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Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research

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ABSTRACT

The amphibian fungal disease chytridiomycosis, which affects species across all continents, recently emerged as one of the greatest threats to biodiversity. Yet, many aspects of the basic biology and epidemiology of the pathogen, *Batrachochytrium dendrobatidis* (*Bd*), are still unknown, such as, when and from where did *Bd* emerge and what is its true ecological niche? Here, we review the ecology and evolution of *Bd* in the Americas and highlight controversies that make this disease so enigmatic. We explore factors associated with variance in severity of epizootics focusing on the disease triangle of host susceptibility, pathogen virulence, and environment. Reevaluating the causes of the panzootic is timely given the wealth of data on *Bd* prevalence across hosts and communities and the recent discoveries suggesting co-evolutionary potential of hosts and *Bd*. We generate a new species distribution model for *Bd* in the Americas based on over 30,000 records, and suggest a novel future research agenda. Instead of focusing on pathogen "hot spots" we need to identify pathogen "cold spots" so that we can better understand what limits the pathogen's distribution. Finally, we introduce the concept of "the Ghost of Epizootics Past" to discuss expected patterns in post epizootic host communities.

INTRODUCTION

Infectious diseases emerge because of changes in host-pathogen-environment interactions and, increasingly, anthropogenic habitat alterations are directly affecting these interactions (Jones *et al.* 2008). The number of emerging diseases caused by fungi relative to other types of pathogens has risen steeply during the last two decades, though the causes for this bias are unclear (Fisher *et al.* 2012). Among these emerging fungal diseases is the amphibian-killing chytrid fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*; (Longcore *et al.* 1999), which has, in the last 15 years, captured the attention of scientists and conservationists. Although CHYTRIDIOMYCOSIS (**Box 1**) is one of a long list of fungal diseases of animals and plants that have emerged in the last century (Fisher *et al.* 2012), over a thousand studies (1,088) have already been published on *Bd* and its effects on amphibians (Web of Science search using term "chytridiomycosis"on June 3, 2015). Most of these studies have been descriptive in scope: establishing baseline patterns of *Bd* distribution (summarized

in (Olson *et al.* 2013)), correlating disease prevalence with abiotic and biotic factors (Kriger & Hero 2007; Liu *et al.* 2013), and documenting pathogen genetic variation to identify its mode of spread (James *et al.* 2009; Rosenblum *et al.* 2013).

A major reason for this focus on *Bd* is that it is a generalist amphibian pathogen and close to 41% of amphibians are threatened, making them one of the most threatened vertebrate lineages (Monastersky 2014). *Bd* also confirms that host-pathogen interactions can play a major role in species declines and even extinctions (Crawford *et al.* 2010). *Bd* has now been reported from over 500 amphibian host species, has a cosmopolitan distribution, and has been detected at 48% of localities that have been surveyed (Olson *et al.* 2013). This wide distribution has been documented just since chytridiomycosis was first described (Longcore *et al.* 1999), when it was the first known vertebrate pathogen from an obscure phylum of fungi whose mechanism of pathogenesis and life cycle (**Figure 1**) were incompletely known. This obscurity has meant studies on the biology of the pathogen have lagged behind those of hosts. Indeed, most of the questions that the research community set out to answer when the disease was first described, including "Where did it come from?", "How does it spread?", "Why are some species resistant or tolerant?", "Does it have an alternate host or environmental stage?" and "Why now?" have yet to be definitively answered (Collins & Crump 2009; Kilpatrick *et al.* 2010).

The chytridiomycosis research community has struggled to reconcile geographic and host patterns of EPIZOOTICS that result from complex interactions among host, pathogen, and the environment. Overlain upon a pan-global distribution of *Bd* is a set of more restricted geographic regions where *Bd* has caused massive loss of amphibian biodiversity, such as eastern Australia, Central America, north-western South America, and western North America (Berger *et al.* 1998; Lips *et al.* 2006; Vredenburg *et al.* 2010). Surprisingly, all continents, whether they have declining or persisting amphibian populations, harbor highly similar pathogen genotypes (the GLOBAL PANZOOTIC LINEAGE [GPL]) that are highly VIRULENT in animal models (Fisher *et al.* 2009b). However, numerous advances in the study of *Bd* in recent years, have radically altered our perspective of a homogeneous pathogen and a homogeneous host response across the globe. For example, the discovery of multiple ENZOOTIC *Bd* lineages (Farrer *et al.* 2011; Schloegel *et al.* 2012; Bataille *et al.* 2013), a new,

related species of *Batrachochytrium* specific to salamanders (Martel *et al.* 2013), and the suggestion of alternative pathogen niches, such as in the GI tracts of crayfish (McMahon *et al.* 2012) challenge the notion of pathogen homogeneity. Variation in host responses to infection is also now better understood. The idea that amphibian species are equivalent targets for *Bd* has been challenged by results that indicate frogs may be capable of acquiring immunity (McMahon *et al.* 2014) and that frog immune-genotype matters for population persistence (Savage & Zamudio 2011). Recent research has also focused on identification of mechanisms leading to variation in effects of *Bd* across hosts or communities, such as understanding how behavior (Venesky *et al.* 2011), environmental contaminants (Hanlon & Parris 2014), microbial skin communities (Bletz *et al.* 2013), seasonality (Longo *et al.* 2010), and community structure (Becker *et al.* 2014) influence a species' susceptibility.

Here we review how recent research has helped explain patterns of chytridiomycosis across space, time, and host. We highlight advances in the field that accompany a shift from the panzootic phase, where emphasis was placed on surveys of the disease, to the post-panzootic phase, in which mechanistic questions are being addressed. We provide a comprehensive species distribution model for *Bd* in the Americas to summarize what we know about the geographic and environmental factors that control *Bd* distribution. We chose to emphasize the distribution of *Bd* in the Americas for several reasons. First, the Americas have good geographic coverage with respect to disease surveillance, genetic data, and contemporary amphibian surveys. Second, the response of host populations to *Bd* in the Americas is highly variable. Finally, host and pathogen diversity are both high in the Americas, and the region contains many endangered species, making the study of *Bd* important from a conservation perspective. Our review is framed using the concept of the disease triangle (environment, host, and pathogen interactions), which highlights interactions among hosts and pathogens under different environmental conditions and is therefore an excellent framework for explaining complex infection outcomes. We outline potential research areas that will help explain the mosaic geographic patterns of morbidity left in the wake of an apparent global panzootic, and we discuss lessons learned that could be useful when considering other emerging infectious diseases.

ENVIRONMENTAL FACTORS PREDICTING CHYTRIDIOMYCOSIS EPIDEMICS

The disease triangle model is commonly used to explain how variation in environmental factors, host susceptibility, and pathogen virulence lead to varying disease outcomes, yet only a small parameter space in the model results in an epidemic (Scholthof 2007; Gurr *et al.* 2011). We know that some aspects of chytridiomycosis epizootics show environmental correlates (Olson *et al.* 2013), and these are expected because temperature and precipitation affect *Bd*-amphibian dynamics by physiologically limiting vital processes such as pathogen growth and host immune responses. All amphibians need moist skin for water uptake and to maintain electrolyte balance, and many species also require standing water for reproduction. Because *Bd* reproduces by zoospores, it requires at least water films to disperse. *Bd* has a surprisingly narrow optimal growth range of 17 to 25 °C, but can tolerate temperatures between 4 and 28 °C (Piotrowski *et al.* 2004), matching most amphibian temperature tolerance ranges.

These physiological limits likely influence *Bd* distribution and disease outcome, but the data reveal complex and even conflicting patterns. At the individual level, preferences for higher temperatures among hosts correlate with reduced probability of *Bd* infection (Rowley & Alford 2013) and warmer or drier areas serve as refugia from *Bd* (Puschendorf *et al.* 2009). At the population level, epidemics in Central America were found in the middle or end of the rainy season (Lips 1998), but the drier months of the year were associated with higher *Bd* prevalence in Puerto Rico (Longo *et al.* 2010). At the landscape level, temperature likely is responsible for a positive correlation between prevalence and both elevation (Gründler *et al.* 2012) and latitude (Kriger *et al.* 2007). On the other hand, a study in the Sierra Nevada demonstrated no relationship between elevation or temperature on *Bd* prevalence (Knapp *et al.* 2011).

Since its discovery, much effort has been spent on mapping the spatial distribution of *Bd* to provide a landscape view of areas with high environmental suitability. Olson *et al.* (2013) provided the most recent overview of the global distribution of *Bd*, analyzing the host and geographic patterns of 4,281 individually swabbed frogs from 56 countries, of which 1,814 (48%) were *Bd* positive by molecular detection methods. Using logistic regression

Olson *et al.* examined associations between *Bd* occurrence at a site and latitude, elevation, biome, amphibian species richness, and global temperature and precipitation metrics. While these methods are powerful to detect environmental correlations, they cannot determine whether an unsampled site may be environmentally suitable for *Bd*. Species distribution models (SDMs), on the other hand, predict the geographic extent of a species and identify the contribution of habitat parameters in explaining that distribution. (Rödder *et al.* 2009) and Liu *et al.* (2013) developed the first *Bd* SDMs that were global in scope, based on 365 and 1,829 *Bd* records, respectively. Here we present a comprehensive SDM for *Bd* in the Americas (**Figure 2**), based on 6,071 *Bd* positives from 30,382 analyzed swabs from fieldwork of the authors plus an intensive literature review (**Box 2)**. The most intensively surveyed areas include western and eastern US, Costa Rica, Panama, Puerto Rico, the Andes, and the Brazilian Atlantic Forest. Knowledge gaps include the central US, northern Mexico, the Amazon Basin, the Brazilian Cerrado and Pantanal, and large regions in Argentina and Bolivia (**Figure 2**).

In comparison to previous SDMs for *Bd*, our new model employs an ensemble model approach, which has superior performance when compared to single algorithms (Meller *et al.* 2014). This enables the prediction of both hot spots and cold spots of environmental suitability for *Bd*. The new SDM predicts that most parts of eastern and western USA, mountainous areas in Central America, the Northern Andes, lowlands of Chile, the Brazilian Atlantic Forest, and adjacent areas in Uruguay and Argentina provide suitable environmental conditions for *Bd* (**Figure 2**). Most parts of the Amazon basin are not predicted to be suitable for *Bd*, most likely because their comparatively high annual mean temperatures exceed the critical thermal maximum of the pathogen. Our results highlight similar hot spots for *Bd* as those predicted by Rödder *et al.* (2009) and Liu *et al.* (2013), but allow more differentiation between suitable and unsuitable sites because of increased sampling efforts and the use of analytical methods that are an ensemble of different algorithms (**Box 2**). A major difference between this new SDM and the previous projections is the lower suitability for *Bd* in the Amazon basin and higher suitability for *Bd* in western North America relative to the earlier model by Rödder *et al.* (2009). Our model also differs from that of Liu *et al.* (2013) in showing less suitability for eastern North America. We compared results of the SDM with

prevalence of *Bd* at given localities for which we had population level data (**Figure 3**). Overall, *Bd* prevalence correlates well with the SDM, but neither picture is able to completely explain the epidemiological patterns observed. For example, eastern US amphibian populations have among the lowest mean prevalence (mean = 12.4%), whereas eastern Brazilian populations display among the highest prevalence (mean = 28.5%) (**Figure 3**). Yet, both regions show limited evidence for *Bd*-related declines.

The first 15 years of chytridiomycosis surveys have limited the space of the disease triangle to a set of environmental parameters that are coincident with hot spots on the SDM. Altogether, our SDM provides a strong prediction of where *Bd* will occur on the basis of climate and land cover (**Box 2**). Yet, currently, environmental factors alone fail to provide a clear explanation for why *Bd* is such a problem right now or what species will be affected. Beyond basic climatic variables, we clearly need more data on how other physical, chemical, and biotic characteristics of environments predict *Bd* presence and disease outcome. For example, the SDM suggests significant impact of "human footprint" on *Bd* prevalence (**Box 2**), yet the mechanisms underlying this footprint need greater investigation.

PATHOGEN EVOLUTION, LIFE HISTORY, AND PREDICTIONS OF CHYTRIDIOMYCOSIS EPIDEMIOLOGY

Another vertex on the disease triangle is the virulence or propagule pressure of the pathogen (Scholthof 2007). Here, we explore how both pathogen genotype and environmental niche influence the virulence and transmission of the pathogen. Early *Bd* studies speculated that a recent change in pathogen virulence occurred, leading to rapid spread of the pathogen across the landscape. If true, this would support the NOVEL PATHOGEN HYPOTHESIS (NPH) over the alternate ENDEMIC PATHOGEN HYPOTHESIS (EPH), which posits that *Bd* was endemic and emerged because of environmental change (Rachowicz *et al.* 2005; Fisher *et al.* 2009b). Here, we review evidence supporting both NPH and ancient endemism and progress towards identifying a source population.

The earliest genetic studies of *Bd* isolates found low global variation at both microsatellite and sequenced loci, with both types of markers having only two alleles

(Morgan *et al.* 2007; James *et al.* 2009). However, sampling in these initial studies was biased to localities with documented die-offs, such as the Sierra Nevada of California, USA, Coclé, Panama, and Queensland, Australia. The absence of allelic diversity, however, supported the NPH model in which the GPL arose from a single diploid genotype, and its descendants were shaped by LOSS OF HETEROZYGOSITY (LOH). This tight bottleneck also suggested a recent emergence of chytridiomycosis from a single source population, but the lack of any geographic pattern left ample room for speculation about the source population.

Because of this potential recent emergence, researchers turned to museum specimens to connect *Bd* occurrence to a place and time, initially with histology (Weldon *et al.* 2004), and subsequently with molecular methods (Cheng *et al.* 2011). Based on museum specimens from southern Africa, where massive capture and export of *Xenopus laevis* occurred in the 20th century, Weldon *et al.* (2004) suggested a possible African origin of *Bd*, correlating the earliest occurrence of *Bd* in the museum record (1938) with the onset of frog exportation. This hypothesis, however, rested solely on the specimens from Africa being the oldest infected specimens known at that time. Subsequent studies have discredited the African origin by demonstrating infected museum amphibians collected considerably earlier: 1894 in the Atlantic Forest of Brazil (Rodriguez *et al.* 2014) and 1888 in central USA (Talley *et al.* 2015).

The picture of global pathogen genetic homogeneity stood until Goka *et al.* (2009) identified a phylogenetically novel lineage of *Bd* from the giant salamander in Japan. This study was the first to indicate that additional genetic diversity might exist and led to the hypothesis of an Asian origin of panzootic *Bd*. Soon thereafter, screening of non-declining populations was intensified and combined with thorough molecular analyses (**Box 3**). Within a span of 3 years, novel genotypes putatively endemic to the Cape of South Africa (*Bd*-Cape), Switzerland (*Bd*-CH), Brazil (*Bd*-Brazil), and Korea (*Bd*-Korea) (Farrer *et al.* 2011; Schloegel *et al.* 2012; Bataille *et al.* 2013), were described. By increasing the sampling of *Bd* strains from regions where populations were not declining, it became apparent that the earlier perspective on genetic diversity was biased towards epizootic strains. Now, in addition to the globally prevalent *Bd*-GPL, we know of several divergent lineages of *Bd*, distributed on each continent that has been surveyed. Another major breakthrough was the recent discovery of a congeneric species *B. salamandrivorans* (*Bsal*). The new species is morphologically,

genetically, and functionally distinct from *Bd*. It was discovered as a pathogen of fire salamanders (*Salamandra salamandra*) in northwestern Europe (Martel *et al.* 2013), but was probably introduced from eastern Asia (Martel *et al.* 2014). The discovery of this new form pushes the association of the *Batrachochytrium* genus as an amphibian parasite to an age of at least 25 million years, showing that the emergence of *Bd* is not associated with a recent host jump to amphibians.

The finding of enzootic lineages of *Bd* that are more restricted in their distribution contrasts with the broad distribution and spread of the virulent genotype (*Bd*-GPL). Enzootic genotypes appear to be rarer and may represent a pattern of historical genetic diversity that is in the process of being erased or outcompeted by the current panzootic. Only in Korea has *Bd*-GPL been shown to have low prevalence, which might indicate that the enzootic genotype can outcompete other *Bd* lineages in this environment or that GPL has only recently been introduced (Bataille *et al.* 2013). Because >90% of strains isolated from nature are *Bd*-GPL (Schloegel *et al.* 2012), a leading hypothesis is that *Bd*-GPL is a hyper-virulent genotype that is replacing the rarer, enzootic lineages. The replacement of enzootic with panzootic genotypes in nature is also consistent with infection experiments showing that strains of *Bd*-Cape have lower virulence than the *Bd*-GPL (Farrer *et al.* 2011), but several studies demonstrate that even *Bd*-GPL genotypes vary in virulence when tested on common hosts (Berger *et al.* 2005; Fisher *et al.* 2009a). Unfortunately, at this point we cannot generalize that certain lineages or genotypes are less virulent because so few host species and *Bd* genotypes have been tested. More standardized studies are needed to quantify the PATHOGENICITY of *Bd* genotypes across various hosts and continents, focusing on standardized housing, doses, and using strains with low passaging history (Kilpatrick *et al.* 2010; Langhammer *et al.* 2013). Other phenotypes such as temperature optima, growth rates, and morphology are worth investigating across genotypes. For example, the optimum growth temperature of the newly described salamander parasite *Bsal* (Martel *et al.* 2013) is markedly lower (15 C) than that of *Bd* (17–25 C). Unfortunately, growth rates and temperature optima for other lineages or other isolates of *Bd*-GPL have not yet been determined.

The basis for these phenotypic distinctions among lineages may be a product of genomic differences among them. A prominent feature of the GPL lineages is the presence of

particular LOH events that must have occurred before the global dispersal of the GPL because they occur in every strain (Rosenblum *et al.* 2013); other LOH events occurred after GPL began diversifying and distinguish two clades within GPL, GPL-1 and GPL-2 (Schloegel *et al.* 2012). Based on the LOH model, we infer that GPL-1 is the more ancestral variant because it differs from GPL-2 by the absence of particular LOH events. GPL-2 is the most common lineage in the tropics (**Figure 4**) and is the genotype isolated from massive die-offs in Central America and Australia (Berger *et al.* 1998). In contrast, GPL-1 is most common in North America, and is the lineage associated with epizootics of *Rana muscosa* in the Sierra Nevada (Schloegel *et al.* 2012). GPL-1 also predominates in Europe, but has not been found in Australia or Africa. If we are correct in our inference that GPL-1 is the ancestral panzootic lineage, this indicates that GPL first emerged in the northern temperate zone and later dispersed into the tropics. Interestingly, only rarely is the dominant tropical form (GPL-2) found in temperate regions, and vice versa for GPL-1. Importantly, the spatial distribution of GPL-2 points in North America suggests a role for anthropogenic movement of frogs or pathogen (**Figure 4**), because most of the GPL-2 points represent isolates from animals in captivity, including one from *Dendrobates azureus* at the National Zoo in Washington, D.C. (the type strain JEL197), and another isolate from a *Xenopus laevis* strain imported to U.C. Berkeley from Africa in the 1980's (Morgan *et al.* 2007).

We know surprisingly little about the requirements of various *Bd* life cycle stages; might free-living stages, environmental resting stages, or alternate hosts exist? This information is crucial when considering any mitigation or reintroduction program. *Bd* DNA was recently reported from the GI tract and the surface of crayfish (*Procamberus alleni*, *P. clarkia* and *Oronectes virilis*; (McMahon *et al.* 2012). Further, the authors inoculated crayfish with *Bd* and found higher mortality and gill recession than for controls. They also documented that *Bd* could be transmitted from crayfish to larval amphibians. The possibility of a SAPROBIC reservoir for *Bd* has been discussed since the description of the species, when it was noted that because *Bd* was able to grow in pure culture on nutrient media and limitedly on snake skin, it might also be able to live saprobically in nature (Longcore *et al.* 1999). *Bd* has survived in autoclaved lake water for 6 weeks, and for 3 weeks in autoclaved tap water (Johnson & Speare 2003). The lack of living microbes, protists and small invertebrates in these

experiments, however, makes inferences about *Bd* survival in nature difficult. Although tested in sterile conditions, the ability of *Bd* to remain viable in natural sources of water and on keratineacous substrates supports the view that the environment can, at least temporarily, support the viability of *Bd* outside of amphibian hosts. Moreover, the detection of *Bd* DNA throughout the entire year from filtered North American water samples suggests the persistence of the fungus during a time when amphibians are dormant (Chestnut *et al.* 2014). In pure culture *Bd* sporangia develop from a zoospore without a germ tube first being formed (**Figure 1**); this differs from development *in vivo* with a germ tube, as shown by transmission electron microscopy (Greenspan *et al.* 2012). Development without forming a germ tube is a feature of many chytrid species that form a sporangium on top of their substrate, whereas formation of a germ tube is characteristic of chytrid species that develop a sporangium within their substrate. The presence of these two developmental pathways, the ability to grow in pure culture, and the presence of *Bd* DNA in environmental sources all predict saprobic reproduction, yet a non-living reservoir for *Bd* has yet to be identified. Identifying such reservoirs will be important to understanding the effects of *Bd* on amphibian populations, as demonstrated by model predictions of the long-term dynamics of *Bd* with and without a hypothesized saprobic phase (Mitchell *et al.* 2008).

The past decade of studies on the pathogen have uncovered deeper complexity in pathogen genotype, phenotype, and novel biotic interactions. Combined, the data indicate a hyper-virulent lineage that is primarily responsible for epizootics; however we still lack a clear indication of why and from where the *Bd*-GPL lineage emerged, and we lack good studies on the phenotypes and virulence of newly discovered enzootic lineages. Genetic studies of *Bd* and other pathogens suggest the origin of *Bd*-GPL will not be found by looking at sites of die-offs, and therefore the hunt for genetically diverse source populations from non-declining populations is a high research priority. Ideally, these genetic diversity surveys would use markers ascertainable from DNA extracted from swabbed animals, but such a marker system has proven difficult to implement (Velo-Anton *et al.* 2012) (**Box 3**). Other priorities involve investigating pathogen genotype dynamics (such as hybridization and competition) in regions where multiple genotypes coexist, such as Mallorca and the Brazilian Atlantic Forest (Walker *et al.* 2008; Farrer *et al.* 2011; Schloegel *et al.* 2012). Studies are also

needed to confirm if non-amphibian hosts are part of the life cycle or just a dead end, and what substrates might support saprobic life styles.

VARIATION IN HOST DISEASE SUSCEPTIBILITY AND DISEASE DYNAMICS

The final vertex on the disease triangle is the host, and here we review evidence that variance in host traits can explain susceptibility to *Bd*. Despite our greater knowledge of amphibian hosts, with over 7,400 species described, the immunology, distribution, and ecology of many species are still poorly characterized. Field and lab studies document large variation in susceptibility and RESISTANCE to *Bd* across species (Lips *et al.* 2006; Crawford *et al.* 2010; Searle *et al.* 2011b; Gahl *et al.* 2012). Multiple factors most likely lead to this variation, including host differences in innate and acquired immunological response, host associated microbes, and behavioral and life history traits. Behavioral, life history, and habitat traits have taken center stage because these characteristics are easy to measure. The emerging consensus is that life history traits matter, such as lower susceptibility in direct developers that lack an aquatic larval stage (Kriger & Hero 2007; Bielby *et al.* 2008). However, these generalizations do not sufficiently capture differences in host range that could be predictive of epizootics. Here we focus on recent findings showing that community composition and individual species genetic variation may influence not only the outcome of infection but also the potential for evolution of resistance.

The role of amphibian community composition in regulating *Bd* dynamics has been addressed from the perspectives of host diversity and identity. One potential consequence of high species richness is a "DILUTION EFFECT" resulting in reduced risk of disease (Keesing *et al.* 2006). Dilution effects occur because more diverse communities should be buffered from epizootics of generalist pathogens because encounters and potential transmission will often occur between susceptible and resistant hosts. Tests for dilution effects in the amphibian-*Bd* system have been conducted by several authors with results showing host diversity decreases (Searle *et al.* 2011a; Becker *et al.* 2014; Venesky *et al.* 2014), increases (Becker & Zamudio 2011), or no impact (Liu *et al.* 2013) on the risk of infection. All lab studies have shown a dilution effect, whereas these effects are more difficult to detect in the field, perhaps due to

the differences in both host diversity and habitat complexity. One lab study (Becker *et al.* 2014) found that host diversity decreased *Bd* infection due to changes in species interactions, specifically by reducing shared habitat use and transmission among hosts. Additionally, one particular terrestrial species showed reduced infection loads in diverse assemblages at the expense of neighboring aquatic hosts becoming heavily infected. Therefore, despite the fact that *Bd* is a highly generalist pathogen, these findings show the importance of understanding community-wide transmission dynamics and species-specific interactions for predicting disease outcome.

The idiosyncratic results from testing the dilution effect suggest that species diversity may be less important than the presence of particular species in a community. Amphibians that are highly susceptible to *Bd*, like *Atelopus zeteki*, can function as "acute supershedders" thus amplifying disease transmission (DiRenzo *et al.* 2014). Non-native, *Bd*-tolerant species, such as the American bullfrog (*Lithobates catesbeianus*) and the African clawed frog (*Xenopus laevis*) may function as reservoir species, those carrier species that are highly tolerant of infections. Importantly, these two invasive species have been implicated in the global spread of the disease (Daszak *et al.* 2001; Vredenburg *et al.* 2013). In Colorado, where *L. catesbeianus* is invasive, the density of *L. catesbeianus* was positively correlated with *Bd* infection prevalence and load in co-occuring native fauna (Peterson & McKenzie 2014). On the other hand, a recent study failed to find evidence of increased *Bd* infection on native UK fauna due to presence of invasive and *Bd*-infected *Xenopus* (Tinsley *et al.* 2015). These different results across regions highlight the important interactions between amphibian communities and environmental factors in determining infection outcomes. Though it would seem from lab studies that the presence of certain key species could alter chytridiomycosis dynamics in the field, only one study has made a link between the presence of particular host genera and chytridiomycosis prevalence, with the highly susceptible *Bufo* spp. increasing community-level *Bd* prevalence and the suspension feeder *Gastrophryne* reducing *Bd* prevalence (Venesky *et al.* 2014). Similar studies are needed to test the role of invasive species on the spread of *Bd* globally.

Moreover, variable outcomes of infection are observed in the field, with some populations persisting after the arrival of *Bd*, while others go extinct (Briggs *et al.* 2010).

These variable outcomes suggest potential differences in host genotype and prompt the question of the potential for host evolution of increased resistance or TOLERANCE to *Bd*. Amphibians can rely on innate and adaptive immune responses to manage *Bd* infections, and at least some of these immune responses have a genetic basis (Savage & Zamudio 2011; Ellison *et al.* 2014), suggesting host genotypic variation may be an important factor explaining persistence or mortality. For instance, alleles of the major histocompatibility complex (MHC), an important family of genes in the adaptive immune response, were significantly associated with resistance and survival in *Lithobates yavapaiensis* (Savage & Zamudio 2011) and *Litoria verreauxii* (Bataille *et al.* 2015). Various immunogenetic studies have reported either a strong or weak adaptive immune response post-*Bd* infection (Rosenblum *et al.* 2012; Ellison *et al.* 2014), underscoring variation among species in their potential for evolution of resistance or tolerance. Recently, a study suggested both adaptive behavioral avoidance and partial immunity could be acquired following *Bd* exposure (McMahon *et al.* 2014), though vaccination by prior infection has not proven effective in at least one species (Cashins *et al.* 2013). Altogether, the data suggest natural variation in both pathogen virulence and host immunity, but the interactions between these two components have not been adequately addressed to allow predictions of which species or communities have the potential to recover after exposure to *Bd*.

The epizootic space of the disease triangle largely excludes direct developing species lacking a larval stage, aggressive invasive species, and those with a large clutch size (Bielby *et al.* 2008). However, communities diverse and species-poor alike have suffered declines, and predicting the outcome of *Bd* infection for any given species remains elusive. Rare species may have lower genetic variation for parasite resistance, and the role of genetic variation in buffering disease through genetic fitness correlations needs to be better explored (Allentoft & O'Brien 2010). What happens in a resistant response is largely unknown, but now that we know that there is meaningful variation in immunological response, further research can also address variation in immunogenetic diversity across species with a range of susceptibilities (Ellison *et al.* 2015).

FUTURE RESEARCH PROGNOSIS: COLD SPOTS, RATHER THAN HOT SPOTS, MAY BE THE KEY TO UNDERSTANDING ENIGMATIC DISEASE

Improved modeling techniques and additional survey data have refined our understanding of *Bd* distribution and prevalence across the globe. *Bd* has a broad distribution that is correlated with colder temperatures and more moist environments (**Figure 2**), yet, distribution maps and SDMs highlight important, yet often enigmatic details about the distribution of *Bd*. First, the SDM identifies a number of hot spots including high elevation forests in Central America, the Sierra Nevada, and the Brazilian Atlantic Forest (**Figure 2**). These are regions of the world where we know *Bd* is already present at high prevalence, and the disease largely seems to have become enzootic, though not all of these regions have suffered declines. Second, the *Bd* distribution model contains several cold spots, such as the Amazon Basin and the Great Plains region of North America. Although the Amazon Basin is an apparent cold spot for *Bd* based on climatic variables, *Bd* has been detected there (McCracken *et al.* 2009). Based on the results of our SDM, we suggest the time is right to rephrase questions regarding distribution of *Bd* to: "Where are the cold spots in *Bd* distribution?" and "Why are they cold?" Now, studies are needed to identify regions and populations where *Bd* is absent to learn about the biotic and abiotic mechanisms underlying this distribution. More surveys in tropical regions are clearly warranted given the diversity of amphibians in these regions and the relative paucity of studies. Moreover, studies at smaller spatial and temporal scales are needed to understand environmental regulation and transmission patterns that lead to variation in community level prevalence.

We have defined cold spots as regions where the pathogen is absent or predicted to be absent, or where it occurs at low prevalence; these spots may exist for a number of reasons. First, they could be artifacts due to limited sampling of habitats within that specific niche parameter space. However, as global *Bd* survey has progressed in the last decade, these potential sampling artifacts are becoming less likely. Second, *Bd* may never have dispersed there. Third, environmental conditions may be outside of *Bd*'s tolerance window. Lastly, *Bd* might historically have been present but the frogs have evolved defenses (or only the resistant

507 species remain through pathogen driven selection), and *Bd* later disappeared or persists at low
508 population prevalence.

509 The existence of *Bd* cold spots raise a number of questions. If they are the result of
510 environmental restriction, why is the fungus unable to adapt to higher temperatures? More
511 experimental work is needed to understand physiological plasticity and propensity for local
512 adaptation in *Bd*. In the laboratory, changes in virulence and other phenotypes have been
513 noted over time in culture, indicating that the fungus can adapt rapidly (Langhammer *et al.*
514 2013; Voyles *et al.* 2014a). The distribution of *Bd*, which includes warm tropical lowland
515 forests, is inconsistent with the high level of growth inhibition seen in the lab at 28 °C. A
516 major research need is characterizing differences in the fungus by the collection, genotyping,
517 and temperature profiling of strains from these habitats seemingly outside of the *Bd*
518 physiological envelope.

519 A second type of cold spot is an area within the distribution of *Bd* that appear to be hot
520 in terms of prevalence, but cold in terms of negative effects on the fitness of the amphibian
521 hosts. These regions, including eastern Brazil, Chile, and eastern North America, apparently
522 have not experienced species declines despite widespread *Bd* occurrence and high prevalence
523 of infection. Because *Bd* has only been known for 15 years, however, missing baseline data
524 may be obscuring proper inferences, and the absence of mass mortalities is certainly not
525 evidence that disease-related declines did not occur. Indeed, species in Brazil, Chile, and
526 Wisconsin of the United States underwent enigmatic declines near the time that *Bd* was
527 implicated in declines in nearby areas (Hine *et al.* 1981; Eterovick *et al.* 2005; Soto-Azat *et*
528 *al.* 2013). Gradual declines of amphibian species are especially hard to detect because
529 populations naturally fluctuate and quantitative population data are lacking (Adams *et al.*
530 2013). Some extant amphibian communities might be remnants, i.e., amphibian communities
531 affected by epizootics before the last three decades of high vigilance. If so, could these
532 surviving communities show signs of "The Ghost of Epizootics Past" and how could we
533 distinguish them?

534 The theory of disease ecology predicts that when a disease enters a naïve population,
535 lack of host immunity often results in epizootics, characterized by high intensity and high
536 prevalence of disease; in contrast, in enzootic scenarios the pathogen is predicted to be present

at lower levels of infection once hosts and pathogens have reached an equilibrium state in susceptibility and infection (Ewald 1994). Over time, enzootic pathogens are expected to coevolve with their hosts and adapt to their shared environment. Given the long term co-existence of *Bd* and amphibians (Rodriguez *et al.* 2014; Talley *et al.* 2015) and the presence of putative enzootic *Bd* lineages, the amphibians we see today may be post-epizootic relics that have adapted to coexist with their now enzootic *Bd* lineage.

If the Ghost of Epizootics Past exists, we should be able to detect it using community and population genetic data. These methods can be tuned to identify signatures expected in communities that suffered epizootics by comparisons with control communities for which we have clear evidence *against* disease related declines. Possible regions for defining control expectations are far-east Asia and the Amazon basin, which have well-studied amphibian fauna with no evidence of declines. Korea is one compelling control population because it essentially only has enzootic *Bd* genotypes present, and exhibits high prevalence with low loads, suggesting that it has not suffered epizootics (Bataille *et al.* 2013).

We expect that communities that have undergone declines may have lost lineages or species with increased susceptibility to the disease, which can be tracked by comparative analyses across communities. One expected signature is the loss of specific susceptible hosts from amphibian communities. For example, post-enzootic communities may be enriched for terrestrial breeders, large clutch sizes, and small body size, given the relationship of these variables and *Bd* infection observed across communities (Kriger & Hero 2007; Bielby *et al.* 2008). Analyses of community diversity using traits as variables could allow identification of outliers with less diversity than expected in particular traits (e.g., breeding behaviors) relative to control communities. Other less typical phenotypes could also be analyzed, such as correlates of innate immunity (e.g., anti-microbial peptide production), skin microbial communities, or average genetic diversity (heterozygosity) of populations or species. As an example, phenotype frequencies from killing assays where immune cells of animals are challenged with bacteria have been shown to change following an epizootic in Florida scrub jays caused by an unknown pathogen (Wilcoxen *et al.* 2010).

Phylogenetic methods analyzing the distribution of species in regional amphibian fauna may also help identify communities that are phylogenetically overdispersed or

underdispersed relative to expectations (Cavender-Bares *et al.* 2009), as if many of the leaves had been pruned by disease. Similar phylogenetic methods could be applied to candidate genes, such as the MHC genes (Savage & Zamudio 2011), where tree-based methods could detect patterns that deviate from the default signature of balancing selection (Schierup *et al.* 2001), which is expected to occur if epizootics selected for particular alleles by directional selection as evidenced in field studies (Savage & Zamudio 2011; Bataille *et al.* 2015). A particularly exciting prospect is that community-level approaches may have the advantage of leveraging museum collections, which extend deep into the early days of exploration of the New World, to characterize control populations. Shifts in taxonomic richness over time can test for selective loss of clades, as *Bd* prevalence appears to be non-randomly phylogenetically distributed (Baláž *et al.* 2014). Testing the predictors we outline could utilize Panamanian and Peruvian communities where declines and extirpations are well-documented (Crawford *et al.* 2010; Catenazzi *et al.* 2011).

These approaches are not easy to implement, so what other signatures might allow us to detect an earlier, community changing epizootic? While we cannot resurrect extinct populations that have experienced historical declines, we can utilize animals in extant communities with this signature of enzootic disease, and test their current susceptibility to the pathogen. Under predictions of the Ghost of Epizootics Past model, the surviving animals should be adapted to resist endemic pathogen genotypes, but testing them against foreign genotypes should reveal higher susceptibility. This calls for inoculation studies, for example, of Brazilian endemic herpetofauna, using enzootic strains of *Bd* from all groups: *Bd*-GPL, *Bd*-Brazil, *Bd*-Cape, *Bd*-Korea. If coevolution following epizootics has occurred, the surviving amphibians in Brazil will be highly susceptible only to the latter two strain types, which are absent from the native range. In contrast, if the populations have not experienced adaptation, the lineages present will be those with the highest virulence. Determining the traits responsible for this adaptation may be key to understanding the signatures of the Ghost of Epizootics Past.

LESSONS LEARNED

Despite the many unanswered questions remaining regarding *Bd* and its interaction with amphibian hosts, major advances have occurred in the last 15 years. These advances have improved our preparedness to document and prevent future emergence of infectious diseases of wildlife (Voyles *et al.* 2014b). We end this review with four unanticipated lessons learned.

Generalist pathogens can be a cause of extinction

The most important lesson is that an infectious disease can be a major cause of biodiversity loss. Before chytridiomycosis emerged we had only a handful of examples where infectious disease was linked to severe declines or extinctions, including the American elm, the American chestnut, and six animal cases (Collins & Crump 2009). As a result, conservation biologists generally ignored pathogens as a cause of extinction. During the amphibian chytridiomycosis crisis we may have lost dozens of species and witnessed severe population declines of hundreds more. That a pathogen can decimate vertebrate populations of multiple species has highlighted the importance of studies in taxonomy and systematics, not only in terms of urgency, but because knowledge on all aspects of biodiversity is critical in preparing us for future outbreaks.

Most emerging infectious diseases (EIDs) of wildlife are host generalists: chytridiomycosis, rabies, white nose syndrome (WNS), West Nile virus, avian cholera, and snake fungal disease all have a wide number of hosts, making them a significant threat to both biodiversity and ecosystem function. However, impacts across host species are highly variable due to biotic and abiotic determinants. For example, just as with chytridiomycosis, WNS occurs in seven bat species but only populations of the four most gregarious species are endangered by the disease (Langwig *et al.* 2012). However, as we have highlighted here, EIDs caused by host generalists require consideration of the whole host community because of the presence of amplifier species and the possibility of dilution effects.

Fungal diseases are on the rise

Though the underlying causes are unclear, fungal EIDs of wildlife (including WNS, snake fungal disease, sea fan disease, and chytridiomycosis) appear to be emerging faster than

626 those caused by bacteria, viruses, and protozoa (Fisher *et al.* 2012). Common themes of
627 fungal EIDs include being host generalists, invaders of soft tissues rather than blood, and
628 typically on ectotherms or the colder extremities of endotherms (such as the wings and noses
629 of hibernating bats). As is the case for *Bd*, environmental filtering also plays a role in other
630 wildlife fungal diseases, such WNS, where the pathogen grows optimally at the same
631 temperatures typically found in bat hibernacula (Blehert *et al.* 2009). The presence of
632 facultative saprobic life cycles or environmental reservoirs may also be a common thread that
633 ties together fungal EIDs, allowing pathogens to continue transmission even after causing host
634 mortality (Fisher *et al.* 2012). These saprobic phases would also facilitate dispersal because
635 they eliminate the need for hosts. As many of the fungal EIDs (e.g., WNS, chytridiomycosis,
636 and snake fungal disease) appear to have evolved from saprobic ancestors this may perhaps
637 explain their necrotrophic pathogenicity with gross tissue destruction.
638 The emergences of a number of fungal EIDs, such as chytridiomycosis, WNS, and
639 sudden oak death, are hypothesized to follow a recent introduction and movement of
640 pathogens. Therefore, it may be that a sudden increase in transmissibility or movement of
641 fungi is what has led to this increase in fungal EIDs. Though not readily obvious, these
642 diseases can spread through international movement of infected hosts, such as when infected
643 chestnut trees were brought to the New York area and led to the epidemic that nearly led to
644 their extinction (Anagnostakis & Hillman 1992). Evidence to support these point
645 introductions comes from characterization of low pathogen genetic diversity. As observed for
646 *Bd*, the rapid emergence of WNS in eastern North America is due to a single clonal genotype
647 of *Pseudogymnoascus destructans*, presumably introduced from Europe (Ren *et al.* 2012).
648 These examples of recent spread indicate that we should consider the ever-increasing
649 potential for anthropogenic movement of pathogen propagules as an explanation for the
650 global rise in fungal EIDs.
651
652 **Baseline data are vital for defining epidemics**
653 Generally, we suffer from a lack of long term data on wildlife populations, which
654 severely impedes the detection of epidemics. How often do declines occur? A meta-analysis
655 of frog declines conducted early in the days of *Bd* research (Houlahan *et al.* 2000) was only

able to draw from data from 1950-2000. These data suggested a downward slide of amphibian populations since 1960, but in the absence of data from before 1960, it is difficult to know how early this trend began. Wildlife and plant monitoring programs need to be supported so that when declines and epizootics occur, baseline data are in place for comparison. The relatively new North American Amphibian Monitoring Program aims at cataloging trends in amphibian populations across the continent using citizen scientists. However, this program is solely based on frog calls, and we need more efforts in monitoring of disease and its effects using molecular diagnostics and mark-recapture studies. Such efforts would expedite our response to epizootics and form the basis for proactive rather than reactive science.

Baseline population and community data can also be drawn from museum collections, and examples exist in which new species of birds have been described from museum specimens which have already gone locally extinct in the Brazilian Atlantic Forest (Lees & Pimm 2015). Museum specimens not only yield locality records, but also allow detection of time periods in which *Bd* increased in prevalence (Cheng *et al.* 2011). Recently, museum specimens have been used to time introductions of particular genotypes of *Aphanomyces astaci* causing epizootics of crayfish plague in Norway beginning in 1971 (Vrålstad *et al.* 2014). Studies of crayfish plague and of *Bd* in the Brazilian Atlantic Forest (Rodriguez *et al.* 2014) that utilize genetic markers provide information regarding gene flow and a more precise migration history. To increase the utility of material for microbial work, collectors should make sure to preserve the integrity of samples for pathogen DNA analysis and histology, and given the importance of amphibian skin and its microbial communities, future amphibian collectors should consider archiving swabs or skin samples with accessioned specimens.

Never stop sampling

Major breakthroughs in understanding the genetic diversity of *Bd* occurred only after years of sampling. Understanding biases in sampling and geographic coverage is essential for identifying source populations and rare, but informative, genotypes. Likewise, important information on the history and virulence of *Bd* will require sampling additional genes (**Box 3**). Meaningful genetic variation goes deeper than sequence polymorphism. Both chromosome number variation and LOH are highly variable in *Bd* at the same time that sequence variation

686 is extremely low. We recently characterized GPL-1 and GPL-2 sublineages, and these, as well
687 as the enzootic lineages, have geographic and genomic patterns in great need of further
688 exploration. The first 10 years of sampling only revealed GPL (James *et al.* 2009), the last
689 five years uncovered five additional lineages of *Bd*, including a clearly sexually produced
690 isolate (Schloegel *et al.* 2012), and a new species with a different host range (Martel *et al.*
691 2013). Sampling biases should also serve as a cautionary tale. For example, failure to detect
692 *Bsal* in North America does not necessarily mean that the species, or a close relative, is not
693 present. In fact, it seems unlikely that *Bsal* or a closely related species is not in North America
694 given that it diverged from *Bd* so long ago and thus has existed for at least 25 million years?
695 An alternative is that we just are unable to detect it, and that the Old World *Bsal* is analogous
696 to *Bd*-GPL, only restricted to salamanders. If anything, our previous experience indicates that
697 we need to keep sampling to find additional lineages of *Bsal* that we may likely be missing.
698
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709 discussions on the dilution effect.
710
711 **Box 1. Glossary**
712
713 ANEUPLOIDY: having an atypical number of chromosomal homologs, not multiples of the
714 baseline haploid number. May be represented by additional or fewer copies of a homologous
715 chromosome(s).

CHYTRIDIOMYCOSIS: the disease of amphibians caused by the fungus *Batrachochytrium dendrobatidis*. Animals that test positive for the presence of *Bd* may show no symptoms of the disease.

DILUTION EFFECT: The concept that disease risk of a generalist pathogen is ameliorated with increased biodiversity through mechanisms that reduce the probability of transmission.

ENDEMIC PATHOGEN HYPOTHESIS (EPH): Posits that *Bd* co-existed with its host in equilibrium before the panzootic was triggered by some other factor, such as environmental change.

ENZOOTIC: Describes host-pathogen dynamics that support coexistence over time.

EPIZOOTIC: Describes pathogens that are increasing in frequency, i.e., have not reached a stable equilibrium.

GLOBAL PANZOOTIC LINEAGE (GPL): The most frequently encountered lineage of *Bd*, which is highly virulent in the lab, genetically depauperate with only two alleles per locus, and the only genotype that has been associated with amphibian dieoffs in the field. GPL contains two sub-lineages, GPL-1 (that predominates in North America and Europe) and GPL-2 (that predominates in the Neotropics, Australia, and Africa).

LOSS OF HETEROZYGOSITY: In diploid or polyploid organisms, genotypes may lose heterozygosity during mitosis through the action of nondisjunction of chromosomes, crossing over, or gene conversion.

NOVEL PATHOGEN HYPOTHESIS (NPH): posits that pathogens emergence by the translocation of a virulent strain into a new geographic location or into a host species that has no evolved resistance.

PATHOGENICITY: Describes the ability of an organism to cause disease.

RESISTANCE: Refers to the natural ability of an organism to resist microorganisms or toxins produced in disease.

RIBOSOMAL INTERNAL TRANSCRIBED SPACER: The DNA region used for diagnostic *Bd* PCR detection that lies between the large and small subunits of ribosomal DNA. Multiple variants per strain make it problematical for use in population genetics.

SAPROBIC: Describes microbes and fungi that feed on dead or decaying organic matter.

TOLERANCE: Refers to the development of the host capacity to endure and become less responsive to a substance or a physiological insult especially with repeated exposure.

VIRULENCE: Describes the degree to which an organism can cause damage to a host.

ZOOSPORE: Flagellated motile spore. In *Bd*, the zoospore possesses a single flagellum and lacks a rigid cell wall.

Box 2. Species Distribution Model for *Bd* in the Americas

Based on the most up-to-date information on the realized distribution of *Bd*, we developed an updated SDM based on predictions of an ensemble of eight different algorithms and both environmental factors (temperature, humidity) and land cover information (Normalized Differenced Vegetation Index, NDVI), thus capturing the pathogen's Grinnellian niche as well as anthropogenic factors such as Human Footprint (Liu *et al.* 2013). The ensemble techniques within the *biomod2* framework (Thuiller *et al.* 2014) which were employed here represent recent advances in SDM methodology, acknowledging that no single algorithm provides the best solution and that uncertainties in different steps of SDM development are best acknowledged in a comparative framework.

Environmental data: As a first step, we obtained a comprehensive set of 19 bioclimatic variables with a spatial resolution of 2.5 arc min from www.wordclim.org as well as variables characterizing seasonal changes in the Normalized Differentiated Vegetation Index (NDVI; derived from the data set GIMMS [Global Inventory Modeling and Mapping Studies] NDVI: 1981-2006; available through www.edc.uri.edu/ATMT-DSS/data_gateway/modis/gimms.zip; NDVI scores are coded as 8bit integer ranging from 0:255), Potential Evapotranspiration (Trabucco & Zomer 2009), and "Human Footprint" (Sanderson *et al*. 2002). The Human Footprint ranges from 0 (no human influence) to 100 (strongest human influence), and characterizes the human influence on land surface based on accessibility, anthropogenic land transformation, human population density, and electrical power infrastructure. As multi-co-linearity of environmental predictors may violate statistical assumptions of SDM algorithms, we computed pair-wise Spearman rank correlations among all variables and selected among those pairs with $R^2 < 0.75$ the putatively most relevant for *Bd*. The final set of predictors included "Mean Diurnal Temperature Range" (Bio2; Mean of monthly (max temperature - min temperature)), "Temperature Annual Range" (Bio7), "Mean Temperature of Warmest Quarter" (Bio10), "Annual Precipitation" (Bio12), "Precipitation Seasonality" (Bio15; Coefficient of Variation of monthly Precipitation), "Precipitation of Warmest Quarter" (Bio18), "Annual Mean NDVI" (NDVI_Bio1), "NDVI Annual Range" (NDVI_Bio7), "Minimum Monthly Potential Evapotranspiration" (PET_HE_Bio6), "Annual Range of Potential Evapotranspiration" (PET_HE_Bio7), modified from monthly raw data in (Trabucco & Zomer 2009), and the "Human Footprint".

Based on our review we compiled a set of 6,071 georeferenced *Bd* positives from 30,382 swabs from adult amphibians of 749 named species. However, for successful SDM development the spatial structure of *Bd* records needs to be taken into account as spatial autocorrelation may hamper inference of *Bd* environmental niche from distribution data. Therefore, the spatial autocorrelation structure of *Bd* records was assessed via a semivariogram based on Moran's I and *Bd* records were subsequently spatially subsampled to a minimum distance between two records of 12.11 km leaving 765 records for model development. *Bd* prevalence in amphibian populations was visualized by aggregating *Bd*

positive and negative records within a distance of four km and computing the percentage of positives from the total sample size.

For full details regarding biomod2 algorithms and model parameters see **Supplementary Material**. To account for inherent uncertainties arising from the modeling and evaluation procedures, we 1) created ten different random subsets of *Bd* records which were used for model calibration (70%) and evaluation (30%), 2) created three different sets of pseudo-absences which were randomly sampled from the environmental space available within the Americas, but outside of the realized environmental space for *Bd* records (SRE option in *biomod2*). From the 240 single models (8 algorithms * 3 pseudo-absence data sets * 10 evaluation runs) 180 SDMs had TSS scores > 0.6 ($TSS_{average}$ = 0.67; $Kappa_{average}$ = 0.43, $ROC_{average}$ = 0.90). On average, "Minimum Monthly Potential Evapotranspiration" had the highest contribution to the *Bd* SDMs (20.3%), followed by "Human Footprint" (14.4%), "Annual Precipitation" (11.5%), "Mean Temperature of Warmest Quarter" (11.7%), "Precipitation of Warmest Quarter" (8.7%), "Annual Range of Potential Evapotranspiration" (7.7%), "Temperature Annual Range" 7.2%), "Mean Diurnal Temperature Range" (7.2%) and "Annual Mean NDVI" (7.1%). The remaining variables contributed less than 5%. Response curves of the final ensemble SDM are shown in Supplementary Figure 1.

Box 3. Advanced molecular methods for *Bd* population genetics

Our understanding of *Bd* population genetics in the last five years has improved through deeper sampling of isolates and an increased sampling of genetic loci. Although PCR amplification of the RIBOSOMAL INTERNAL TRANSCRIBED SPACER (ITS) region has been the workhorse for diagnosing *Bd* infections; each strain contains multiple and variable copies of the locus (Schloegel *et al.* 2012; Longo *et al.* 2013), limiting its utility as a population genetic marker. On the other hand, multi-locus sequence typing or microsatellite markers provide low resolution among strains for the level of effort/expense (Morgan *et al.* 2007; James *et al.* 2009). With the reduced cost of next-generation sequencing, population genetics by genome resequencing is starting to replace marker-based studies (Farrer *et al.* 2011; Rosenblum *et al.* 2013). These genome resequencing studies reveal rampant loss of heterozygosity and ANEUPLOIDY or polyploidy (Rosenblum *et al.* 2013); however, with respect to geographic

conclusions, critical patterns within the GPL are unclear because of low sample sizes and geographically disparate isolates grouping together. Currently, sequencing a single locus (e.g., BdC24 (James *et al.* 2009)) can typically distinguish each of the major groups of strains and increasing this to a handful of marker loci may be useful for identifying clones within populations. Extensive clonal reproduction, however, suggests that genome sequencing is unnecessary at the local level (e.g., within a stream). Nonetheless, given the limitations to obtaining cultures, it seems probable that future studies will work toward genome resequencing of many isolates. These studies must be carried out with improved methods, however, and with high sequence coverage to deal with the variable and high ploidy common in *Bd*. Insufficient coverage, can lead to low quality genotyping, which increases the noise:signal ratio.

A major need is to develop marker approaches for population genetics using skin swabs. When infection levels are high, MLST markers can be genotyped from swabs (Garner *et al.* 2006; Velo-Anton *et al.* 2012) with modest success rates. Enrichment techniques to increase the recovery of *Bd* DNA by hybridization to *Bd*-specific probes attached to magnetic beads, show promise to increase the success of genotyping from swabs when coupled with whole genome amplification (Rodriguez *et al.* 2012). A danger with low DNA input methods is the inability to distinguish allele drop out from true loss of heterozygosity (LOH), and LOH is exactly the information being targeted. As single cell genomic methods continue to develop it is likely that future epidemiological studies can be carried out from swabs. However, firstly, by comparing genotypes of cultures to relevant phenotypes (e.g., morphometrics and virulence testing), we as a community must determine what genotypic information we really are after.

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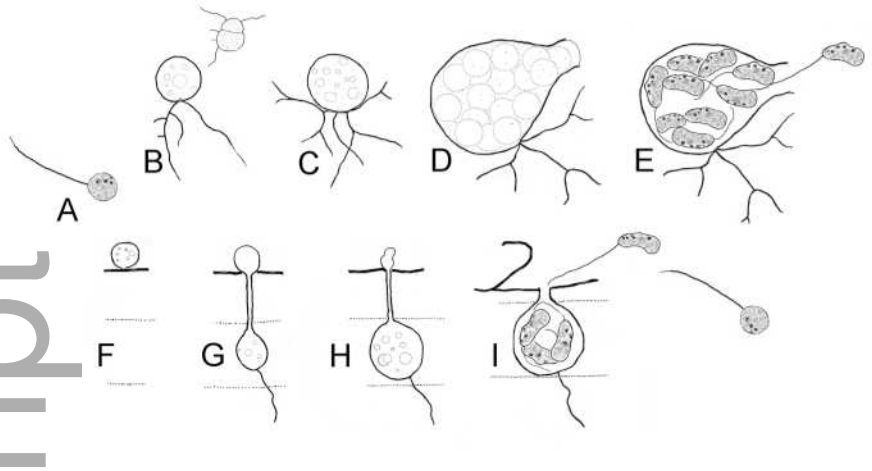
Figure Legends

Figure 1. *Batrachochytrium dendrobatidis* develops by either of two pathways, depending on whether growth is on nutrient agar (Figs. A–E; Longcore *et al.* 1999) or inside of amphibian cells (Figs. A, F–I; (Greenspan *et al.* 2012). On agar the ZOOSPORE (Fig. A) encysts and forms anucleate rhizoids. Over the course of 4 days (Figs. A–D), the zoospore cyst matures into a zoosporangium that releases zoospores through discharge papillae (Fig. E). Colonial thalli occur occasionally (Fig. B′), and their presence has been used to confirm identity of *B. dendrobatidis*. On skin, the zoospore encysts on the surface of a cell (Fig. F), and forms a germ tube, which grows through one or more host cell layers (Fig. G). The zoosporangium with sparse rhizoids forms from a swelling of the germ tube (Figs. G, H). By the time zoospores are released, the outer skin layer has sloughed (Fig. I).

Figure 2. Positive records of *Batrachochytrium dendrobatidis* and potential distribution of the fungus according to an ensemble species distribution model. Warmer colors indicate higher probability of environmental suitability. Areas exceeding the environmental training range of the SDM are indicated in grey.

Figure 3. Prevalence of *Batrachochytrium dendrobatidis* and potential distribution of the fungus according to an ensemble species distribution model. Warmer colors indicate higher probability of environmental suitability. Prevalence was computed only for those grid cells with more than 10 samples, wherein the size of the circles represent sample size. Areas exceeding the environmental training range of the SDM are indicated in grey.

Figure 4. Population genetic distribution of *Batrachochytrium dendrobatidis* genotypes determined from multi-locus sequence typing of cultured isolates. *Bd* clades are identified by color, and the captive status of the host amphibians is indicated by shape. The area of each shape represents the sample size of genotypes from each locality. Notable samples include: a captive isolate of *Bd*-GPL-2 from *Xenopus laevis* imported to U.C. Berkeley, California (1), isolate of a novel *Bd*-Brazil strain from *Lithobates catesbeianus* in a Michigan market (2), captive isolates from the National Zoo, Washington D.C. (3), and the Bronx Zoo, New York (4), and a region of high genetic heterogeneity in the Atlantic Forest of southeastern Brazil (5). Isolates are considered GPL-1 if they are heterozygous at loci BdC24, R6046, or both. Data are compiled from published sources (Morgan *et al.* 2007; James *et al.* 2009; Schloegel *et al.* 2012; Velo-Anton *et al.* 2012), and unpublished data.



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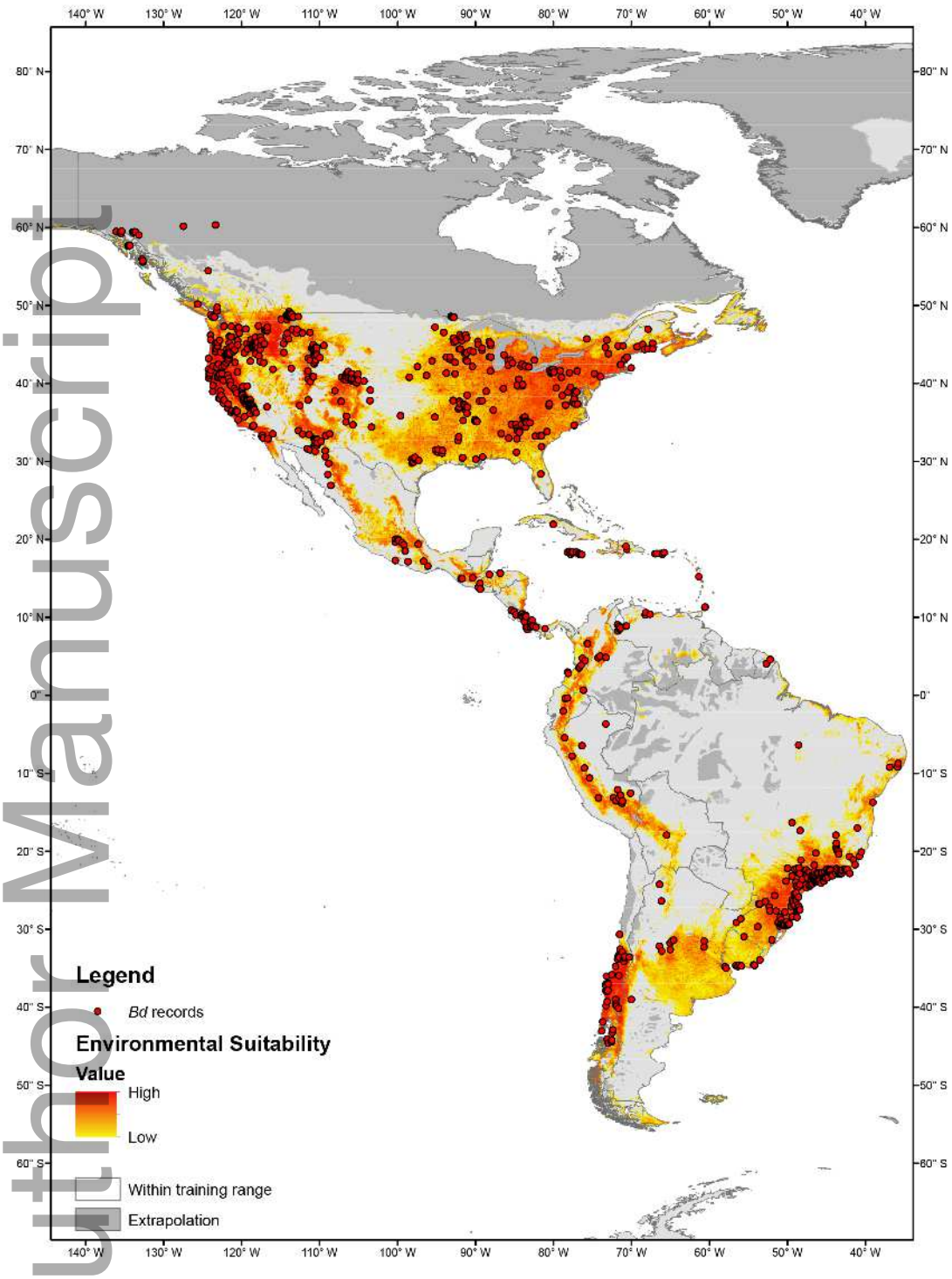

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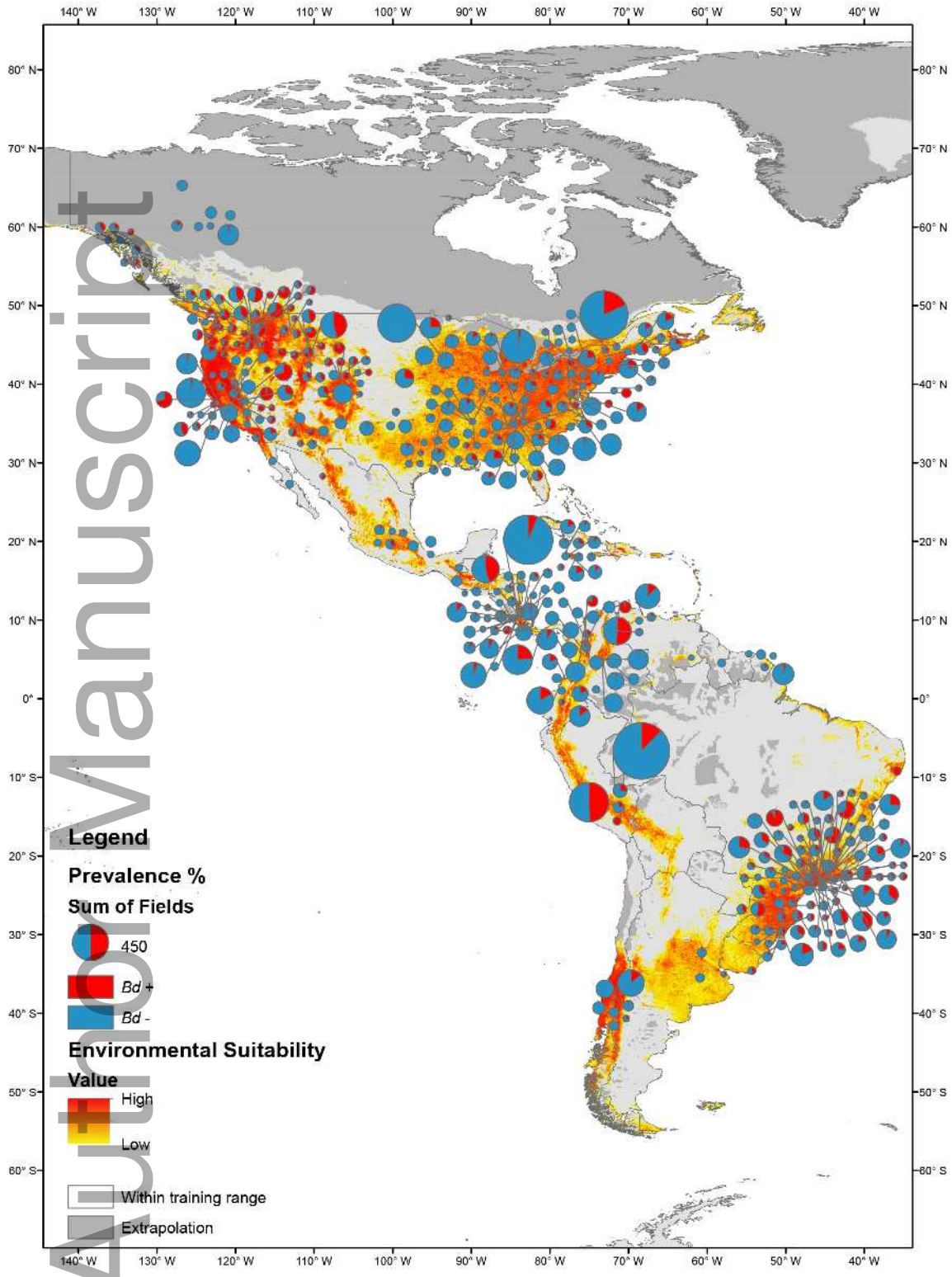

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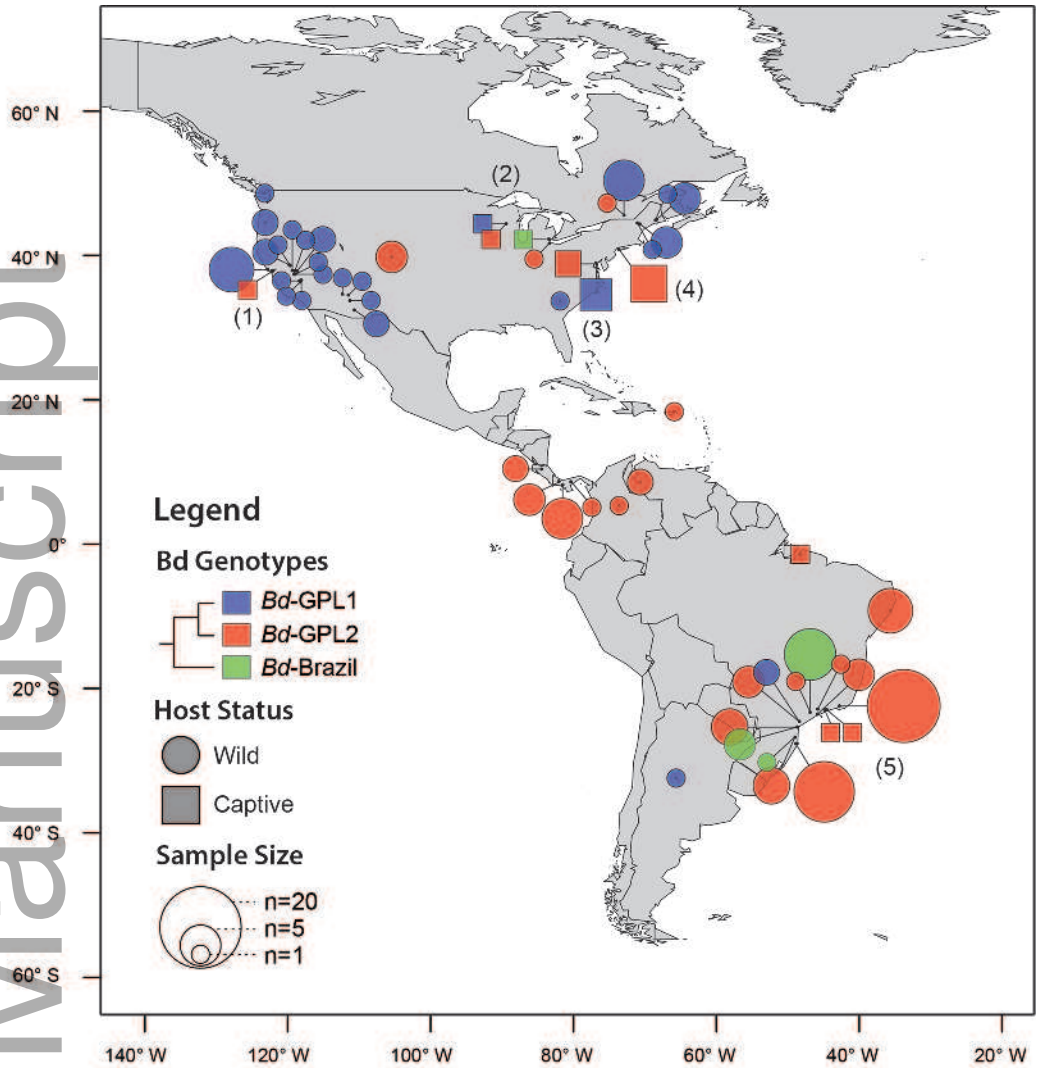

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