

The amphibian-killing fungus in a biodiversity hotspot: identifying and validating high-risk areas and refugia

LEONARDO D. BACIGALUPE,^{1,†} I. A. VÁSQUEZ,¹ SERGIO A. ESTAY,^{1,2} ANDRÉS VALENZUELA-SÁNCHEZ,^{1,3}
MARIO ALVARADO-RYBAK,^{4,5} ALEXANDRA PEÑAFIEL-RICAURTE,^{4,5} ANDREW A. CUNNINGHAM,⁵ AND
CLAUDIO SOTO-AZAT⁴

¹Facultad de Ciencias, Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile

²Center of Applied Ecology and Sustainability, Pontificia Universidad Católica de Chile, Santiago, Chile

³ONG Ranita de Darwin, Santiago, Chile

⁴Centro de Investigación para la Sustentabilidad, Facultad de Ciencias de la Vida & Doctorado en Medicina de la Conservación, Universidad Andrés Bello, Santiago, Chile

⁵Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY UK

Citation: Bacigalupe, L. D., I. A. Vásquez, S. A. Estay, A. Valenzuela-Sánchez, M. Alvarado-Rybak, A. Peñafiel-Ricaurte, A. A. Cunningham, and C. Soto-Azat. 2019. The amphibian-killing fungus in a biodiversity hotspot: identifying and validating high-risk areas and refugia. *Ecosphere* 10(5):e02724. 10.1002/ecs2.2724

Abstract. Amphibian chytridiomycosis, due to infection with the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been associated with the alarming decline and extinction crisis of amphibians worldwide. It is essential for conservation management to identify regions with high or low suitability for *Bd*. We use a species distribution model to estimate the environmental suitability of *Bd* in the Chilean Winter Rainfall–Valdivian Forest biodiversity hotspot. Fourteen environmental variables were used as predictors in the statistical modeling (Maxent, generalized linear models, random forest) which also included 56 independent *Bd*+ localities. High-risk areas (i.e., suitability above a defined threshold) were validated through prospective field surveys conducted in 2017. As results from Maxent, which only uses presence data, were the only results retained, refugia (i.e., suitability below a defined threshold) were validated with the *Bd* absences (N = 12) used in the GLM and RF modeling. Our results showed that (1) suitability for *Bd* increased with human footprint and with shorter distances to urban centers and water bodies and decreased with elevation; (2) climate was not a major factor shaping the current distribution of *Bd*; and (3) the model predicted high-risk and refugia areas fairly well. Surveys of 24 new localities in high-risk areas confirmed that 23 were *Bd*+; hence, these areas warrant consideration for long-term *Bd* surveillance, population monitoring, and disease mitigation. In addition, five localities with apparent *Bd* absence were found in the predicted high-risk areas. Our models showed that refugia can exist near high-risk areas and *Bd*+ sites. Four localities with apparent *Bd* absence were located within the refugia predicted by the model. Preventing *Bd* transmission to such refugia is of paramount importance for persistence of *Bd*-susceptible amphibian populations. The identification and validation through prospective field surveys of high-risk areas and refugia are imperative to develop strategies to prevent further arrival and establishment of *Bd* and also, by identifying amphibian species or populations of conservation concern in such areas, will help to guide specific actions to reduce the biodiversity loss caused by chytridiomycosis.

Key words: *Batrachochytrium dendrobatidis*; chile; chytrid fungus; chytridiomycosis; emerging infectious diseases; maxent; pathogen mitigation strategy; species distribution model.

Received 24 July 2018; revised 20 March 2019; accepted 29 March 2019. Corresponding Editor: Sarah M. Zohdy.

Copyright: © 2019 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** lbacigal@gmail.com

INTRODUCTION

Emerging infectious diseases (EIDs) are increasingly recognized as a major threat to biodiversity (Daszak et al. 2000, Cunningham et al. 2017). Furthermore, given the current scenario of rapid environmental change driven by anthropogenic activity, there is growing interest in understanding the role of global change in the emergence and spread of infectious diseases (Jones et al. 2008, O'Hanlon et al. 2018). This is particularly true for amphibians, where population declines and extinctions at the global scale reported in Australia, Costa Rica, and Chile have been linked to the EID chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*; Lips et al. 2006, Schloegel et al. 2006, Skerratt et al. 2007, Wake and Vredenburg 2008, Soto-Azat et al. 2013a, b, Valenzuela-Sánchez et al. 2017).

At the local scale, *Bd* prevalence (i.e. number of positive animals/number sampled) and the host response to *Bd* exposure are likely the result of the complex interaction between the host, the pathogen, and the environmental conditions they encounter (Murray and Skerratt 2012, Spitzen-Van Der Sluijs et al. 2014, Blaustein et al. 2018). Overall, our understanding of the factors underlying the distribution and prevalence of *Bd* has greatly increased over recent years (Berger et al. 2016). Globally, climate, elevation, and species richness have been shown to be associated with *Bd* occurrences (Olson et al. 2013, Xie et al. 2016). At regional levels, it is known that *Bd* prevalence varies with latitude (Kriger et al. 2007), climate (Murray et al. 2011), human activities (James et al. 2009, Adams et al. 2010, Liu et al. 2013a,b), and with the interaction of these factors (Bacigalupe et al. 2017). By analyzing *Bd* distribution and spread at regional scales, it is possible to identify localities that are likely to be highly suitable (high-risk) and those that are unsuitable for *Bd*. The latter might represent potential disease refugia for amphibians (Keppel and Wardell-Johnson 2012). Such information regarding *Bd* habitat suitability would be useful to conservation managers, especially if areas can be confidently classified as high-risk for, or as refugia from, this important threat. For example, *Bd* refugia may protect susceptible amphibians from the constant pressure of *Bd* spread, or potentially

serve as source or destiny areas for amphibian conservation-based translocations.

Correlative species distribution models (SDMs) are extensively used tools that evaluate the association between the presence of a species in a set of locations and environmental data to predict its potential distribution in terms of habitat suitability across landscapes (Guisan and Thuiller 2005, Elith and Leathwick 2009, Estay et al. 2014). In this context, SDMs have been used to predict the global (Rödder et al. 2009, Liu et al. 2013a,b, Xie et al. 2016), continental (Ron 2005, Murray et al. 2011, James et al. 2015), and regional (Puschendorf et al. 2009, Ghirardi et al. 2011, Moriguchi et al. 2015, Flechas et al. 2017) distribution of *Bd*. Although the value of *Bd* environmental suitability to local conservation managers has been disputed (Riley et al. 2013), its utility has also been demonstrated (Murray et al. 2011). For example, Murray et al. (2011) showed that areas of high-predicted environmental suitability for *Bd* in Australia (i.e., Queensland and New South Wales) were indeed areas where *Bd*-related declines have been documented. Nevertheless, Riley et al. (2013) reported that infected populations of *Crinia georgiana* in southwestern Australia inhabiting areas with high-predicted environmental suitability for *Bd* show no evidence of declines. However, it should be noted that conclusions from Riley et al. (2013) came from studying *Crinia georgiana*, a non-threatened, non-declining species, which is also not susceptible to chytridiomycosis. Although SDMs are not without their shortcomings (Meineri et al. 2015, Peterson et al. 2015, Qiao et al. 2015), they provide an important baseline of key information to guide future sampling efforts or to test specific hypotheses regarding disease risk (Murray et al. 2011).

Here, we use an SDM to estimate the environmental suitability for *Bd* of the Chilean Winter Rainfall-Valdivian Forest biodiversity hotspot (Mittermeier et al. 2011), which has high amphibian species richness and endemism, including a large number of amphibian species threatened with extinction (Vidal and Díaz-Páez 2012). This hotspot incorporates the most urbanized, dense, and economically productive areas of Chile, as well as natural environments (Barbosa and Villagra 2015). We have recently shown species-specific patterns of *Bd* prevalence and a

non-random geographic distribution of *Bd* throughout this hotspot; prevalence decreases with latitude and increases with economic development, interacting with some components of climate (Bacigalupe et al. 2017). Given the inherent complexity of the relationship between *Bd*, its host, and the environment, we used field surveys to validate the SDM-modeled refugia and the high-risk areas (i.e., suitability below and above defined thresholds, respectively). To the best of our knowledge, such ground-truthing of a *Bd* SDM has not been previously conducted. This novel approach for *Bd*-refugia identification offers a powerful management tool to sustain *Bd*-vulnerable amphibian host populations.

METHODS

Bd occurrence

In order to construct an SDM for *Bd* in the Chilean biodiversity hotspot, *Bd* occurrences were compiled through an extensive survey (2008–2017, Bacigalupe et al. 2017) of the Mediterranean and Valdivian forest ecoregions, which together form the Chilean biodiversity hotspot (30°–46°S; Fig. 1, Mittermeier et al. 2011). In addition, an extensive literature search was carried out in www.bd-maps.net (Olson et al. 2013), Google Scholar, and ISI Web of Knowledge using the following search term combinations: *Batrachochytrium dendrobatidis* OR chytrid* AND Chile. Cited literature in all retrieved articles was screened for further relevant publications. Only studies that used molecular methods (PCR and qPCR) as a means of *Bd* detection were included. From these resources, we constructed a database of known *Bd* occurrence for the biodiversity hotspot (Appendix S1: Table S1).

Predictor variables

Fourteen environmental variables derived from landscape-scale geographic layers that could potentially influence the occurrence of *Bd* were used as predictors in the statistical modeling. These comprised bioclimate, elevation, distance to nearest water body, topographic wetness index, net primary production of biomass, amphibian species richness, and several human developmental variables (see Table 1 for a full description and for data sources). Layers of all predictor variables were cropped to our

study area and resampled to 1-km resolution using ArcGIS 10.1. A principal component analysis of the bioclimate variables was conducted to reduce their high collinearity (i.e., the first 4 principal components accounted for 97.7% of the bioclimatic variability and thus were used as the final bioclimatic predictors; Appendix S1: Table S2).

Modeling methods

In order to increase the spatial independence between sites and reduce the effect of the heterogeneous distribution of the sampling effort, sites that were within 3-km of each other were grouped, with the centroid of each grouped site assigned as the new georeferenced location (Muths et al. 2008, Rohr et al. 2011). We considered a grouped site to be *Bd* positive if at least one individual showed evidence of *Bd* infection at any of the individual sites.

The SDM was conducted using Maxent, generalized linear models (GLM, logit link), and random forest (RF) under default settings, with prevalence (i.e. the proportion of sampled sites where a species is present, Santika 2011) set to 0.8, as we have 56 *Bd*+ localities out of a total of 66 (see *Results*). *Bd* absences (N = 10) for GLM and RF were based in localities where more than 21 individuals were sampled and all were *Bd*-negative by qPCR. This minimum sample size was based on an expected infection prevalence of 13.3% (Bacigalupe et al. 2017), an assumed test sensitivity of 100%, and a confidence interval of 95%. For each modeling method, random sampling of 25% of the data was used to evaluate the model performance, while the remaining 75% was used to fit the SDM. This procedure was repeated 10 times, and the AUC and jackknife values reported represent the mean of the 10 tests (Phillips et al. 2017). In Maxent, a geographical sampling bias correction was applied, including a 50-km buffer around each surveyed site. The area outside this buffer was automatically excluded (Syfert et al. 2013). In Maxent, the gain is defined as the improvement in penalized average log-likelihood compared to a null model (Elith et al. 2011) and we used it to evaluate the relative importance of each predictor variable by (1) jackknifing the observed value of gain; (2) comparing the decrease in gain when fitting a model using all but one variable with the gain of

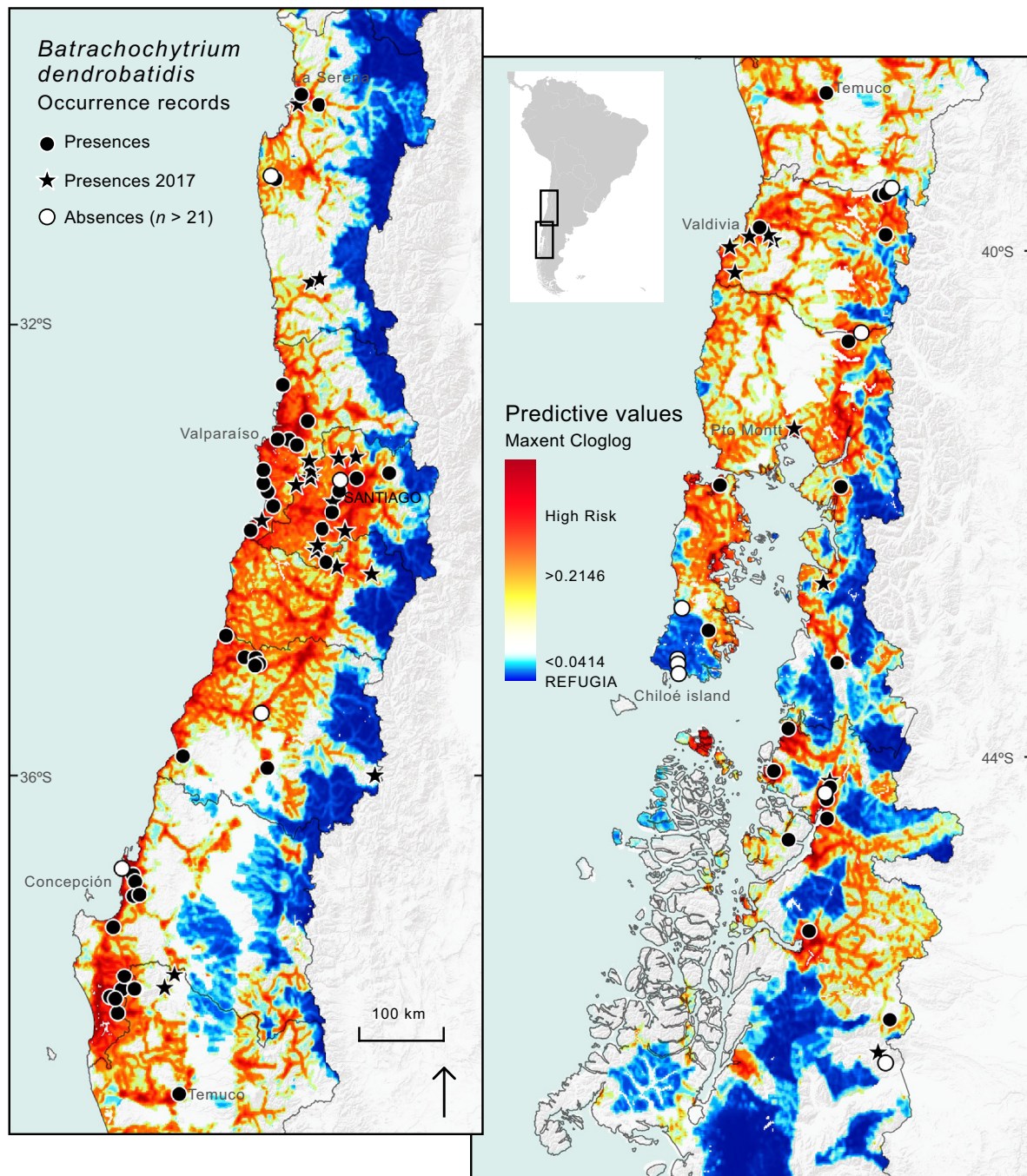


Fig. 1. Map of the biodiversity hotspot in Chile showing environmental suitability for *Batrachochytrium dendrobatidis* (*Bd*) based on Maxent projections. High-risk areas and refugia are shown in tones of red and blue, respectively. Black dots are the presences of *Bd* used in Maxent niche modeling. White dots (sample size ≥ 21) are the absences of *Bd* used to validate the refugia, while black stars are the new sampled localities in 2017 used to validate the high-risk areas.

Table 1. Variables used in the species distribution modeling.

Variable	Description	Source
Bioclimate variables: Annual Mean Temperature Mean Diurnal Range Isothermality Temperature Seasonality Max Temperature of Warmest Month Min Temperature of Coldest Month Temperature Annual Range Mean Temperature of Wettest Quarter Mean Temperature of Driest Quarter Mean Temperature of Warmest Quarter Mean Temperature of Coldest Quarter Annual Precipitation Precipitation of Wettest Month Precipitation of Driest Month Precipitation Seasonality Precipitation of Wettest Quarter Precipitation of Driest Quarter Precipitation of Warmest Quarter Precipitation of Coldest Quarter	Representative of 1960–1990, obtained from the Worldclim database with a resolution of 30-arc seconds	Hijmans et al. (2005)
Altitude	Digital elevation model with a resolution of 30 m	USGS, and Japan ASTER program
Distance to water bodies	Euclidean distance to water bodies boundaries	MMA-CEA (2012)
Topographic wetness index	Used to quantify topographic effect on hydrological processes and calculated in R through the package RSAGA (Brenning 2008)	Calculated from altitude
Net primary production	Net primary production of biomass from 1951 to 2000	Grieser et al. (2006)
Amphibian species richness	Obtained by overlaying the distribution range maps from the IUCN Red List Global Amphibian Assessment	IUCN (2017)
Population density	Population density of each municipality, obtained from the national population census	INE (2009)
Road density	Density of the road network in a 10-km radius	MOP (2012)
Distance to urban centers	Euclidean distance to urban land cover boundaries	CONAF-CONAMA-BIRF (1999)
Gross domestic product	Gross domestic product of each administrative Region	INE (2009)
Human footprint	A composite index which characterizes human influence on the land based on accessibility, anthropogenic land use, population density, and infrastructure	Sanderson et al. (2002)

the full model (i.e., including all variables); and (3) comparing the increase in gain when fitting a model using only one variable with the gain of the full model. Models were evaluated using the area under the curve (AUC) of the receiver operating characteristic, which measures the ability of the model to discriminate between sites with *Bd* presence and background sites. All modeling analyses were performed using Maxent 3.3.4 (Phillips et al. 2017) and the R package biomod2 (version 3.3-7; Thuiller et al. 2016).

Identification of thresholds to determine high-risk areas and refugia

Most SDMs give the results of the occurrence probability or environmental suitability for the target species as a continuous prediction in the study area. However, for many conservation management applications, discrete or presence/absence information is more useful (Liu et al. 2013a,b). In the context of our work, we considered the identification of two areas: (1) those with a high probability of *Bd* occurrence, which

we defined as high-risk; and (2) those with a low probability of *Bd* occurrence, which we defined as refugia (Puschendorf et al. 2009). Thus, threshold values were needed to transform the continuous output of each model into a discrete representation. For high-risk areas, we tested the performance of maximizing the sum of specificity and sensitivity (mSSS) and the prevalence of the training model, which maximizes the discrimination between presences and absences (Phillips et al. 2017). These thresholds have been shown to perform very well for presence-only models (Liu et al. 2013a,b) as only results from Maxent were retained (see *Results*). For refugia, we tested a very conservative threshold in order to be confident that an area identified with low suitability had a minimal probability of *Bd* occurrence. Therefore, we used the fixed cumulative value 1, which rejected only the lowest 1% of possible predicted values (Phillips et al. 2017).

Validation of SDM predictions

In addition to the SDM results, high-risk areas were validated via prospective field surveys conducted in 2017. These surveys collected 692 new *Bd* samples from 24 randomly chosen independent localities that were not included in the model training but were identified as high risk for *Bd* by our model. As we only retained the results from Maxent (see *Results*) which only uses presence data, we evaluated whether localities with apparent *Bd* absence (N = 12, 620 individual samples) were located within the refugia or high-risk areas predicted by the model. Two of these localities were found during the field surveys in 2017.

RESULTS

The compiled dataset for *Bd* occurrence comprised samples from 3881 individual amphibians (3539 from our field sampling, and 342 obtained from the literature) and represented 29 species (50% of Chilean amphibian richness), 20 of which had at least one individual positive for *Bd* (Appendix S1: Table S1). The dataset comprised 143 sites surveyed between 2005 and 2017, 85 (59%) of which had confirmed *Bd* presence. We used 56 independent *Bd*+ occurrence localities to carry out environmental niche models (Fig. 1).

Environmental niche modeling

Maxent had the highest average AUC compared with RF and GLM models (Maxent = 0.82; RF = 0.64; GLM = 0.61). As the predictive capacity of both RF and GLM was just above a random model (i.e., AUC = 0.5), the information they provide regarding the coefficients and the relative importance of the independent variables is unreliable (Qiao et al. 2015). Therefore, Maxent was the only modeling procedure retained for further analyses. The exclusion of human footprint caused the greatest reduction in both the regularized training gain and the test gain (Appendix S1: Fig. S1). The environmental variables with the highest gains when used in isolation were human footprint, elevation, distance to nearest water body, and distance to nearest urban center (Appendix S1: Fig. S1). Climatic variables were not primary drivers of *Bd* environmental suitability. The response curves for the predictors with the highest gains showed that environmental suitability for *Bd* increased with human footprint and shorter distances to urban centers and water bodies. In addition, environmental suitability for *Bd* decreased with elevation.

High-risk areas and refugia: Validation of SDM predictions

The threshold performance in the classification of training presences (true positives and false negatives), testing absences (true negatives, false positives), and testing presences (true positives, false negatives) for *Bd* is summarized in Fig. 2; Appendix S1: Table S3. In brief, a higher proportion of *Bd*+ sites were found from the prevalence of the training model threshold than from mSSS. In addition, compared to what was found with mSSS, prevalence of the training model threshold yielded a higher accuracy and a substantially lower omission error when classifying presences surveyed during 2017 (Fig. 2; Appendix S1: Table S3). Therefore, the prevalence of the training model threshold was the one we used to define high-risk areas. Likewise, the fixed 1 threshold had zero omission error (Appendix S1: Table S3), thus providing a reliable indicator for *Bd* absence. Overall, the areas identified as refugia and high-risk for *Bd* covered 22.6 and 27.1% of the study area, respectively. Additionally, mean amphibian species richness in the high-risk

areas was 6.1 ± 2.6 SD while in the refugia was 4.3 ± 3.1 SD. Despite this slight difference, both types of areas show a relatively high diversity of amphibians.

Twenty-three out of 24 sites surveyed during 2017 in the predicted high-risk areas were positive for *Bd*, which represents a 96% validation of this aspect of the model predictions (Fig. 1). The AUC from the Maxent model for these new occurrences was 0.815. Of the 12 sites with apparent *Bd* absences, four were located within the refugia predicted by the model (i.e., Chiloé Island) and five were located within the predicted high-risk areas (Fig. 1). The remaining three sites were located between the defined thresholds.

DISCUSSION

Amphibian chytridiomycosis is recognized as a primary driver factor of the global amphibian decline crisis (Skerratt et al. 2007, Wake and Vredenburg 2008, Collins and Crump 2009, Fisher et al. 2012). For effective conservation management, therefore, it is prudent to identify areas with high or low suitability for *Bd*. This knowledge can also inform where to allocate amphibian population monitoring and disease surveillance efforts (Bosch et al. 2015, Langwig et al. 2015). For the Chilean biodiversity hotspot, our results show that (1) suitability for *Bd* increased with human footprint and shorter distances to urban centers and water bodies and decreased with elevation; (2) climate is not a major factor shaping the current distribution of *Bd*, and (3) the model predicted high-risk and refugia areas fairly well, as they were validated with new data.

A recent regional analysis of *Bd* prevalence in the Chilean biodiversity hotspot indicated that, once temporal and spatial effects are taken into account, *Bd* occurrence tends to be higher in regions with higher gross domestic product, particularly near large urban areas (Bacigalupe et al. 2017). Our SDM model found a positive association between *Bd* occurrence and proximity to urban centers and, more general, with increasing human footprint. At least two different processes might produce this pattern. First, it might be indicative of human-mediated transmission of the fungus (e.g., intentional or accidental

introduction of *Bd* with infected animals or via the transportation of contaminated water or damp substrates; e.g., Fisher and Garner 2007, Soto-Azat et al. 2016). This is consistent with other studies that have also found a positive relationship between *Bd* occurrence and human influence at both local (Adams et al. 2010) and regional (James et al. 2015) scales, but not at the global scale (Liu et al. 2013a,b). Second, this pattern might occur if the human footprint translates into reduced habitat quality for amphibians or increases other stressors, which may increase their susceptibility to *Bd* infections (Daszak et al. 2003, Gray et al. 2007, Ramsey et al. 2010, Becker et al. 2016, Blaustein et al. 2018), potentially increasing pathogen occurrence.

Distribution patterns associated with populated centers and roads are common in the first stages of many biological invasions (Pauchard and Alaback 2004, Christen and Matlack 2009, Skultety and Matthews 2017). Therefore, our results might indicate that *Bd* invasion in Chile is fairly recent, a hypothesis supported by two additional lines of evidence. First, a retrospective epidemiological study based on museum specimens showed that the earliest record of *Bd* in Chile is from a four-eyed toad collected from Concepción in 1970 (Soto-Azat et al. 2013b). Second, more recently, Valenzuela-Sánchez et al. (2018) conducted a phylogenomic study of *Bd* isolates from Chile and showed that all belong to the global pandemic lineage, possibly indicating a single, recent introduction of the pathogen into Chile. The global panzootic lineage of *Bd* emerged from East Asia during the past century and has been rapidly dispersed globally since then by the international amphibian trade (O'Hanlon et al. 2018).

A recent invasion, or invasions, of *Bd* into Chile would suggest that the distribution of *Bd* has not yet reached equilibrium with its environment. That is, an organism is considered to be at equilibrium if it occurs in all suitable locations and is absent from all unsuitable ones (Araújo and Pearson 2005). Thus, under equilibrium settings it would be reasonable to expect that climate (or other non-human environmental factors) were the main drivers of a species distribution. It is important mentioning that non-equilibrium settings violate one of the key assumptions of environmental niche modeling,

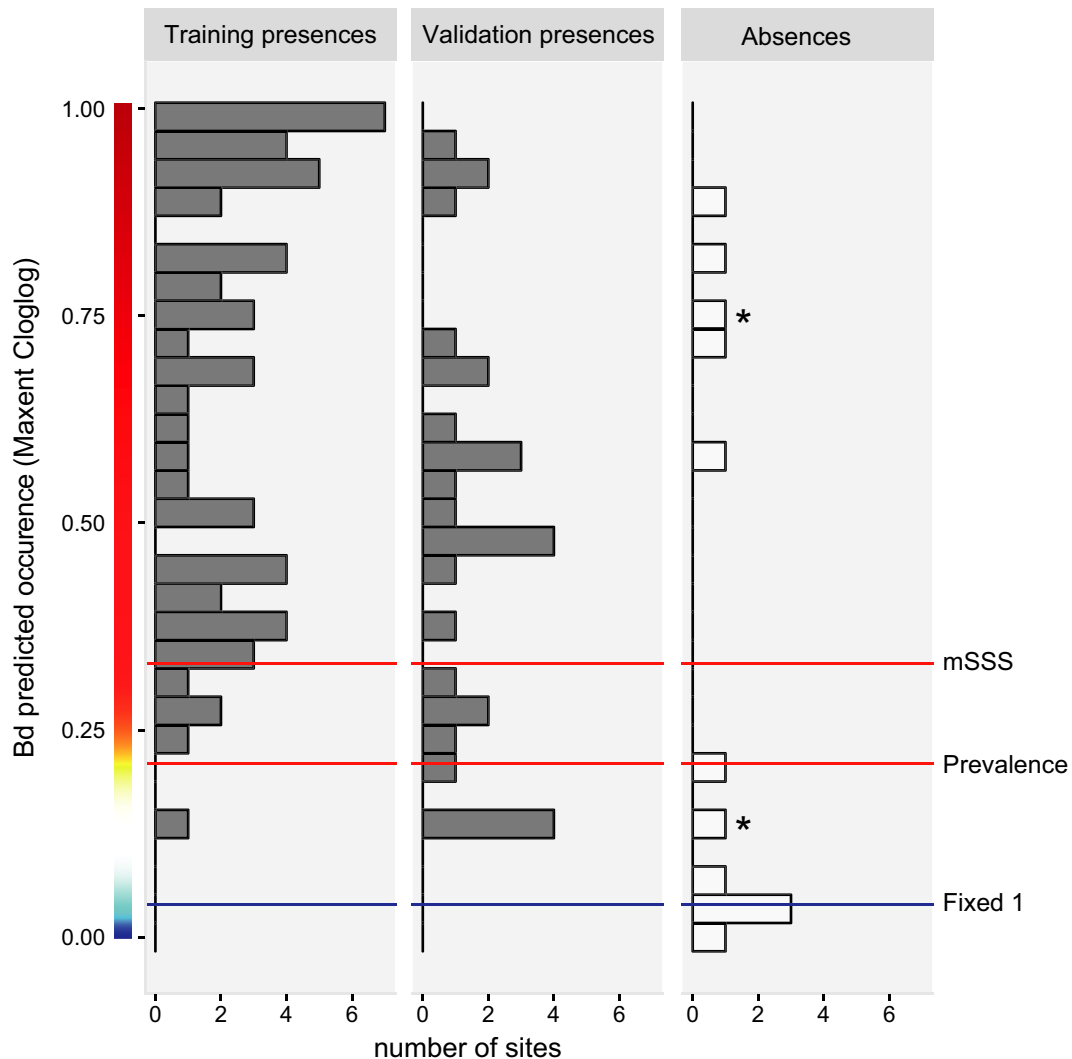


Fig. 2. Frequency distribution of occurrence records in relation to the Maxent predicted probability of *Bd* occurrence. The thresholds for fixed accumulative value 1 (F1), prevalence of the training model (Prev), and for maximizing the sum of sensitivity and specificity (mSSS) used for refugia and high-risk delimitation are highlighted in blue (refugia) and red (high-risk) horizontal lines. Training presences were the *Bd*⁺ occurrences used to fit the model. Validation presences were the *Bd*⁺ occurrences obtained during the prospective fields surveys in 2017. Twenty-three of those occurrences are above the Prevalence threshold (high-risk areas), while the remaining 5 occurred in areas between thresholds. Absences with an * represent the *Bd*⁻ localities obtained during the 2017 field surveys.

because they usually involve extrapolating to novel environments, well beyond where the species is found (Jeschke and Strayer 2008, Elith and Leathwick 2009). However, this might not represent a problem in our study, as our data set has *Bd* occurrences fairly well distributed throughout the biodiversity hotspot (Fig. 1),

encompassing the wide range of climates, environments, and human impact factors along its latitudinal range. Therefore, the extrapolated area is located within the same latitudinal range of the biodiversity hotspot, although beyond the 50-km buffer that we constructed around *Bd* occurrence locations.

A recent *Bd* introduction could explain why climatic or other environmental factors do not play a dominant role predicting the current distribution of *Bd* in our study area. This result does not agree to what similar studies have shown for other regions (Puschendorf et al. 2009, Murray et al. 2011, Flechas et al. 2017). However, those studies have only used climatic variables as predictors, possibly because the *Bd* distribution in those regions is in equilibrium with the environment. In our study, only the PC2, which was positively associated with mean temperatures (i.e., annual mean, minimum temperature of coldest month, and mean temperature of coldest and wettest quarter, Appendix S1: Table S2), showed some test gain when used as the only predictor in fitting the model (Appendix S1: Fig. S1). Nevertheless, once the effect of the invasion stabilizes and *Bd* reaches an equilibrium with the environment, climatic factors would take more prominence in explaining its distribution and therefore in predicting high-risk and refugia areas. It was not completely surprising that precipitation variables, incorporated mostly within PC1, were not important in predicting suitability for *Bd*. This is because distance to water bodies encompasses a much more reliable proxy of the presence of permanent water, certainly an important component that facilitates *Bd* persistence and transmission. Our results also indicate that suitability for *Bd* decreases with elevation, which is opposite to what other authors have found regarding the best conditions for *Bd* growth (Woodhams and Alford 2005, Scheele et al. 2017). Nevertheless, the high altitude areas where suitability is indeed very low (Andes mountains in blue in Fig. 1) are actually areas where human impact is very low, strengthening the fact of a recent invasion of *Bd* in the hotspot.

Management implications

Although correlative SDMs have been applied to a broad range of ecological questions, there has been considerable interest in predicting possible ranges of invasive species (Peterson 2003). Therefore, predicting the occurrence of *Bd* with accuracy represents a key tool for spatially explicit, landscape-based conservation planning.

Our surveys of 24 new localities in high-risk areas predicted by the model confirmed *Bd* occurrence in 23 of them. Identified high-risk

areas were mainly situated along the coast and central valley of Chile (Fig. 1). While the prediction of a site being high-risk (i.e., high suitability for *Bd*) does not necessarily mean that *Bd* is already present, it identifies areas where *Bd* surveillance and amphibian population monitoring efforts are needed (Langwig et al. 2015). In addition, five localities with apparent *Bd* absence were found in the predicted high-risk areas (Fig. 1). The identification of naïve populations of *Bd*-susceptible species in these areas should be a priority, either to develop management strategies to prevent *Bd* arrival or to rapidly identify novel *Bd* occurrences and consider *Bd* mitigations strategies. This is particularly relevant given that most of the identified high-risk areas are regions of high endemic amphibian diversity, such as the Nahuelbuta range (Vidal and Díaz-Páez 2012). Although refugia are areas with low *Bd* suitability accordingly to our model, our results indicate that suitability in our system is more related with the potential of *Bd* to disperse into an area, mediated by human impact, rather than with environmental variables such as climate. This would suggest that the identified refugia are such because *Bd* has not yet reached them and therefore, preventing its eventual arrival is of paramount importance. In this context, refugia provide a critical line of defense because, at least in the Chilean biodiversity hotspot, they can exist not only nearby high-risk areas but also close to sites with known *Bd* presence (Fig. 1). Four localities with apparent *Bd* absence were located within refugia predicted by the model, all within southern Chiloé Island (Fig. 1). Low *Bd* suitability areas, therefore, represent potentially valuable pathogen refuge for native amphibians, as well as candidate sites for translocating animals, in the framework of reintroduction programs. Predicted refugia warrant systematic monitoring to identify potential *Bd* incursion and to enable rapid responses to prevent pathogen establishment (Garner et al. 2016, Hudson et al. 2016). However, as only 24.4% of the areas predicted to be refugia are under some formal aspect of land protection, education and outreach are key to engage the public about the importance of these sites and to ensure the protection of these important amphibian habitats. Furthermore, the lack of legislative and organizational governance to address emerging wildlife

diseases might limit the range of potential actions that can be implemented. For example, a response strategy currently exists in the United States for the potential invasion of *Batrachochytrium salamandrivorans* (*Bsal*) aimed at identifying, among other things, the monitoring needs that could better inform management and conservation strategies for the country (Gray et al. 2015, Grant et al. 2016). In this context, it would be highly relevant to hold meetings and workshops with relevant stakeholders, including government agencies, private land owners (protected and non-protected areas), and local communities in order to design a monitoring plan and implement national preventive biosecurity protocols and campaigns such as the one employed with the invasion of the freshwater algae *Didymosphenia geminata* (*Didymo*) in south Chile (e.g., <http://didymo.sernapesca.cl>).

In this study, we developed a feasible approach for guiding conservation management to help mitigate the impact of the EID amphibian chytridiomycosis. Using Chile as a model system, we present key baseline information to enable the prioritization of efforts to detect and manage *Bd* in the Chilean biodiversity hotspot. We clearly identify which areas are priorities for monitoring for conservation purposes (e.g., refugia in southern Chiloé, *Bd*-negative high-risk areas) by using a fairly simple and well-known statistical methodology that can be readily applied elsewhere. The statistical methodology we used (i.e., SDM) is standard and has been already proposed as a tool to guide conservation efforts against chytridiomycosis (e.g., Puschen-dorf et al. 2009, Murray et al. 2011, Flechas et al. 2017). Although environmental suitability for *Bd* is context-dependent, SDMs could be used to identify areas of concern in other regions (particularly in Central and South America, two areas severely impacted by chytridiomycosis) where prioritized *Bd* surveillance efforts and amphibian population monitoring could be implemented. New information about *Bd* is rapidly emerging; hence, adaptive management of response efforts will likely be needed. Results from SDMs should be regularly updated once new data on *Bd* presence becomes available or as more precise and relevant information about environmental factors that enable or suppress *Bd* establishment is obtained.

ACKNOWLEDGMENTS

Leonardo D. Bacigalupe, C. Soto-Azat, and Andrés Valenzuela-Sánchez acknowledge funding from FONDECYT grants 1150029, 1181758, and 3180107, respectively. Sergio A. Estay acknowledges funding from FONDECYT 1160370 and CAPES FB-0002-2014 line 4. The authors declare they do not have any conflict of interest. We would like to thank Johara Bourke and Virginia Moreno who gave us unpublished information on *Bd* sampling sites and The Nature Conservancy for collaborating with us in sampling within the Reserva Costera Valdiviana. Special thanks to two anonymous reviewers whose comments greatly improved the manuscript. LDB and CSA conceived the idea and secured the funding for this project; LDB, IAV, and SAE designed the methodology; CSA, AVS, MAR, AAC, IAV, and APR completed the fieldwork, molecular analyses, and interpretation of laboratory results; IAV performed the analyses with input from SAE and LDB; LDB and IAV led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. We declare we have no competing interests.

LITERATURE CITED

- Adams, M. J., et al. 2010. Using occupancy models to understand the distribution of an amphibian pathogen, *Batrachochytrium dendrobatidis*. *Ecological Applications* 20:289–302.
- Araújo, M. B., and R. G. Pearson. 2005. Equilibrium of species' distributions with climate. *Ecography* 28:693–695.
- Bacigalupe, L. D., C. Soto-Azat, C. García-Vera, I. Barriá-Oyarzo, and E. L. Rezende. 2017. Effects of amphibian phylogeny, climate and human impact on the occurrence of the amphibian-killing chytrid fungus. *Global Change Biology* 23:3543–3553.
- Barbosa, O., and P. Villagra. 2015. Socio-ecological studies in urban and rural ecosystems in Chile. Pages 297–311 in R. Rozzi, F. S. Chapin III, J. B. Callicott, S. Pickett, M. E. Power, J. J. Armesto, and R. H. May Jr, editors. *Earth stewardship, linking ecology and ethics in theory and practice*. Springer, New York, New York, USA.
- Becker, C. G., D. Rodríguez, C. Lambertini, L. F. Toledo, and C. F. Haddad. 2016. Historical dynamics of *Batrachochytrium dendrobatidis* in Amazonia. *Ecography* 39:954–960.
- Berger, L., A. A. Roberts, J. Voyles, J. E. Longcore, K. A. Murray, and L. F. Skerratt. 2016. History and recent progress on chytridiomycosis in amphibians. *Fungal Ecology* 19:89–99.

- Blaustein, A. R., J. Urbina, P. W. Snyder, E. Reynolds, T. Dang, J. T. Hoverman, B. Han, D. H. Olson, C. Searle, and N. M. Hambalek. 2018. Effects of emerging infectious diseases on amphibians: a review of experimental studies. *Diversity* 10:81.
- Bosch, J., E. Sanchez-Tomé, A. Fernandez-Loras, J. A. Oliver, M. C. Fisher, and T. W. J. Garner. 2015. Successful elimination of a lethal wildlife infectious disease in nature. *Biology Letters* 11:20150874.
- Brenning, A. 2008. Statistical geocomputing combining R and SAGA: the example of landslide susceptibility analysis with generalized additive models. *Hamburger Beiträge zur Physischen Geographie und Landschaftsökologie* 19:23–32.
- Christen, D. C., and G. R. Matlack. 2009. The habitat and conduit functions of roads in the spread of three invasive plant species. *Biological Invasions* 11:453–465.
- Collins, J. P., and M. L. Crump. 2009. Extinction in our times: global amphibian decline. Oxford University Press, Oxford, UK.
- CONAF (Corporación Nacional Forestal, CL), CONAMA (Comisión Nacional del Medio Ambiente, CL), BIRF (Banco Internacional de Reconstrucción y Fomento), Universidad Austral de Chile, Pontificia Universidad Católica de Chile, Universidad Católica de Temuco. 1999. Catastro y evaluación de recursos vegetacionales nativos de Chile. Informe nacional con variables ambientales.
- Cunningham, A. A., P. Daszak, and J. L. N. Wood. 2017. One health, emerging infectious diseases, and wildlife: two decades of progress? *Philosophical Transactions of the Royal Society B* 372:20160167.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife - Threats to biodiversity and human health. *Science* 287:443–449.
- Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* 9:141–150.
- Elith, J., and J. R. Leathwick. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics* 40:677–697.
- Elith, J., S. J. Phillips, T. Hastie, M. Dudík, Y. E. Chee, and C. J. Yates. 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions* 17:43–57.
- Estay, S. A., F. A. Labra, R. D. Sepúlveda, and L. D. Bacigalupe. 2014. Evaluating habitat suitability for the establishment of *Monochamus* spp. through climate-based niche modelling. *PLoS ONE* 9:e102592.
- Fisher, M. C., and T. W. J. Garner. 2007. The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. *Fungal Biology Reviews* 21:2–9.
- 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.
- Flechas, S. V., et al. 2017. Current and predicted distribution of the pathogenic fungus *Batrachochytrium dendrobatidis* in Colombia, a hotspot of amphibian biodiversity. *Biotropica* 49:685–694.
- Garner, T. W. J., B. R. Schmidt, A. Martel, F. Pasmans, E. Muths, A. A. Cunningham, C. Weldon, M. C. Fisher, and J. Bosch. 2016. Mitigating amphibian chytridiomycoses in nature. *Philosophical Transactions of the Royal Society: Biological Sciences* 371:20160207.
- Ghirardi, R., M. G. Perotti, M. M. Steciow, M. L. Arelano, and G. S. Natale. 2011. Potential distribution of *Batrachochytrium dendrobatidis* in Argentina: implications in amphibian conservation. *Hydrobiologia* 659:111–115.
- Grant, E. H. C., et al. 2016. Salamander chytrid fungus (*Batrachochytrium salamandrivorans*) in the United States—Developing research, monitoring, and management strategies: U.S. Geological Survey Open-File Report 2015–1233. <https://doi.org/10.3133/ofr20151233>
- Gray, M. J., D. L. Miller, A. C. Schmutz, and C. A. Baldwin. 2007. Frog virus 3 prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA. *Diseases of Aquatic Organisms* 77:97–103.
- Gray, M. J., et al. 2015. *Batrachochytrium salamandrivorans*: the North American Response and a Call for Action. *PLoS Pathogens* 11:e1005251.
- Grieser, J., R. Gommers, S. Cofield, and M. Bernardi. 2006. World maps of climatological net primary production of biomass, NPP. UN/FAO, Environment, Climate Change and Bioenergy Division, FAO-Climpag, Rome, Italy.
- Guisan, A., and W. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters* 8:993–1009.
- Hijmans, R. J., S. E. Cameron, and J. L. Parra. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965–1978.
- Hudson, M. A., et al. 2016. In-situ itraconazole treatment improves survival rate during an amphibian chytridiomycosis epidemic. *Biological Conservation* 195:37–45.
- INE, Instituto Nacional de Estadísticas, MINSAL, SRCEL. 2009. Estadísticas vitales, informe anual

2009. INE, Instituto Nacional de Estadísticas, MIN-SAL, SRCeL, Santiago, Chile.
- IUCN. 2017. The IUCN Red List of Threatened Species. Version 2017-3. <http://www.iucnredlist.org>
- James, T. Y., A. P. Litvintseva, R. Vilgalys, J. A. T. Morgan, J. W. Taylor, M. C. Fisher, L. Berger, C. Weldon, L. du Preez, and J. E. Longcore. 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *PLoS Pathogens* 5: e1000458.
- James, T. Y., et al. 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.
- Jeschke, J. M., and D. L. Strayer. 2008. Usefulness of bioclimatic models for studying climate change and invasive species. *Annals of the New York Academy of Sciences* 1134:1–24.
- Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. *Nature* 451:990–994.
- Keppel, G., and G. W. Wardell-Johnson. 2012. Refugia: keys to climate change management. *Global Change Biology* 18:2389–2391.
- Kruger, K. M., F. Pereoglou, and J. M. Hero. 2007. Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. *Conservation Biology* 21:1280–1290.
- Langwig, K. E., et al. 2015. Context-dependent conservation responses to emerging wildlife diseases. *Frontiers in Ecology and the Environment* 13:195–202.
- Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of USA* 103:3165–3170.
- Liu, X., J. R. Rohr, and Y. Li. 2013a. Climate, vegetation, introduced hosts and trade shape a global wildlife pandemic. *Proceedings of the Royal Society B* 280:20122506.
- Liu, C., M. White, and G. Newell. 2013b. Selecting thresholds for the prediction of species occurrence with presence-only data. *Journal of Biogeography* 40:778–789.
- Meineri, E., A. S. Deville, D. Grémillet, M. Gauthier-Clerc, and A. Béchet. 2015. Combining correlative and mechanistic habitat suitability models to improve ecological compensation. *Biological Reviews* 90:314–329.
- Mittermeier, R. A., W. R. Turner, and F. W. Larsen. 2011. Global biodiversity conservation: the critical role of hotspots. Pages 3–22 *in* Biodiversity hotspots. Springer, New York, New York, USA.
- MMA - CEA. 2012. Inventario nacional de humedales. Ministerio de Medio Ambiente, Santiago, Chile.
- MOP (Ministerio de Obras Públicas). 2012. Red vial – Dirección de Vialidad.
- Moriguchi, S., A. Tominaga, K. J. Irwin, M. J. Freake, K. Suzuki, and K. Goka. 2015. Predicting the potential distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in East and Southeast Asia. *Diseases of Aquatic Organisms* 113: 177–185.
- Murray, K. A., R. W. R. Retallick, R. Puschendorf, L. F. Skerratt, D. Rosauer, H. I. McCallum, L. Berger, R. Speare, and J. VanDerWal. 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.
- Murray, K. A., and L. F. Skerratt. 2012. Predicting wild hosts for amphibian chytridiomycosis: integrating host life-history traits with pathogen environmental requirements. *Human and Ecological Risk Assessment: an International Journal* 18:200–224.
- Muths, E., D. S. Pilliod, and L. J. Livo. 2008. Distribution and environmental limitations of an amphibian pathogen in the Rocky Mountains, USA. *Biological Conservation* 141:1484–1492.
- O’Hanlon, S. J., et al. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. *Science* 360:621–627.
- Olson, D. H., D. M. Aanensen, K. L. Ronnenberg, C. I. Powell, S. F. Walker, J. Bielby, T. W. J. Garner, G. Weaver, The Bd Mapping Group, and M. C. Fisher. 2013. Mapping the Global Emergence of *Batrachochytrium dendrobatidis*, the Amphibian Chytrid Fungus. *PLoS ONE* 8:e56802.
- Pauchard, A., and P. B. Alaback. 2004. Influence of elevation, land use, and landscape context on patterns of alien plant invasions along roadsides in protected areas of South Central Chile. *Conservation Biology* 18:238–248.
- Peterson, A. T. 2003. Predicting the geography of species’ invasions via ecological niche modeling. *Quarterly Review of Biology* 78:419–433.
- Peterson, A. T., M. Papeş, and J. Soberón. 2015. Mechanistic and correlative models of ecological niches. *European Journal of Ecology* 1:28–38.

- Phillips, S. J., R. P. Anderson, M. Dudík, R. E. Schapire, and M. E. Blair. 2017. Opening the black box: an open-source release of Maxent. *Ecography* 40:887–893.
- 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. *Diversity and Distributions* 15:401–408.
- Qiao, H., J. Soberón, and A. T. Peterson. 2015. No silver bullets in correlative ecological niche modelling: insights from testing among many potential algorithms for niche estimation. *Methods in Ecology and Evolution* 6:1126–1136.
- Ramsey, J. P., L. K. Reinert, L. K. Harper, D. C. Woodhams, and L. A. Rollins-Smith. 2010. Immune defenses against *Batrachochytrium dendrobatidis*, a fungus linked to global amphibian declines, in the South African Clawed frog, *Xenopus laevis*. *Infection & Immunity* 78:3981–3992.
- Riley, K., O. F. Berry, and J. D. Roberts. 2013. Do global models predicting environmental suitability for the amphibian fungus, *Batrachochytrium dendrobatidis*, have local value to conservation managers? *Journal of Applied Ecology* 50:713–720.
- Rödger, D., J. Kielgast, J. Bielby, S. Schmidlein, J. Bosch, T. W. J. Garner, M. Veith, S. Walker, M. C. Fisher, and S. Lötters. 2009. Global amphibian extinction risk assessment for the Panzootic Chytrid fungus. *Diversity* 1:52–66.
- Rohr, J. R., N. T. Halstead, and T. R. Raffel. 2011. Modelling the future distribution of the amphibian chytrid fungus: the influence of climate and human-associated factors. *Journal of Applied Ecology* 48:174–176.
- Ron, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. *Biotropica* 37:209–221.
- Sanderson, E. W., M. Jaiteh, M. A. Levy, K. H. Redford, A. V. Wannebo, and G. Woolmer. 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* 52:891–904.
- Santika, T. 2011. Assessing the effect of prevalence on the predictive performance of species distribution models using simulated data. *Global Ecology and Biogeography* 20:181–192.
- Scheele, B. C., et al. 2017. After the epidemic: ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis. *Biological Conservation* 206:37–46.
- Schloegel, L. M., J. M. Hero, L. Berger, R. Speare, K. McDonald, and P. Daszak. 2006. The decline of the sharp-snouted say frog (*Taudactylus acutirostris*): the first documented case of extinction by infection in a free-ranging wildlife species? *EcoHealth* 3:35–40.
- Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.
- Skultety, D., and J. W. Matthews. 2017. Urbanization and roads drive non-native plant invasion in the Chicago Metropolitan region. *Biological Invasions* 19:2553.
- Soto-Azat, C., A. Peñafiel-Ricaurte, S. J. Price, N. Sallaberry-Pincheira, M. P. García, M. Alvarado-Rybak, and A. A. Cunningham. 2016. *Xenopus laevis* and emerging amphibian pathogens in Chile. *EcoHealth* 13:775–783.
- Soto-Azat, C., A. Valenzuela-Sánchez, B. Collen, J. M. Rowcliffe, A. Veloso, and A. A. Cunningham. 2013a. The population decline and extinction of Darwin's Frogs. *PLoS ONE* 8:e66957.
- Soto-Azat, C., A. Valenzuela-Sánchez, B. T. Clarke, K. Busse, J. C. Ortiz, C. Barrientos, and A. A. Cunningham. 2013b. Is chytridiomycosis driving Darwin's frogs to extinction? *PLoS ONE* 8:e79862.
- Spitzen-Van Der Sluijs, A., A. Martel, C. A. Hallmann, W. Bosman, T. W. J. Garner, P. Van Rooij, F. Haesebrouck, and F. Pasmans. 2014. Environmental determinants of recent endemism of *Batrachochytrium dendrobatidis* infections in Amphibian Assemblages in the absence of disease outbreaks. *Conservation Biology* 28:1302–1311.
- Syfert, M. M., M. J. Smith, and D. A. Coomes. 2013. The effects of sampling bias and model complexity on the predictive performance of MaxEnt species distribution models. *PLoS ONE* 8:e55158.
- Thuiller, W., D. Georges, R. Engler, and F. Breiner. 2016. Package 'Biomod2': ensemble Platform for Species Distribution Modeling. R Package Version 3.3-7.
- Valenzuela-Sánchez, A., S. J. O'Hanlon, M. Alvarado-Rybak, D. E. Uribe-Rivera, A. A. Cunningham, M. C. Fisher, and C. Soto-Azat. 2018. Genomic epidemiology of the emerging pathogen *Batrachochytrium dendrobatidis* from native and invasive amphibian species in Chile. *Transboundary and Emerging Diseases* 65:309–314.
- Valenzuela-Sánchez, A., B. R. Schmidt, D. E. Uribe-Rivera, F. Costas, A. A. Cunningham, and C. Soto-Azat. 2017. Cryptic disease-induced mortality may cause host extinction in an apparently stable host-parasite system. *Proceedings of the Royal Society B* 284:20171176.

- Vidal, M. A., and H. Díaz-Páez. 2012. Biogeography of Chilean herpetofauna: biodiversity hotspot and extinction risk. Pages 137–154 in L. Stevens, editor. *Global advances in biogeography* InTech, Rijeka, Croatia.
- Wake, D. B., and V. T. Vredenburg. 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 of USA* 105:11466–11473.
- Woodhams, D. C., and R. A. Alford. 2005. Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conservation Biology* 19:1449–1459.
- Xie, G. Y., D. H. Olson, and A. R. Blaustein. 2016. Projecting the global distribution of the emerging amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, based on IPCC climate futures. *PLoS ONE* 11:e0160746.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2724/full>