Effects of amphibian phylogeny, climate and human impact on the occurrence of the amphibian-killing chytrid fungus

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Abstract

Chytridiomycosis, due to the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been associated with the alarming decline and extinction crisis of amphibians worldwide. Because conservation programs are implemented locally, it is essential to understand how the complex interactions among host species, climate and human activities contribute to *Bd* occurrence at regional scales. Using weighted phylogenetic regressions and model selection, we investigated geographic patterns of *Bd* occurrence along a latitudinal gradient of 1500 km within a biodiversity hot spot in Chile (1845 individuals sampled from 253 sites and representing 24 species), and its association with climatic, socio-demographic and economic variables. Analyses show that *Bd* prevalence decreases with latitude although it has increased by almost 10% between 2008 and 2013, possibly reflecting an ongoing spread of *Bd* following the introduction of *Xenopus laevis*. Occurrence of *Bd* was higher in regions with high gross domestic product (particularly near developed centers) and with a high variability in rainfall regimes, whereas models including other bioclimatic or geographic variables, including temperature, exhibited substantially lower fit and virtually no support based on Akaike weights. In addition, *Bd* prevalence exhibited a strong phylogenetic signal, with five species having high numbers of infected individuals and higher prevalence than the average of 13.3% across all species. Taken together, our results highlight that *Bd* in Chile might still be spreading south, facilitated by a subset of species that seem to play an important epidemiological role maintaining this pathogen in the communities, in combination with climatic and human factors affecting the availability and quality of amphibian breeding sites. This information may be employed to design conservation strategies and mitigate the impacts of *Bd* in the biodiversity hot spot of southern Chile, and similar studies may prove useful to disentangle the role of different factors contributing to the emergence and spread of this catastrophic disease.

Introduction

Emerging infectious diseases (EIDs) have been increasingly recognized as a threat to biodiversity (Daszak *et al.*, 2000). Disease emergence results from a change in the interplay between host, pathogen and environment (Smith *et al.*, 2009; Engering *et al.*, 2013). Under the current scenario of rapid environmental changes having direct impacts on hosts and pathogens, there is a growing interest to understand the role of global change drivers in the emergence and spread of infectious diseases (Jones *et al.*, 2008). In this context, amphibians are one of the most representative symbols of the current biodiversity crisis (Wake & Vredenburg, 2008; Collins &

Crump, 2009). Almost one-third of the world's amphibian species are threatened by extinction, with around 40% having declining populations and 122 species having disappeared since 1980 (Stuart *et al.*, 2004). These daunting trends have been driven primarily by land change use, leading to habitat loss and fragmentation, but other factors including over-exploitation, competition and/or predation by introduced species, several types of contaminants, global warming and EIDs have been also recognized as accelerating factors to the current declines (Sodhi *et al.*, 2008; Collins & Crump, 2009; Hof *et al.*, 2011).

In recent years, evidence for the critical involvement of EIDs in the amphibian decline phenomenon has grown and become more convincing, especially in the case of amphibian chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*). This disease has

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been associated with amphibian population declines at the global scale and is implicated in the extinction of several amphibian species from Australia, Costa Rica and Chile (Pounds *et al.*, 1997; Daszak *et al.*, 1999; Lips *et al.*, 2006; Pounds *et al.*, 2006; Schloegel *et al.*, 2006; Skerratt *et al.*, 2007; Bielby *et al.*, 2008; Wake & Vredenburg, 2008; Collins & Crump, 2009; Soto-Azat *et al.*, 2013a,b). Less than two decades exist for *Bd* investigation (Berger *et al.*, 1998; Longcore *et al.*, 1999), and although a substantial amount of research has been accomplished, several aspects of its basic biology, epidemiology and interaction with their hosts are still unknown (Kilpatrick *et al.*, 2010). Knowledge of the epidemiology and pathogenesis of *Bd* is essential in attempt to prevent further amphibian declines (Skerratt *et al.*, 2007; Berger *et al.*, 2016). The pathogen has been detected in 695 amphibian species, but appears to be capable of infecting the entire class Amphibia (Olson & Ronnenberg, 2014). Many lineages of *Bd* have now been described, including enzootic genotypes from Asia, Africa, Europe and South America, but one clade termed the global pandemic lineage (*Bd*GPL), globally widespread and hypervirulent, is believed to be the main lineage responsible for the observed catastrophic declines (James *et al.*, 2009; Farrer *et al.*, 2011; Liu *et al.*, 2013; Rosenblum *et al.*, 2013).

The susceptibility to the pathogenic effects of *Bd* differs across amphibian life stages, populations and species (Blaustein *et al.*, 2005; Fisher *et al.*, 2009; Searle *et al.*, 2011; Bradley *et al.*, 2015). Tadpoles, as well as adults of many species, are tolerant to *Bd* infection but resistant to developing chytridiomycosis, which potentially would make them to act as competent reservoirs of the fungus (Daszak *et al.*, 2004; Hanselmann *et al.*, 2004; Fisher & Garner, 2007; Schloegel *et al.*, 2010). It is important to note that this resistance can be reversed under certain environmental conditions, for example those that can exert stress and immunosuppression (Ramsey *et al.* 2010). Thus, the prevalence of *Bd* (i.e., number of positives/number sampled) in different populations is likely reflecting the interaction between the host, the pathogen and the environmental conditions they encounter (Murray *et al.*, 2010; Murray & Skerratt, 2012; Spitzen-Van der Sluijs *et al.*, 2014). Determining how these factors interact and explain the distribution patterns of *Bd* should prove extremely useful in developing population monitoring programs and management practices to reduce the impacts of this EID (Haydon *et al.*, 2002; Reeder *et al.*, 2012; Scheele *et al.*, 2014). For instance, information on whether *Bd* prevalence varies geographically (Kriger *et al.*, 2007; Spitzen-Van der Sluijs *et al.*, 2014) with climate (Murray *et al.*, 2011) and/or across amphibian species can be employed to determine areas that harbor *Bd* and

species that might act as reservoirs and, ultimately, predict areas under risk of further *Bd* spread. Interestingly, it has recently been found that human-associated factors are good predictors of *Bd* occurrence both at the local (Murray *et al.*, 2011; Rohr *et al.*, 2011; Spitzen-Van der Sluijs *et al.*, 2014) and at the global level (Liu *et al.*, 2013). To the best of our knowledge, such a thorough large-scale analysis has never been carried out before in a single study.

Although global analyses of *Bd* occurrence are extremely useful to target important associated macroscale drivers, it is well known that the processes leading to disease outbreaks are variable among geographic regions (Briggs *et al.*, 2005; Bosch *et al.*, 2007; Raffel *et al.*, 2010; Walker *et al.*, 2010). As a consequence, evaluating regional and local patterns of *Bd* occurrence is key to unraveling cold (i.e., low or zero infection) as well as hot spots of disease occurrence (Walker *et al.*, 2010; Spitzen-Van der Sluijs *et al.*, 2014). Furthermore, at the local level it is possible to assess on a much more precise scale the combined effects that result from the complex interactions among host species, climate and human activities. For instance, it is known that physiology mediates almost all host–parasite interactions, which in turn are also influenced by temperature, particularly in ecotherms (Angilletta, 2009; Ruiz-Aravena *et al.*, 2014). Although there is yet no agreement regarding the negative impacts of climate change on biodiversity through the emergence and spread of EIDs (Rhor *et al.*, 2013), unraveling the role of climate might prove crucial to understand the mechanistic aspects of the disease dynamics at the regional level. This is particularly important as the evidence for associations between levels of *Bd* infection with temperature- and precipitation-related variables is far from conclusive (Raffel *et al.*, 2015 and references therein).

Here we investigate geographic patterns of *Bd* prevalence along the Chilean Winter Rainfall – Valdivian Forest biodiversity hot spot (Mittermeier *et al.*, 2011), which concentrates richness, endemism and a high number of threatened amphibians in Chile (Vidal & Díaz-Páez, 2012). This range also includes from pristine habitats to the most urbanized, dense and economic productive areas of the country (Barbosa & Villagra, 2015) where the impact of human activities is, therefore, very high. Furthermore, central-south Chile has been identified as a *Bd* hot spot, with climatic conditions favorable for the development of this fungus (Rödder *et al.*, 2009; Liu *et al.*, 2013; James *et al.*, 2015). In particular, we evaluated the joint effects of phylogenetic history, latitude, altitude, climate and human-associated factors in the prevalence of *Bd*. Specifically, we assessed: (i) whether there is a phylogenetic signal of *Bd* prevalence, (ii) whether *Bd* prevalence is associated

with latitude and climate and (iii) whether *Bd* prevalence is explained by human-associated factors.

Materials and methods

Bd sampling

Chile is politically organized into 15 Regions, which are further divided into 54 Provinces subdivided into 356 Communes (Library National Congress, 2013), and its biodiversity hot spot spans 12 Regions, 41 Provinces and 314 Communes. Between 2008 and 2013, we sampled a total of 1845 individuals from 24 amphibian species (Table 1) along a latitudinal gradient of ~1500 km encompassing 9 Regions, 22 Provinces and 40 Communes within the hot spot (Fig. 1). Collections were performed primarily during austral summer and spring

(69.3% and 21.4%, respectively), with additional samples obtained in fall (6.8%) and winter (2.4%). Sites were chosen on the basis of having amphibian populations, good accessibility, and that contributed to a good coverage of the whole study area. In each, a search effort of one hour by two researchers was conducted using a standardized methodology, as previously described (Soto-Azat *et al.*, 2013a,b). In each locality, we performed exhaustive searches across land and aquatic environments and recorded the altitude and geographic coordinates. Each individual was handled using a new pair of disposable gloves and skin swabbed for *Bd* detection following Soto-Azat *et al.* (2013b). To minimize any contamination of samples or the spread of pathogens within or between study sites by researchers, equipment or materials, a strict field sampling and disinfection protocol was followed, with reference to Phillott *et al.* (2010).

Table 1 Amphibian species sampled and occurrence of *Batrachochytrium dendrobatidis* infection detected by quantitative PCR across central-south Chile

Administrative regions	V	RM	VI	VII	VIII	IX	XIV	X	XI
Latitude	33.2°S	33.6°S	34.7°S	35.5°S	37.3°S	38.4°S	39.9°S	42.7°S	44.3°S
Species									
Alsodes tumultuosus		12/26 (3)							
Rhinella spinulosa		0/19 (2)							
Xenopus laevis	10/114 (9)	48/143 (7)		0/23 (1)					
Alsodes nodosus	4/13 (2)	1/12 (1)							
Calyptocephalella gayi	13/13 (2)	0/1 (1)		15/15 (1)			9/9 (1)		
Eupsophus septentrionalis				2/12 (1)					
Pleurodema thaul	14/23 (6)			20/39 (3)	42/123 (14)	3/13 (1)			0/1 (1)
Telmatobufo bullocki					0/3 (3)				
Eupsophus contulmoensis					6/24 (10)	0/4 (3)			
Eupsophus roseus					3/16 (7)		0/1 (1)	0/1 (1)	
Eupsophus nahuelbutensis					1/11 (5)	4/98 (8)			
Batrachyla taeniata			0/1 (1)	3/30 (7)	0/9 (3)	0/4 (1)		0/38 (12)	
Alsodes barrioi						0/27 (6)			
Alsodes verrucosus						0/3 (3)			
Eupsophus vertebralis					0/1 (1)		0/5 (1)	0/1 (1)	0/1 (1)
Batrachyla leptopus					0/3 (2)	0/7 (2)		0/14 (3)	0/13 (7)
Eupsophus migueli						0/1 (1)			
Eupsophus altor						0/5 (3)			
Rhinoderma darwinii					3/37 (7)	2/21 (8)	2/189 (33)	3/356 (55)	0/162 (19)
Eupsophus calcaratus						0/3 (3)	2/5 (2)	2/31 (16)	2/23 (10)
Eupsophus emiliopugini								0/4 (3)	0/5 (4)
Alsodes australis								0/2 (2)	0/8 (3)
Hylorina sylvatica								0/1 (1)	3/6 (4)
Batrachyla antartandica								0/9 (2)	16/63 (15)

RM, Región Metropolitana.
Values correspond to: *Bd* positives/total (number of different samples; e.g., including season, year or developmental stage).
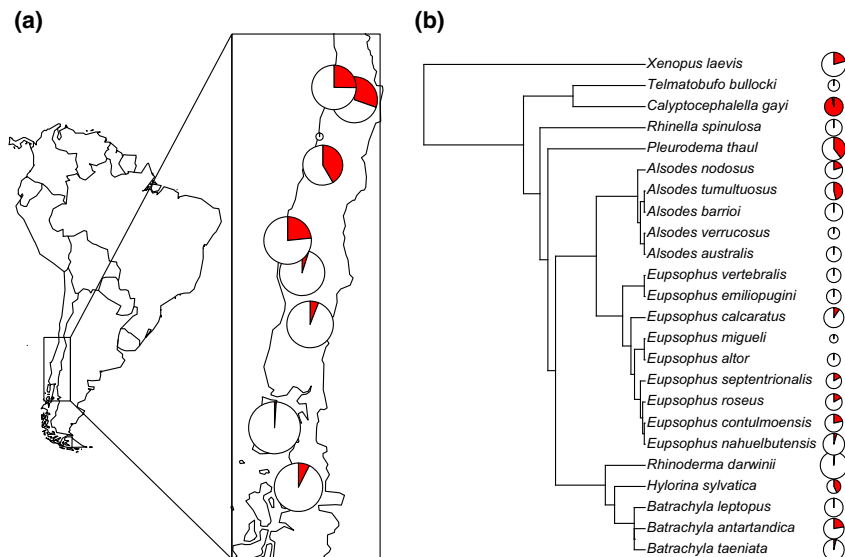
Fig. 1 Sampling across studied regions (a) and amphibian species (b) in Chile. Number of samples that were positive for *Batrachochytrium dendrobatidis* within each region or species was pooled and is represented in red (see Table 1). The size of the pie chart is proportional to the number of individuals sampled within each region or species.

Bd diagnostic analysis

Extraction of DNA from swab tips was carried out following the protocol of Boyle *et al.* (2004). Extracted DNA was diluted (1:10) in double-distilled water and analyzed using a quantitative real-time PCR Taqman assay (qPCR) with primers specific for the ITS-1/5.8S ribosomal DNA region of *Bd*, according to Soto-Azat *et al.* (2013b). In addition, bovine serum albumin (BSA) was included in the Taqman mastermix to minimize PCR inhibition (Garland *et al.*, 2010). For each sample, diagnostic assays were performed in duplicate, and standards of known zoospore concentration were included within each PCR plate, as were negative controls.

Climate, socio-demographic and economic data

Climate data for each sampled site was obtained from the WorldClim high-resolution (30 arc seconds) maps (Hijmans *et al.*, 2005). We obtained 19 climatic variables such as annual mean temperature and precipitation, temperature extremes during the cold and warmest months and precipitation during the wettest and driest months (see http://www.worldclim.org/bioclim for a complete list of the variables).

Using ArcGIS® 10.0 (ESRI, Redlands, CA, USA) we obtained and mapped for each commune, its total surface (Library National Congress, 2013), population (INE, 2009), the urban and industrial area (CONAF, 2011), the human development index (a composite index of health, education and income indicators, MIDEPLAN and PNUD Chile 2004) and the average (2008–2012) gross domestic product (GDP) from the corresponding region (Central Bank of Chile, 2014). For each locality, a detailed description of the socio-demographic, economic and land-use characterization (as a proportion of land use) was obtained from commune records. Population density and GDP were log-transformed to improve normality.

Statistical analyses

For operational purposes, the prevalence of *Bd* at each site was quantified as the proportion of infected individuals of each species per sampling site (number of positives/total samples; Table 1) and expressed separately for different species, developmental stages and sampling period to account for these factors in our statistical model (see below). As a result, we obtained a total of $N = 336$ estimates of *Bd* prevalence, ranging from 1 to 75 sampled individuals (mean of 5.4 ± 7.6 SD). Uncertainty associated with low sample size was incorporated in the model (see below).

To determine which factors best explain the variation in prevalence of *Bd* across species and sites, we employed weighted phylogenetic generalized least squares (PGLS). *Bd* prevalence was arcsine square-root-transformed prior to analyses. Although logistic regressions and general linear models are generally preferred to analyze binomial data (Warton & Hui, 2011), we employed this approach because no statistical package could accommodate the phylogenetic analysis required here to account for differences in sample size across sites. To control for sample size in our PGLS, we assumed a variance of $1/n^3$ around each estimate of *Bd* prevalence, where n corresponds to the total number of individuals measured in each site. This so-called Wilson–Hilferty cubic transformation, widely employed to normalize a chi-square distribution (Carroll & Ruppert, 1988), substantially improved the fit of our models (analyses not shown). This is not surprising because, unless the prevalence approaches 0 or 1, the estimation error of *Bd* around the expected value (i.e., its real proportion in the population) should be normally distributed and its variance should consequently approximate a chi-square distribution. Thus, with this approach, we were able to include all species and sampled sites, while taking into account the uncertainty surrounding estimates of *Bd* prevalence when sample sizes were small.

To account for phylogenetic relatedness, we built a dated phylogeny based on the hypotheses proposed by Pyron & Wiens (2011) and Blotto *et al.* (2013), employing the divergence times between major amphibian groups described by Marjanović & Laurin (2007). The root node describing the split between *Xenopus* in Pipoidea and the remaining species belonging to Neobatrachia was set at 170 mya, and branch lengths within genera *Eupsophus* and *Alsodes* were arbitrarily assigned according to Pagel's (1992) method and rescaled to 65 mya divergence from *Telmatobius* (Marjanović & Laurin, 2007). The resulting phylogeny also included multiple tips per species, which corresponded to the fraction of infected individuals at any given sampling site (range between 1 site for *Eupsophus migueli* and *Alsodes tumultuosus* and 79 sampling sites for *Rhinoderma darwinii*), with branch lengths equal to zero (Ives *et al.*, 2007; Felsenstein, 2008).

We employed model selection and an information-theoretic approach (Burnham & Anderson, 2002) to contrast the adequacy of different working hypotheses explaining the geographic occurrence of *Bd* in central-south Chile. Exploratory analyses suggest that the prevalence of *Bd* infection varies across species and developmental stages (see below) and exhibits a well-defined spatial structure: prevalence decreased with latitude (Fig. 1; see also Soto-Azat *et al.*, 2013a). Consequently, we employed the following standard model to control for the remaining confounding effects:

$$Bd \sim \mathrm{sp} + \mathrm{stage} + \mathrm{season} + \mathrm{year} + \mathrm{lat} \times \mathrm{long}$$

where *Bd* is prevalence (number of positives/total sample), sp and stage correspond to factors accounting for species identity (24 spp) and developmental stage (tadpoles, juveniles and adults), season and year included as factor and covariable, respectively, account for temporal effects of the sampling and lat × long specify spatial covariates (both main effects and interaction) that account for the geographic structure of the data.

To reduce the number of candidate models, thereby minimizing the likelihood of spurious results (Burnham & Anderson, 2002; Lukacs *et al.*, 2010), we first assessed the collinearity between environmental variables on the one hand (including altitude), and human-associated factors on the other, with principal component analyses (PCA). For the environmental variables, the first and second principal components accounted for 97.1% and 94.3% of the total variation, respectively. Given the high collinearity detected, we selected annual mean temperature and its standard deviation, as well as annual precipitation and its coefficient of variation as environmental descriptors of average conditions and variability. These variables explained 99.99% and 99.97% of the variation in the first and second components, according to the r^2 of a multiple regression. For human-associated factors, the first and second principal components accounted for 87.3% and 94.3% of the total variation, respectively. Subsequently, the regional GDP and urban and industrial area (expressed as a proportion of commune area), explaining together 96.3% and 97.7% the variation in the first and second components, were selected as general descriptors of human-associated factors. We ran PGLS models including each variable to the standard model described above and subsequently models combining one environmental and one human impact variable. We employed Akaike information criterion (AIC), which takes into consideration the likelihood (*LogLik*) of each model while penalizing for the number of parameters (*K*) to obtain the best-fitted model, and Akaike weights (w_i) to quantify the relative support of each model in the set (Burnham & Anderson, 2002; Turkheimer *et al.*, 2003). Because models included different fixed effects, Pagel's λ – an index of phenotypic covariation due to phylogeny varying between 0 and 1, with $\lambda = 0$ implying no resemblance between related species and $\lambda = 1$ indicating that patterns of resemblance closely match the expectation of a Brownian model of evolution (Pagel, 1999) – was estimated with restricted maximum likelihood (REML; range between 0.42 and 0.71) and set to 0.59 in subsequent model comparisons employing maximum likelihood (ML). This multistep approach of employing REML for variance-covariance optimization and ML for model selection is discussed in detail elsewhere (see Ngo & Brand, 1997 and references therein). All analyses were performed in R version 3.1.3 employing packages *ape* (Paradis *et al.*, 2004) and *picante* (Kembel *et al.*, 2010).

Results

Species sampled, proportion of *Bd* infection and number of sample per region are presented in Table 1 and Fig. 1. In summary, 24 species (ca. 40% of Chilean amphibian diversity) belonging to seven families were sampled along the 33°–44° latitudinal gradient. In total, 1845 samples were taken, from which 245 individuals resulted positive obtaining an average *Bd* prevalence in the country of 13.3%.

As *Bd* prevalence is affected by species identity (Pagel's $\lambda = 0.59$, $P < 0.001$), all evaluated models included the phylogenetic relatedness among them. Additionally, all evaluated models controlled for phylogenetic relatedness and the confounding effects of spatial structure, season and year (standard model, Table 2). The model selection procedure indicated that, from the 15 candidate models tested, the best model included both precipitation seasonality ($\mathrm{PCP_{cv}} = $ coefficient of variation in monthly precipitation over the course of the year) and regional gross domestic product (GDP) with a relative support of virtually 100% given by its Akaike weight w_i (Table 2). Interestingly, the model including the interaction between $\mathrm{PCP_{cv}}$ and GDP exhibited a substantially better fit than the one taking only main effects into consideration: whereas the later exhibited a AICc = 1153.80 (Table 2), inclusion of the interaction resulted in an AICc = 1149.68 in spite of the addition of an extra parameter (Table 3). Recalculation of Akaike weights w_i in light of this model provided a relative support of 89% to the inclusion of the interaction vs. 11% to the model including only main effects.

We employed the r^2 of weighed linear regressions of the predicted values against the observed *Bd* prevalence to evaluate the performance of these models (Piñeiro *et al.*, 2008), which performed considerably well. Whereas the standard model accounting for species identity, developmental stage, spatial structure and temporal effects explained 78.9% of the variation in *Bd* prevalence across sites ($r^2 = 0.789$), inclusion of PCP_{cv}, GDP and their interaction resulted in a model explaining 82.4% of the observed variation (Fig. 2a). The intercept and slope of this regression (0.0273 ± 0.0070 and 0.829 ± 0.021, respectively; mean ± SE) only slightly underestimate the prevalence of *Bd* in species/sites in which all individuals were infected (Fig. 2a), and the geographic distribution of the fitted values clearly match the empirical data of *Bd* prevalence (Fig. 2c, d).

This model shows that *Bd* prevalence differs significantly between species, developmental stages, seasons and years (Table 3). After controlling for these effects, the model suggests that regions with a high GDP exhibit a substantially higher prevalence of *Bd* (Fig. 2b), hence the importance of GDP among anthropogenic and environmental variables in our best model (Table 3). In regions with lower GDP prevalence of *Bd* increases with PCP_{cv} (Fig. 2b), which explains the higher support of the model including the interaction between these variables.

With regard to species differences in *Bd* prevalence, the high λ observed in preliminary analyses (see

Section Statistical analyses) indicates that this variable exhibits phylogenetic signal and suggests that the susceptibility to *Bd* is specific for each amphibian taxa (see Table 1). Consequently, we tested for a posteriori differences in the proportion of *Bd* infection across species employing Tukey HSD test controlling for the remaining covariables in the full model (Table 3). In total, 66 of 276 pairwise differences were statistically significant

Table 3 Significance test in the model with the best fit (*) for the prevalence of *Batrachochytrium dendrobatidis* (*Bd*) infection among anurans of central-south Chile

	df	F	P
Species	23, 300	18.549	<0.0001
Developmental stage	2, 300	16.031	<0.0001
Season	3, 300	18.162	<0.0001
Year	1, 300	4.262	0.0398
Latitude	1, 300	4.190	0.0415
Longitude	1, 300	1.495	0.2223
Latitude: longitude	1, 300	4.393	0.0369
PCP_{cv}	1, 300	1.918	0.1671
GDP	1, 300	10.753	0.0012
PCP_{cv}: GDP	1, 300	5.991	0.015

PCP_{cv}, precipitation variability; GDP, gross domestic product. Akaike information criterion: $K = 37$, AICc = 1149.68 and LogLik = −533.12.
*Model: *Bd* ~ sp + stage + season + year + lat × long + PCP_{cv} × GDP.

Table 2 Models accounting for the prevalence of *Batrachochytrium dendrobatidis* (*Bd*) infection among anurans of central-south Chile

Model*	K	AICc	ΔAICc	w_i	LogLik
Standard	34	1195.05	41.25	0	−559.57
Standard + T	35	1165.90	12.10	0	−543.75
Standard + T_{sd}	35	1191.33	37.53	0	−556.46
Standard + PCP	35	1197.53	43.73	0	−559.56
Standard + PCP_{cv}	35	1169.24	15.44	0	−545.42
Standard + Urb	35	1190.99	37.19	0	−556.29
Standard + GDP	35	1187.99	34.19	0	−554.79
Standard + T + Urb	36	1167.48	13.69	0	−543.29
Standard + T_{sd} + Urb	36	1177.42	23.62	0	−548.26
standard + PCP + Urb	36	1193.50	39.70	0	−556.29
Standard + PCP_{cv} + Urb	36	1163.25	9.45	0.01	−541.17
Standard + T + GDP	36	1167.88	14.08	0	−543.49
Standard + T_{sd} + GDP	36	1188.19	34.39	0	−553.64
Standard + PCP + GDP	36	1189.45	35.66	0	−554.27
Standard + PCP_{cv} + GDP	36	1153.80	0	0.99	−536.44

T, annual mean temperature; T_{sd}, temperature seasonality; PCP, annual precipitation; PCP_{cv}, precipitation variability; Urb, commune urban and industrial area (%); GDP, regional gross domestic product; K, number of parameters; AICc, AIC values corrected for small sample sizes; w_i, Akaike weights (the probability that the model is the correct one of those tested).
*All models control for species identity, developmental stage, seasonal and yearly effects and spatial structure. Estimates of *Bd* prevalence are weighed for sample size n by specifying a heterogeneous variance $= 1/n^3$ (Wilson–Hilferty transformation). Pagel's $\lambda = 0.59$ for all models, $N = 336$.
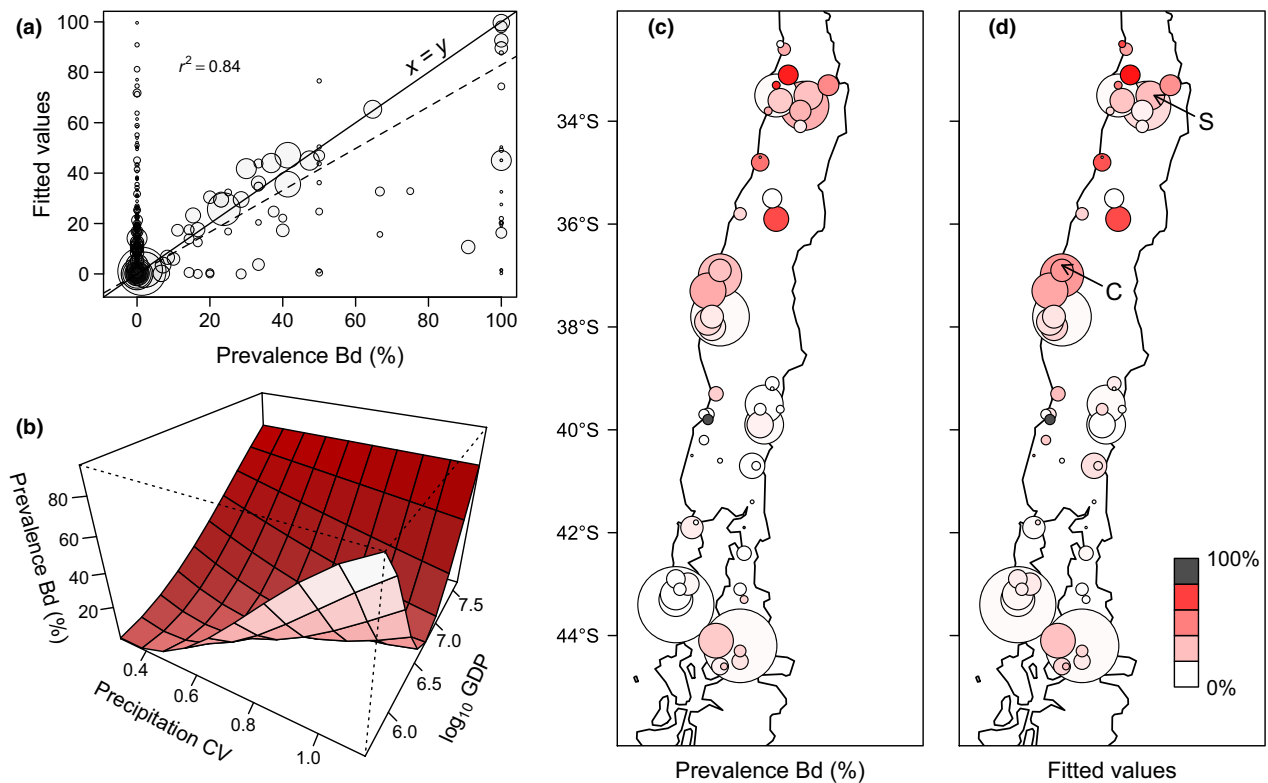
Fig. 2 (a) Fitted values against empirical estimates of *Batrachochytrium dendrobatidis* (*Bd*) prevalence for the model with the best fit (standard + PCP$_{cv}$ × GDP). (b) This model suggests that the interaction between PCP$_{cv}$ and GDP best explains the prevalence of *Bd* along the Chilean geography, with developed regions with high GDP exhibiting the highest prevalence in *Bd*. This can be seen when contrasting (c) empirical estimates vs. (d) fitted values, with the largest cities Santiago and Concepción shown by their initials. Data points in panels (a), (c) and (d) represent the proportion of infected individuals of a given species within a given site, and their size is proportional to the cube of the number of sampled individuals (n^3), which was employed to assign weights in our PGLS (see Section Statistical analyses). Note the close correspondence between fitted values and empirical estimates for most sites with large sample sizes, which explains the high r^2 of our model ($r^2 = 0.824$). GDP, gross domestic product; PGLS, phylogenetic generalized least squares.

after correcting for multiple comparisons (two-tailed $P < 0.05$), reflecting to a large extent the disproportionally high occurrence of *Bd* in *Calyptocephalella gayi* (97.4%), which was significantly higher than adjusted estimates of other 19 species (see Fig. 1, Table 1). Interestingly, the number of individuals infected, which reflects the interaction between the relative abundance of different species and their susceptibility to *Bd* infection, was disproportionally high in *C. gayi*, *X. laevis*, *Pleurodema thaul*, *Batrachyla antartandica* and *A. tumultuosus*, which together accounted for 82.4% of all *Bd*-positive frogs detected in our survey (202 of a total of 245 infected individuals; Fig. 3).

Discussion

Batrachochytrium dendrobatidis has been associated with the alarming decline and extinction crisis of amphibians at the global scale (Stuart *et al.*, 2004; Sodhi *et al.*, 2008; Wake & Vredenburg, 2008; Collins & Crump,

2009; Hof *et al.*, 2011). From a conservation point of view, it is essential to understand, at the regional or local level, whether species differ in their susceptibility to *Bd* infection as this may suggest that some are acting as reservoirs, and whether *Bd* occurrence is non-randomly distributed to provide guidelines for population management and disease mitigation measures (Bosch *et al.*, 2015; Langwig *et al.*, 2015). Our results show that *Bd* occurrence: (i) has a strong phylogenetic signal, (ii) decreases with latitude (although we cannot disentangle the effects of latitude *per se* from a potential ongoing spread of *Bd* in the country, see below), (iii) varies significantly across seasons and years and (iv) increases with PCP$_{cv}$ and GDP.

This is, to the best of our knowledge, the first study to explicitly evaluate for phylogenetic signal on *Bd* prevalence. Nonetheless, the relatively strong signal observed was not completely surprising. Several experimental studies have described substantial variation across species on their susceptibility to *Bd* infection and
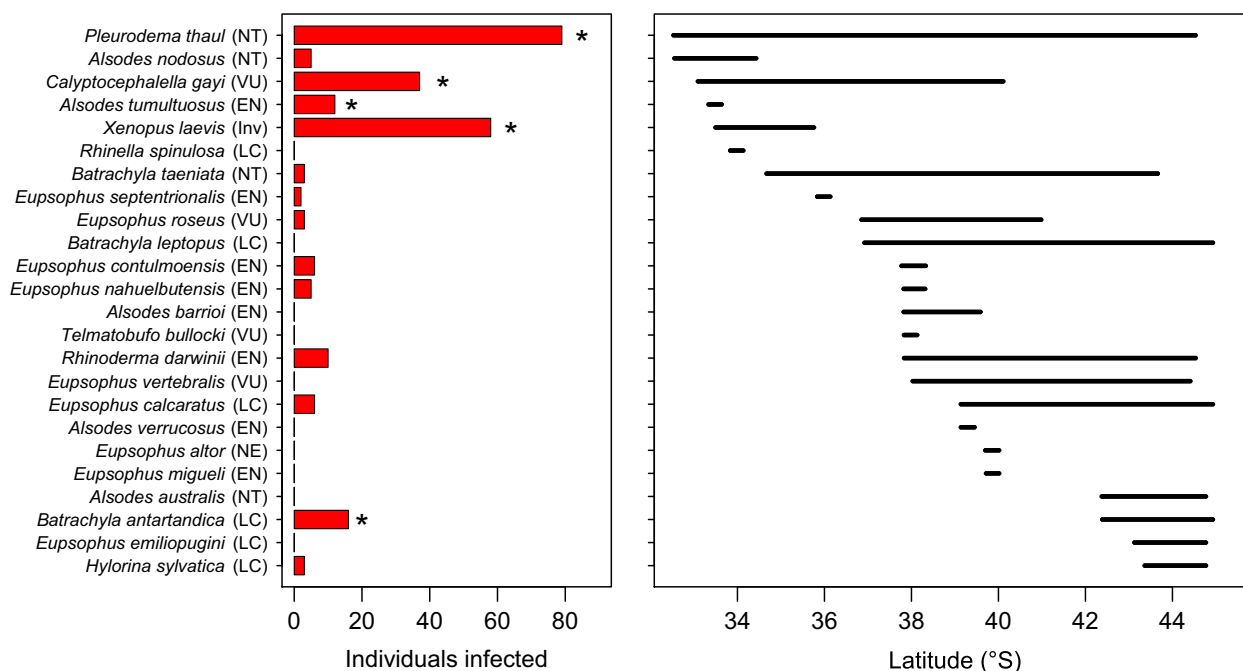
Fig. 3 The number of individuals infected by *Batrachochytrium dendrobatidis* and the latitudinal range of anuran species in this study. The conservation status of each species according to IUCN is shown between parentheses, with acronyms representing from low to high extinction risk: LC, least concern; NT, near threatened; VU, vulnerable; EN, endangered. The status of *Eupsophus altor* was not evaluated (NE) and *Xenopus laevis* was included as an invasive species (Inv). Asterisks denote species with high numbers of infected individuals (see text).

development of lethal chytridiomycosis (Blaustein *et al.*, 2005; Woodhams *et al.*, 2007; Vazquez *et al.*, 2009; Searle *et al.*, 2011; Ohmer *et al.*, 2013). It is also known that *Bd* susceptibility and its potential population consequences might be mediated by amphibian behavior and life history (Berger *et al.*, 2016 and references therein). Our analyses suggest that even though individuals of *P. thaul*, *X. laevis*, *C. gayi*, *B. antartandica* and *A. tumultuosus* have not been found showing signs of chytridiomycosis, these are species of particular concern as they show high numbers of infected individuals (Fig. 3). Thus, in spite of the multifactorial nature of *Bd* prevalence occurrence (see Introduction), the phylogenetic signal detected in our study indicates that lineage-specific attributes such as differences in susceptibility to *Bd* infection and/or resistance to developing chytridiomycosis seem to be major drivers of the epidemiological patterns described here. It should also be noted that these five species are highly associated with water, and thus, the higher number of infected individuals might be a reflection of a higher contact rate with *Bd* zoospores found in water compared to terrestrial environments (Longcore *et al.*, 1999; Johnson & Speare, 2003; Piotrowski *et al.*, 2004).

The negative latitudinal trend that we found in *Bd* prevalence (with a decrease of 0.69% in *Bd* prevalence per degree of latitude obtained from the model with the best fit; Table 3) is in agreement with the pattern reported at the intraspecific level in Darwin's frogs (*Rhinoderma* spp.; Soto-Azat *et al.*, 2013a), although contrary to what Kriger *et al.* (2007) reported for Australia (i.e., higher *Bd* prevalence toward lower latitudes). These seemingly opposite patterns could be explained by the history of the introduction and spread of *Bd* in Chile and Australia, differences in *Bd* strains (however, endemic strains have not been described so far for these regions) and/or differences in climates. In this context, we believe that the observed latitudinal trend partly reflects an ongoing spread of *Bd* in Chile. Evidence from a retrospective epidemiological study (Soto-Azat *et al.*, 2013a) and from the genetic characterization of three *Bd* strains obtained from different sites within the study area (CS-A unpublished) suggests that *Bd* has been introduced in Chile circa 1970, possibly through the introduction and subsequent invasion of *X. laevis* (Solís *et al.*, 2010). This invasive frog has been associated with the global spread of *Bd* (Weldon *et al.*, 2004; Kilpatrick *et al.*, 2010; Soto-Azat *et al.*, 2010) and is currently spreading south from its original site of introduction near Santiago (Lobos & Jaksic, 2005). Importantly, an ongoing expansion of *Bd* is further supported by yearly effects detected in our best model (Table 3):

back-transformed adjusted data suggest that *Bd* prevalence has increased from 12.5% in 2008 to 21.1% in 2013, everything else being equal (species identity, developmental stage, etc.).

Once we accounted for temporal and spatial effects, the interaction between human-associated factors and climate becomes important in explaining *Bd* prevalence (Tables 2 and 3). In particular, *Bd* occurrence tends to be higher in those regions with higher GDP, particularly near developed centers such as Santiago and Concepción (Fig. 2). Different factors may account for this result. Regions with higher GDP are more impacted by human activities, particularly through habitat modification and degradation (Barbosa & Villagra, 2015), possibly restricting the number of available amphibian breeding sites and increasing the rate of encounters between infected and uninfected animals (Fortuna *et al.*, 2006). However, an explicit evaluation of this relationship requires further research, particularly considering that species interactions are important at affecting distributions at lower spatial scales (Cohen *et al.*, 2016). Pathogen dispersal mediated by humans, associated with a high traffic of people, cattle and motor vehicles (Skerratt *et al.*, 2007; James *et al.*, 2009; Schloegel *et al.*, 2009; Adams *et al.*, 2010; Liu *et al.*, 2013), may also be higher in these regions. Additionally, the reduced habitat quality in these sites may have an impact on the aquatic microfauna (Schmeller *et al.*, 2014) and on amphibians' immune function (Daszak *et al.*, 2003; Gray *et al.*, 2007; Ramsey *et al.*, 2010), potentially increasing pathogen occurrence and impacts of disease. Whereas other authors have proposed that reduced habitat quality (e.g., due to pollutants) might prevent the fungus from finding its host (Van den Wyngaert *et al.*, 2014), this is not supported by our data. Interestingly, a reduced availability and quality of amphibian breeding sites during periods of droughts might also explain the high *Bd* occurrence found in both regions with high GDP as well as in more pristine regions with low GDP but high PCP$_{cv}$ (Fig. 2b) (Fortuna *et al.*, 2006).

Our findings of species-specific patterns on *Bd* prevalence and the non-random geographic distribution of *Bd* emphasizes the importance of targeting the appropriate scale to evaluate the risk of pathogens threatening wildlife (Fisher *et al.*, 2012; Doddington *et al.*, 2013; Spitzen-Van der Sluijs *et al.*, 2014). This is of particular concern as usually conservation programs are implemented at the regional or local level (Langwig *et al.*, 2015). Thus, although it is known that host susceptibility to *Bd* is context-dependent, phylogenetic analyses of *Bd* prevalence in other regions and species should provide useful information on those species and genus of concern where efforts on *Bd* monitoring should be focused. Furthermore, our results strongly suggest that human-associated factors should not be overlooked when trying to understand patterns of *Bd* occurrence. These findings are particularly relevant for the biodiversity hot spot in central-southern Chile, because the drying conditions the country is experiencing since the late 1970s (Boisier *et al.*, 2016) are not projected to decline in the future. The variation in precipitation regimes are expected to increase in the regions studied here, which, combined with the ongoing rate of land conversion and associated habitat degradation for forestry and agriculture use (Oficina de Estudios y Políticas Agrarias, 2011; Manuschevich & Beier, 2016), might contribute to increased levels of *Bd* occurrence in the near future (see also Adams *et al.*, 2010; Murray *et al.*, 2011; Spitzen-Van der Sluijs *et al.*, 2014). Nonetheless, our study clearly delineates which species and regions should be monitored for conservation purposes, and similar efforts in Chile and elsewhere might point out relatively simple conservation strategies to mitigate the impacts and reduce the spread of a disease with such relevance for biodiversity.

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Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main article.
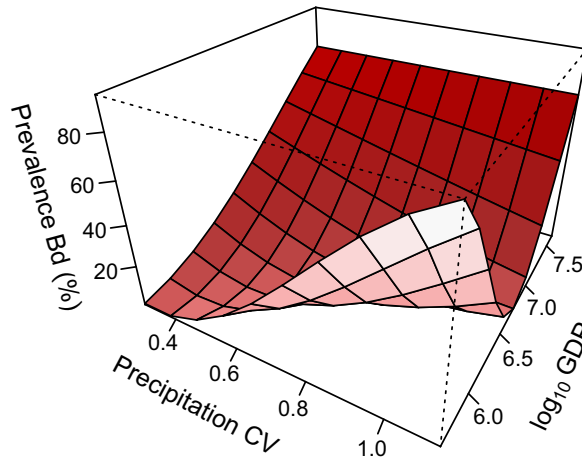
Chytridiomycosis, due to the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been associated with the alarming decline and extinction crisis of amphibians worldwide. Because conservation programs are implemented locally, it is essential to understand how the complex interactions among host species, climate and human activities contribute to *Bd* occurrence at regional scales. Analyses show that *Bd* prevalence decreases with latitude although it has increased by almost 10% between 2008 and 2013, possibly reflecting an ongoing spread of *Bd* following the introduction of *Xenopus laevis*. Occurrence of *Bd* was higher in regions with high gross domestic product (particularly near developed centers) and with a high variability in rainfall regimes. Taken together, our results highlight that *Bd* in Chile might still be spreading south, facilitated by a subset of species that seem to play an important epidemiological role maintaining this pathogen in the communities, in combination with climatic and human factors affecting the availability and quality of amphibian breeding sites.