				X		0–300
Order Caudata 1 [3] (53)									
Plethodontidae [3] (53)									
Bolitoglossa alvaradoi	X	3	EN	X		X			15–1116
*Bolitoglossa aurae**	X	NE	NE			X			1300
*Bolitoglossa aureogularis**	X	NE	LC		X				1680–2100
Bolitoglossa bramei		NE	LC		X				1900–3200
Bolitoglossa cerroensis	X	3	LC		X				2100–3300
Bolitoglossa colonnea		2	LC	X	X	X		X	2–1600
Bolitoglossa compacta		2	LC		X				1650–2780
Bolitoglossa diminuta	X	3	DD		X				1555
Bolitoglossa epimela	X	3	DD		X	X			775–1555
Bolitoglossa gomezi		NE	LC		X				1170–2400
Bolitoglossa gracilis	X	3	LC		X	X			1225–1380
*Bolitoglossa kamuk**	X	NE	DD		X				2870–3126
Bolitoglossa lignicolor		2	LC			X		X	2–1050
Bolitoglossa marmorea		2	LC		X				1920–3444
Bolitoglossa minutula		2	LC		X	X			1670–2660
Bolitoglossa nigrescens	X	3	EN		X				1650–3000
Bolitoglossa obscura	X	3	DD		X				1555
Bolitoglossa pesrubra	X	3	VU		X				1875–3620
*Bolitoglossa pygmaea**		NE	NE		X				3000–3335
Bolitoglossa robinsoni		NE	LC		X				2450–3335
Bolitoglossa robusta		2	LC		X	X			500–2400
Bolitoglossa schizodactyla		2	NA	X		X			300–850
Bolitoglossa sombra		3	VU		X	X			1500–2300
Bolitoglossa sooyorum	X	2	EN		X				2355–3100
*Bolitoglossa splendida**	X	NE	DD		X				1825
Bolitoglossa striatula		0	LC	X		X	X	X	10–1380
Bolitoglossa subpalmata	*X*	3	LC			X	X		1054–2900

Table B.3. Continued

Bolitoglossa tica	*X*	NE	LC		X	X			1745–2500
Nototriton abscondens	X	3	LC			X	X		960–2500
*Nototriton costaricense**	X	NE	NE		X				1500
Nototriton gamezi	X	3	LC			X			1550–1650
Nototriton guanacaste	X	3	VU			X			1400–1580
Nototriton major	X	3	LC			X			870–1200
*Nototriton matama**	X	NE	LC			X			1300
Nototriton picadoi	X	3	LC			X			1200–2200
Nototriton richardi	X	3	LC			X			1370–1800
Nototriton tapanti	X	3	LC			X			1300
Oedipina alfaroi		2	VU	X					19–850
Oedipina alleni	X	2	LC					X	2–880
Oedipina altura	X	3	CR		X				2286–2320
*Oedipina berlini**	X	NE	NE	X		X			540–850
Oedipina carablanca	X	3	LC	X					60–750
Oedipina collaris		1	DD			X			600
Oedipina cyclocauda		0	LC	X					0–600
Oedipina gracilis	X	2	EN	X					3–710
Oedipina grandis		2	LC		X	X			1810–1950
*Oedipina nimaso**	X	NE	DD			X			1093
Oedipina pacificensis	X	2	LC				X	X	0–750
Oedipina paucidentata	X	3	CR		X				2286
Oedipina poelzi	X	3	EN			X	X		775–2050
Oedipina pseudouniformis	X	3	LC	X		X			19–1253
Oedipina savagei	X	3	LC			X		X	1260–1400
Oedipina uniformis	X	3	LC	X	X	X	X		750–2150
Order Gymnophiona 2 [4] (8)									
Caeciliidae [2] (2)									
*Caecilia volcani**		NE	NE	X					50–600
Oscaecilia osae	X	3	DD					X	3–240
Dermophiidae [2] (6)									
Dermophis costaricense	X	3	DD			X			1000 –1300
Dermophis glandulosus		0	LC		X	X		X	10–1200
Dermophis gracilior		2	DD		X	X		X	404–2000
Dermophis occidentalis	X	3	LC			X	X	X	0–1000
Dermophis parviceps		0	LC	X					40–1220
Gymnopis multiplicata		0	LC	X		X	X	X	1–1400

*New addition

Table B.4. List of species that have been screened for *Batrachochytrium dendrobatidis* (*Bd*) in Costa Rica. For every species, the table shows the method used for detection of *Bd*.

Species	Histology	Conventional PCR	qPCR	qPCR museum specimens
Agalychnis annae				x
Agalychnis callidryas		X	x	
Agalychnis lemur	X		x	x
Agalychnis spurrelli			x	
Allobates talamancae		x	x	
Atelopus chiriquiensis	X			x
Atelopus senex	X			x
Atelopus varius	X		x	x
Boana rosenbergi			x	
Boana rufitela			x	
Bolitoglossa colonnea			x	
Cochranella granulosa			x	
Craugastor andi	X			x
Craugastor angelicus				x
Craugastor bransfordii		x	x	
Craugastor catalinae				x
Craugastor crassidigitus		x	x	
Craugastor escoces	X			x
Craugastor fitzingeri			x	
Craugastor fleischmanni				x
Craugastor gabbi			x	
Craugastor gollmeri		x		
Craugastor megacephalus		x	x	
Craugastor melanostictus	X			x
Craugastor mimus			x	
Craugastor noblei		x		
Craugastor obesus				x
Craugastor podiciferus			x	
Craugastor ranoides			x	x
Craugastor rhyacobatrachus				x
Craugastor stejnegerianus			x	
Craugastor talamancae	X			
Craugastor taurus			x	x
Craugastor underwoodi			x	
Cruziohyla calcarifer			x	
Dendrobates auratus		x	x	
Dendropsophus ebraccatus			x	
Dendropsophus microcephalus			x	
Dendropsophus phlebodes			x	

Table B.4. Continued

Diasporus diastema		x	x	
Diasporus hylaeformis	X			
Diasporus tigrillo			x	
Diasporus vocator			x	
Duellmanohyla rufioculis	X		x	x
Duellmanohyla uranochroa	X			x
Engystomops pustulosus			x	
Espadarana prosoblepon			x	
Hyalinobatrachium colymbiphyllum			x	
Hyalinobatrachium fleischmanni			x	
Hyalinobatrachium valerioi		x	x	
Hyloscirtus colymba				x
Hyloscirtus palmeri			x	x
Hypopachus variolosus			x	
Incilius coccifer			x	
Incilius coniferus		x	x	
Incilius fastidiosus	X			x
Incilius holdridgei			x	x
Incilius luetkenii			x	
Incilius melanochlorus			x	
Incilius periglenes				x
Isthmohyla angustilineata	X			x
Isthmohyla calypsa	X			x
Isthmohyla pictipes				x
Isthmohyla pseudopuma	X		x	
Isthmohyla rivularis	X			x
Isthmohyla tica				x
Isthmohyla xanthosticta				x
Leptodactylus fragilis			x	
Leptodactylus insularum			x	
Leptodactylus melanonotus			x	
Leptodactylus poecilochilus			x	
Leptodactylus savagei		x	x	
Lithobates forreri			x	
Lithobates taylori			x	
Lithobates vaillanti			x	
Lithobates vibicarius			x	x
Lithobates warszewitschii		x	x	x
Oedipina gracilis			x	
Oophaga granulifera			x	
Oophaga pumilio		x	x	

Table B.4. Continued

Phyllobates lugubris		x		
Pristimantis altae			x	
Pristimantis caryophyllaceus	X			x
Pristimantis cerasinus		x	x	
Pristimantis cruentus			x	
Pristimantis ridens			x	
Ptychohyla legleri			x	x
Rhaebo haematiticus			x	
Rhinella horribilis		x	x	
Sachatamia ilex			x	
Scinax boulengeri			x	
Scinax elaeochroa			x	
Scinax staufferi			x	
Silverstoneia flotator		x		
Silverstoneia nubicola				x
Smilisca baudinii			x	
Smilisca manisorum		x	x	
Smilisca phaeota	X	x		
Smilisca sila			x	
Smilisca sordida	X	x		
Teratohyla pulverata			x	
Teratohyla spinosa			x	
Tlalocohyla loquax			x	
Trachycephalus typhonius			x	
Triprion spinosus			x	

Table B.5. List of species where *Batrachochytrium dendrobatidis* was surveyed in Costa Rica in our combined dataset. For every species, the table shows the positive number of samples (total sample size) and the overall percentage of infection (with 95% binomial CI).

Species		Percentage of infection (95% CI)
Agalychnis callidryas	4 (20)	20 (6-44)
Agalychnis lemur	(0) 5	0 (0-52)
Agalychnis spurrelli	5 (12)	42 (15-72)
Allobates talamancae	0 (14)	0 (0-23)
Boana rosenbergi	0 (1)	0 (0-98)
Boana rufitela	8 (12)	67 (35-90)
Bolitoglossa colonnea	1 (1)	100 (25-100)
Cochranella granulosa	1 (7)	14 (0-58)
Cochranella spinosa	0 (2)	0 (0-84)
Craugastor bransfordi	24 (107)	22 (15-32)
Craugastor crassidigitus	8 (56)	14 (6-26)
Craugastor fitzingeri	51 (176)	29 (22-36)
Craugastor gabbi	0 (2)	0 (0-84)
Craugastor gollmeri	0 (1)	0 (0-98)
Craugastor megacephalus	1 (19)	5 (0-26)
Craugastor mimus	9 (12)	75 (43-95)
Craugastor noblei	1 (2)	50 (1-99)
Craugastor podiciferus	0 (3)	0 (0-70)
Craugastor ranoides	3 (116)	3 (1-7)
Craugastor stejnegerianus	2 (6)	33 (4-78)
Craugastor taurus	12 (24)	50 (29-71)
Craugastor underwoodi	2 (2)	1 (16-100)
Cruziohyla calcarifer	1 (1)	1 (25-100)
Dendrobates auratus	1 (16)	6 (0-30)
Dendropsophus ebraccatus	38 (81)	47 (36-58)
Dendropsophus microcephalus	0 (7)	0 (0-41)
Dendropsophus phlebodes	0 (1)	0 (0-98)
Diasporus diastema	3 (31)	10 (2-26)
Diasporus tigrillo	0 (5)	0 (0-52)
Diasporus vocator	0 (1)	0 (0-98)
Duellmanohyla rufioculis	2 (7)	29 (37-71)
Engystomops pustulosus	11 (46)	24 (13-39)
Espadarana prosoblepon	5 (15)	33 (12-62)
Hyalinobatrachium colymbiphyllum	1 (4)	25 (1-91)
Hyalinobatrachium fleischmanni	0 (2)	0 (0-84)
Hyalinobatrachium valerioi	4 (30)	13 (3-31)
Hyloscirtus palmeri	1 (1)	100 (25-100)
Hypopachus variolosus	0 (9)	0 (0-34)

Table B.5 Continued

Incilius coccifer	0 (29)	0 (0-12)
Incilius coniferus	0 (8)	0 (0-37)
Incilius luetkenii	0 (12)	0 (0-26)
Incilius melanochlorus	2 (8)	25 (3-65)
Isthmohyla pseudopuma	1 (12)	8 (0-38)
Leptodactylus fragilis	0 (1)	0 (0-98)
Leptodactylus insularum	0 (4)	0 (0-60)
Leptodactylus melanonotus	4 (23)	17 (5-38)
Leptodactylus poecilochilus	1 (35)	3 (0-15)
Leptodactylus savagei	2 (28)	7 (0-24)
Lithobates forreri	2 (30)	7 (1-22)
Lithobates taylori	16 (21)	76 (53-92)
Lithobates vaillanti	0 (5)	0 (0-52)
Lithobates warszewitschii	15 (41)	52 (32-71)
Oedipina gracilis	0 (1)	0 (0-98)
Oophaga granulifera	9 (11)	82 (48-98)
Oophaga pumilio	35 (80)	44 (33-55)
Phyllobates lugubris	1 (4)	25 (1-91)
Pristimantis altae	0 (2)	0 (0-84)
Pristimantis cerasinus	9 (26)	35 (17-56)
Pristimantis cruentus	0 (11)	0 (0-29)
Pristimantis ridens	7 (27)	26 (11-46)
Ptychohyla legleri	0 (1)	0 (0-98)
Rhaebo haematiticus	22 (51)	43 (29-0.58)
Rhinella horribilis	0 (90)	0 (0-4)
Sachatamia ilex	0 (2)	0 (0-84)
Scinax boulengeri	1 (6)	17 (0-64)
Scinax elaeochroa	5 (39)	13 (4-27)
Scinax staufferi	0 (3)	0 (0-70)
Silverstoneia flotator	0 (15)	0 (0-22)
Smilisca baudinii	0 (31)	0 (0-11)
Smilisca manisorum	0 (2)	0 (0-84)
Smilisca phaeota	4 (18)	22 (6-48)
Smilisca sila	0 (10)	0 (0-31)
Smilisca sordida	50 (133)	38 (29-46)
Teratohyla pulverata	2 (26)	7 (1-25)
Teratohyla spinosa	5 (17)	29 (10-56)
Tlalocohyla loquax	11 (16)	69 (41-89)
Trachycephalus typhonius	0 (13)	0 (0-25)
Triprion spinosus	1 (1)	100 (25-100)
Total		23 (21-25)

Table B.6. Candidacy generalized linear models (GLMs) and linear models (LMs) used to determine the best predictors of prevalence and infection intensity of *Batrachochytrium dendrobatidis* in amphibian assemblages from Costa Rica. Predictors were species, herpetological province (region), Holdridge's altitudinal belt (elevation), and the foraging-reproduction habitat index (FRHI). The most robust GLMs were selected according to the Akaike Information Criteria (AIC). For LMs, ANOVA was used to test significance of predictors.

Predictors	Evaluation	Significant predictors (P<0.05)
GLMs	AIC	
Region + FRHI + Elevation	1701	Region, FRHI
Region + FRHI	1700*	Region, FRHI
LM	(ANOVA)	
Region + FRHI + Elevation	($F_{11,339}$=7.44, P<0.01, k=3	FRHI
Region + FRHI	($F_{11,339}$=7.44, P<0.01, k=2)	FRHI
FRHI	($F_{8,342}$=7.91, P<0.01, k =1)*	FRHI

*Selected models.

APPENDIX C. CHAPTER 3 SUPPLEMENTARY MATERIALS

Table C.1. Specific contribution of bioclimatic variables used for modelling the range of *Batrachochytrium dendrobatidis* in Costa Rica. All the 19 bioclimatic layers were downloaded from WorldClim v1.4 (http://www.worldclim.org/bioclim) at a spatial resolution of 30 arc-secs (Hijmans et al., 2005).

Bioclimatic variables	Contribution of variables (%)	
	Histology dataset	Combined dataset
BIO_1 = Annual Mean Temperature	0.00	0.0
BIO_2 = Mean Diurnal Range	0.00	2.6
BIO_3 = Isothermality	0.00	1.1
BIO_4 = Temperature Seasonality	0.00	0.0
BIO_5 = Max Temperature of Warmest Month	0.00	0.0
BIO_6 = Min Temperature of Coldest Month	0.00	13.9
BIO_7 = Temperature Annual Range	0.00	1.7
BIO_8 = Mean Temperature of Wettest Quarter	67.74	12.2
BIO_9 = Mean Temperature of Driest Quarter	0.00	0.0
BIO_{10} = Mean Temperature of Warmest Quarter	0.00	1.1
BIO_{11} = Mean Temperature of Coldest Quarter	0.00	1.2
BIO_{12} = Annual Precipitation	0.00	0.0
BIO_{13} = Precipitation of Wettest Month	0.00	0.0
BIO_{14} = Precipitation of Driest Month	25.35	41.5
BIO_{15} = Precipitation Seasonality	6.91	0.0
BIO_{16} = Precipitation of Wettest Quarter	0.00	0.0
BIO_{17} = Precipitation of Driest Quarter	0.00	0.8
BIO_{18} = Precipitation of Warmest Quarter	0.00	13.8
BIO_{19} = Precipitation of Coldest Quarter	0.00	10.1

Table C.2. List of studies where polymerase chain reaction (PCR) methods were used to detect *Batrachochytrium dendrobatidis* (*Bd*) in Costa Rica and then added to the histology dataset to generate the combined dataset. The table also includes the year of collection, percentage of infection and sample size, and estimated arrival of *Bd* to the study site.

Reference	Year of collection	Study region (Elevation m)	% Infection (n)	*B. dendrobatidis* arrival*
Picco & Collins, 2007	2005	Tilarán Volcanic Range (800–18420)	12.2 (41)	1987
		Southern Talamanca Range (1120–1385)	9.3 (43)	1993
Goldberg et al. 2009	2006	South Pacific Lowlands (0–100)	0.1 (91)	1993
		South Pacific Lowlands (0–100)	0.0 (62)	1993
		South Pacific Lowlands (0–100)	0.1 (25)	1993
Whitfield, et al., 2012	2007-2008	Caribbean Lowlands (0–200)	6.1 (836)	1987-1989
Zumbado-Ulate et al., 2014	2007-2008	Santa Elena Peninsula (0–200)	0.0 (310)	Before 1987
		Santa Rosa Dry Forest (0–200)	9.0 (100)	Before 1987
Saenz, et al.,2009+	2008	Caribbean Lowlands (0–100)	7.9 (126)	1987-1989
Whitfield et al., 2013	2011	Caribbean Lowlands (0–200)	21.3 (253)	1987-1989
Zumbado-Ulate, et al., 2019	2011	South Pacific Dry Lowlands (0–100)	68.6 (35)	1993
		South Pacific Lowlands (0–100)	0.0 (25)	1993
		Caribbean Lowlands (0–200)	67.4 (144)	1987-1989
		Caribbean Lowlands (400–600)	47.9 (144)	1987-1989
Whitfield et al., 2017	2012	Santa Elena Peninsula (0–200)	60.0 (20)	1993
		South Pacific Dry Lowlands (0–100)	80.0 (20)	1993
		Central Volcanic Range (1100–1300)	90.0 (20)	1987-1988
		Central Volcanic Range (2000–2300)	0.0 (7)	1987-1988
		Caribbean Lowlands (400–600)	10.0 (20)	1987
		Tilarán Volcanic Range (400–2300)	31.8 (22)	1987
		Southern Talamanca Range (1120–1385)	92.9 (14)	1993

*Estimated arrival of *Bd* was based on published literature (Cheng et al., 2011; Lips et al., 2008).
+Only this study used standard PCR. The remaining studies used quantitative PCR (qPCR).

Table C.3. List of 72 candidate tuned models for the combined dataset. Best fit model is shown in bold font. Details on settings, mean AUC, AUC difference (AUC Diff), minimum training presence omission rate (mtpOR), and number of parameters are provided.

Model	Settings	Mean AUC	AUC Diff	mtpOR	Parameters
1	L_1	0.80	0.06	0.05	5
2	Q_1	0.82	0.04	0.05	5
3	H_1	0.83	0.07	0.04	15
4	**LQ_1**	**0.83**	**0.04**	**0.05**	**6**
5	LH_1	0.83	0.07	0.04	15
6	LQH_1	0.83	0.07	0.08	17
7	LQHP_1	0.83	0.07	0.04	14
8	LQHPT_1	0.83	0.07	0.04	14
9	L_1.5	0.79	0.06	0.05	5
10	Q_1.5	0.82	0.04	0.05	5
11	H_1.5	0.83	0.06	0.13	10
12	LQ_1.5	0.82	0.04	0.05	6
13	LH_1.5	0.83	0.06	0.13	10
14	LQH_1.5	0.83	0.06	0.13	11
15	LQHP_1.5	0.83	0.06	0.13	9
16	LQHPT_1.5	0.83	0.06	0.13	9
17	L_2	0.78	0.05	0.05	4
18	Q_2	0.80	0.04	0.05	5
19	H_2	0.83	0.06	0.14	11
20	LQ_2	0.80	0.05	0.05	5
21	LH_2	0.83	0.06	0.10	8
22	LQH_2	0.83	0.06	0.14	9
23	LQHP_2	0.83	0.06	0.10	9
24	LQHPT_2	0.83	0.06	0.10	9
25	L_2.5	0.77	0.06	0.00	4
26	Q_2.5	0.80	0.04	0.05	4
27	H_2.5	0.83	0.05	0.14	11
28	LQ_2.5	0.79	0.05	0.05	4
29	LH_2.5	0.83	0.05	0.10	11
30	LQH_2.5	0.83	0.05	0.10	5
31	LQHP_2.5	0.83	0.05	0.10	11
32	LQHPT_2.5	0.83	0.05	0.10	11
33	L_3	0.76	0.06	0.00	3
34	Q_3	0.79	0.04	0.05	4
35	H_3	0.82	0.05	0.10	7
36	LQ_3	0.79	0.05	0.05	4
37	LH_3	0.82	0.05	0.10	8
38	LQH_3	0.82	0.04	0.10	5
39	LQHP_3	0.82	0.05	0.10	7
40	LQHPT_3	0.82	0.05	0.10	7

Table. C.3. Continued

41	L_3.5	0.75	0.06	0.00	3
42	Q_3.5	0.78	0.05	0.00	3
43	H_3.5	0.81	0.05	0.05	10
44	LQ_3.5	0.78	0.05	0.00	3
45	LH_3.5	0.81	0.05	0.05	10
46	LQH_3.5	0.81	0.04	0.10	8
47	LQHP_3.5	0.81	0.05	0.05	10
48	LQHPT_3.5	0.81	0.05	0.05	10
49	L_4	0.75	0.06	0.00	3
50	Q_4	0.76	0.05	0.00	3
51	H_4	0.80	0.04	0.10	11
52	LQ_4	0.77	0.05	0.00	3
53	LH_4	0.80	0.04	0.10	11
54	LQH_4	0.81	0.04	0.10	4
55	LQHP_4	0.80	0.04	0.10	11
56	LQHPT_4	0.80	0.04	0.10	11
57	L_4.5	0.75	0.06	0.00	3
58	Q_4.5	0.75	0.05	0.00	2
59	H_4.5	0.79	0.05	0.10	6
60	LQ_4.5	0.75	0.06	0.00	3
61	LH_4.5	0.79	0.05	0.10	6
62	LQH_4.5	0.80	0.04	0.10	4
63	LQHP_4.5	0.79	0.05	0.10	6
64	LQHPT_4.5	0.79	0.05	0.10	6
65	L_5	0.75	0.05	0.00	2
66	Q_5	0.75	0.05	0.00	2
67	H_5	0.79	0.04	0.10	5
68	LQ_5	0.75	0.05	0.00	3
69	LH_5	0.79	0.04	0.10	5
70	LQH_5	0.79	0.05	0.10	3
71	LQHP_5	0.79	0.04	0.10	5
72	LQHPT_5	0.79	0.04	0.10	5

Table C.4. List of 72 candidate tuned models for the histology dataset. Best fit model is shown in bold font. Details on settings, mean AUC, AUC difference (AUC Diff), minimum training presence omission rate (mtpOR), and number of parameters are provided.

Model	settings	Mean AUC	AUC Diff	mtpOR	Parameters
1	L_1	0.68	0.08	0.06	5
2	Q_1	0.66	0.07	0.06	5
3	H_1	0.66	0.16	0.08	31
4	**LQ_1**	**0.68**	**0.07**	**0.06**	**3**
5	LH_1	0.66	0.16	0.08	30
6	LQH_1	0.65	0.17	0.08	33
7	LQHP_1	0.66	0.16	0.08	31
8	LQHPT_1	0.66	0.16	0.08	31
9	L_1.5	0.67	0.08	0.06	5
10	Q_1.5	0.66	0.07	0.06	5
11	H_1.5	0.64	0.14	0.08	24
12	LQ_1.5	0.67	0.08	0.06	4
13	LH_1.5	0.64	0.13	0.08	10
14	LQH_1.5	0.64	0.14	0.08	10
15	LQHP_1.5	0.64	0.14	0.08	24
16	LQHPT_1.5	0.64	0.14	0.08	24
17	L_2	0.67	0.08	0.06	4
18	Q_2	0.66	0.07	0.06	5
19	H_2	0.63	0.14	0.08	14
20	LQ_2	0.67	0.08	0.06	4
21	LH_2	0.64	0.12	0.08	9
22	LQH_2	0.64	0.12	0.08	10
23	LQHP_2	0.63	0.14	0.08	14
24	LQHPT_2	0.63	0.14	0.08	14
25	L_2.5	0.67	0.08	0.06	4
26	Q_2.5	0.65	0.07	0.06	4
27	H_2.5	0.62	0.14	0.08	11
28	LQ_2.5	0.67	0.08	0.06	4
29	LH_2.5	0.64	0.12	0.08	10
30	LQH_2.5	0.65	0.11	0.08	8
31	LQHP_2.5	0.62	0.14	0.08	11
32	LQHPT_2.5	0.62	0.14	0.08	11
33	L_3	0.66	0.08	0.06	4
34	Q_3	0.65	0.07	0.06	4
35	H_3	0.61	0.15	0.08	11
36	LQ_3	0.66	0.08	0.06	4
37	LH_3	0.64	0.12	0.08	7
38	LQH_3	0.64	0.11	0.08	6
39	LQHP_3	0.61	0.15	0.08	11
40	LQHPT_3	0.61	0.15	0.08	11

Table C.4 Continued

41	L_3.5	0.66	0.08	0.06	4
42	Q_3.5	0.65	0.07	0.06	4
43	H_3.5	0.61	0.14	0.06	9
44	LQ_3.5	0.66	0.08	0.06	4
45	LH_3.5	0.63	0.12	0.08	7
46	LQH_3.5	0.64	0.11	0.06	8
47	LQHP_3.5	0.61	0.14	0.06	9
48	LQHPT_3.5	0.61	0.14	0.06	9
49	L_4	0.66	0.08	0.06	4
50	Q_4	0.65	0.07	0.06	4
51	H_4	0.60	0.14	0.06	8
52	LQ_4	0.66	0.08	0.06	3
53	LH_4	0.62	0.12	0.08	7
54	LQH_4	0.63	0.11	0.06	6
55	LQHP_4	0.60	0.14	0.06	5
56	LQHPT_4	0.60	0.14	0.06	5
57	L_4.5	0.64	0.09	0.06	4
58	Q_4.5	0.65	0.07	0.06	4
59	H_4.5	0.58	0.15	0.12	9
60	LQ_4.5	0.65	0.08	0.06	3
61	LH_4.5	0.61	0.12	0.08	6
62	LQH_4.5	0.62	0.11	0.06	6
63	LQHP_4.5	0.58	0.15	0.12	8
64	LQHPT_4.5	0.58	0.15	0.12	8
65	L_5	0.63	0.09	0.06	3
66	Q_5	0.64	0.07	0.06	4
67	H_5	0.56	0.15	0.12	6
68	LQ_5	0.65	0.07	0.06	3
69	LH_5	0.61	0.11	0.06	4
70	LQH_5	0.62	0.11	0.06	5
71	LQHP_5	0.56	0.15	0.12	6
72	LQHPT_5	0.56	0.15	0.12	6

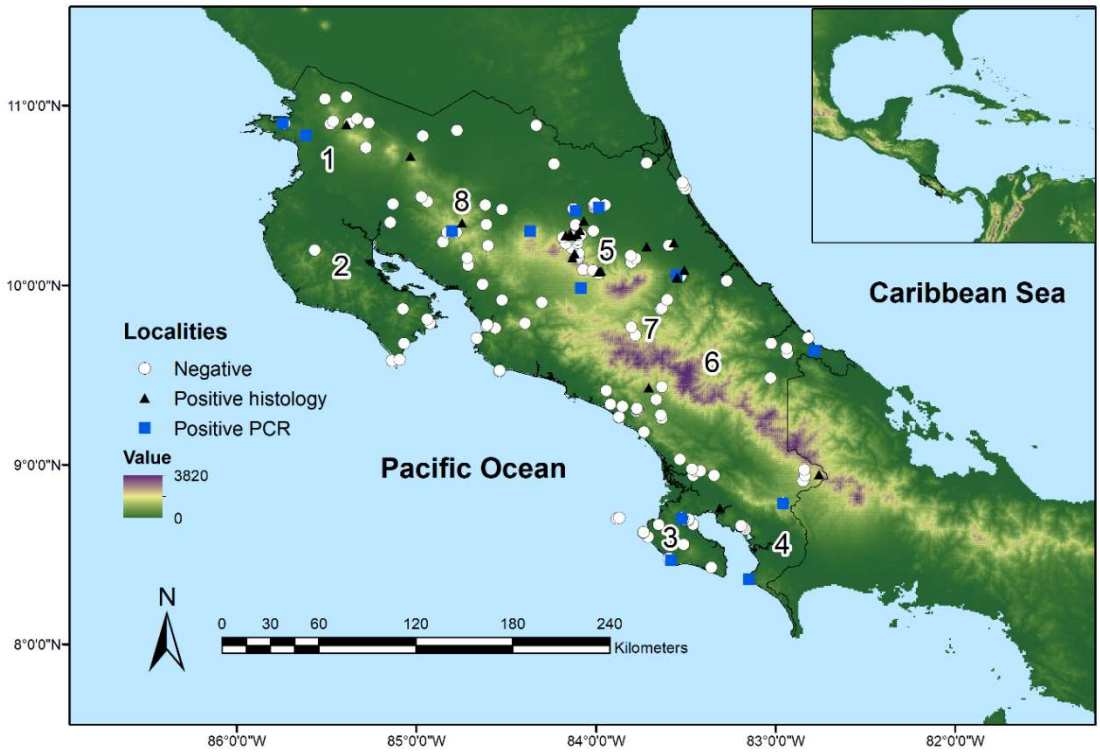

Figure C.1. Map of the 172 localities screened for *Batrachochytrium dendrobatidis* in Costa Rica. Localities are shape-coded by negative and positive detection: histology or polymerase chain reaction (PCR). Numbers show localities mentioned in text: 1- Santa Elena Peninsula; 2- Nicoya Peninsula; 3- Osa Peninsula; 4-Punta Banco-Burica; 5-Caribbean Lowlands; 6- Talamanca Range; 7-Central Volcanic Range; 8- Tilarán Volcanic Range.

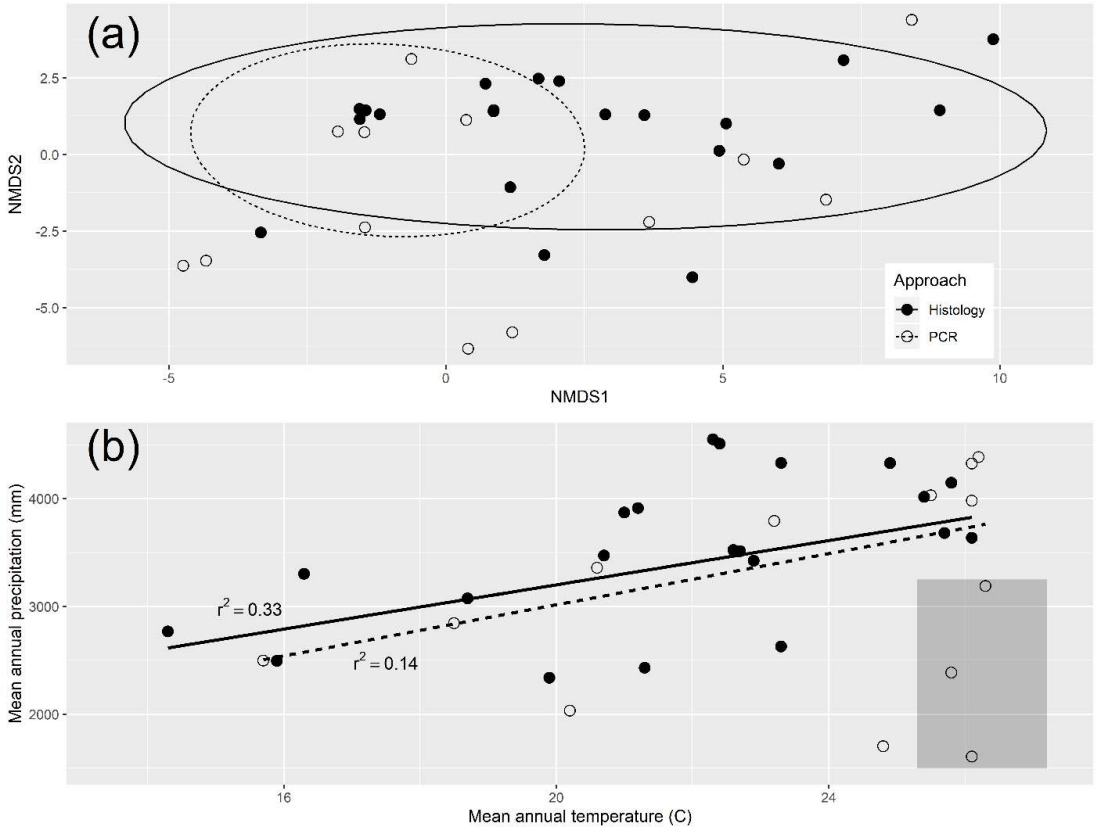

Figure C.2. Abiotic distribution of the 34 localities where *Batrachochytrium dendrobatidis* (*Bd*) has been detected in Costa Rica (21 through histology and 13 through PCR). The abiotic space was generated from the 19 bioclimatic layers of WorldClim (Hijmans et al., 2005). Closed circles and solid lines represent the spatial range of detection of histology. Open circles and dotted lines represent the spatial range of detection of polymerase chain reaction (PCR) methods. (a) The range of detection of both methods was compared with a permutational multivariate analyses of variance (PERMANOVA) with Euclidean dissimilarity and 10,000 permutations. The nonmetric multidimensional scaling (NMDS) shows that histology exhibited a more homogeneous environmental spatial distribution, represented by a wider ellipse compared to PCR methods. However, PCR methods led to higher environmental dispersion (points outside the ellipse) compared with histology, representing the detection of *Bd* in dry and semi-dry Pacific lowlands where histology failed to detect *Bd* (PERMANOVA: $F_{1,450} = 32.9$, $p<0.01$). The ellipses surround the 95% of abiotic space where *Bd* detection occurs. (b) Environmental plot shows the distribution of the 34 *Bd*-positive localities regarding temperature (°C) and precipitation (mm). The gray box displays the environmental conditions previously suggested to be unsuitable for *Bd* in Costa Rica.

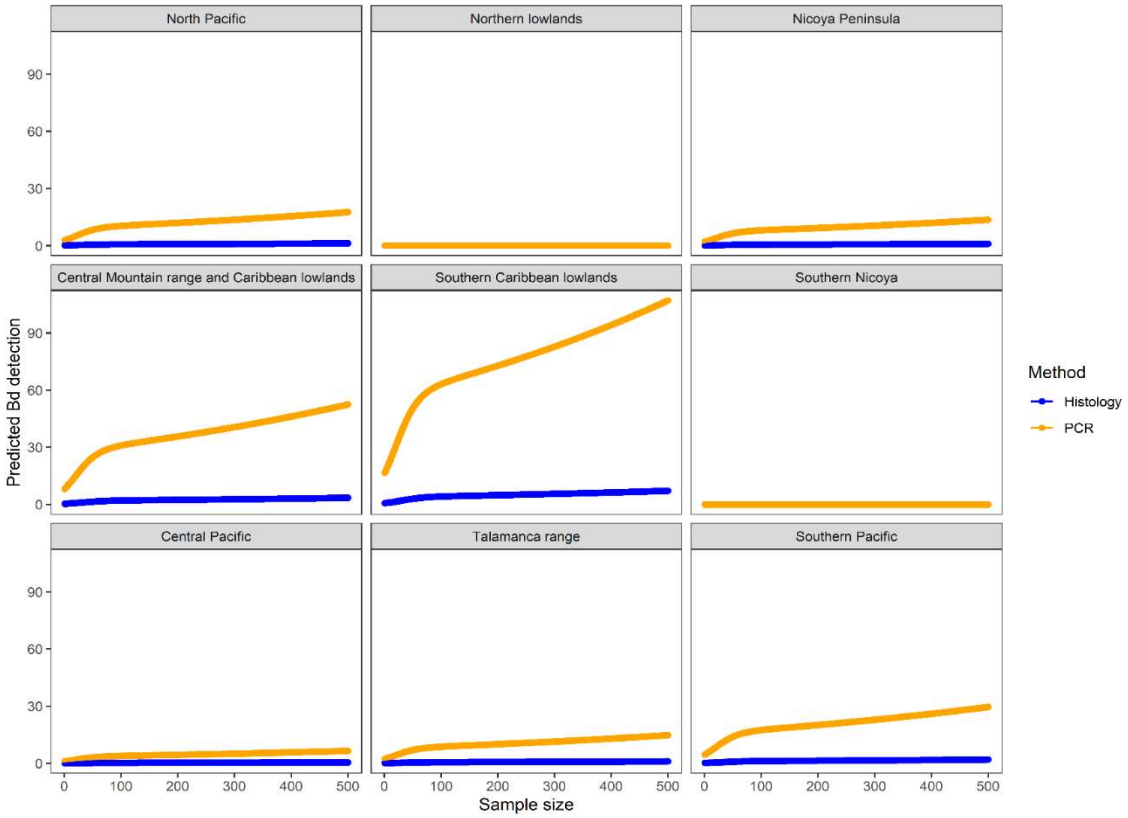

Figure C.3. Detection hotspots for *Batrachochytrium dendrobatidis* (*Bd*) in Costa Rica. The figure shows that most detection of *Bd* positives is predicted to occur in regional hotspots across the Central Mountain range and Caribbean lowlands. The detection of *Bd* is also favored by an increase in sample size and use of PCR methods**.**

REFERENCES

Cheng TL, Rovito SM, Wake DB, Vredenburg VT (2011) Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences* 108:9502–9507.

Goldberg, C. S., Hawley, T. J., & Waits, L. P. (2009). Local and regional patterns of amphibian chytrid prevalence on the Osa Peninsula, Costa Rica. *Herpetological Review* 40(3), 309–311.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978.

Lips KR, Diffendorfer J, Mendelson JR, Sears MW (2008) Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLOS Biology* 6:e72.

Picco AM, Collins JP (2007) Fungal and viral pathogen occurrence in Costa Rican amphibians. *Journal of Herpetology* 41:746–749.

Puschendorf R, Carnaval AC, VanDerWal J, et al (2009) Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: Proposing climatic refuges as a conservation tool. *Diversity and Distributions* 15:401–408.

Saenz D, Adams CK, Pierce JB, Laurencio D (2009) Occurrence of *Batrachochytrium dendrobatidis* in an anuran community in the southeastern Talamanca region of Costa Rica. *Herpetological Review* 40:311–313

Whitfield SM, Alvarado G, Abarca J, et al (2017) Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Diseases of Aquatic Organisms* 126:33–41.

Whitfield SM, Geerdes E, Chacon I, et al (2013) Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. *Diseases of Aquatic Organisms* 104:173–178.

Zumbado-Ulate H, Bolaños F, Gutiérrez-Espeleta G, Puschendorf R (2014) Extremely low prevalence of *Batrachochytrium dendrobatidis* in frog populations from Neotropical dry forest of Costa Rica supports the existence of a climatic refuge from disease. *EcoHealth* 11:593–602.

Zumbado-Ulate H, García-Rodríguez A, Vredenburg VT, Searle CL (2019a) Infection with *Batrachochytrium dendrobatidis* is common in tropical lowland habitats: Implications for amphibian conservation. *Ecology and Evolution* 9:4917–4930.

APPENDIX D. CHAPTER 4 SUPPLEMENTARY MATERIALS 1

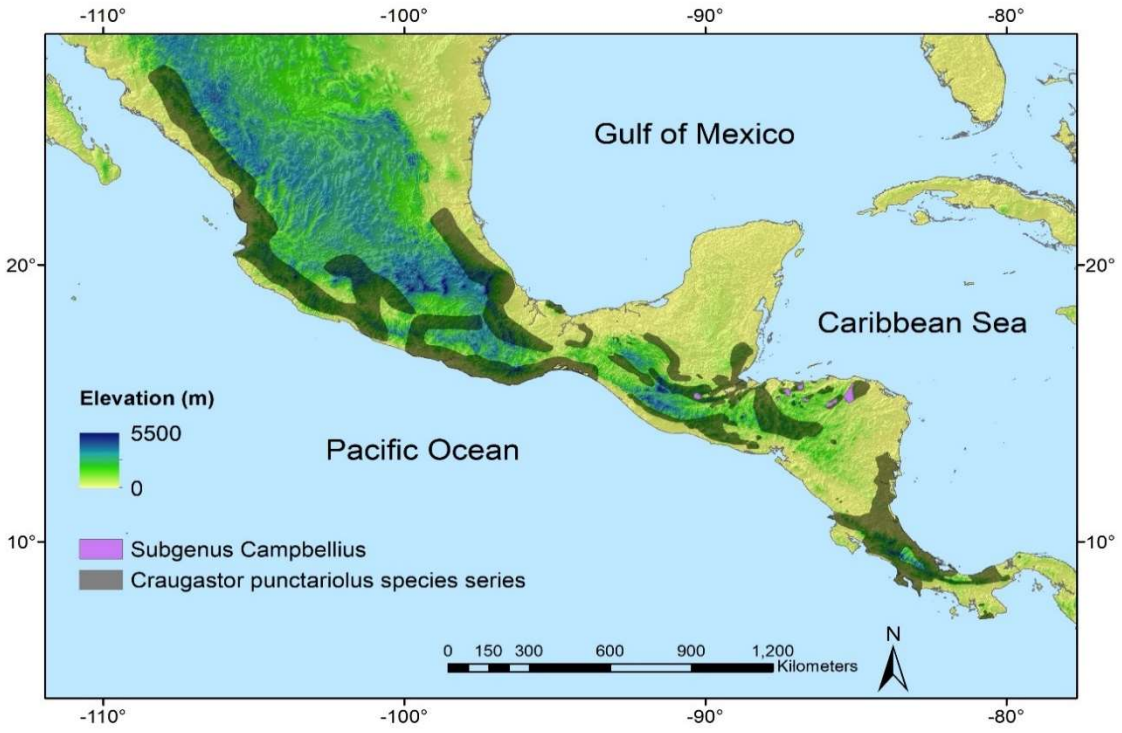

Figure D.1. Range of 'robber frogs' in Mesoamerica, a group of 46 stream-dwelling species that range from Mexico to Panama. Robber frogs are classified into two clades, the *Craugastor punctariolus species* series (gray polygons) and the Subgenus *Campbellius* (former *C. milesi* group, pink polygons).

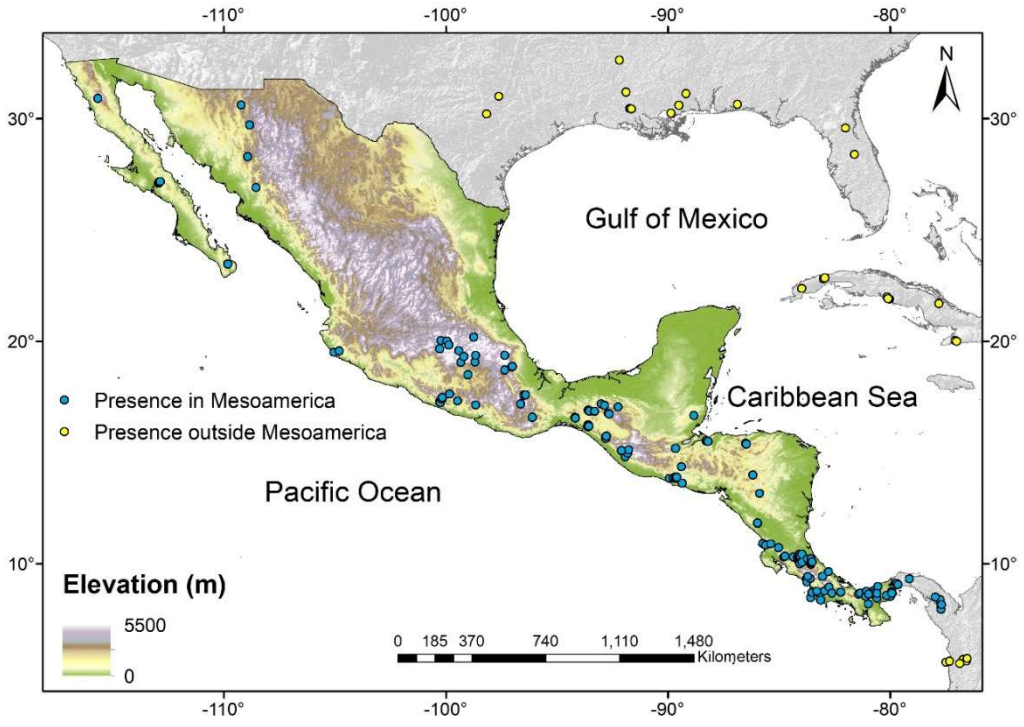

Figure D.2. Distribution of positive localities for the pathogen *Batrachochytrium dendrobatidis* (Bd) across Mesoamerica (blue dots). The map also shows Bd-positive localities from the USA, Colombia, and Cuba (yellow dots) that were used as calibration points for the distribution model.

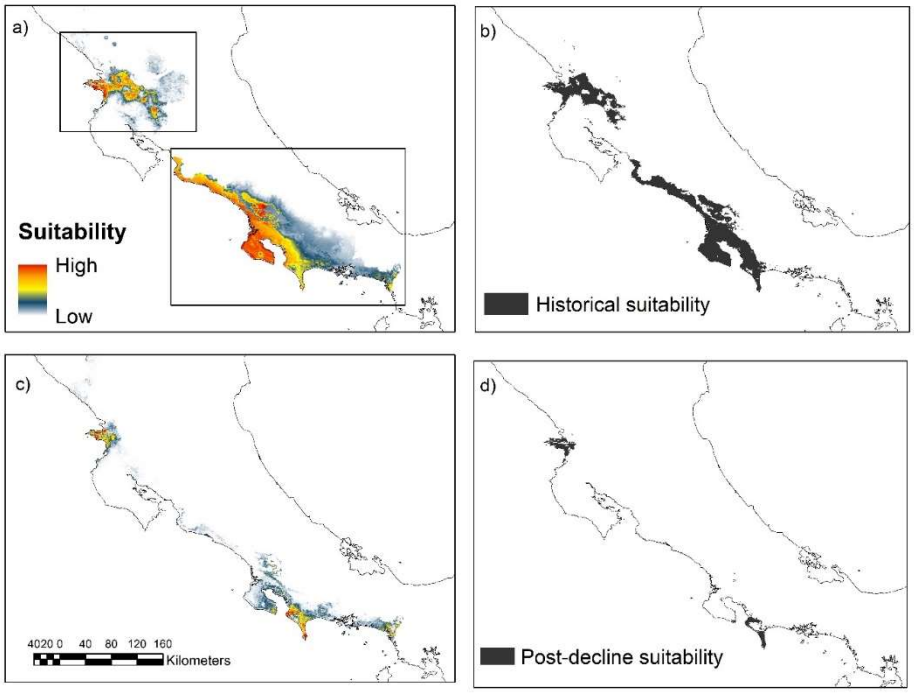

Figure D.3. The historical and present habitat suitability and range of two Critically Endangered amphibian species. a) Historical range of the dry forest robber frog (subspecies of *Craugastor ranoides* in the Santa Elena Peninsula, upper left rectangle) and the Golfito robber frog (*C. taurus*, bottom right rectangle) shows the strong impact of environmental threats during *Batrachochytrium dendrobatidis* enzootic times; the historical suitability for the dry forest robber frog is high in the lowlands and highlands in northwestern Costa Rica and the lowlands in southwestern Nicaragua (99.8% in Costa Rica). For the Golfito robber frog the historical habitat suitability is high in the lowlands and midlands across the Central and South Pacific area of Costa Rica, and along the Pacific Coast of Chiriquí Province in Panamá (94% in Costa Rica); b) The historical range shows an AOO of approximately 2600 km^2 for the dry forest robber frog and 6200 km^2 for the Golfito robber frog; c) The present suitability of both species of robber frogs is restricted to dry and semidry regions in the Santa Elena Peninsula and Punta Burica; d) The present range shows a reduction of 85% of the AOO for the dry forest robber frog and 95% for the Golfito robber frog.

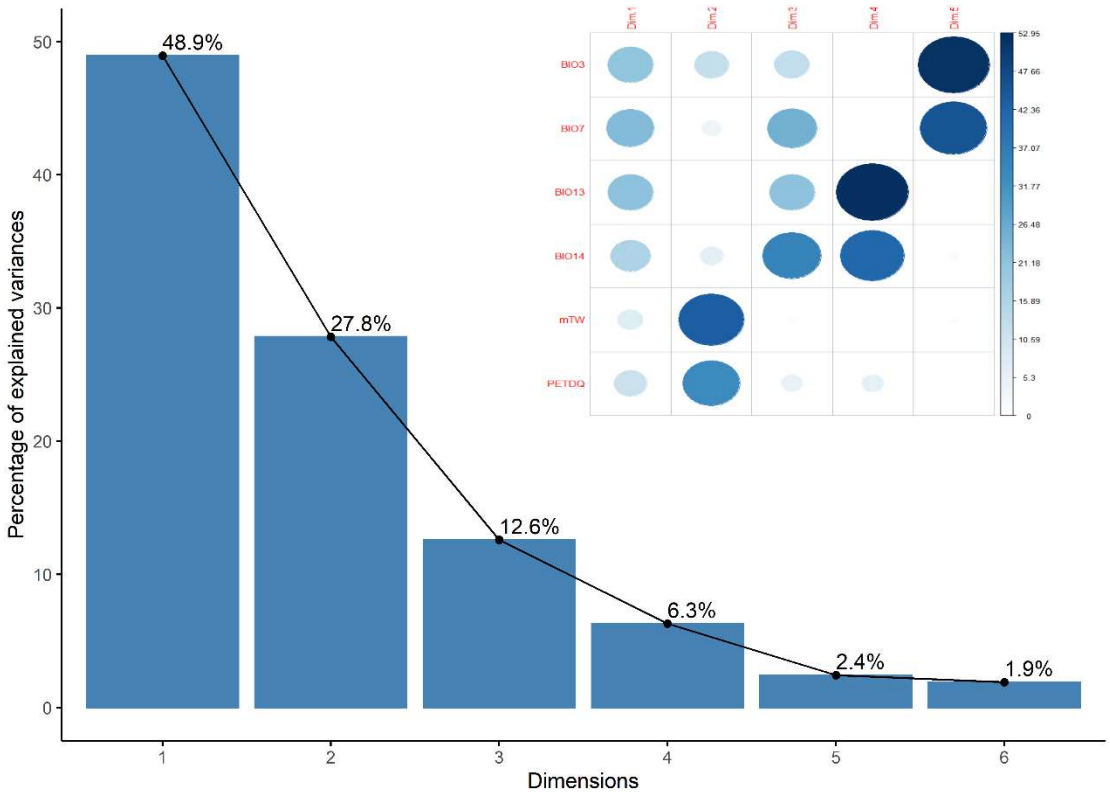

Figure D.4. Scree plot of PCA conducted to analyze the climatic difference among study locations. The figure also shows the contribution of environmental predictors across dimensions. The scale on the right indicates percent of contribution of each predictor on each dimension, with dark blue showing highest contributions and white the lowest contributions. The climatic predictors included 'isothermality' (BIO3), 'temperature annual range' (BIO7), 'precipitation of the wettest month' (BIO13), 'precipitation of driest month (BIO14)', 'minimum temperature of the warmest month' (mTW), and 'mean monthly potential evapotranspiration of driest quarter' (PETDQ).

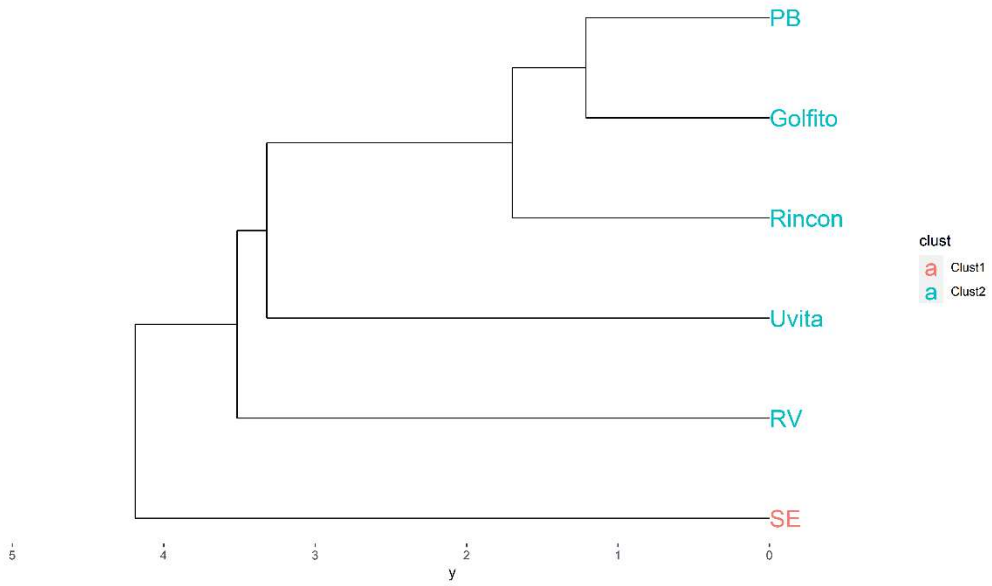

Figure D.5. Cluster analysis shows that study locations group according to versants (North Pacific and South Pacific), with the highest dissimilarities between the locations that sustain remnant population of robber frogs (Santa Elena and Punta Banco. Localities: Punta Banco (PB),Golfito, Rincón de Osa (Rincón), Uvita, Rincón de la Vieja Volcano (RV), and Santa Elena Peninsula (SE).

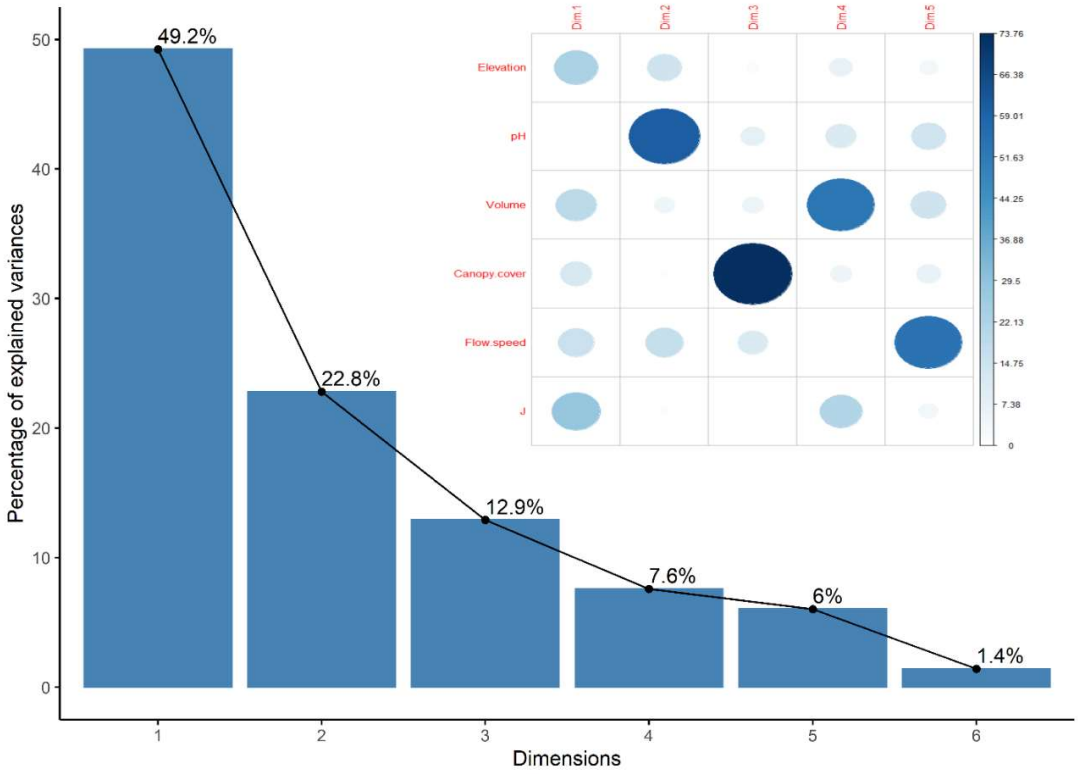

Figure D.6. Scree plot of PCA conducted to analyze specific stream predictors among study streams. The figure also shows the contribution of predictors across dimensions. The scale on the right indicates percent of contribution of each predictor on each dimension, with dark blue showing highest contributions and white the lowest contributions. The climatic predictors included elevation, pH, Community heterogeneity (J), Volume, canopy cover, and flow speed.

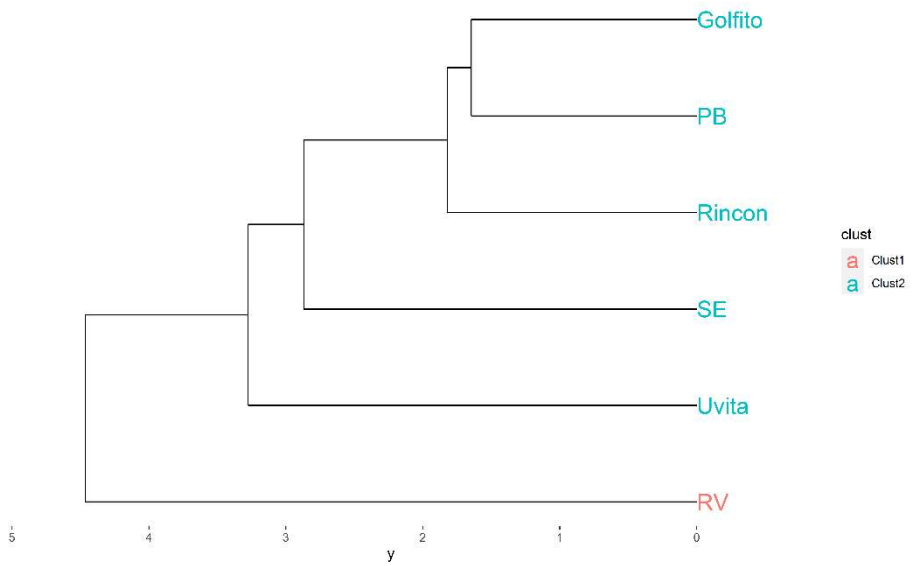

Figure D.7. Cluster analysis shows dissimilarities among study streams. Localities: Golfito, Punta Banco (PB), Rincón de Osa (Rincon), Santa Elena Peninsula (SE), Uvita, and Rincón de la Vieja Volcano (RV).

Figure D.8. An individual of the species *Craugastor amniscola* found in La Pera State Park, Chiapas, México. Photographed by L. Ochoa-Ochoa.

Table D.1. List of the 46 species of robber frogs in Mesoamerica included in our study. For each species, we present the countries of distribution: B = "Belize", C = "Costa Rica, E= "El Salvador", G = "Guatemala", H = "Honduras", M= "Mexico", P = "Panama"; and status according to the International Union of Conservation of Nature (IUCN) as follows: DD = "Data Deficient," LC = "Least Concern," NT = "Near Threatened," VU = "Vulnerable," EN = "Endangered," CR = "Critically Endangered,", "CR (PE)" = Critically Endangered (Possibly Extinct), and EX = "Extinct in the wild". Also shown are environmental vulnerability scores (EVS), a regional vulnerability index that classifies amphibians and reptiles into four levels of risk: 1 = "no immediate risk" (EVS < 3), 2 = "low vulnerability" (EVS of 3–9), 3 = "medium vulnerability" (EVS of 10–13), and 4 = "high vulnerability" (EVS of 14–17). A high EVS indicates species that have restricted ranges, occur in a single life zone, and have a highly derived reproductive mode.

Species name	Common name	Country	Status (IUCN)	EVS
Craugastor punctariolus species series				
Craugastor amniscola	Rivulet rainfrog	M, G	VU	4
*Craugastor anciano**	Corquin robber frog	H	EX	4
Craugastor angelicus	Angel robber frog	C	CR	4
Craugastor aurilegulus	Rio Viejo robber frog	H	VU	4
Craugastor azueroensis	Azuero robber frog	P	EN	4
Craugastor berkenbuschii	Berkenbusch's streamfrog	M	NT	4
Craugastor brocchi	Brocchi's rainfrog	M, G	VU	4
*Craugastor catalinae**	Las Tablas robber frog	C, P	CR (PE)	4
Craugastor charadra	Mountain river rainfrog	G, H	VU	4
Craugastor emleni	Honduras robber frog	H	EN	4
Craugastor escoces	Heredia robber frog	C	CR	4
Craugastor evanesco	Vanishing robber frog	P	CR	4
Craugastor fleischmanni	Fleischmann's robber frog	C	CR	4
Craugastor inachus	Guatemala robber frog	G	CR	4
Craugastor laevissimus	Jungle streamfrog	H, N	EN	3
*Craugastor merendonensis**	San Pedro robber frog	H	CR (PE)	4
*Craugastor obesus**	Caribbean robber frog	C, P	CR	4
*Craugastor olanchano**	Olancho robber frog	H	CR (PE)	4
Craugastor palenque	Palenque robber frog	M	VU	4
Craugastor pechorum	NCN	H	EN	4
Craugastor pelorus	Monstrous rainfrog	M	VU	4
Craugastor pozo	Turipache rainfrog	M	CR (PE)	4
Craugastor psephosypharus	Limestone rainfrog	M,G, B	NT	4
Craugastor punctariolus	Bob's robber frog	P	EN	4
Craugastor ranoides	Lowland robber frog	C, N, P	CR (PE)	4
*Craugastor rhyacobatrachus**	Talamanca robber frog	C, P	CR (PE)	4
Craugastor rivulus	Coban robber frog	G	VU	4
Craugastor rugulosus	Rugulose rainfrog	M	LC	4
Craugastor rupinius	Cliffy streamfrog	M, G, H, E	LC	4
Craugastor sabrinus	Long-legged streamfrog	G, B	NT	4
Craugastor sandersoni	Sanderson's streamfrog	G, B	EN	4

Table D.1. Continued

Craugastor sandersoni	Sanderson's streamfrog	G, B	EN	4
Craugastor taurus	Golfito robber frog	C, P	CR	4
Craugastor vocalis	Taylor's streamfrog	M	LC	4
Craugastor vulcani	San Martin Tuxtla robber frog	M	EN	4
Subgenus *Campbellius* (former *Craugastor milesi* group)				
*Craugastor adamastus**	NCN	G	DD	4
*Craugastor chrysozetetes**	McCranie's robber frog	H	CR (PE)	4
*Craugastor cruzi**	Cruz robber frog	H	CR (PE)	4
*Craugastor daryi**	Dary's robber frog	G	EN	4
*Craugastor epochthidius**	NCN	H	CR (PE)	4
*Craugastor fecundus**	Nombre de Dios robber frog	H	CR (PE)	4
Craugastor matudai	Pine-oak forest robber frog	M, G	VU	4
Craugastor milesi	Miles' robber frog	H	CR	4
*Craugastor myllomyllon**	NCN	G	DD	4
*Craugastor omoaensis**	Omoa robber frog	H	EX	4
*Craugastor saltuarius**	NCN	H	CR (PE)	4
Craugastor stadelmani	Stadelman's robber frog	H	CR	4

***Not detected after 2005 or before**
NCN=No common name

Table D.2. Permutation importance (%) of the eight environmental predictors used to model the range of the chytrid fungus *Batrachochytrium dendrobatidis* in Mesoamerica. The climatic predictors include 'isothermality' (BIO 3), 'max temperature of warmest month' (BIO 5), 'precipitation seasonality' (BIO 15), 'precipitation of warmest quarter' (BIO 18), 'precipitation of coldest quarter' (BIO 19), 'mean monthly potential evapotranspiration of driest quarter' (PETDQ), 'mean monthly potential evapotranspiration of warmest quarter' (PETWQ), and 'sum of mean monthly temperature for months with mean temperature greater than 5 ℃ multiplied by number of days' (gDD5).

Predictor	Permutation importance (%)
BIO 3	1.4
BIO 5	74.1
BIO 15	0.1
BIO 18	0.1
BIO 19	14.2
PETDQ	2.6
PETWQ	0.0
gDD5	7.5

Table D.3. List of countries and localities (181) where the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) has been detected in Mesoamerica. Also included are 41 localities in Colombia, Cuba, and the USA where Bd has been detected, which were used as calibration points for the model.

Country	Localities	Source
Occurrence points		
Belize	1	Kaiser & Pollinger, 2012
Costa Rica	42	Zumbado-Ulate et al., 2019; Zumbado-Ulate et al., 2020
El Salvador	10	Felger et al., 2007; Lawson et al., 2011
Guatemala	4	Mendelson et al., 2014; Rovito et al., 2009
Honduras	6	Kolby et al., 2010; Puschendorf et al., 2006
México	78	Bolom-Huet et al., 2019
Nicaragua	4	García-Roa et al., 2014; Stark et al., 2017
Panamá	36	Brem & Lips, 2008; Kilburn et al., 2010; Rebollar et al., 2014; Richards-Zawacki, 2009; Rodríguez-Brenes et al., 2016; Woodhams et al., 2008
Calibration points		
Colombia	8	Flechas et al., 2017
Cuba	8	Cádiz et al., 2019; Sabino-Pinto et al., 2017
USA	25	Chatfield et al., 2012; Gaertner et al., 2009; Gaertner et al., 2009; Rizkalla, 2010; Rothermel et al., 2008

Table D.4. Permutation importance (%) of six environmental predictors in the four best-fitted species distribution models describing the historical and present range of the dry forest robber frog (subspecies of *Craugastor ranoides* in the Santa Elena Peninsula) and the Golfito robber frog (*C. taurus*). The climatic predictors include 'isothermality' (BIO3), 'temperature annual range' (BIO7), 'precipitation of the wettest month' (BIO13), 'precipitation of driest month (BIO14)', 'minimum temperature of the warmest month' (mTW), and 'mean monthly potential evapotranspiration of driest quarter' (PETDQ).

SDM	BIO 3	BIO 7	BIO 13	BIO 14	mTW	PETDQ
Dry forest robber frog (historic)	26.7	30.0	4.9	19.2	12.0	7.2
Dry forest robber frog (present)	1.5	4.8	0.0	93.4	0.0	0.3
Golfito robber frog (historic)	0.1	16.3	0.4	0.9	0.4	81.9
Golfito robber frog (present)	23.4	0.3	0.4	0.3	8.9	66.7

Table D.5. Average (SD) of six environmental predictors used to describe the abiotic environment of six study stream networks: Santa Elena Peninsula (SE), Rincón de la Vieja Volcano (RV), Punta Banco (PB), Rincón de Osa (Rincón), Golfito, and Uvita. The climatic predictors include 'isothermality' (BIO 3), 'temperature annual range' (BIO 7), 'precipitation of the wettest month' (BIO 13), 'precipitation of driest month (BIO 14)', 'minimum temperature of the warmest month' (mTW), and 'mean monthly potential evapotranspiration of driest quarter' (PETDQ).

Locality	BIO 3	BIO 7	BIO 13	BIO 14	mTW	PETDQ
SE	72.7 (0.5)	11.5 (0.5)	352.4 (26.5)	1.2 (2.6)	209.6 (8.5)	152.2 (4.5)
RV	75.2 (0.7)	11.5 (0.4)	384.2 (25.7)	21.0 (15.2)	173.9 (18.7)	140.9 (8.0)
PB	75.2 (0.7)	12.6 (0.2)	607.8 (100.8)	38.6 (15.7)	210.8 (7.0)	151.3 (1.2)
Rincón	77.8 (0.3)	13.3 (0.2)	508.9 (80.6)	44.3 (24.2)	207.9 (8.9)	154.1 (0.9)
Golfito	77.6 (0.4)	12.7 (0.3)	573.9 (62.6)	44.2 (14.1)	212.9 (8.4)	151.6 (1.0)
Uvita	80.1 (0.9)	13.9 (0.4)	499.5 (66.8)	32.7 (11.7)	187.9 (19.8)	149.6 (5.5)

Table D.6. Biotic and abiotic predictors measured in our six study stream networks: the Santa Elena Peninsula (SE), Rincón de la Vieja Volcano (RV), Punta Banco (PB), Rincón de Osa (Rincón), Golfito, and Uvita. Predictors: elevation, pH, community heterogeneity (J), Volume, canopy cover, and flow speed.

stream network	Predictor						
	Transects	Elevation (m)	pH	J (%)	Volume (m3)	Canopy cover (%)	Flow speed (m/s)
SE	23	63.7	8.0	67.3	3.1	15.1	0.2
RV	14	829.6	8.0	22.6	42.4	77.5	0.6
PB	16	35.1	8.5	60.1	18.0	73.3	0.4
Rincón	16	66.4	8.3	54.6	26.1	43.4	0.5
Golfito	8	29.0	8.1	43.8	10.9	64.6	0.4
Uvita	8	244.5	7.5	64.6	7.9	80.2	0.2

Table D.7. List of species and abundance per species found in linear transects in our six study stream networks: Santa Elena Peninsula (SE), Rincón de la Vieja Volcano (RV), Punta Banco (PB), Rincón de Osa (Rincón), Golfito, and Uvita.

Species	stream network					
	SE	RV	PB	Rincón	Golfito	Uvita
Bolitoglossa lignicolor	0	0	0	0	0	1
Cochranella granulosa	0	0	32	2	6	1
Craugastor bransfordi	0	5	0	0	0	0
Craugastor fitzingeri	2	0	312	71	23	81
Craugastor ranoides	142	0	0	0	0	0
Craugastor stejnegerianus	0	0	4	3	3	3
Craugastor taurus	0	0	263	0	0	0
Diasporus diastema	0	0	0	0	2	0
Duellmanohyla rufioculis	0	1	0	0	0	0
Engystomops pustulosus	8	0	0	0	0	0
Hyalinobatrachium colymbiphyllum	0	0	0	0	3	0
Hypsiboas rosenbergi	0	0	0	4	0	0
Incilius aucoinae	0	0	0	2	47	0
Incilius coniferus	0	0	0	0	4	0
Incilius luetkenii	33	0	0	0	0	0
Leptodactylus fragilis	0	0	0	3	0	0
Leptodactylus melanonotus	62	0	0	0	0	0
Leptodactylus poecolochilus	1	0	0	3	0	0
Leptodactylus savagei	0	0	8	12	1	1
Lithobates forreri	6	0	0	0	0	0
Lithobates warszewitschii	0	165	0	0	0	0
Oophaga granulifera	0	0	0	0	0	58
Pristimantis cerasinus	0	4	0	0	0	0
Pristimantis ridens	0	2	0	0	0	0
Rhaebo haematiticus	0	0	0	0	0	3
Rhinella marina	80	3	45	15	10	1
Sachatamia albomaculata	0	1	0	3	0	17
Silverstoneia flotator	0	0	0	1	0	0
Smilisca baudinii	3	1	0	0	0	0
Smilisca sila	0	0	10	0	0	35
Smilisca sordida	0	0	6	171	268	6
Teratohyla pulverata	0	0	16	12	4	0

Notes: The dominant species in Santa Elena was the dry forest robber frog (subspecies of *Craugastor ranoides* in the Santa Elena Peninsula) comprising 42% of the total records. In both, Punta Banco and Uvita, the dominant species was the Fitzingeri's frog (*Craugastor fitzingeri*) accounting for 45% and 39% of the records, respectively. The amphibian abundance in Rincón de la Vieja Volcano was dominated by the Warszewitsch's frog (*Lithobates warszewitschii*) making up 91% of the total records. In Golfito and Rincón de Osa, the dominant species was the drab tree frog (*Smilisca sordida*) with 72% and 57% of the records respectively.

Table D.8. Evaluation values of candidate models selected to generate our suitability maps for the enzootic range of the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) in Mesoamerica, and the historic and present range (post-decline) of two species of robber frog in Mesoamerica. Feature class (FC), Regularization multiplier (RM), Average test of the area under the curve (AUC mean), and omission rate at minimum training presence (orMTP).

Suitability map	FC	RM	AUC mean	orMTP
Bd in Mesoamerica	LQ	3	0.82	0.05
Dry forest robber frog (historic)	H	0.5	0.98	0.09
Dry forest robber frog (present)	LQHP	0.5	0.99	0.12
Golfito robber frog (historic)	H	3	0.89	0.05
Golfito robber frog (present)	LQH	1	0.99	0.17

Table D.9. Loadings of environmental predictors used to describe the climate and stream microhabitat of six study stream networks. The climatic predictors include 'isothermality' (BIO 3), 'temperature annual range' (BIO 7), 'precipitation of the wettest month' (BIO 13), 'precipitation of driest month (BIO 14)', 'minimum temperature of the warmest month' (mTW), and 'mean monthly potential evapotranspiration of driest quarter' (PETDQ). The microhabitat predictors include elevation, water pH, volume, canopy cover, flow speed, and community heterogeneity (J).

Predictor	PC1	PC2
Climate		
BIO3	0.79	-0.45
BIO7	0.82	-0.26
BI013	0.79	-0.04
BIO14	0.69	-0.31
mTW	0.45	0.86
PETDQ	0.57	0.75
Streams		
Elevation	0.83	-0.45
Water pH	0.03	0.91
Volume	0.77	0.26
Canopy cover	0.61	-0.10
Flow speed	0.69	0.48
J	-0.91	0.13

References

Bolom-Huet, R., Pineda, E., Díaz-Fleischer, F., Muñoz-Alonso, A. L., & Galindo-González, J. (2019). Known and estimated distribution in Mexico of *Batrachochytrium dendrobatidis*, a pathogenic fungus of amphibians. *Biotropica*, 51(5), 731–746.

Brem, F., & Lips, K. (2008). *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Diseases of Aquatic Organisms,* 81, 189–202.

Cádiz, A., Reytor, M. L., Díaz, L. M., Chestnut, T., Burns, J. A., & Amato, G. (2019). The chytrid fungus, *Batrachochytrium dendrobatidis*, is widespread among Cuban amphibians. *EcoHealth*, 16(1), 128–140.

Chatfield, M. W. H., Moler, P., & Richards-Zawacki, C. L. (2012). The amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in fully aquatic salamanders from southeastern North America. *PLOS ONE,* 7(9), e44821.

Felger, J., Enssle, J., Mendez, D., & Speare, R. (2007). Chytridiomycosis in El Salvador. SALAMANDRA-BONN-, 43(2), 122.

Flechas, S. V., Paz, A., Crawford, A. J., Sarmiento, C., Acevedo, A. A., Arboleda, A., Bolívar-García, W., Echeverry-Sandoval, C. L., Franco, R., Mojica, C., Muñoz, A., Palacios-Rodríguez, P., Posso-Terranova, A. M., Quintero-Marín, P., Rueda-Solano, L. A., Castro-Herrera, F., & Amézquita, A. (2017). Current and predicted distribution of the pathogenic fungus *Batrachochytrium dendrobatidis* in Colombia, a hotspot of amphibian biodiversity. *Biotropica,* 49(5), 685–694.

Gaertner, J. P., Gascon, C., Spontak, D., Forstner, M., & Hahn, D. (2009). Seasonal variation in the detection of *Batrachochytrium dendrobatidis* in a Texas population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*). *Herpetological Review,* 40(2), 184–187.

Gaertner, J. P., Forstner, M. R. J., O'Donnell, L., & Hahn, D. (2009). Detection of *Batrachochytrium dendrobatidis* in endemic salamander species from central Texas. *EcoHealth,* 6(1), 20.

García-Roa, R., Sunyer, J., Fernández-Loras, A., & Bosch, J. (2014). First record of *Batrachochytrium dendrobatidis* in Nicaragua. *The Herpetological Journal,* 24(1), 65–68.

Kaiser, K., & Pollinger, J. (2012). *Batrachochytrium dendrobatidis* shows high genetic diversity and ecological niche specificity among haplotypes in the Maya Mountains of Belize. *PLOS ONE,* 7(2), e32113.

Kilburn, V. L., Ibáñez, R., Sanjur, O., Bermingham, E., Suraci, J. P., & Green, D. M. (2010). Ubiquity of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in anuran communities in Panamá. *EcoHealth,* 7(4), 537–548.

Kolby, J. E., Padgett-Flohr, G. E., & Field, R. (2010). Amphibian chytrid fungus *Batrachochytrium dendrobatidis* in Cusuco National Park, Honduras. *Diseases of Aquatic Organisms,* 92(2–3), 245–251.

Lawson, T. D., Jones, M. L., Komar, O., & Welch, A. M. (2011). Prevalence of *Batrachochytrium dendrobatidis* in *Agalychnis moreletii* (Hylidae) of El Salvador and association with larval jaw sheath depigmentation. *Journal of Wildlife Diseases,* 47(3), 544–554.

Mendelson, J. R., Jones, M. E. B., Pessier, A., Toledo, G., Grant, E. H., & Campbell, J. A. (2014). On the timing of an epidemic of amphibian chytridiomycosis in the highlands of Guatemala. South American *Journal of Herpetology,* 9(2), 151–153.

Puschendorf, R., Castañeda, F., & McCranie, J. R. (2006). Chytridiomycosis in wild frogs from Pico Bonito National Park, Honduras. *EcoHealth,* 3(3), 178–181.

Rebollar, E. A., Hughey, M. C., Harris, R. N., Domangue, R. J., Medina, D., Ibáñez, R., & Belden, L. K. (2014). The lethal fungus *Batrachochytrium dendrobatidis* is present in lowland tropical forests of far eastern Panamá. *PLOS ONE,* 9(4), e95484.

Richards-Zawacki, C. L. (2009). Effects of slope and riparian habitat connectivity on gene flow in an endangered Panamanian frog, *Atelopus varius. Diversity and Distributions,* 15(5), 796–806.

Rizkalla, C. (2010). Increasing detections of Batrachochytrium dendrobatidis in central Florida, USA. *Herpetological Review,* 41(2), 180.

Rodríguez-Brenes, S., Rodriguez, D., Ibáñez, R., & Ryan, M. J. (2016). Spread of amphibian chytrid fungus across lowland populations of túngara frogs in Panamá. *PLOS ONE,* 11(5), e0155745.

Rothermel, B. B., Walls, S., Mitchell, J., Dodd, C. J., Irwin, L., Green, D., Vazquez, V., Petranka, J., & Stevenson, D. (2008). Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. *Diseases of Aquatic Organisms,* 82(1), 3–18.

Rovito, S. M., Parra-Olea, G., Vásquez-Almazán, C. R., Papenfuss, T. J., & Wake, D. B. (2009). Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of the National Academy of Sciences,* 106(9), 3231.

Sabino-Pinto, J., Bletz, M. C., Iturriaga, M., Vences, M., & Rodríguez, A. (2017). Low infection prevalence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Chytridiomycetes: Rhizophydiales) in Cuba. *Amphibia-Reptilia,* 38(2), 243–249.

Stark, T., Laurijssens, C., Weterings, M., Martel, A., Köhler, G., & Pasmans, F. (2017). Prevalence of *Batrachochytrium dendrobatidis* in a Nicaraguan, micro-endemic Neotropical salamander, *Bolitoglossa mombachoensis*. *Amphibia-Reptilia,* 38(1), 102–107.

Woodhams, D. C., Kilburn, V. L., Reinert, L. K., Voyles, J., Medina, D., Ibáñez, R., Hyatt, A. D., Boyle, D. G., Pask, J. D., Green, D. M., & Rollins-Smith, L. A. (2008). Chytridiomycosis and amphibian population declines continue to spread eastward in Panama. *EcoHealth,* 5(3), 268–274.

Zumbado-Ulate, H., García-Rodríguez, A., & Searle, C. L. (2020). Species distribution models predict the geographic expansion of an enzootic amphibian pathogen. *Biotropica,* 53(1), 221–321.

Zumbado-Ulate, H., Nelson, K. N., García-Rodríguez, A., Chaves, G., Arias, E., Bolaños, F., Whitfield, S. M., & Searle, C. L. (2019). Endemic infection of *Batrachochytrium dendrobatidis* in Costa Rica: Implications for amphibian conservation at regional and species level. *Diversity,* 11(8), 129.

APPENDIX E. CHAPTER 4 SUPPLEMENTARY MATERIALS 2

Since the early 2000s amphibian monitoring programs led by researcher and conservationists across Mesoamerica have resulted in the rediscovery of a number of amphibian species once thought to be extinct, including several species within the *C. punctariolus* species series and the subgenus *Campbellius* (former *C. milesi* group). To date, we have documented verified reports of remnant populations of the following species: *C. amniscola* (L. Ochoa-Ochoa this study), *C. angelicus* (Kubicki, 2016), *C. aurilegulus* (Puschendorf et al., 2006), *C. azueroensis* (Köhler et al., 2012), *C. escoces* (Jiménez & Alvarado, 2017), *C. emleni* (McCranie et al., 2010), *C. evanesco* (Ryan, et al., 2010b), *C. fleischmanni* (Ryan et al., 2010a), *C. inachus* (Ariano-Sánchez & Campbell, 2018), *C. laevissimus* (Ryan et al., 2013), *C. milesi* (Kolby & McCranie, 2009), *C. palenque* (Percino-Daniel et al. 2014), *C. psephosypharus* (Hernández-Ordóñez et al., 2017), *C. ranoides* (Puschendorf et al., 2005; based on reports of Sasa & Solórzano, 1995), *C. rupinius* (Townsend et al., 2015), *C. stadelmani* (McCranie et al., 2010), and *C. taurus* (Chaves et al., 2014).

Here we present specific actions at the regional and species levels to aid in the conservation of the *C. punctariolus* species series and the subgenus *Campbellius* (former *C. milesi* group). For each species, we propose areas where efforts towards population rediscovery is most likely and therefore where survey effort should be targeted. In general, these areas are highly unexplored and tend to have low levels of anthropogenic disturbance, making them suitable environments for robber frogs.

Craugastor punctariolus species series

1) *Craugastor amniscola* (Rivulet rainfrog). Guatemala: Searches for remnant populations should be targeted in the dry forest remnants surrounding Nentón and Rio Azul rivers at Nentón valley in the department of Huehuetenango. Preservation of the remnants of riparian dry forest all along the seasonal streams in Nentón valley is necessary. Also, the implementation of eco-friendly methods for agrochemical waste disposal is needed to avoid pollution of the seasonal streams and surrounding habitats inhabited by this species. **México:** Searches for remnant populations should be targeted in the Biosphere Reserve of El Ocote, and the state parks of La Pera, Rancho Nuevo, and Cerro Mactumatza, state of Chiapas. Stop the removal of leaf-litter understory vegetation and

illegal selective logging of wood in the forests where has been/still is found. Leaf-litter extraction represents a huge problem for the maintenance of robber frog populations because, in addition to the known removal of organisms at the time of extraction, shelters are lost, and it alters the soil moisture that is essential for robber frogs. Lastly, the species should be included in the NOM-059-SEMARNAT-2010, which is the official list of protected species in México (SEMARNAT, 2010).

2) *Craugastor anciano* (Corquin robber frog). Honduras: Extensive surveys have been carried out in suitable habitat at historical and nearby sites. At the Honduras IUCN Red List workshop in April 2019, experts expressed that is unlikely to be present in any other areas of Honduras and likely already extinct.

3) *Craugastor angelicus* (Angel robber frog). Costa Rica: Searches for remnant populations should be concentrated in Santa Elena de Monteverde, Puntarenas province (Kubicki, 2016) and the vicinity of Cataratas del Ángel, Varablanca, on the Central Volcanic Range (García-Rodríguez et al., 2012; Savage, 2002).

4) *Craugastor aurilegulus* (Río Viejo robber frog). Honduras: All populations above 900 m appear to have disappeared or are in steep decline. Surveys should focus in the remaining habitat patches between known isolated localities in the Nombre de Dios Mountain Range.

5) *Craugastor azueroensis* (Azuero robber frog). Panamá: Searches for remnant populations should target areas within the El Montuoso Forest Reserve (MFR), province of Azuero, where a remnant population was discovered in 2011 (Köhler et al., 2012). A field survey to the area by one of the authors (A. Hertz) in 2018 failed to detect additional individuals. Although the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) was not detected in *C. azueroensis* at MFR in 2011, it was found in high prevalence (46%) in the species *Smilisca sila* in 2018 (A. Hertz, unpublished data). This proves that *Bd* is present on the Azuero Peninsula at least after 2011 and might have affected the Azuero robber frog population at MFR. The Cerro Hoya in Azuero province is another region that should be explored.

6) *Craugastor berkenbuschii* (Berkenbusch's stream frog). México: A monitoring program should be initiated for the remnant populations found along the southern part of the Sierra Madre Oriental Mountain Range in the states of Veracruz, Oaxaca and Puebla (Pineda, 2014). Continued monitoring in the eco-touristic complex "Agua Selva", municipality of Humanguillo, state of Tabasco is needed (Ríos-Rodas et al., 2020).

7) *Craugastor brocchi* (Brocchi's rain frog). Guatemala: A monitoring program should be initiated for the remnant populations found in the cloud forest of Purulhá and Salamá in the department of Baja Verapaz, Guatemala. Searches for remnant populations should be focused in the department of El Quiché. **México:** monitoring program should be initiated for the populations at San Cristobal, the Biosphere Reserve of Nahá, and the state park of La Pera (Muñoz-Alonso, 2010; Ochoa-Ochoa & Whittaker, 2014), state of Chiapas. The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

8) *Craugastor catalinae* (Las Tablas robber frog). Costa Rica: Searches for remnant populations should be concentrated in areas from Las Tablas to Cerro Pando, on the Pacific side of the Talamanca Mountain Range (García-Rodríguez et al., 2012; Savage, 2002). **Panamá:** Continued search efforts are recommended along the adjacent Pacific slope of the Talamanca Mountain Range, between Cerro Pando and Volcán Barú (although several expeditions in search of this species have been conducted between 2008 and 2018 have failed to find the species).

9) *Craugastor charadra* (Mountain river rain frog). Guatemala: Searches for remnant populations should targed the vicinity of La Unión, department of Zacapa. **Honduras:** Search effort should focus in lower elevations below Cusuco National Park, San Pedro Sula, to determine how much further away from the park its populations might extend. Remnant populations might survive within Cerro Azul National Park, department of Copán, so increased survey effort is needed in the area.

10) *Craugastor emleni* (Honduras robber frog). Honduras: Monitoring of remnant populations in the dry forests in and around Tegucigalpa is recommended.

11) *Craugastor escoces* (Heredia robber frog). Costa Rica: Continued monitoring of the remnant population at Juan Castro Blanco National Park, Alajuela is necessary (Jiménez & Alvarado, 2017). Searches for additional remnant populations should be directed along the Pacific slope of Volcán Barva, Bajo la Hondura, and Cascajal, on the Central Volcanic Range from 1200 to 1700 m. **Potential captive-breeding species**.

12) *Craugastor evanesco* (Vanishing robber frog). Panamá: Continued monitoring of remnant populations in the Donoso region, Colón is needed. Studies conducted on five individuals kept in the *ex-situ* facility in the town of Gamboa suggest that this species is highly susceptible to *Bd* (A. Hertz, unpublished data).

13) *Craugastor fleischmanni* (Fleischmann's robber frog). Costa Rica: effort should be focused along the Pacific slope of Volcán Barva, on the Central Volcanic Range (Ryan et al., 2010a) and across the Pacific slope of the Volcan Poás, and Volcan Irazú, on the Central Volcanic Range from 1000 to 2000 m (Savage, 2002).

14) *Craugastor inachus* (Guatemala robber frog). Guatemala: A monitoring program should be initiated for the remnant population at Heloderma Natural Reserve and surrounding dry forest areas in Cabañas, department of Zacapa (Ariano-Sánchez & Campbell 2018). Searches for remnant populations should be concentrated in the riparian dry forest at upper Motagua valley (Guatemala and El Progreso departments). Preservation of the remnants of riparian dry forest all along the seasonal streams in Motagua valley is necessary. Also, the implementation of eco-friendly methods for agrochemical waste disposal is needed to avoid pollution of the seasonal streams and surrounding habitats inhabited by this species. **Potential captive-breeding species.**

15) *Craugastor laevissimus* (Jungle stream frog). Honduras: Continued monitoring of populations in the department of Olancho and Yoro, and Cerro Guanacuare, department of Choluteca is necessary (Lovich et al., 2010). **Nicaragua:** Continued monitoring of populations across the Caribbean versant (North Caribbean Coast Autonomous Region, South Caribbean Coast Autonomous Region, and Río San Juan provinces) is necessary.

16) *Craugastor merendonensis* (San Pedro robber frog). Honduras: Searches for a remnant population should be focused at the type locality, in the lower Santa Ana Canyon on the Atlantic slope near San Pedro Sula, between 150-200 m, as well as above 250 m where the habitat is better protected, although these frogs have not previously been found above 200 m.

17) *Craugastor obesus* (Caribbean robber frog). Costa Rica: effort should be carried out along the Atlantic slope of Cerro Uthyum and Kamuk, on the Costa Rican side of the Talamanca Mountain Range from 400 to 1700 (García-Rodríguez et al., 2012; Savage, 2002). **Panamá:** Continued searches for remnant populations are recommend, specifically along the Caribbean slopes of western Panama, in the protected areas La Amistad International Park, Palo Seco Forest, and Fortuna Forest Reserve, province of Chiriquí.

18) *Craugastor olanchano* (Olancho robber frog). Honduras: Searches for a remnant population should be concentrated in Refugio de Vida Silvestre El Armado, department of Olancho.

19) *Craugastor palenque* (Palenque robber frog). México: A monitoring program should be initiated for the existing populations in the Palenque and new populations in Montes Azules reserves, state of Chiapas (Percino-Daniel et al. 2014; Hernández-Ordóñez et al. 2019). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

20) *Craugastor pechorum* (No common name). Honduras: Continued monitoring is needed for populations occurring on the Atlantic versant of the north-eastern and eastern portions of the departments of Olancho, Colón, and Gracias a Dios.

21) *Craugastor pelorus* (Monstrous rain frog). México: A monitoring program should be initiated for the populations at the Biosphere Reserve of El Ocote and Tenejapa, state of Chiapas (Muñoz-Alonso, 2010). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

22) *Craugastor pozo* (Turipache rain frog). México: Search effort for remnant populations should be targeted (AmphibiaWeb, 2020). A monitoring program should be initiated for the populations at the Biosphere Reserve of El Ocote (Luna-Reyes et al., 2017), and the state park of La Pera, state of Chiapas (Muñoz-Alonso, 2010). Stop the removal of leaf-litter understory vegetation and illegal selective logging of wood in the forests where has been/still is found. The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

23) *Craugastor psephosypharus* (Limestone rain frog). Belize: Continued monitoring of populations across the Maya Forest is recommended. **Guatemala:** A monitoring program should be initiated for the remnant population in Cerro San Gil, department of Izabal. Searches for remnant populations should be concentrated in the Sierra Chinajá (department of Alta Verapaz) and Sierra Santa Cruz (department of Izabal). **México:** Continued monitoring of the population recently described in the Lacandona region of Chiapas, is necessary (Hernández-Ordóñez et al., 2017).

24) *Craugastor punctariolus* (Bob's robber frog). Panamá: Continued searching for remnant populations between Fortuna Forest Reserve, province of Chiriquí, and Altos de Campana National Park, province of Panamá is recommended (although extensive work between 2008 and 2018 has failed to detect the species). Studies conducted on one individual kept in the *ex-situ* facility in in the town of Gamboa suggest that this species is highly susceptible to *Bd* (A. Hertz, unpublished data).

25) *Craugastor ranoides* (Lowland robber frog). Costa Rica: Continued monitoring of remnant populations in the Santa Elena Peninsula, Guanacaste province is desired (Zumbado-Ulate & Willink, 2011). Search effort for remnant populations should target the dry and semidry areas across the Central Pacific (Puntarenas Province), and Nicoya Peninsula Mountain Range, Guanacaste Province. **Nicaragua:** Searches should be conducted in San Juan del Sur, especially in the dry ecosystems and tributaries of San Juan river. **Panamá:** Search effort is needed along the Caribbean coast in Bocas del Toro province. **Potential captive-breeding species.**

26) *Craugastor rhyacobatrachus* (Talamanca robber frog). Costa Rica: Searches for remnant populations should be focused along the Pacific slope of Cerro Chirripó, on the Costa Rican side of the Talamanca Mountain Range from 400 to1700 m (García-Rodríguez et al., 2012; Savage, 2002). **Panamá:** Continued searching for remnant populations in the adjacent Pacific slope of the Talamanca Mountain Range, between Cerro Pando and Volcán Barú is necessary (although several expeditions in search of this species have been conducted between 2008 and 2018 have failed to find the species).

27) *Craugastor rivulus* (Coban robber frog). Guatemala: A monitoring program should be initiated for remnant populations at San Cristobal Verapaz, department of Alta Verapaz. Search effort should be concentrated in areas surrounding San Pedro Carchá (department of Alta Verapaz) and Chajul (department of El Quiché).

28) *Craugastor rugulosus* (Rugulose rain frog). México: A monitoring program should be initiated for the existing populations in the Mixteca Baja Poblana reserve in Puebla (Hernández-Ayotla, 2019) and new populations found in Jocotepec, Cerro San Juan, in Oaxaca (Mata-Silva et al., 2019). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

29) *Craugastor rupinius* (Cliffy stream frog). El Salvador: Continued monitoring of populations across the country is recommend (Greenbaum & Komar, 2005). **Guatemala:** A monitoring program should be initiated for the remnant populations at the network of private natural reserves in the western portion of the volcanic chain in southwestern Guatemala and searches for other remnant populations should focus in the eastern portion of the volcanic chain in southeastern Guatemala. **Honduras:** Monitoring efforts are needed for the recently rediscovered population at Cerro Montecristo, within the boundaries of Montecristo-Trinio National Park, department of Ocotepeque (Townsend et al., 2015). **México:** Continued monitoring of remnant populations in the state of Chiapas is desirable (Muñoz-Alonso, 2010). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

30) *Craugastor sabrinus* (Long-legged streamfrog). Belize: Continued population monitoring should be carried out across the Maya Forest. **Guatemala:** A monitoring program should be initiated for the remnant population in Cerro San Gil, Izabal, Guatemala. Searches for remnant populations should target the Eastern portion of Sierra de las Minas and Sierra de Santa Cruz.

31) *Craugastor sandersoni* (Sanderson's streamfrog). Belize: Continued population monitoring should be carried out across the Maya Forest. **Guatemala:** A monitoring program should be initiated for the remnant population in Cerro San Gil, Izabal, Guatemala. Searches for remnant populations should target the Sierra de Xucaneb, and at the eastern portion of Sierra de las Minas.

32) *Craugastor taurus* (Golfito robber frog). Costa Rica: Continued monitoring of the remnant populations at Punta Banco, Costa Rica is needed (Chaves et al., 2014). Searches for remnant populations should be focused in the semidry areas of Drake, Osa Peninsula in Costa Rica, and the foothills of the southern side of the Talamanca Mountain Range (across the counties of Golfito and Corredores). **Panamá:** The occurrence of the species in Puerto Armuelles requires confirmation. Searches for remnant populations should concentrate on the Panamanian side of Talamanca Mountain Range, across the province of Chiriquí, and along the Pacific coast, from Punta Burica to the origin of Azuero Peninsula.

33) *Craugastor vocalis* (Taylor's stream frog). México: A monitoring program should be initiated for the populations discovered in the states of Durango, Jalisco and Nayarit (Luja et al., 2014; Valdés-Lares et al., 2013). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

34) *Craugastor vulcani* (San Martin Tuxtla robber frog). México: A monitoring program should be initiated for remnant populations in the Biological Station of Los Tuxtlas, of the National Autonomous University of Mexico, state of Veracruz (UNAM; Pineda & Rodríguez-Mendoza 2010). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

Subgenus *Campbellius* (former *Craugastor milesi* species series)

35) *Craugastor adamastus* (No common name). Guatemala: Searches for remnant populations should be focused at the eastern portion of Sierra de las Minas Mountain Range. It is unlikely to be present in any other areas of Guatemala and there are very strong reasons to believe it is extinct.

36) *Craugastor chrysozetetes* (McCranie's robber frog). Honduras: Remnant populations might be found inside the core zone of Pico Bonito National Park (department of Atlántida), and especially on the northern slopes of the Nombre de Dios Mountain Range, where suitable habitat remains.

37) *Craugastor cruzi* (Cruz robber frog). Honduras: Search effort for remnant populations should be concentrated inside the core zone of Pico Bonito National Park, department of Atlántida.

38) *Craugastor daryi* (Dary's robber frog). Guatemala: Searches for remnant populations should be conducted at Tamahú (department of Alta Verapaz) and the western portion of Sierra de las Minas.

39) *Craugastor epochthidius* (No common name). Honduras: Surveys for remnant populations should be carried out within Río Plátano Biosphere Reserve (departments of Olancho, Colón, and Gracias a Dios) and Sierra de Agalta National Park (Department of Olancho).

40) *Craugastor fecundus* (Nombre de Dios robber frog). Honduras: Search effort for remnant populations should be focused in the core zone of Pico Bonito National Park (department of Atlántida) and Capiro y Calentura National Park (department of Colón).

41) *Craugastor matudai* (Pine-oak forest robber frog). Guatemala: Searches for remnant populations should be focused near San Marcos, and San Pedro, department of Sacatepéquez. **México:** Continued monitoring of remnant populations in Tacana, Monte Ovando, and Tres Picos, state of Chiapas, is necessary (Muñoz-Alonso, 2010). The species should be included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010).

42) *Craugastor milesi* (Miles' robber frog). Honduras: This species was previously declared extinct at both known sites in Cusuco National Park (San Pedro Sula) and Cerro Azul National Park (department of Copán), but has been rediscovered in Cusuco National Park. Additional efforts to search for a remnant population in Cerro Azul National Park should be conducted.

43) *Craugastor myllomyllon* (No common name). Guatemala: Searches for remnant populations should be concentrated at the Sierra de Xucaneb. It is unlikely to be present in any other areas of Guatemala and there are very strong reasons to believe it is extinct.

44) *Craugastor omoaensis* (Omoa robber frog). Honduras: Extensive surveys have been carried out in suitable habitat at historical and nearby sites during the appropriate season within the known range. It is unlikely to be present in any other areas of Honduras and there are very strong reasons to believe it is extinct.

45) *Craugastor saltuarius* (No common name). Honduras: Additional surveys for remnant populations should be performed at Cerro Corre viento, in the department of Choluteca and also in remote areas of Texiguat Wildlife Refuge, department of Atlántida.

46) *Craugastor stadelmani* (Stadelman's robber frog). Honduras: Searches for remnant populations are needed at all three known localities: La Muralla National Park (department of Olancho), Pico Pijol National Park (department of Yoro), and Texiguat Wildlife Refuge (department of Atlántida). It is possible that it might already be extinct range-wide.

References

AmphibiaWeb. (2020). AmphibiaWeb. https://amphibiaweb.org

Ariano-Sánchez, D., & Campbell, J. A. (2018). A new species of *Rhadinella* (Serpentes: Dipsadidae) from the dry forest of Motagua Valley, Guatemala. *Zootaxa,* 442(2), 338–344.

Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V. T., & Ryan, M. J. (2014). Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Tropical Conservation Science,* 7(4), 628–638.

García-Rodríguez, A., Chaves, G., Benavides-Varela, C., & Puschendorf, R. (2012). Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions,* 18(2), 204–212.

Greenbaum, E., & Komar, O. (2005). Threat assessment and conservation prioritization of the herpetofauna of El Salvador. *Biodiversity & Conservation,* 14(10), 2377–2395.

Hernández-Ayotla, L. I. (2019). Diversidad del ensamble de anfibios y reptiles en la Mixteca Baja Poblana [Licenciatura]. Benemérita Universidad Autónoma de Puebla.

Hernández-Ordóñez, O., Cervantes-López, M. de J., González-Hernández, A., Andresen, E., & Reynoso, V. H. (2017). First record of the limestone rainfrog *Craugastor psephosypharus* (Amphibia: Anura: Craugastoridae) in Mexico. *Revista Mexicana de Biodiversidad,* 88(1), 260–264.

Hernández-Ordóñez, O., Santos, B. A., Pyron, R. A., Arroyo-Rodríguez, V., Urbina-Cardona, J. N., Martínez-Ramos, M., Parra-Olea, G., & Reynoso, V. H. (2019). Species sorting and mass effect along forest succession: Evidence from taxonomic, functional, and phylogenetic diversity of amphibian communities. Ecology and Evolution, 9(9), 5206–5218.

Jiménez, R., & Alvarado, G. (2017). *Craugastor escoces* (Anura: Craugastoridae) reappears after 30 years: rediscovery of an "extinct" Neotropical frog. *Amphibia-Reptilia,* 38(2), 257–259.

Köhler, G., Batista, A., Carrizo, A., & Hertz, A. (2012). Field notes on *Craugastor azueroensis* (Savage, 1975) (Amphibia: Anura: Craugastoridae). *Herpetological Notes*, 5, 157.

Kolby, J. E., & McCranie, J. R. (2009). Discovery of a surviving population of the montane streamside frog *Craugastor milesi* (Schmidt). *Herpetological Review*, 40(3), 282.

Kubicki, B. (2016). Rediscovery of the Critically Endangered frog, Craugastor angelicus, in Costa Rica. Mesoamerican Herpetology, 3(4), 1070–1071.

Lovich, R., Akre, T., Ryan, M. J., Nuñez, S., Cruz, G., Borjas, G., Scott, N., Flores, S., del Cid, W., Flores, A., Luque-Montes, I. R., Rodriguez, C., & Ford, R. (2010). New Herpetofaunal records from southern Honduras. *Herpetological Review,* 41(1), 112.

Luja, V. H., Ponce-Campos, P., Ahumada-Carrillo, I., & Figueroa-Esquivel, E. (2014). Checklist of amphibians of Nayarit, western Mexico. *Check List,* 10, 1336.

Luna-Reyes, R., Cundapí-Pérez, C., Pérez-López, P. E., López-Villafuerte, A., Rodríguez-Reyes, M. Á., & Luna-Sánchez, J. A. (2017). Riqueza y diversidad de anfibios y reptiles en Nuevo San Juan Chamula y Veinte Casas, Reserva de la Biosfera Selva El Ocote. In Vulnerabilidad social y biológica ante el cambio climático en la Reserva de la Biósfera Selva El Ocote (pp. 356–393). Ecosur.

Mata-Silva, V., Rocha, A., Mata-González, S., Ramirez-Bautista, A., Berriozabal-Islas, C., García-Padilla, E., Johnson, J. D., & Wilson, L. D. (2019). First municipality records and additional information on the herpetofauna of Oaxaca, Mexico. *Herpetological Review*, 50(3), 533–536.

McCranie, J. R., Orellana, L. V., & Himes, J. G. (2010). Rediscovery of two Honduran endemic streamside frogs, *Craugastor emleni* (Dunn) and *Craugastor stadelmani* (Schmidt). *FrogLog,* 94, 12–15.

Muñoz-Alonso, A. (2010). Riqueza, diversidad y estatus de los anfibios amenazados en el sureste de México; una evaluación para determinar las posibles causas de la declinación de sus poblaciones (p. 55). Ecosur.

Ochoa-Ochoa, L. M., & Whittaker, R. J. (2014). Spatial and temporal variation in amphibian metacommunity structure in Chiapas, Mexico. *Journal of Tropical Ecology,* 30(6), 537–549.

Pineda, E. (2014). Situación actual de ocho especies de anfibios en categoría de riesgo en Veracruz (p. 18) [Informe final SNIBCONABIO proyecto No. HK006]. Instituto de Ecología A. C.

Pineda, E., & Rodríguez-Mendoza, C. A. (2010). Distribución y abundancia de Craugastor vulcani: Una especie de rana en riesgo de Los Tuxtlas, Veracruz, México. *Revista Mexicana de Biodiversidad,* 81, 133–141.

Puschendorf, R., Castañeda, F., & McCranie, J. R. (2006). Chytridiomycosis in wild frogs from Pico Bonito National Park, Honduras. *EcoHealth,* 3(3), 178–181.

Puschendorf, R., Chaves-Cordero, G. A., Crawford, A. J., & Brooks, D. R. (2005). *Eleutherodactylus ranoides* (NCN). Dry forest population, refuge from decline? *Herpetological Review*, 36(1), 53.

Ríos-Rodas, L., Zenteno Ruíz, C. E., Pérez de la Cruz, M., Arriaga-Weiss, S. L., Jiménez-Pérez, N. del C., & Bustos-Zagal, M. G. (2020). Anfibios riparios en dos ecosistemas tropicales del sureste de México. *Ecosistemas,* 29(3), 2098.

Percino-Daniel, R., García del Valle, Y., & Campbell, J. A. (2014). Rediscovery and additional records for *Craugastor palenque* (Anura: Craugastoridae) from the archaeological Mayan site of Palenque, Chiapas, Mexico. *The Southwestern Naturalist,* 59(1), 139–141.

Ryan, M. J., Poe, S., Latella, I. M., & Davis, J. (2013). *Craugastor laevissimus* (NCN). New population. *Herpetological Review,* 44, 471.

Ryan, Mason J., Bolaños, F., & Chaves, G. (2010a). Museums help prioritize conservation goals. *Science,* 1272, 1273.

Ryan, Mason J., Savage, J. M., Lips, K. R., & Giermakowski, J. T. (2010b). A New Species of the *Craugastor rugulosus* Series (Anura: Craugastoridae) from West-Central Panama. *Copeia,* 2010(3), 405–409.

Sasa, M., & Solórzano, A. (1995). The reptiles and amphibians of Santa Rosa National Park, Costa Rica, with comments about the herpetofauna of xerophytic areas. *Herpetological Natural History*, 3(2), 113–126.

Savage, J. M. (2002). The Amphibians and Reptiles of Costa Rica: A Herpetofauna Between Two Continents, Between Two Seas. The University of Chicago Press.

SEMARNAT. (2010). NOM-059-SEMARNAT-2010. Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio. Lista de especies en riesgo. SEMARNAT.

Townsend, J. H., Firneno Jr, T. J., Escoto, D. L., Flores-Girón, E. A., Medina-Flores, M., & Oyuela, O. W. (2015). The first record of the streamside frog *Craugastor rupinius* (Anura: Craugastoridae) in Honduras, confirmed by 16S DNA barcoding. *Alytes,* 32(3), 55.

Valdés-Lares, R., Martin-Muñoz De Cote, G. A., Muñiz, R., & Santos-Barrera, G. (2013). New distributional records for amphibians from Durango, Mexico. *Herpetological Review,* 44(4), 646–649.

Zumbado-Ulate, H., & Willink, B. (2011). *Craugastor ranoides* (NCN) geographic distribution. *Herpetological Review,* 42, 236.