




## Current and predicted distribution of the pathogenic fungus *Batrachochytrium dendrobatidis* in Colombia, a hotspot of amphibian biodiversity

Sandra V. Flechas<sup>1,12,\*</sup> , Andrea Paz<sup>1,2,\*</sup>, Andrew J. Crawford<sup>1,3,4</sup>, Carolina Sarmiento<sup>1,3</sup>, Aldemar A. Acevedo<sup>5</sup>, Alejandro Arboleda<sup>6,†</sup>, Wilmar Bolívar-García<sup>7</sup>, Claudia L. Echeverry-Sandoval<sup>8</sup>, Rosmery Franco<sup>5</sup>, Cindy Mojica<sup>7</sup>, Amanda Muñoz<sup>6</sup>, Pablo Palacios-Rodríguez<sup>1,8</sup>, Andrés M. Posso-Terranova<sup>9</sup>, Paulina Quintero-Marín<sup>1</sup>, Luis A. Rueda-Solano<sup>10</sup>, Fernando Castro-Herrera<sup>11</sup>, and Adolfo Amézquita<sup>1</sup>

<sup>1</sup> Department of Biological Sciences, Universidad de los Andes, Bogotá, AA 4976, Colombia

<sup>2</sup> Department of Biology, Graduate Center, City University of New York, New York, NY 10016, USA

<sup>3</sup> Smithsonian Tropical Research Institute, Apartado, 0843-03092, Panama City, Republic of Panama

<sup>4</sup> Círculo Herpetológico de Panamá, Apartado, 0824-00122, Panama City, Republic of Panama

<sup>5</sup> Grupo de Investigación en Ecología y Biogeografía (GIEB), Universidad de Pamplona, Pamplona, Colombia

<sup>6</sup> Universidad del Cauca, Popayán, Colombia

<sup>7</sup> Grupo de Investigación en Ecología Animal, Universidad del Valle, Cali, Colombia

<sup>8</sup> Universidad Tecnológica del Chocó 'Diego Luis Córdoba', Quibdó, Colombia

<sup>9</sup> Universidad Nacional de Colombia, Sede Palmira, Colombia

<sup>10</sup> Universidad del Magdalena, Santa Marta, Colombia

<sup>11</sup> Departamento de Ciencias Fisiológicas, Facultad de Salud, Universidad del Valle, Cali, Colombia

### ABSTRACT

Global amphibian declines have been attributed to several factors including the chytrid fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), that infects hosts' skin and causes death by inhibiting immune response and impairing osmoregulatory function. Here, we integrate extensive new field data with previously published locality records of *Bd* in Colombia, a megadiverse and environmentally heterogeneous country in northwestern South America, to determine the relative importance of environmental variables and reproductive mode for predicting the risk of *Bd* infection in amphibians. We surveyed 81 localities across Colombia and sampled 2876 individual amphibians belonging to 14 taxonomic families. Through a combination of end-point PCR and real-time PCR analyses, *Bd* was detected in 338 individuals (12%) representing 43 localities (53%) distributed from sea level to 3200 m. We found that annual mean temperature and variables related with seasonality in precipitation and temperature appeared to define the most suitable areas for the establishment of the pathogen. In addition, prevalence of infection appeared to be higher in species with a terrestrial reproductive mode. Our study provides the first large-scale study of the current and potential distribution of *Bd* in the biodiversity hotspot centered on Colombia. We hope the newly provided information on the extent of the distribution of the pathogen and the potential areas where *Bd* may impact the amphibian fauna will inform decision making by environmental authorities and future conservation action.

Abstract in Spanish is available with online material.

**Key words:** amphibians; chytridiomycosis; habitat suitability; species distribution model; temperature; water dependence.

AMPHIBIANS HAVE EXPERIENCED DRAMATIC DECLINES IN MANY AREAS OF THE GLOBE, WITH AT LEAST TWO HUNDRED SPECIES SUFFERING MAJOR POPULATION DECREASES (Stuart *et al.* 2004, Hoffman *et al.* 2010). Population declines and extinctions have been attributed to several factors including habitat loss, increased ultraviolet radiation, chemical pollution, overexploitation, and epizootic diseases (Alford & Richards 1999, Blaustein & Kiesecker 2002, Collins &

Storfer 2003, Hof *et al.* 2011, Martel *et al.* 2013, Price *et al.* 2014, Tarvin *et al.* 2014). The latter threat arises principally from emerging fungal and viral pathogens, including *Batrachochytrium dendrobatidis* (hereafter, *Bd*), *B. salamandrorans*, and viruses of the genus *Ranavirus*. Although chytrid fungi rarely attack vertebrates, *Bd* is the known etiological agent of the amphibian disease, chytridiomycosis (Berger *et al.* 1998, Daszak *et al.* 2003, Fisher *et al.* 2012). Single-celled *Bd* attacks the superficial keratinized areas of the skin of juvenile and adult amphibians, and the mouthparts of tadpoles (Longcore *et al.* 1999). *Bd* then reproduces asexually inside dermal skin cells, impairing the

Received 25 May 2016; revision accepted 22 March 2017.

\*These authors contributed equally to this work.

†This is a posthumous publication.

<sup>12</sup>Corresponding author; e-mail: vickyflechas@gmail.com

osmoregulatory function of the skin (Voyles *et al.* 2009, Campbell *et al.* 2012) and affecting the host's immune response (Fites *et al.* 2013).

*Bd*-related declines were first noted in tropical areas (Lips *et al.* 2006), especially montane sites (Bradford 1991, Wake 1991, Laurance *et al.* 1996, Berger *et al.* 1998, Lips 1998). *Bd* thrives at cool temperatures between 17 and 25°C but cannot tolerate desiccation at any point in its life cycle (Piotrowski *et al.* 2004). Both characteristics may explain, in part, why chytridiomycosis appears particularly problematic for mid- to high-elevation populations (Daszak *et al.* 1999) and for riparian species (Daszak *et al.* 1999, Lips *et al.* 2003, Woodhams & Alford 2005, Kriger & Hero 2007). In addition, amphibians inhabiting higher elevations may be more susceptible to chytridiomycosis due to reduced or suppressed immune responses at lower ambient temperatures, where thermoregulatory opportunities are restricted (Carey *et al.* 1999). Although most research on amphibian declines has focused on montane environments, a growing body of evidence suggests that *Bd* may also be widespread in lowlands (Heyer *et al.* 1988, Kilburn *et al.* 2010, Flechas *et al.* 2015) and causing population declines (Whitfield *et al.* 2007, 2012). However, these events are rarely observed in tropical lowlands.

Two main approaches have been used to understand the presence and prevalence of *Bd* around the globe. The first approach seeks to explain the taxonomic variation among hosts in *Bd* prevalence and in amphibian declines by looking at host-specific traits to understand why some amphibians readily succumb to chytridiomycosis, while other species may act as asymptomatic carriers (*i.e.*, reservoirs) of the pathogen. For example, close association with water bodies has been linked with increased vulnerability to the disease (Lips *et al.* 2003, Hero *et al.* 2005, Bielby *et al.* 2008). In addition, various studies suggest that variation among amphibian hosts in their response to *Bd* infection has a phylogenetic component (Hero *et al.* 2005, Bielby *et al.* 2008, Guayasamin *et al.* 2014). Within a single community or locality, however, declines may be random with respect to shared evolutionary history among host species, and affect a subset of species within each taxonomic family (Crawford *et al.* 2010). Thus, the phylogenetic patterns may be confounded with geographic distribution or environmental variables.

The second approach to understanding the distribution of *Bd* and chytridiomycosis seeks to explain spatial variation in *Bd* prevalence by considering climatic parameters to predict the probability of *Bd* presence through abiotic climatic envelope modeling (Peterson 2001). Previous studies have shown that temperature may account for a large fraction of among-locality variation in the prevalence of the pathogen (Puschendorf *et al.* 2009, Rohr & Raffel 2010), as expected given the thermal biology of *Bd* (Piotrowski *et al.* 2004, Woodhams *et al.* 2008), *i.e.*, cooler temperatures promote the growth of the pathogen to the detriment of the host (Russell *et al.* 2010). In the field, however, *Bd* may be affected by the interaction of multiple environmental factors, especially in climatically heterogeneous regions.

In Colombia, recent reports have begun to shed light upon the extent of *Bd* prevalence, but these studies have been restricted

in geographical scope (Ruiz & Rueda-Almonacid 2008, Velásquez-Escobar *et al.* 2008, Urbina & Galeano 2011, Flechas *et al.* 2012, 2015, Vasquez-Ochoa *et al.* 2012, Acevedo *et al.* 2016a,b). Colombia harbors the second most diverse amphibian fauna in the world (AmphibiaWeb 2016) and is also host to the recently evolved strain of *Bd* known as the global panzootic lineage (GPL) (Flechas *et al.* 2013, Rosenblum *et al.* 2013). Despite these recent reports, little is yet known about the geographic distribution of the pathogen or the spatial extent of disease. Given these gaps in our knowledge, we have undertaken a broad survey of *Bd* in wild amphibian populations using non-invasive molecular techniques. Moreover, because differences in susceptibility may be due to characteristics of the host or of the environment, we used natural history traits of the host along with spatial climatic parameters to predict the probability of *Bd* presence. Specifically, we tested (1) whether amphibian species breeding in close association with water are more likely to carry *Bd*, and (2) whether climatic variables influence the probability of *Bd* occurrence. The performance of predictive models is known to depend on the natural range of variation of the predictor variables. The extreme environmental heterogeneity presented by our sampling sites across Colombia, including lowland sites, should thus improve the chances of identifying biologically relevant predictors for *Bd* presence in the country.

## METHODS

**FIELD SAMPLING.**—In this study, six research teams obtained 2876 swab samples from 81 localities (Fig. 1, Table S1), particularly, in Andean areas, where the majority of amphibian species are found (Lynch *et al.* 1997). Swabbing protocols in the field were standardized among the surveying teams following the protocol by Hyatt *et al.* (2007). To determine infection status, adult and post-metamorphic juvenile amphibians were sampled during diurnal and nocturnal surveys between June 2007 and February 2012 using visual and acoustic searches. Whenever possible, several microhabitats were surveyed within each locality, including streams, ponds, open areas, and forests. To avoid cross contamination, animals were collected using clean, decontaminated equipment, individually handled with fresh disposable gloves, and placed in new plastic bags prior to obtaining skin samples. Each animal was sampled by running a synthetic cotton swab (MW100; Medical Wire & Equipment, Corsham, UK) ten times over the drink patch, the inner thigh area, and the plantar surface of the hind feet webbing for a total of 50 strokes (Hyatt *et al.* 2007). Skin swabs were preserved in the field either in 96 percent ethanol or dry and then stored at  $-20^{\circ}\text{C}$  until processing. To avoid repeated sampling of the same individual, all frogs were left in plastic bags for up to 6h during sample collection and released upon completion of each local survey. At eight localities, a subsample of frogs was collected as voucher specimens and tissue samples to confirm field identifications through morphological and molecular taxonomic procedures. Results of DNA barcoding surveys are published in Guarnizo *et al.* (2015).

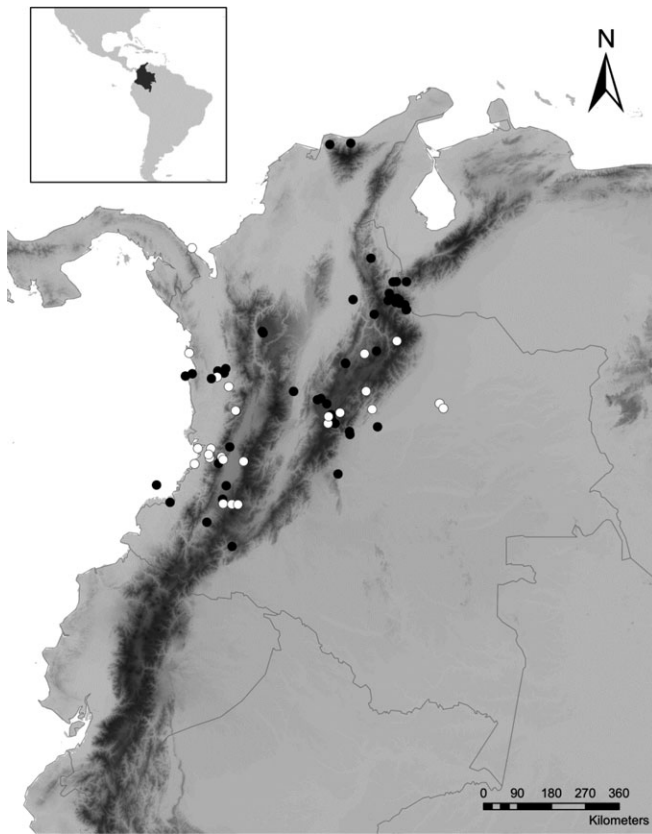


FIGURE 1. Sampled localities in Colombia. Black dots represent localities that included at least one amphibian that tested positive for *Batrachochytrium dendrobatidis* (*Bd*). White dots represent sampled localities where *Bd* was not detected. Eighty-one localities were surveyed and *Bd* was detected in 43 localities in altitudes ranging from 0 to 3200 m.

**DIAGNOSING *Bd* INFECTION.**—Our study integrated efforts from several research groups, which led to the use of two techniques to diagnose *Bd* infection: end-point PCR and real-time PCR (*i.e.*, quantitative or qPCR). Most of the samples were analyzed at Universidad de los Andes in Bogotá using either diagnostic techniques. A total of 94 samples from Chocó were diagnosed at Universidad Nacional de Colombia sede Palmira using qPCR. We ran end-point PCR tests on samples from 13 localities above 1000 m in elevation and from 23 localities under 1000 m. We conducted qPCR on samples from 29 localities above 1000 m in elevation and on samples representing 16 localities under 1000 m. For end-point PCR, DNA was extracted using GeneReleaser<sup>®</sup> (Bioventures Inc., Murfreesboro, Tennessee, U.S.A.). We used the following primers developed by Annis *et al.* (2004) to amplify the ITS1-ITS2 region in *Bd*: Bd1a (5′-CAGTGTGC CATATGTCACG-3′) and Bd2a (5′-CATGGTTCATATCTGTC CAG-3′). Amplifications were performed using an MJ Research Peltier Thermal Cycler (PTC-200; Hercules, California, U.S.A.), as follows: an initial 2 min denaturation at 95°C, followed by 35 cycles of DNA amplification (45 sec at 95°C, 45 sec at 55°C, and 1 min at 72°C), and a final extension at 72°C for 10 min. Each reaction consisted of 0.5 μL of each primer (1 μM), 3.0 μL

of doubly distilled DNA-free water, 6 μL of GoTaq<sup>®</sup> Green Master Mix (1X; Promega, Madison, Wisconsin, U.S.A.), and 2 μL of the DNA extraction. Amplified fragments were separated by electrophoresis in 1 percent agarose gels. For qPCR, we followed the recommendations of Hyatt *et al.* (2007) and Boyle *et al.* (2004). DNA was extracted using PrepMan Ultra (ThermoFisher Scientific, Waltham, Massachusetts, U.S.A.). Each real-time PCR reaction consisted of 0.8 μL (18 μM) of each primer ITS1 (5′-CCTTGATATAATACAGTGTGCCATATGTC-3′) and 5.8S (5′-AGCCAAGAGATCCGTTGTCAAA-3′), 10 μL of TaqMan, 1.0 μL (5 μM) of MGB Probe, 2.0 μL Exo IPC mix, 0.4 μL Exo IPC DNA (ThermoFisher Scientific), and 5.0 μL of the DNA extract. Amplification conditions consisted of 2 min at 50°C, 15 min at 95°C followed by 50 cycles of 15 sec at 95°C, and 1 min 60°C. Products were run on a BIO-RAD CFX96 PCR machine (Hercules, California, U.S.A.) and a BIO-RAD DNA Engine with CHROMO 4 Alpha block assembly.

**REPRODUCTIVE MODES AND *Bd* PRESENCE.**—The degree of water dependence during an amphibian’s lifetime varies with the reproductive mode of the species, and those with aquatic reproductive strategies appear to be more susceptible to decline (Bielby *et al.* 2008). Following Crump (1974, 2015) and Duellman and Trueb (1986), we categorized our study species into four reproductive modes: (1) eggs and feeding tadpoles living in lentic water ( $N = 1164$  individuals in 49 species); (2) eggs in foam nests with aquatic feeding tadpoles ( $N = 179$  individuals in 19 species); (3) terrestrial eggs and aquatic feeding tadpoles ( $N = 554$  individuals in 46 species); and (4) terrestrial eggs that hatch into froglets ( $N = 979$  individuals in 98 species). Prior to performing a chi-square test, we reclassified these reproductive modes in two broad categories: (A) Aquatic, including reproductive modes 1–3 ( $N = 1897$  individuals in 114 species), and (T) Terrestrial including reproductive mode 4 ( $N = 979$  individuals in 98 species). We estimated prevalence of infection and 95 percent Bayesian credible intervals (CIs) using the ‘pwr’ (Champlsey 2015) and ‘binom’ (Dorai-Raj 2014) packages in R v. 3.1.2 (R Development Core Team 2008).

**PREDICTING *Bd* PRESENCE USING SPECIES DISTRIBUTION MODELS.**—Species distribution models (SDMs) allow for predicting geographic distributions based on data about an organisms’ presence combined with a set of environmental layers. To predict which areas within Colombia present abiotic environmental conditions suitable for *Bd*, we built an SDM based on geographic localities that tested positive for the pathogen. In order to minimize bias due to spatial clustering of presence localities (Peterson *et al.* 2011), we randomly selected and removed 80 percent of points in two regions with numerous geographically proximal presences (*i.e.*, Norte de Santander and Chocó departments for which 14 and 2 localities were removed, respectively). The remaining points were at a minimum distance of 5 km from each other. Thus, we were left with 42 *Bd*-positive localities for model construction, including 32 localities representing new locality records in Colombia plus ten published localities based on

histology (Ruiz & Rueda-Almonacid 2008, Velásquez-Escobar *et al.* 2008) or PCR (Urbina & Galeano 2011, Flechas *et al.* 2012, Vásquez-Ochoa *et al.* 2012, Acevedo *et al.* 2016a,b). We limited our study to Colombia which encompasses a tremendous variety of environments that can be considered representative of at least some of the environments found in neighboring countries that might share the same or closely related strains of *Bd*. Furthermore, almost no surveys on chytrid presence exist in the eastern lowlands of Colombia (Llanos Orientales) or the Colombian Amazon, except two *Bd*-negative localities sampled in Casanare (Table S1). Since undersampled areas may bias the SDM output (Barbet-Massin *et al.* 2012), we decided to exclude these areas from our study. Thus, we recognized that with limited geographic coverage, our models may be underpredictions, but as our environmental sampling was relatively wide, we expected this underprediction to be minor.

Our environmental variables consisted of the 19 bioclimatic variables obtained from the WorldClim data base (Hijmans *et al.* 2005). These variables are derived from just two types of climatic data, temperature and rainfall, which are spatially correlated (Xu & Hutchinson 2010). We extracted values for all 19 variables from 10,000 random points over the study landscape and performed a two-tailed Spearman correlation test between all pairwise combinations of variables. We considered pairs of variables with a correlation coefficient  $\geq 0.75$  as containing a redundant variable. For each pair of redundant variables, we preferred to remove those variables that (a) were more often correlated with additional variables, (b) related to averages rather than extremes, or (c) were regarded as less relevant to host or pathogen biology. The new dataset of ten variables included the following: annual mean temperature (AMT, BIO1), precipitation seasonality (PS, BIO15), precipitation of wettest quarter (PWeQ, BIO16), mean diurnal temperature range (MDTR, BIO2), isothermality (IT, BIO3), temperature seasonality (TS, BIO4), mean temperature of warmest quarter (MTWaq, BIO10), annual precipitation (AP, BIO12), precipitation of driest month (PDM, BIO14), and precipitation of warmest quarter (PwaQ, BIO18).

The models were fit using a machine learning algorithm implemented in MAXENT 3.3.3 (Phillips *et al.* 2006) based on 80 percent of the data for model construction and 20 percent for model testing (Phillips & Dudík 2008). We used the area under the curve (AUC) (Phillips *et al.* 2006) to measure the predictive accuracy of the model in terms of sensitivity and specificity. AUC values generally range from 0.5 (prediction no better than random) to 1 (perfect discrimination) (Fielding & Bell 1997). For easier interpretation of estimated probabilities of occurrence, we present the predicted distribution of *Bd* as a binary map of presence/absence. For this, we selected as our threshold the lowest prediction for training data above the 10th percentile (T10, Pearson *et al.* 2007), *i.e.*, selecting the lowest prediction after eliminating 10 percent of the points as a correction for potential sink populations and georeferencing errors (Barbet-Massin *et al.* 2012). Climatic preferences of *Bd* were inferred by extracting values of our ten bioclimatic variables from 10,000 random points from the binary map and comparing the values

of those taken from sites predicted as suitable versus those predicted as unsuitable.

## RESULTS

We surveyed 81 localities for *Bd* and found 2876 individual amphibians comprised 2812 frogs and toads, 63 salamanders, and one caecilian (Table S2). The sampling represented 14 taxonomic families and included 170 identified species plus 49 unidentified morphospecies (recorded as ‘sp.’). *Bd* was detected in 43 localities in altitudes ranging from 0 to 3200 m (Fig. 1). A total of 338 (11.7%) individuals from 70 species (47 identified species plus 23 undetermined but unique sp.) in 12 taxonomic families tested positive for the pathogen (Fig. 2). *Bd* was diagnosed through end-point PCR ( $N = 1743$  swabbed amphibians with 257 positives for a prevalence = 14.7%, CI = 13–16%) and qPCR ( $N = 1133$  animals including 81 positives for a prevalence = 7.14%, CI = 5.0–8.0%).

The chytrid fungus was detected on frogs with reproductive modes ranging from total independence from standing water bodies to species that lay eggs in water where the tadpoles remain until metamorphosis. Thirteen salamanders (genus *Bolitoglossa*) were diagnosed as *Bd* positive. We found significant differences between species with terrestrial and aquatic reproductive modes, with the highest proportion of infected individuals in species with terrestrial reproductive strategies ( $P < 0.001$ ). Fifteen percent (CI = 12.9–17.5%) of individuals with terrestrial reproductive mode were diagnosed as *Bd* positive, versus 10 percent (CI = 8.7–11.4%) of individuals with aquatic reproductive modes.

According to the SDM, the potential distribution of *Bd* in Colombia is broad, and, although predicted absences are more frequent at lower altitudes (~0–300 m), *Bd* presence is predicted

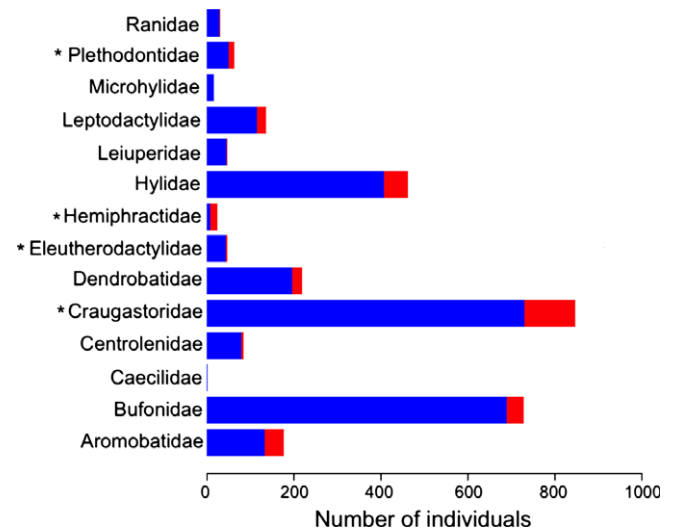


FIGURE 2. Number of infected individuals per taxonomic family of amphibian. Red bars denote the number of individuals that tested positive for *Batrachochytrium dendrobatidis* (*Bd*). Blue bars denote the number of individuals for which *Bd* was not detected. *Bd* was detected in 338 of 2876 individuals sampled. Asterisks denote families with terrestrial reproductive mode.

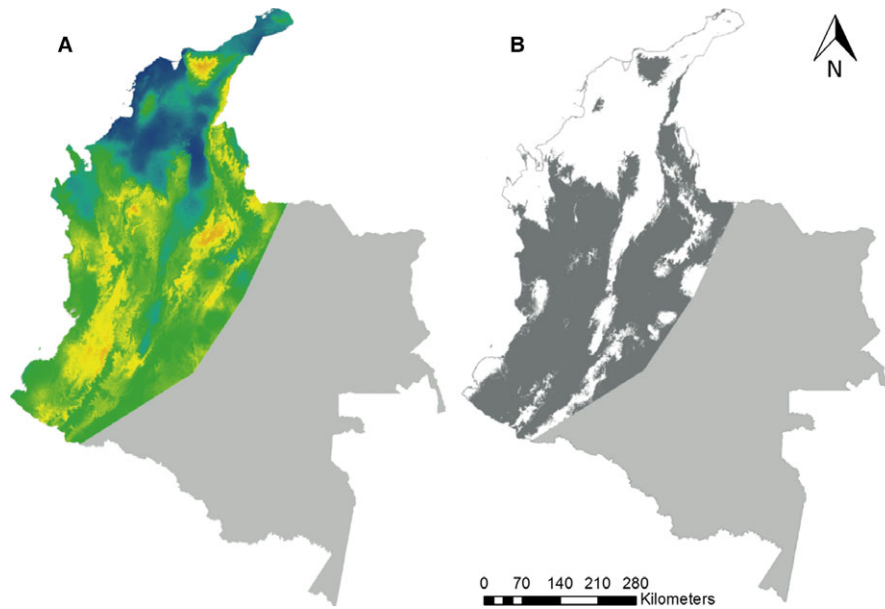


FIGURE 3. Species distribution model (SDM) of *Batrachochytrium dendrobatidis* (*Bd*) in Colombia. (A) Habitat suitability map, where warmer colors represent higher suitability and colder colors denote habitat with less suitable areas for *Bd*. (B) Binary presence–absence map obtained by using the T10 threshold method such that observed presence localities are ranked according to suitability scores and the minimum suitability for a presence site is identified (excluding the lowest 10% of presence sites). Pixels with suitability values higher than the identified minimum are considered as the presence of the species. White indicates *Bd* absence, while dark gray indicates *Bd* presence. Areas in light gray correspond to areas that were not included in the model because of the lack of sampling.

at all elevations including the lowlands (Fig. 3A). The SDM based on ten bioclimatic variables was a sufficient predictor of *Bd* presence: the AUC was 0.736 for training data and 0.768 for test data, which is above the 0.75 threshold for models considered to have good predictive power (Fielding & Bell 1997). Furthermore, our models had 0 test omission rates when using both the minimum training presence threshold and the T10 threshold. Four variables together had a 91 percent contribution to the SDM, *i.e.*, AMT, PDM, TS, and MDTR, with the first variable alone explaining 56.4 percent of variation in the model, and the latter variables explaining 17.8 percent, 9.1 percent, and 7.5 percent, respectively. Using the binary prediction of *Bd* presence with the T10 threshold (Fig. 3B), we were able to estimate the climatic space suitable versus unsuitable for *Bd*. Our analyses suggest that the strain or strains of *Bd* in Colombia tolerate a broad range of temperatures, with minimum mean annual temperatures around 0°C and maximum around 30°C. Predicted absences accumulated at higher mean annual temperatures (Fig. 4A–C). In Colombia, *Bd* differentially occurs at sites with higher precipitation during the driest month (Fig. 4C,D,F), lower temperature seasonality (Fig. 4B,E,F), and tolerates a wide range of variation in diurnal temperature (Fig. 4A,D,E). However, these variables also show a high level of interaction in determining the preferred climatic conditions of *Bd* (Fig. 4).

## DISCUSSION

Our study represents the first attempt to identify geographical areas with higher environmental suitability for *Bd* and thus higher

estimated risk of chytridiomycosis for amphibians in Colombia, one of the most species-rich countries in the world. We found *Bd* in essentially all regions surveyed and ranging vertically from 0 to 3200 m. Not surprisingly, surveyed sites in the Andean mountains were broadly infected with *Bd*, as were the lowlands of the Pacific Coast and Andean foothills. Specific patterns of climatic co-variation with *Bd* presence included finding that annual mean temperature (AMT), precipitation of the driest month (PDM), mean diurnal temperature range (MDTR), and temperature seasonality (TS) were the most important environmental correlates of *Bd* occurrence (Fig. 4). In addition, our data reveal an effect of reproductive mode on the probability of *Bd* infection, suggesting that species with terrestrial reproduction are more prone to infection than species for which reproduction occurs in close association with bodies of water. In our dataset, terrestrial-breeding species are mostly represented by the predominantly montane genus, *Pristimantis*. Thus, elevation and reproductive mode may be somewhat confounded. In addition, the pattern of higher *Bd* prevalence in terrestrial breeders found for the full dataset holds only for samples diagnosed with qPCR (26% terrestrial vs. 4% aquatic). For samples diagnosed with end-point PCR, the difference in prevalence is slight but reversed (13% terrestrial vs. 16% aquatic). The diagnostic technique could thus be confounding the pattern of prevalence of *Bd* between reproductive modes.

Temperature has been hypothesized to be the most important parameter affecting the distribution and prevalence of *Bd* in the wild (Lips *et al.* 2008, Woodhams *et al.* 2008). In support of this hypothesis, we found that annual mean temperature and mean diurnal temperature range (AMT and MDTR) are the two

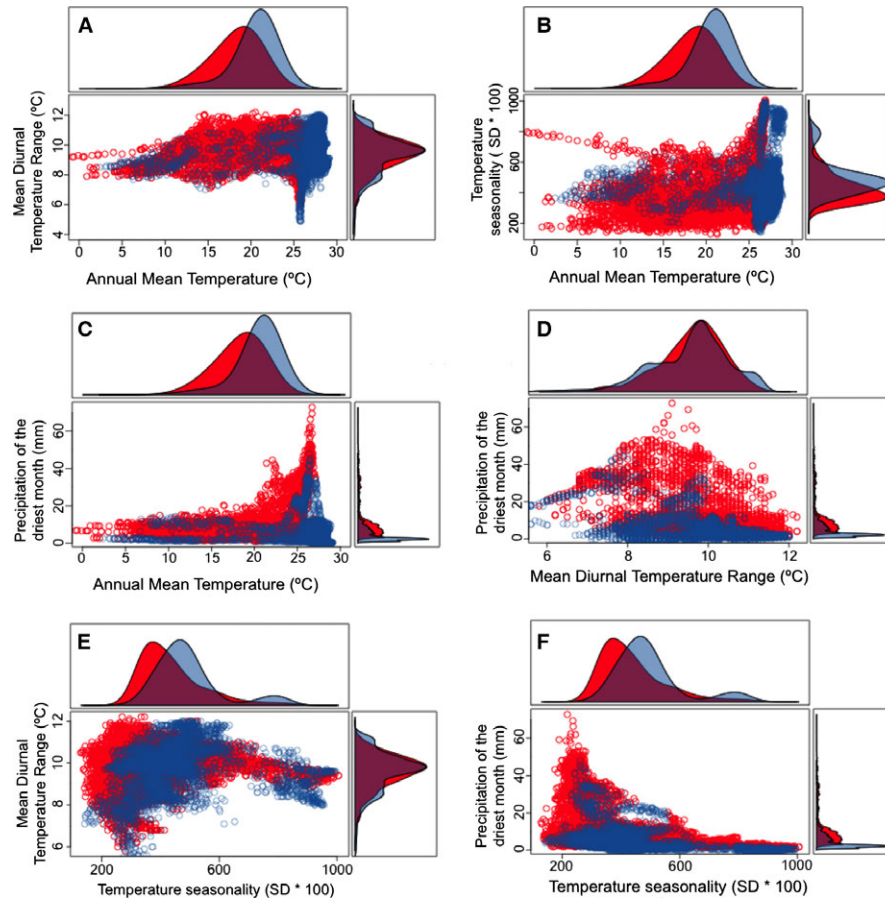


FIGURE 4. Bivariate climatic spaces predicted to be occupied (red) and not occupied (blue) by the fungal pathogen, *Batrachochytrium dendrobatidis*, according to a species distribution model. Kernel density curves indicate the predicted probability of absence (blue) or presence (red) of the fungus along the environmental gradient of the corresponding variable.

most important predictors, with AMT being the single most important parameter predicting *Bd* occurrence in Colombia, explaining 56.4 percent of variation. In addition, our study predicts a high incidence of the fungal pathogen in areas where AMT exceeds the physiological limit previously reported from laboratory experiments (Piotrowski *et al.* 2004). We detected a wide range of temperatures suitable for *Bd* in the wild (Fig. 4A–C). Assumptions about the thermal biology of *Bd* are based on limited information from laboratory experiments and based on a single strain (Piotrowski *et al.* 2004). However, Colombia might harbor more than one strain, and if so, it is important to determine if their physiological requirements vary, especially in environmentally diverse regions of the Neotropics. Furthermore, the temperature in the microhabitats where frogs and their pathogens actually live might be considerably cooler than the macroscale temperatures reported in WorldClim (Kearney *et al.* 2014). Thus, more detailed research is needed on microclimates to better understand why *Bd* is surviving and infecting species that occur in areas where thermal conditions exceed the optimal requirements for its survival (Puschendorf *et al.* 2009). In addition, the physiological ability of some amphibians to tolerate higher

temperatures might impose restrictions on the pathogen's distribution, making some areas unsuitable for its establishment, and thus imposing low infection intensities or low prevalence of *Bd* (Nowakowski *et al.* 2016).

In addition to AMT and MDTR, our results suggest that low precipitation (*i.e.*, precipitation of the driest month, PDM) plus seasonality in temperature (TS) and precipitation (PS) are good predictors of the presence (or, rather, absence) of the fungal pathogen. *Bd* seems to be more prevalent in areas with lower temperature seasonality and higher precipitation of the driest month. As a consequence of the imminent global climate change, climatic seasonality will potentially increase in coming years, as well as the frequency of extreme events such as droughts (IPCC 2014). These unusual events might have an impact on *Bd* survival and performance. Therefore, additional research that evaluates how changes in climatic conditions, especially seasonality in temperature and precipitation, affect the performance of different strains of the pathogen will become extremely relevant. Alterations in climate may promote the evolution of *Bd* (*e.g.*, faster growth or higher pathogenicity). Thus, some amphibian populations that appear to be stable might suffer indirectly from

environmental fluctuation through its effect on *Bd*, while other amphibian populations may occupy ‘refuges’ where *Bd* cannot persist because of its physiological restrictions (Puschendorf *et al.* 2011).

Most field research and surveys of *Bd* have been conducted at middle and high elevations, where declines were initially observed (Lips 1998), but recent reports of *Bd* in the lowlands suggest we need to survey more widely (Brem & Lips 2008, Kriger & Hero 2008, McCracken *et al.* 2009, Kilburn *et al.* 2010, Flechas *et al.* 2012, Whitfield *et al.* 2012, Zumbado-Ulate *et al.* 2014). *Bd* should be considered a threat to species occurring at low elevations as well, although population declines have been rarely reported (Kilburn *et al.* 2010, Flechas *et al.* 2015). In this study, 19 of 32 surveyed localities in the lowlands below 1000 m, and 13 of 25 below 500 m tested positive for *Bd* (59% and 52% of sites, respectively). Since accurate SDMs require sampling that covers the gradient of host species’ environmental tolerances (Boria *et al.* 2014), including these lowland localities in our analyses should increase the predictive accuracy in the Andean region, which is recognized for its topographic heterogeneity leading to complex environmental conditions.

To identify the suite of environmental conditions optimal for *Bd* growth and dispersal, various studies have modeled *Bd* distribution at local and global scales. Ron (2005) suggested that montane (above 1000 m) forests of the Colombian Andes represent suitable regions for *Bd*, in contrast to the Colombian savannas (Llanos) and the Choco tropical rainforests. More recently, James *et al.* (2015) also predicted the northern Andes should be one of the most suitable areas for the establishment of the pathogen. Here, we predict low suitability for *Bd* in the Caribbean region (Fig. 3), probably due to relatively dry and highly seasonal conditions, although this result might also be biased because the low sampling in this area (only two localities). In contrast to previous models (Ron 2005, James *et al.* 2015), however, our model predicted relatively high suitability for *Bd* in the lowlands of the wet Pacific coast, suggesting that *Bd* is more widely distributed than previously appreciated. This information emphasizes the need to regularly survey lowland areas, especially humid lowlands, and assess the effects of the pathogen on amphibian populations.

In addition to abiotic climatic parameters, natural history attributes intrinsic to amphibian species have been hypothesized to influence their susceptibility to *Bd* infection and possible declines. These traits include clutch size, habitat specialization, and water dependence (Williams & Hero 1998, Hero *et al.* 2005, Bielby *et al.* 2008). Given that *Bd* is an aquatic pathogen and that its motile zoospores need a moist substrate to move between hosts (Berger *et al.* 2005), species that spend more time in water are expected to have higher exposure to the pathogen, and thus are considered to be at greater risk of chytridiomycosis (Lips *et al.* 2005). Previously, species with reproductive strategies associated with water were thought to be more prone to infection than those not needing water to complete their life cycles (Bielby *et al.* 2008, Lötters *et al.* 2009), in part, because most declines and extinctions of amphibians have been observed in species with aquatic reproduction (Bustamante *et al.* 2005, La Marca *et al.*

2005, Coloma *et al.* 2010, Catenazzi *et al.* 2011). Although water dependence has been considered a key factor that increases the probability of infection, some recent studies have found the opposite scenario, where species with terrestrial reproductive modes exhibited higher *Bd* prevalence than aquatic ones (Gründler *et al.* 2012, Guayasamin *et al.* 2014). Our study supports the latter pattern, where species with direct development exhibited a higher prevalence of infection.

Although our initial goal was to predict *Bd* occurrence for the entire country of Colombia, the paucity of records from the eastern savannas (Llanos) and the Amazon basin constrained our geographic scope. Nonetheless, our study provides a reliable overview of the potential distribution of *Bd* in Colombia and draws the attention to the need for additional surveys in these poorly sampled areas. Our limited sampling revealed interactions among climatic variables that together define *Bd* presence. For example, we found that regions with temperatures above 25°C are most likely to be unsuitable for *Bd*, yet if precipitation during the driest month rises to 10–20 mm, *Bd* could persist in that area (Fig. 4C). If global climate change led to increased rainfall in these warm regions, *Bd* could potentially expand its geographic range and thus infect new populations or species of amphibians.

Despite its limited geographic scope, our study provides a dense sampling that allowed us to recognize heretofore-unappreciated variation in observed and predicted *Bd* prevalence among lowland sites. For example, the Choco lowlands of the Pacific coast, previously thought to have a very low probability of *Bd* occurrence, may in fact be facing a major threat with many positive localities (Flechas *et al.* 2015). Given that the Choco region hosts high amphibian biodiversity, we highlight the urgent need to develop a monitoring program for the Pacific lowlands of Colombia. Finally, and according to the results reported by Becker *et al.* (2016), where *Bd*-positive samples with high infection loads were found in areas of predicted low suitability in the Amazonia, we suggest that future *Bd* surveys should be extended into the Colombian Amazon, which remain undersampled. Our work here shows that for humid lowland sites, this assumption is false. Thus, humid regions of Amazonia may also be adequate for the establishment of *Bd*. The information presented here should be used as a baseline to develop new studies that examine undersampled areas and evaluate amphibian population status in places where *Bd* is already present and inform future conservation actions.

## ACKNOWLEDGMENTS

This study was completed with financial support from ECOPE-TROL (grant 156-09 to AJC, SVF, and AA), US Fish & Wildlife Service—Wildlife without Borders (96200-0-G228 to AJC), Association of Zoos and Aquariums—Conservation Endowment Fund (08-836 to AA and SVF), Conservation International Critically Endangered Species Fund (to SVF), Conservation Leadership Program (Project ID 0621310 to AAA, and Project ID 070207 to LARS), COLCIENCIAS (1121-659-44242, agreement 553 to AAA), Vicerrectoría de Investigaciones at the Universidad

del Valle, Vicerrectoría de Investigaciones at the Universidad Tecnológica de Chocó, Vicerrectoría de Investigaciones at Universidad de Pamplona, Conservation International Colombia (Agreement 545), and Corporación Autónoma Regional del Valle del Cauca (CVC). For assistance in the field, we thank L. M. Arenas, O. Armesto, L. S. Barrientos, A. Batista, C. Burbano, R. Coral, G. Corredor, A. Dapper, L. M. Escobar, T. Escovar, C. Esquivel, D. M. Galindo, J. A. Hernández, D. J. Lizcano, F. J. López, J. Méndez, J. S. Mendoza, F. L. Meza, D. Moen, A. Muñoz, J. E. Ortega, J. Ospina, N. R. Pinto-Sánchez, E. P. Ramos, C. Silva, K. L. Silva, F. Vargas-Salinas, R. S. Ramírez-Valdez, A. A. Velásquez, and B. E. Velásquez-Escobar. We thank V. Ramírez for assistance in the laboratory. We thank F. Brown, C. González, and L. M. Arenas for helpful comments and suggestions that greatly improved this article. We thank Proaves Foundation, San Pedro de la Sierra community, and the Indigenous community from La Sierra Nevada de Santa Marta for their support during the fieldwork. Research permits were provided by the Colombian National Parks authority, Ministry of the Environment, and the National Authority for Environmental Licenses (ANLA), under permits DTSO 019-09, DTSO 001-09, and No 10-07032012 (to SVF), permit No 15, July 26th, 2010 (to AJC), resolution No 0255, March 12th, 2014 (to AMPT), resolution CorpMag No 425, February 2015 (to LARS), and DTAN 001-13 (to AAA).

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

TABLE S1. *Summary of sampled sites and infection prevalence per site.*

TABLE S2. *Results of Bd diagnoses for individual species including locality, reproductive mode, classification (terrestrial/aquatic), and diagnostic technique used.*

## LITERATURE CITED

- ACEVEDO, A. A., M. MARTINEZ, L. O. ARMESTO, L. SOLANO, K. SILVA, AND D. J. LIZCANO. 2016b. Detection of *Batrachochytrium dendrobatidis* in amphibians from Northeastern Colombia. *Herpetol. Rev.* 47: 220–226.
- ACEVEDO, A. A., R. F. PALLARES, AND D. A. CARRERO. 2016a. Diversity of Andean amphibians of the Tamá National Natural Park in Colombia: A survey for the presence of *Batrachochytrium dendrobatidis*. *Anim. Biodivers. Cons.* 39: 1–10.
- ALFORD, R. A., AND S. J. RICHARDS. 1999. Global amphibian declines: A problem in applied ecology. *Annu. Rev. Ecol. Syst.* 30: 133–165.
- AmphibiaWeb. 2016. AmphibiaWeb: Information on amphibian biology and conservation. Berkeley. Available from: <http://amphibiaweb.org/> (accessed 3 March 2016).
- ANNIS, S. L., F. P. DASTOOR, H. ZIEL, P. DASZAK, AND J. E. LONGCORE. 2004. A DNA-Based assay identifies *Batrachochytrium dendrobatidis* in amphibians. *J. Wildl. Dis.* 40: 420–428.
- BARBET-MASSIN, M., F. JIGUET, C. H. ALBERT, AND W. THUILLER. 2012. Selecting pseudo-absences for species distribution models: How, where and how many?. *Methods Ecol. Evol.* 3: 327–338.
- BECKER, C. G., D. RODRIGUEZ, C. LAMBERTINI, L. F. TOLEDO, AND C. F. B. HADDAD. 2016. Historical dynamics of *Batrachochytrium dendrobatidis* in Amazonia. *Ecography* 39: 954–960.
- BERGER, L., A. D. HYATT, R. SPEARE, AND J. E. LONGCORE. 2005. Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* 68: 51–63.
- BERGER, L., R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. A. RAGAN, A. D. HYATT, K. R. McDONALD, H. B. HINES, K. R. LIPS, G. MARANTELLI, AND H. PARKES. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forest of Australia and Central America. *Proc. Natl Acad. Sci. USA* 95: 9031–9036.
- BIELBY, J., N. COOPER, A. A. CUNNINGHAM, T. W. J. GARNER, AND A. PURVIS. 2008. Predicting susceptibility to future declines in the world's frogs. *Conserv. Lett.* 1: 82–90.
- BLAUSTEIN, A. R., AND J. M. KIESECKER. 2002. Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecol. Lett.* 5: 597–608.
- BORIA, R. A., L. E. OLSON, S. M. GOODMAN, AND R. P. ANDERSON. 2014. Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. *Ecol. Model.* 275: 73–77.
- BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. T. MORGAN, AND A. D. HYATT. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Organ.* 60: 141–148.
- BRADFORD, D. F. 1991. Mass mortality and extinction in a high-elevation population of *Rana muscosa*. *J. Herpetol.* 25: 174–177.
- BREM, F. M. R., AND K. R. LIPS. 2008. *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. *Dis. Aquat. Organ.* 81: 189–202.
- BUSTAMANTE, M. R., S. R. RON, AND L. A. COLOMA. 2005. Cambios en la diversidad en siete comunidades de anuros en los Andes de Ecuador. *Biotropica* 37: 180–189.
- CAMPBELL, C. R., J. VOYLES, D. COOK, AND A. DINUDOM. 2012. Frog skin epithelium: Electrolyte transport and chytridiomycosis. *Int. J. Climatol.* 44: 431–434.
- CAREY, C., N. COHEN, AND L. ROLLINS-SMITH. 1999. Amphibian declines: An immunological perspective. *Dev. Comp. Immunol.* 23: 459–472.
- CATENAZZI, A., E. LEHR, L. O. RODRIGUEZ, AND V. T. VREDENBURG. 2011. *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the upper Manu National Park, southeastern Peru. *Conserv. Biol.* 25: 382–391.
- CHAMPLEY, S. 2015. pwr: Basic functions for power analysis. R package version 1.1-2. <http://CRAN.R-project.org/package=pwr>
- COLLINS, J. P., AND A. STORFER. 2003. Global amphibian declines: Sorting the hypotheses. *Divers. Distrib.* 9: 89–98.
- COLOMA, L., W. E. DUELLMAN, A. ALMENDÁRIZ, S. RON, A. TERÁN-VALDEZ, AND J. M. GUAYASAMIN. 2010. Five new (extinct?) species of *Atelopus* (Anura: Bufonidae) from Andean Colombia, Ecuador, and Peru. *Zootaxa* 2574: 1–54.
- CRAWFORD, A. J., K. R. LIPS, AND E. BERMINGHAM. 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proc. Natl Acad. Sci. USA* 107: 13777–13782.
- CRUMP, M. L. 1974. Reproductive strategies in a tropical anuran community. *Misc. publ. Univ. Kans. Mus. Nat. Hist.* 61: 1–68.
- CRUMP, M. L. 2015. Anuran reproductive modes: Evolving perspectives. *J. Herpetol.* 49: 1–16.
- DASZAK, P., L. BERGER, A. A. CUNNINGHAM, A. D. HYATT, D. E. GREEN, AND R. SPEARE. 1999. Emerging infectious diseases and amphibian population declines. *Emerg. Infect. Diseases* 5: 735–748.
- DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2003. Infectious disease and amphibian population declines. *Divers. Distrib.* 9: 141–150.



- DORAI-RAJ, S. 2014. binom: Binomial confidence intervals for several parameterizations. R package version 1.1-1. <http://CRAN.R-project.org/package=binom>
- DUELLMAN, W. E., AND L. TRUEB. 1986. Biology of amphibians. McGraw Hill Publ. Co., New York.
- FIELDING, A. H., AND J. F. BELL. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environ. Conserv.* 24: 38–49.
- FISHER, M. C., D. A. HENK, C. J. BRIGGS, J. S. BROWNSTEIN, L. C. MADOFF, S. L. McCRAW, AND S. J. GURR. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186–194.
- FITES, J. S., J. P. RAMSEY, W. M. HOLDEN, S. P. COLLIER, D. M. SUTHERLAND, L. K. REINERT, A. S. GAYEK, T. S. DERMODY, T. M. AUNE, K. OSWALD-RICHTER, AND L. A. ROLLINS-SMITH. 2013. The invasive chytrid fungus of amphibians paralyzes lymphocyte responses. *Science* 342: 366–369.
- FLECHAS, S. V., E. M. MEDINA, A. J. CRAWFORD, C. SARMIENTO, M. E. CÁRDENAS, A. AMÉZQUITA, AND S. RESTREPO. 2013. Characterization of the first *Batrachochytrium dendrobatidis* isolate from the Colombian Andes, an amphibian biodiversity hotspot. *EcoHealth* 10: 72–76.
- FLECHAS, S. V., C. SARMIENTO, AND A. AMÉZQUITA. 2012. *Bd* on the beach: High prevalence of *Batrachochytrium dendrobatidis* in the lowland forests of Gorgona Island (Colombia, South America). *EcoHealth* 9: 298–302.
- FLECHAS, S. V., V. T. VREDENBURG, AND A. AMÉZQUITA. 2015. Infection prevalence in three lowland species of harlequin toads from the threatened genus *Atelopus*. *Herp. Rev.* 46: 528–532.
- GRÜNDLER, M. C., L. F. TOLEDO, G. PARRA-OLEA, C. F. B. HADDAD, L. O. M. GIASSON, R. J. SAWAYA, C. P. A. PRADO, O. G. S. ARAUJO, F. J. ZARA, F. C. CENTENO, AND K. R. ZAMUDIO. 2012. Interaction between breeding habitat and elevation affects prevalence but not infection intensity of *Batrachochytrium dendrobatidis* in Brazilian anuran assemblages. *Dis. Aquat. Organ.* 97: 173–184.
- GUARNIZO, C. E., A. PAZ, A. MUÑOZ-ORTIZ, S. V. FLECHAS, J. MÉNDEZ-NARVÁEZ, AND A. J. CRAWFORD. 2015. DNA Barcoding survey of anurans across the eastern cordillera of Colombia and the impact of the Andes on cryptic diversity. *PLoS One* 10: e0127312.
- GUAYASAMIN, J. M., A. M. MENDOZA, A. V. LONGO, K. R. ZAMUDIO, AND E. BONACCORSO. 2014. High prevalence of *Batrachochytrium dendrobatidis* in an Andean frog community (Reserva Las Gralarias, Ecuador). *Amphib. Reptile Conserv.* 8: 33–44.
- HERO, J.-M., S. E. WILLIAMS, AND W. E. MAGNUSON. 2005. Ecological traits of declining amphibians in upland areas of eastern Australia. *J. Zool.* 267: 221–232.
- HEYER, W. R., A. S. RAND, C. A. G. DA CRUZ, AND O. L. PEIXOTO. 1988. Decimations, extinctions, and colonizations of frog populations in southeast Brazil and their evolutionary implications. *Biotropica* 20: 230–235.
- HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25: 1965–1978.
- HOF, C., M. B. ARAÚJO, AND C. RAHBEK. 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature* 480: 516–519.
- HOFFMAN, M., C. HILTON-TAYLOR, A. ANGULO, M. BÖHM, T. M. BROOKS, AND S. H. M. BUTCHART. 2010. The impact of conservation on the status of the world's vertebrates. *Science* 1503–1509.
- HYATT, A. D., D. G. BOYLE, V. OLSEN, D. B. BOYLE, L. BERGER, D. OBENDORF, A. DALTON, K. KRIGER, M. HERO, H. HINES, R. PHILLOTT, R. CAMPBELL, G. MARANTELLI, F. GLEASON, AND A. COLLING. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* 73: 175–192.
- IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change T. C. W. Team, R. K. Pachauri, and L. A. Meyer (Eds.). Geneva, Switzerland.
- JAMES, T. Y., L. F. TOLEDO, D. RÖDDER, D. SILVA LEITE, A. M. BELASEN, C. M. BETANCOURT ROMÁN, T. S. JENKINSON, C. SOTO AZAT, C. LAMBERTINI, A. V. LONGO, J. RUGGERI, J. P. COLLINS, P. A. BURROWES, K. R. LIPS, K. R. ZAMUDIO, AND J. E. LONGCORE. 2015. Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research. *Ecol. Evol.* 5: 4079–4097.
- KEARNEY, M. R., A. P. ISAAC, AND W. P. PORTER. 2014. microclim: Global estimates of hourly microclimate based on long-term monthly climate averages. *Sci. Data* 1: 1–9.
- KILBURN, V., R. IBÁÑEZ, O. SANJUR, E. BERMINGHAM, J. P. SURACI, AND D. M. GREEN. 2010. Ubiquity of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in anuran communities in Panamá. *EcoHealth* 4: 537–548.
- KRIGER, K. M., AND J.-M. HERO. 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Divers. Distrib.* 13: 781–788.
- KRIGER, K. M., AND J.-M. HERO. 2008. Altitudinal distribution of chytrid (*Batrachochytrium dendrobatidis*) infection in subtropical Australian frogs. *Austral. Ecol.* 33: 1022–1033.
- LA MARCA, E., K. R. LIPS, S. LÖTTTERS, R. PUSCHENDORF, R. IBÁÑEZ, J. V. RUEDA-ALMONACID, R. SCHULTE, C. MARTY, F. CASTRO, J. MANZANILLA-PUPPO, J. E. GARCÍA-PÉREZ, F. BOLAÑOS, G. CHAVES, J. A. POUNDS, E. TORAL, AND B. E. YOUNG. 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37: 190–201.
- LAURANCE, W. F., K. R. McDONALD, AND R. SPEARE. 1996. Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conserv. Biol.* 10: 406–413.
- LIPS, K. R. 1998. Decline of a tropical montane amphibian fauna. *Conserv. Biol.* 12: 106–117.
- LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, L. J. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Natl Acad. Sci. USA* 103: 3165–3170.
- LIPS, K. R., P. A. BURROWES, J. R. I. I. MENDELSON, AND G. PARRA-OLEA. 2005. Amphibian population declines in Latin America: A synthesis. *Biotropica* 37: 222–226.
- LIPS, K. R., J. DIFFENDORFER, J. R. MENDELSON, III, AND M. W. SEARS. 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *PLoS Biol.* 6: e72.
- LIPS, K. R., J. D. REEVE, AND L. R. WITTERS. 2003. Ecological traits predicting amphibian population declines in Central America. *Conserv. Biol.* 17: 1078–1088.
- LONGCORE, J. E., A. P. PESSIER, AND D. K. NICHOLS. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91: 219–227.
- LÖTTTERS, S., J. KIELGAST, J. BIELBY, S. SCHMIDTLEIN, J. BOSCH, M. VEITH, S. F. WALKER, M. C. FISHER, AND D. RÖDDER. 2009. The link between rapid enigmatic amphibian decline and the globally emerging chytrid fungus. *EcoHealth* 6: 358–372.
- LYNCH, J. D., P. M. RUIZ-CARRANZA, AND M. C. ARDILA-ROBAYO. 1997. Biogeographic patterns of Colombian frogs and toads. *Rev. Acad. Colomb. Ciencias Exactas Fis. Nat. XXI*: 237–248.
- MARTEL, A., A. S.-V. DER SLUIJS, M. BLOOI, W. BERT, R. DUCATTELLE, M. C. FISHER, A. WOELTJES, W. BOSMAN, K. CHIERS, F. BOSSUYT, AND F. PASMANS. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proc. Natl Acad. Sci. USA* 110: 15325–15329.
- MCCRACKEN, S., J. P. GAERTNER, M. J. FORSTNER, AND D. HAHN. 2009. Detection of *Batrachochytrium dendrobatidis* in amphibians from the forest floor to the upper canopy of an ecuadorian amazon lowland rainforest. *Herp. Rev.* 40: 190–195.
- NOWAKOWSKI, A. J., S. M. WHITFIELD, E. A. ESKEW, M. E. THOMPSON, J. P. ROSE, B. L. CARABALLO, J. L. KERBY, M. A. DONNELLY, AND B. D.

- TODD. 2016. Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances. *Ecol. Lett.* 19: 1051–1061.
- PEARSON, R. G., C. J. RAXWORTHY, M. NAKAMURA, AND A. T. PETERSON. 2007. Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *J. Biogeogr.* 34: 102–117.
- PETERSON, A. T. 2001. Predicting species' geographic distributions based on ecological niche modeling. *Condor* 103: 599–605.
- PETERSON, A. T., J. SOBERÓN, R. G. PEARSON, R. P. ANDERSON, E. MARTÍNEZ-MEYER, M. NAKAMURA, AND M. B. ARAÚJO. 2011. Ecological niches and geographic distributions. Princeton University Press. Princeton New Jersey. 328 pp.
- PHILLIPS, S. J., R. P. ANDERSON, AND R. E. SCHAPIRE. 2006. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* 190: 231–259.
- PHILLIPS, S. J., AND M. DUDÍK. 2008. Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. *Ecography* 31: 161–175.
- PIOTROWSKI, J. S., S. L. ANNIS, AND J. E. LONGCORE. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96: 9–15.
- PRICE, S. J., T. W. J. GARNER, R. A. NICHOLS, F. BALLOUX, C. AYRES, A. MORACABELLO DE ALBA, AND J. BOSCH. 2014. Collapse of amphibian communities due to an introduced *Ranavirus*. *Curr. Biol.* 24: 2586–2591.
- PUSCHENDORF, R., A. C. CARNAVAL, J. VANDERWAL, H. ZUMBADO-ULATE, G. CHAVES, F. BOLAÑOS, AND R. A. ALFORD. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: Proposing climatic refuges as a conservation tool. *Divers. Distrib.* 15: 401–408.
- PUSCHENDORF, R., C. J. HOSKIN, S. D. CASHINS, K. McDONALD, L. F. SKERRATT, J. VANDERWAL, AND R. A. ALFORD. 2011. Environmental refuge from disease-driven amphibian extinction. *Conserv. Biol.* 25: 956–964.
- R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>
- ROHR, J. R., AND T. R. RAFFEL. 2010. Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proc. Natl Acad. Sci. USA* 107: 8269–8274.
- RON, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the new world. *Biotropica* 37: 2009–2221.
- ROSENBLUM, E. B., T. Y. JAMES, K. R. ZAMUDIO, T. J. POORTEN, D. ILUT, D. RODRIGUEZ, J. M. EASTMAN, K. RICHARDS-HRDLICKA, S. JONESON, T. S. JENKINSON, J. E. LONGCORE, G. PARRA-OLEA, L. F. TOLEDO, M. L. ARELLANO, E. M. MEDINA, S. RESTREPO, S. V. FLECHAS, L. BERGER, C. J. BRIGGS, AND J. E. STAJICH. 2013. Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proc. Natl Acad. Sci. USA* 110: 9385–9390.
- RUIZ, A., AND J. V. RUEDA-ALMONACID. 2008. *Batrachochytrium dendrobatidis* and chytridiomycosis in anuran amphibians of Colombia. *EcoHealth* 5: 27–33.
- RUSSELL, D. M., C. S. GOLDBERG, AND L. P. WAITS. 2010. *Batrachochytrium dendrobatidis* infection dynamics in the Columbia spotted frog *Rana luteiventris* in north Idaho, USA. *Dis. Aquat. Organ.* 92: 223–230.
- STUART, S. N., J. S. CHANSON, N. A. COX, B. E. YOUNG, A. S. L. RODRIGUES, D. L. FISCHMAN, AND R. W. WALLER. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1786.
- TARVIN, R. D., P. PEÑA, AND S. R. RON. 2014. Changes in population size and survival in *Atelopus spumarius* (Anura: Bufonidae) are not correlated with chytrid prevalence. *J. Herpetol.* 48: 291–297.
- URBINA, J. C., AND S. P. GALEANO. 2011. *Batrachochytrium dendrobatidis* detected in amphibians of the Central Andean cordillera of Colombia. *Herp. Rev.* 42: 558–560.
- VÁSQUEZ-OCHOA, A., P. BAHAMÓN-CARMONA, L. D. PRADA-SALCEDO, AND M. FRANCO-CORREA. 2012. Detection and quantification of *Batrachochytrium dendrobatidis* in amphibians of the Central and Eastern Andean, Orinoco and Amazon regions of Colombia. *Herpetotropicos* 8: 13–22.
- VELÁSQUEZ-ESCOBAR, B. E., F. CASTRO, W. BOLÍVAR-G, AND M. I. HERRERA. 2008. Infección por el hongo *Batrachochytrium dendrobatidis* en anuros de la cordillera occidental de Colombia. *Herpetotropicos* 4: 65–70.
- VOYLES, J., S. YOUNG, L. BERGER, C. CAMPBELL, W. F. VOYLES, A. DINODUM, D. COOK, R. WEBB, R. A. ALFORD, L. F. SKERRATT, AND R. SPEARE. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* 326: 582–585.
- WAKE, D. B. 1991. Declining amphibian populations. *Science* 253: 860.
- WHITFIELD, S. M., K. E. BELL, T. PHILIPPI, M. SASA, F. BOLAÑOS, G. CHAVES, J. M. SAVAGE, AND M. A. DONNELLY. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proc. Natl Acad. Sci. USA* 104: 8352–8356.
- WHITFIELD, S. M., J. KERBY, L. R. GENTRY, AND M. A. DONNELLY. 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.
- WILLIAMS, S. E., AND J.-M. HERO. 1998. Rainforest frogs of the Australian wet tropics: Guild classification and the ecological similarity of declining species. *Proc. R. Soc. B* 265: 597–602.
- WOODHAMS, D. C., AND R. A. ALFORD. 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conserv. Biol.* 19: 1449–1459.
- WOODHAMS, D. C., R. A. ALFORD, C. J. BRIGGS, M. JOHNSON, AND L. A. ROLLINS-SMITH. 2008. Life-history trade-offs influence disease in changing climates: Strategies of an amphibian pathogen. *Ecosphere* 89: 1627–1639.
- XU, T., AND M. HUTCHINSON. 2010. ANUCLIM version 6.1. User guide. Australian National University, Canberra.
- ZUMBADO-ULATE, H., F. BOLAÑOS, G. GUTIÉRREZ-ESPELETA, AND R. PUSCHENDORF. 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.