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The Emerging Amphibian Fungal Disease, Chytridiomycosis: A Key Example of the Global Phenomenon of Wildlife Emerging Infectious Diseases

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ABSTRACT The spread of amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, is associated with the emerging infectious wildlife disease chytridiomycosis. This fungus poses an overwhelming threat to global amphibian biodiversity and is contributing toward population declines and extinctions worldwide. Extremely low host-species specificity potentially threatens thousands of the 7,000+ amphibian species with infection, and hosts in additional classes of organisms have now also been identified, including crayfish and nematode worms.

Soon after the discovery of *B. dendrobatidis* in 1999, it became apparent that this pathogen was already pandemic; dozens of countries and hundreds of amphibian species had already been exposed. The timeline of *B. dendrobatidis*'s global emergence still remains a mystery, as does its point of origin. The reason why *B. dendrobatidis* seems to have only recently increased in virulence to catalyze this global disease event remains unknown, and despite 15 years of investigation, this wildlife pandemic continues primarily uncontrolled. Some disease treatments are effective on animals held in captivity, but there is currently no proven method to eradicate *B. dendrobatidis* from an affected habitat, nor have we been able to protect new regions from exposure despite knowledge of an approaching "wave" of *B. dendrobatidis* and ensuing disease.

International spread of *B. dendrobatidis* is largely facilitated by the commercial trade in live amphibians. Chytridiomycosis was recently listed as a globally notifiable disease by the World Organization for Animal Health, but few countries, if any, have formally adopted recommended measures to control its spread. Wildlife diseases continue to emerge as a consequence of globalization, and greater effort is urgently needed to protect global health.

INTRODUCTION: GLOBAL AMPHIBIAN DECLINE

During the latter half of the 20th century, it was noticed that global amphibian populations had entered a state of unusually rapid decline. Hundreds of species have since become categorized as "missing" or "lost," a growing number of which are now believed extinct (1). Amphibians are often regarded as environmental indicator species because of their highly permeable skin and biphasic life cycles, during which most species inhabit aquatic zones as larvae and as adults become semi or wholly terrestrial. This means their overall health is closely tied to that of the landscape. Amphibian declines in recent decades are largely attributed to increases in habitat destruction, pollution, and commercial

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exploitation, but enigmatic declines and mass mortality events began to be observed in seemingly healthy environments, suggesting that an additional factor with considerable negative impact was also influencing declines (2, 3).

Discovery of *Batrachochytrium dendrobatidis*

In 1998, a mass mortality event occurred in a colony of poison-dart frogs (*Dendrobates* spp.) held in a collection at the National Zoo in Washington, DC. During an autopsy, histological examination revealed an unusual fungal infection of the skin. The fungus was soon described as *B. dendrobatidis*, a previously unknown species of parasitic chytrid fungi with a particular appetite for amphibians (4). There are several hundred described species of chytrid fungi, most of which are important decomposers of nonliving organic material in the environment, such as pollen and rotting vegetation. A few exceptions infect living plant or animal cells, with *B. dendrobatidis* becoming the first known species to attack living vertebrate hosts.

Infection with *B. dendrobatidis* and Chytridiomycosis

B. dendrobatidis begins life as an aquatic uniflagellated zoospore released from a mature zoosporangia embedded in the skin of an amphibian (4, 5). *B. dendrobatidis* zoospores are commonly shed into the water, where they can swim short distances and/or are carried by water currents to reach a new host. Upon contact with an amphibian, *B. dendrobatidis* zoospores burrow several layers down into the skin, to the area where keratin is produced. These zoospores remain there, where they grow and mature into new zoosporangia. Through asexual reproduction, multiple new zoospores are produced within the zoosporangia and, when ready, are released from the amphibian's skin via discharge tubules. If the infected amphibian is in a terrestrial location when zoospores are released, they are likely to reinfect that animal and/or be shed onto vegetation or into soil. This growth cycle from zoospore to mature zoosporangium normally takes about five days at optimum temperatures and nutrient conditions.

Infection with *B. dendrobatidis* has various effects upon an amphibian, ranging from asymptomatic presence to the often lethal disease, chytridiomycosis (5). Low host-species specificity threatens potentially thousands of species with disease. As of 2013, 42% of 1,240 amphibian species tested were found to be infected (6). In amphibians susceptible to disease, the presence of *B. dendrobatidis* causes hyperkeratosis and interferes with normal shedding, damaging the animal's ability to osmoregulate and maintain electrolyte balance. In severe infections, this leads to death by cardiac arrest (7). Amphibians also sometimes manifest behavioral symptoms of disease such as lethargy, anorexia, and loss of righting reflex, but these are inconsistent and nonspecific to chytridiomycosis and thus cannot be used alone to confirm infection. The same applies to the presence of amphibian skin lesions sometimes caused by *B. dendrobatidis*. Consequently, diagnosis of chytridiomycosis is challenging and nearly impossible under field conditions. Thus, the presence of seemingly healthy amphibian populations is sometimes misleading. Dead amphibians are infrequently observed in the field despite sometimes high mortality rates (8), because they quickly decompose or become scavenged.

Chytrid Resistance in Nature

While some amphibians readily develop clinical symptoms of chytridiomycosis, others do not express illness. Certain species appear to possess a variable degree of innate resistance to *B. dendrobatidis* infection and/or disease. It is not yet fully understood what provides these species with a greater defense than most others, but it sometimes involves the presence of anti–*B. dendrobatidis* symbiotic bacteria in the skin and/or the amphibian's ability to produce certain skin antimicrobial peptides (9). These species can sometimes resist or clear *B. dendrobatidis* infection or persist with low-intensity infections. Unfortunately, some of these are species known to be invasive outside their native ranges, such as the American bullfrog (*Lithobates catesbeianus*) and African clawed frog (*Xenopus laevis*), and have established feral populations around the world (10). These species serve as asymptomatic *B. dendrobatidis* reservoir hosts that can transmit *B. dendrobatidis* infections to more susceptible species sharing a habitat. The presence of tolerant amphibians in a community of *B. dendrobatidis*–susceptible species can maintain pathogen presence even as vulnerable species decline and become locally extinct.

Detection of *B. dendrobatidis* Infection versus Disease

Distinction between *B. dendrobatidis* presence on a skin swab, *B. dendrobatidis* infection, and the disease chytridiomycosis must be made since these terms are sometimes used interchangeably, but each has a distinct meaning and denotes a different physical presence. The most widely accepted protocol to identify a *B. dendrobatidis*–infected amphibian is the collection of a skin swab sample together with a highly sensitive

and specific quantitative PCR diagnostic test (11). This is effective because *B. dendrobatidis* grows within the amphibians' skin and frequently sheds zoospores back out to the skin surface, where swabbing the highly keratinized regions (i.e., pelvic patch and feet) is likely to collect *B. dendrobatidis* particles that are then identifiable by PCR. It is important to remember that PCR-positive skin swab results alone do not show the condition of infection or disease, but rather show the molecular presence of live or dead *B. dendrobatidis*. Since *B. dendrobatidis* particles are shed by infected animals into the environment, it is possible that some skin swabs test positive from contact with *B. dendrobatidis*–contaminated water droplets or soil on an amphibians' skin (12). Still, skin swabs are highly advantageous over traditional histological analysis in that they are noninvasive and sampling can be performed on rare and endangered species, whereas tissue extraction would be potentially harmful to the animals' well-being. Therefore, although PCR-positive results for *B. dendrobatidis* via skin swabs do not truly prove the animal is infected, researchers agree that this is a generally acceptable assumption since the amount of *B. dendrobatidis* detected on swabs can now be quantified and is often quite high compared to detection outside the host in environmental substrates. For absolute confirmation of infection, tissue sampling and histological examination are needed (13). The presence of *B. dendrobatidis* within the amphibian's skin does indicate infection, but unless there are also clinical signs of detriment to the surrounding tissues, it is possible to have *B. dendrobatidis* infection without the disease chytridiomycosis.

Sampling techniques are also now available to detect the presence of *B. dendrobatidis* in the environment, outside the host. Water samples can be collected and filtered to capture environmental DNA, which includes free-floating zoospores and/or *B. dendrobatidis*-infected animal cells shed into the water (14–16). This technique is useful both independently, to screen for areas of *B. dendrobatidis* presence where swabbing surveys are impossible to perform, or complementary to swabbing surveys to develop greater context for interpretation of the survey results. In either case, it should be noted that detection of *B. dendrobatidis* in water filter samples only proves pathogen presence at a location, and not the infection of amphibians at that location.

Effects of *B. dendrobatidis* on Amphibian Populations

The effect of *B. dendrobatidis* on amphibian populations generally varies by species and region, but population decline attributed to this pathogen has now been documented on every continent where amphibians are found (6). Although all 7,000+ species in the class *Amphibia* are potentially vulnerable to infection, *B. dendrobatidis* seems to cause disease most often in members of the order *Anura*, the frogs and toads. Not only is *B. dendrobatidis* capable of impacting a broad range of host species, but it is also believed to be the first wildlife pathogen to have caused widespread species extinctions (17, 18). In recent years, it has been blamed for the extinction of several Australian frogs, including the sharp-snouted day frog (*Taudactylus acutirostris*) (17), the Northern gastric brooding frog (*Rheobatrachus vitellinus*) (19), and the Southern gastric brooding frog (*Rheobatrachus silus*) (19). Although unconfirmed, it is also suspected to have driven extinction of the golden toad (*Incilius periglenes*) in Costa Rica, a formerly common species endemic to the cloud forest of Monteverde that mysteriously vanished in 1989 (20), around the time a wave of *B. dendrobatidis*–associated disease swept through Central America causing a wave of dramatic decline (21, 22). In Africa, it is believed that *B. dendrobatidis* together with habitat degradation catalyzed the precipitous decline of Tanzania's Kihansi spray toad (*Nectophrynoides asperginis*), declared extinct in the wild by 2009 (23). In the United States, chytridiomycosis has driven the loss of California's yellow-legged frogs (*Rana muscosa* and *Rana sierrae*) from 93% of their historical range over the past few decades (24) and the near-extinction of the endangered Wyoming toad (*Bufo baxteri*) (25).

It would be remiss to speak of amphibian extinctions without also mentioning that some species previously declared extinct have later been rediscovered. Some are suspected to have vanished due to *B. dendrobatidis*, while others disappeared for less certain reasons. Instances of the former include the miles robber frog (*Craugastor milesi*) of Honduras (26), the armored mistfrog (*Litoria lorica*) of Australia (27), and the Rancho Grande harlequin frog (*Atelopus cruciger*) of Venezuela (28). These previously common species were suddenly "lost" for approximately 20 years following the arrival of *B. dendrobatidis*. Each was declared extinct, and then rediscovered and now classified as critically endangered. Other species went missing for much longer, and from places where *B. dendrobatidis* was not suspected, such as the Hula painted frog (*Discoglossus nigriventer*) from Israel (57 years) (29), the Bururi long-fingered frog (*Cardioglossa cyaneospila*) from Burundi (62 years) (30), and the starry shrub frog (*Pseudophilautus stellatus*) from Sri Lanka (160 years) (31). In some instances, the

surviving populations are unsurprisingly found in regions or habitats not previously explored, but curiously, most have been close to where the last known sighting was recorded. Although this phenomenon provides hope that other lost species might not yet be extinct, these instances remain the minority. Judging from the population crashes observed in *B. dendrobatidis*'s wake as it has invaded new regions, and particularly Central America (21, 32, 33), it is reasonable to think that a greater number of missing amphibian species are likely extinct or on the verge.

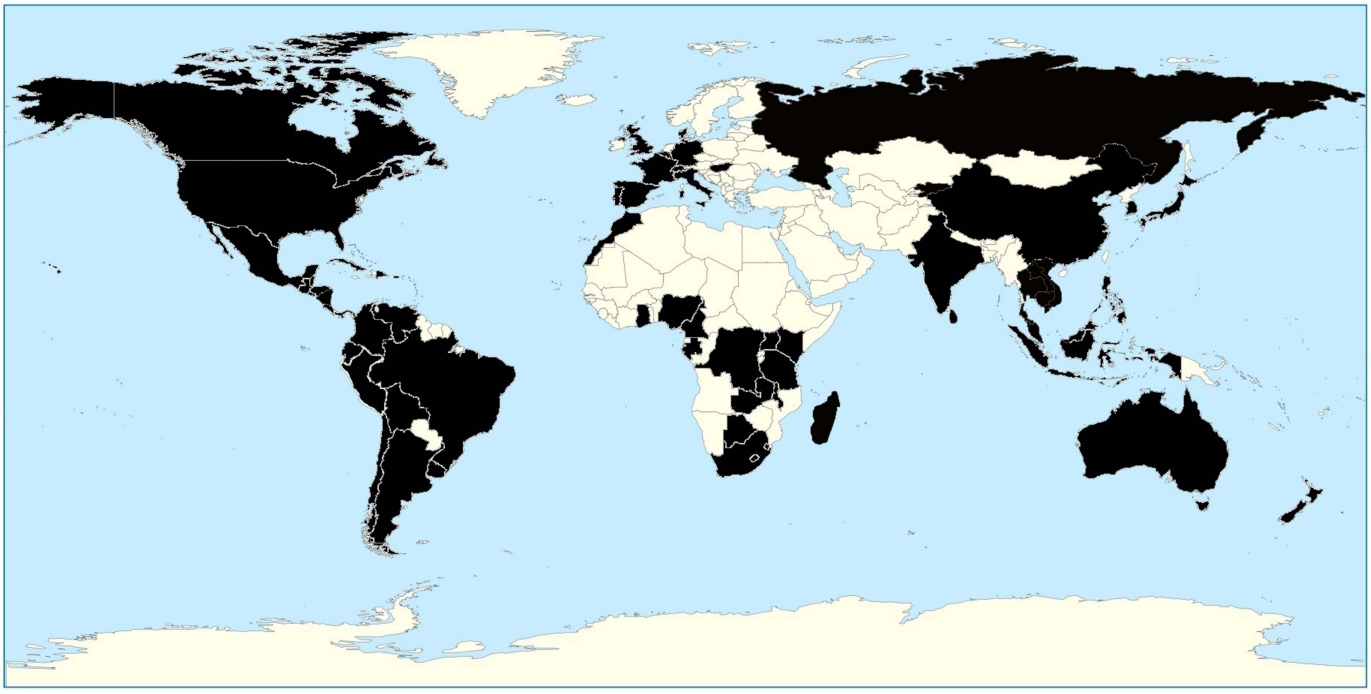
Emerging Infectious Disease or Globally Endemic Pathogen?

The seemingly sudden emergence of *B. dendrobatidis* and its association with global amphibian declines generated uncertainty as to the origin of this pathogen and the reason for disease emergence. A rift within the scientific community developed, and two virtually opposite hypotheses to explain this phenomenon were postulated: (i) the globally endemic pathogen hypothesis and (ii) the emerging infectious disease hypothesis (18). Each conveyed a different reason for disease emergence with a distinct conservation and management undertone. In the former, *B. dendrobatidis* is assumed to have become globally dispersed in historic times, and its presence alone was not a threat to amphibians until recently, when some external influence "changed" *B. dendrobatidis* to become virulent. In this scenario, *B. dendrobatidis* was already everywhere, and something flicked a switch that allowed disease to suddenly emerge from a longstanding commensal relationship with amphibian hosts. The latter hypothesis assumed that the global distribution of *B. dendrobatidis* was heterogeneous and it was still actively spreading, driving a wave of disease as it progressed. Skerratt et al. (18) showed that greater evidence supported the emerging infectious disease hypothesis and advocated the importance of continued surveillance efforts to monitor *B. dendrobatidis*'s spread and for activities that predict and mitigate future biodiversity decline.

Global *B. dendrobatidis* Distribution

Even after 15 years of investigation, the global origin of *B. dendrobatidis* and timeline of emergence remain poorly understood (34). *B. dendrobatidis*'s presence has recently been reported from 52 of 82 countries sampled (6), and it continues to spread (Fig. 1). There remain

FIGURE 1 Detection of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* as of August 2015, as reported in the literature. Black shading represents one or more confirmed detections of *B. dendrobatidis* illustrated at the country level and should be interpreted conservatively.



many countries where sampling for *B. dendrobatidis*'s presence has been limited or not yet performed, and it remains unknown just how many regions have still evaded *B. dendrobatidis* exposure. Demonstrating the presence of *B. dendrobatidis* is relatively straightforward—a few PCR-positive field samples will generally suffice—but proving the absence of *B. dendrobatidis* requires thousands of negative samples, and yet this still only suggests its absence. At present, only two countries have been systematically surveyed for nearly a decade without *B. dendrobatidis* confirmation: Hong Kong (35) and Madagascar (36).

Multiple *B. dendrobatidis* Strains

The true genetic diversity of *B. dendrobatidis* was not fully appreciated until nearly a decade following its initial discovery as a pathogen affecting amphibians. We now know that there exists a diversity of molecularly distinct *B. dendrobatidis* isolates, some of which seem to be associated with particular regions of the world, possibly due to periodic isolation and mutations (34, 37, 38,). Some isolates have been studied in depth and represent distinct "strains" that consistently vary from others by genotype, morphology, and virulence (34, 38, 39). Laboratory exposure experiments have shown that *B. dendrobatidis* strains from different geographic regions differ in virulence (5, 39–41) and that outcomes of exposure to *B. dendrobatidis* can be difficult to predict, especially without knowing the strain identity and characteristics. For example, exposure to *B. dendrobatidis* collected from Spain and the United Kingdom caused significantly greater mortality in European toad (*Bufo bufo*) tadpoles than did an isolate from Majorca; 37.5% survived Majorcan *B. dendrobatidis* exposure compared to only 7.5% and 2.5% for strains from Spain and the United Kingdom, respectively (39). Time until death following exposure to three Australian *B. dendrobatidis* strains also differed significantly; mean time until 100% mortality in juvenile *Litoria caerulea* varied between strains by nearly 19 days (5).

On a global scale, at least 49 genetically distinct isolates of *B. dendrobatidis* have been described that form five lineages (34). Of these lineages, the hypervirulent *B. dendrobatidis* GPL clade is the most broadly distributed strain identified to date, but diverse local isolates likely remain undetected and untested due to a sampling bias toward areas experiencing rapid amphibian declines (38, 42). The global distribution of each *B. dendrobatidis* strain has not yet been identified due to current limitations in diagnostic abilities. If visualized in greater detail—down to the strain level—the global distribution represented in Fig. 1 would likely be much more complex and dynamic, with dozens of overlapping and competing *B. dendrobatidis* boundaries.

Although *B. dendrobatidis* is a clonal organism, it is believed that sexual recombination may have occurred between two different strains to produce novel hybrid offspring (38, 43). This phenomenon has been proposed twice, first between two unidentified strains to produce the hypervirulent *B. dendrobatidis* GPL clade (38) and again between *B. dendrobatidis* GPL and a regionally endemic strain in Brazil (43). The contemporary human-assisted movement of *B. dendrobatidis*–infected amphibians creates numerous opportunities for native and foreign *B. dendrobatidis* isolates to cross historical boundaries, meet, and hybridize. This is of particular concern with respect to animals produced at frog-farming facilities, where groups of amphibians (most often American bullfrogs) are maintained in high densities. These artificially crowded environments provide elevated rates of pathogen transmission, and restocking to replace dead animals might remove a selection pressure that could have otherwise tempered virulence over time.

Global Origin of *B. dendrobatidis*: Initial Hypothesis

To identify the catalyst of this global amphibian disease event, it is important to map the expansion of *B. dendrobatidis*'s distribution over time. *B. dendrobatidis* is an ancient organism, (34), existing for thousands of years without apparent adverse effects. Thus, what sparked the relatively recent emergence of chytridiomycosis? Was it simply the expansion of *B. dendrobatidis*'s range into novel regions where naïve amphibian populations become exposed? Or was this just one factor among many that aligned to catalyze this phenomenon?

An "out of Africa: hypothesis for *B. dendrobatidis*'s origin and global dispersal was developed soon after its discovery, anchored on the detection of *B. dendrobatidis* in a South African specimen of *X. laevis* collected in 1938 (44). In 1935, the discovery of a rudimentary human pregnancy test that involved the use of live *X. laevis* sparked a notable export trade of these frogs to countries around the world, which continued for several decades (44). This species tolerates *B. dendrobatidis* infection without developing chytridiomycosis and is an invasive species outside of Africa, having established feral populations globally after escape or release. These factors, together with their export from Africa shortly preceding global disease emergence framed a compelling argument for Africa as the source of *B. dendrobatidis* and provided a plausible catalyst for this global disease

event—the international wildlife trade. The previous paucity of *B. dendrobatidis* distribution records preceding the onset of significant amphibian trade strengthened the appearance that this activity "unlocked" *B. dendrobatidis* from its global origin, but correlation does not imply causation. Recent information now suggests that Africa might not have been the original global source of *B. dendrobatidis*.

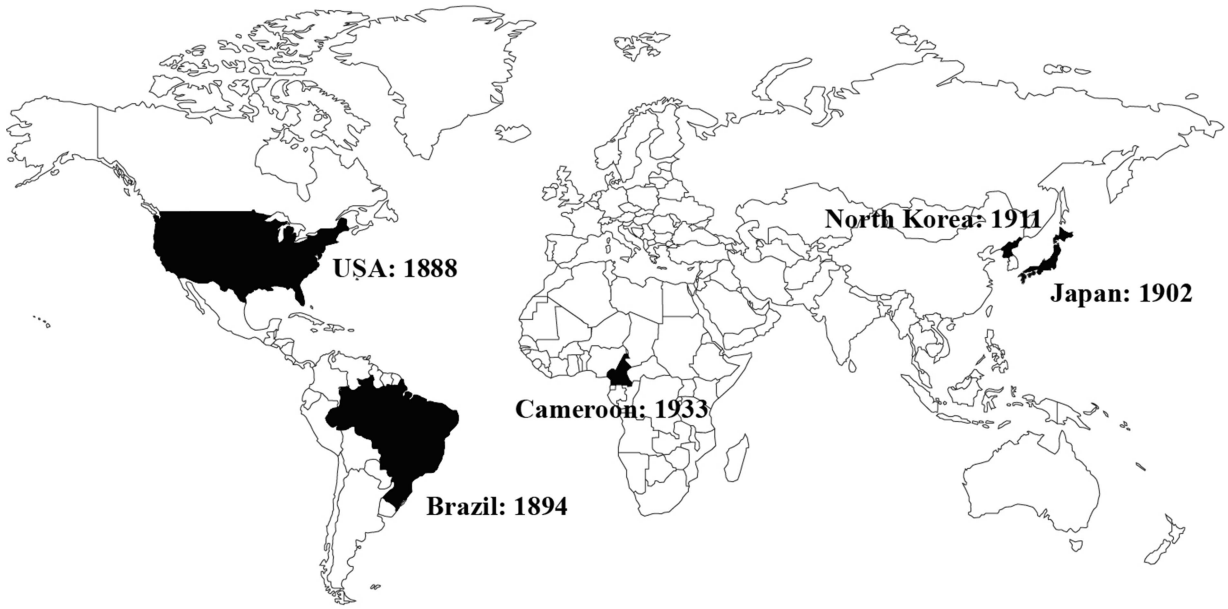
Timeline of Emergence

Our ability to map the historic presence of *B. dendrobatidis* and develop a more accurate timeline of emergence is limited by the quality and quantity of amphibian material held in museum collections available for *B. dendrobatidis* sampling. Advances in diagnostic methods have recently allowed *B. dendrobatidis* sampling to be performed on samples collected long ago and now preserved in museum collections, no longer restricting detection to freshly collected samples (45–47). Retrospective surveillance for the presence of *B. dendrobatidis* has now provided greater insight into its geographic history: it was present in the United States by 1888 (48), in Brazil in 1894 (49), Japan in 1902 (50), North Korea in 1911 (51), and Cameroon in 1933 (52) (Fig. 2). These records collectively show that *B. dendrobatidis*'s presence stretched across at least four continents prior to the 1938 *B. dendrobatidis*–positive detection in *X. laevis* from South Africa. Africa still might be the original source of *B. dendrobatidis*, but the best available data now show that it is equally plausible for the global origin to be North or South America, or even Asia (37, 50). Wherever the true origin lies, viable *B. dendrobatidis* must have successfully traversed oceans multiple times before the 20th century. This is an important amendment to make upon the earlier estimated timeline of *B. dendrobatidis* emergence compared to that of disease emergence. It is now apparent that *B. dendrobatidis* was already globally widespread much earlier than the first observed waves of disease, and this further illustrates that the spread of *B. dendrobatidis* is not always associated with the spread of chytridiomycosis.

MODES OF *B. DENDROBATIDIS* DISPERSAL

While the global origin of *B. dendrobatidis* and timeline of emergence remain obscure, significant research effort has been devoted to understanding mechanisms of contemporary dispersal to identify potential *B. dendrobatidis* mitigation opportunities. The spread of *B. dendrobatidis*

FIGURE 2 Minimum global distribution of amphibian chytrid fungus *Batrachochytrium dendrobatidis* pre-1935. The exportation of *Xenopus laevis* from Africa began in 1935, marking the emergence of the modern international amphibian trade. Black shading represents *B. dendrobatidis* detection in archived museum specimens. Shaded countries and year of *B. dendrobatidis* presence include United States (1888), Brazil (1894), Japan (1902), North Korea (1911), and Cameroon (1933).



involves multiple simultaneous pathways, each varying in likelihood, quantity of pathogen transported, and expected consequence. These mechanisms can be generalized into three main categories: (i) anthropogenic-assisted spread, (ii) natural spread by wildlife, and (iii) natural spread by environmental forces.

Anthropogenic-Assisted Spread: International Amphibian Trade

Contemporary global spread of *B. dendrobatidis* is closely associated with international trade in millions of live amphibians annually, facilitating dispersal between countries and across oceans (43, 53–55). Notable global amphibian commerce first emerged around 1935, sparked by the development of a rudimentary human pregnancy test requiring the use of African clawed frogs (*X. laevis*). International trade in live amphibians escalated over the following decades, with animals becoming popularly traded as exotic pets, biomedical research subjects, and food sources (54, 55). Since highly traded species involve those identified as *B. dendrobatidis* reservoir hosts, it is not surprising that surveys of American bullfrogs (*L. catesbeianus*) imported to the United States have demonstrated *B. dendrobatidis* prevalence of 41 to 62% at markets sampled (43) and 70% in *X. laevis* upon importation for the pet trade (55).

Following importation, *B. dendrobatidis* may spill over into the wild and expose native amphibians, by either the accidental or intentional release of amphibians, and especially in instances where these animals survive and become established. This has been documented on numerous occasions with respect to American bullfrogs and African clawed frogs, both of which are considered invasive species and have developed feral populations both in the United States and globally (10). When invasive species invade new regions, they also bring their pathogens along for the ride and provide them with a greater chance to infect local wildlife than would a less adaptable and persistent host.

Additionally, the shipping materials used to transport or house *B. dendrobatidis*–positive amphibians are liable also to transmit infection to new animals if reused or spread *B. dendrobatidis* into the environment if disposed of untreated (56). These *B. dendrobatidis*–contaminated materials commonly include water or soil, cardboard or plastic boxes, and dead animals. If not treated properly to kill *B. dendrobatidis* prior to disposal, wastewater discarded into storm sewers can introduce pathogens directly into local waterways (57), and solid waste can provide new acute sources of transmission in terrestrial locations.

In recent decades the global trade in live amphibians has grown exponentially, and nearly 5 million live amphibians are now imported into the United States annually, all in the absence of required disease screening or quarantine measures. To remedy this situation, and in recognition of the emerging global disease concern, chytridiomycosis was listed as a notifiable disease by the World Organization for Animal Health (OIE) in 2009 (58). OIE notifiable listing requires its 174 member countries to conduct surveillance for *B. dendrobatidis* within in their borders, report confirmed cases, and implement measures to control its spread. Unfortunately, at the time of writing (August 2015), few if any countries have formally integrated these recommendations into legislation and are following this procedure.

Anthropogenic-Assisted Spread: International Trade (Nonamphibian)

The spread of *B. dendrobatidis* through international trade is not limited to the trade in amphibians. Contrary to conventional perception, *B. dendrobatidis* may be vectored by trade activities in the absence of amphibian hosts. In recent years, alternative nonamphibian *B. dendrobatidis* hosts have been identified, including crayfish (*Procambarus* spp. and *Orconectes virilis*) (59, 60) and the nematode worm *Caenorhabditis elegans* (61). Both crayfish and nematodes became infected following laboratory exposure to *B. dendrobatidis* and also suffered associated disease and mortality. Crayfish are traded live, both for direct consumption and for establishing new aquaculture farms, and the widespread soil-dwelling nematode worm *C. elegans* is likely to be transported within potting substrates spread by the international trade in ornamental plants. This is not meant to suggest that the "silent" dispersal of *B. dendrobatidis* by nonamphibian commerce is of greater concern, but rather demonstrates the complexity involved in tracking the spread of a pathogen now known to be capable of infecting three classes of organisms: *Amphibia*, *Malacostraca*, and *Chromadorea*.

Anthropogenic-Assisted Spread: Fomites

It has been suggested that *B. dendrobatidis* may be spread by people following exposure to affected regions, since the pathogen can survive for some time if protected from complete drying and elevated temperatures (56). Fomites, or nonliving objects that can carry pathogens, may be accidentally spread by human activities. The movement of *B. dendrobatidis*–contaminated footwear by researchers or eco-tourists represents a potentially common opportunity for the translocation of viable

propagules between disconnected habitats. This dispersal pathway has not been formally evaluated, but due to its likelihood, hygiene protocols have been provided to prevent the accidental spread of *B. dendrobatidis* after entering *B. dendrobatidis*–positive locations or performing high-risk activities (56, 62–64). In addition to footwear, *B. dendrobatidis* is also likely spread by other freshwater activity–related fomites, such as recreational boating (nondecontaminated boat hulls) and fishing (bait wastewater).

Nonanthropogenic-Assisted Spread: Dispersal by Wildlife

Within the natural environment and in the absence of human influence, *B. dendrobatidis* spreads through autonomous movement of infected animals. It can be transmitted to other nearby amphibians by direct skin–skin contact (65) during territorial exchanges or when engaged in amplexus—the mating embrace in which a male amphibian grasps a female with his front legs. Additionally, infected animals may carry *B. dendrobatidis* away from water and shed zoospores into the terrestrial environment, leaving a trail of *B. dendrobatidis* on vegetation often shared with other amphibian species (66). This phenomenon may partially explain enigmatic records of this aquatic pathogen in species of terrestrial amphibians that do not enter the water (67–69). It is also possible that the aforementioned crayfish carriers, some of which occasionally disperse over land during periods of heavy rain, may contribute toward the spread of *B. dendrobatidis* between separate water bodies. Aside from these local dispersal opportunities, longer-distance *B. dendrobatidis* spread may involve aerial transport on the feet of waterfowl (70) moving between wetlands.

Nonanthropogenic-Assisted Spread: Dispersal by Environmental Forces

Animals infected with *B. dendrobatidis* frequently shed zoospores into their environment (71, 72). If released into an aquatic habitat, zoospores can swim short distances and/or be carried to new locations by water currents (57). In addition, wind and rain are known to assist the spread of microbes, some of which are pathogenic to animals and plants (73, 74), and may also contribute toward the spread of *B. dendrobatidis*. Recently, *B. dendrobatidis* was detected in rainwater processed by filtration (75), although its viability could not be ascertained from molecular presence alone. Atmospheric and avian dispersal of *B. dendrobatidis* is unpredictable, but occasional viability following aerial transport could help explain *B. dendrobatidis*'s multiple successful transoceanic dispersal events prior to the first commercial cargo flights in the 1930s.

B. DENDROBATIDIS MITIGATION ATTEMPTS AND OPPORTUNITIES

At the time of writing (August 2015), the reason why *B. dendrobatidis* seems to have recently increased in virulence to catalyze this disease event remains unknown. Despite 15 years of investigation, this wildlife pandemic continues to progress largely unabated. There is currently no proven method to eradicate *B. dendrobatidis* from an affected habitat, nor have we been able to control its spread and protect new regions from exposure despite knowledge of an approaching wave of *B. dendrobatidis* and disease. In captivity, there are some options to cure infected amphibians, but there is not yet a single cure-all treatment that can be safely applied to all species. It is becoming increasingly evident that a "silver bullet" solution to stem the tide of *B. dendrobatidis*–driven amphibian declines and extinctions does not exist, despite remarkable efforts. More realistically, the application of multiple case-specific activities may provide the necessary "silver buckshot" solution to prevent amphibian extinctions, although resources are limited with respect to the diversity of species potentially vulnerable to chytridiomycosis.

Government Intervention to Mitigate *B. dendrobatidis* Spread

Although *B. dendrobatidis* spreads through a variety of pathways, it is unquestionable that the international trade in live amphibians is spreading a considerable amount of this pathogen and is contributing toward global amphibian declines and extinctions. In 2009, Defenders of Wildlife submitted a petition to the U.S. Fish and Wildlife Service (USFWS) proposing that all live amphibians be listed as injurious species under the Lacey Act and thus be prohibited from trade into and within the United States, except for specimens proven to be free of *B. dendrobatidis* (76). It is currently impossible to eradicate *B. dendrobatidis* following establishment, so preventing importation of foreign *B. dendrobatidis* strains that may express greater virulence to native amphibians should be considered a high conservation priority. Although expressed as a matter of urgency nearly 6 years ago, the USFWS has yet to announce whether regulations will be proposed to address this concern. Meanwhile, trade continues unabated and continues to introduce *B. dendrobatidis* (55). Similar extended delays

between listing petitions and listing actions are not uncommon. Due to cumbersome risk assessment and review processes, most injurious species listings by USFWS have proceeded slowly and failed to prevent establishment of harmful organisms (77–78).

Mitigation of *B. dendrobatidis*'s Impact

Efforts to mitigate the impact of *B. dendrobatidis* can generally be divided into one of two categories: those targeting the reduction of *B. dendrobatidis* on amphibian hosts and those that strive to remove *B. dendrobatidis* from affected habitats. While treatment of infected amphibians is an immediate challenge for highly vulnerable species collected from the wild or already held in captivity, the continued spread of *B. dendrobatidis* in the wild is shrinking the amount of safe amphibian habitat and jeopardizing long-term successful population recovery. Therefore, while efforts to develop captive assurance populations of amphibians facing immediate risks of extinction have been fairly successful, reintroduction attempts have been minimal because contaminated natural habitats continue to be problematic. Effective methods to mitigate *B. dendrobatidis* both on amphibians and in their habitats are needed to protect amphibian biodiversity. An effective *B. dendrobatidis* mitigation program will likely require multiple complementary actions and be case-specific. Fortunately, complete eradication or removal of *B. dendrobatidis* may not be necessary to control disease, because a significant reduction in pathogen abundance might be enough to tip the scale in favor of amphibian survival.

Amphibian: antifungal chemotherapy

An antifungal itraconazole bath has been implemented as a common treatment for infection with *B. dendrobatidis* in amphibians held in captivity. The itraconazole treatment solution (0.01%) applied for 5 minutes once daily for 10 consecutive days can produce a dramatic or complete reduction in *B. dendrobatidis* infection load. Unfortunately, this treatment is toxic to some species and especially to larval life stages (79). Recently, this protocol has been experimentally tested at a much lower dosage concentration (0.0025% versus 0.01%) and for fewer days (6 versus 10) and still found to be effective, but with fewer instances of negative side effects (80). Nikkomycin Z is another antifungal agent also found to be effective against *B. dendrobatidis*, but exposure dosages necessary to mitigate *B. dendrobatidis* might also reduce the survival of amphibians (81). Anti–*B. dendrobatidis* chemotherapeutics are not restricted to antifungals; antibiotics such as chloramphenicol have also been effective in some circumstances (82), although its exposure has been associated with bone marrow suppression and aplastic anemia in cats and human beings, and it requires treatment lasting 2 to 4 weeks, which terrestrial amphibians might not be able to tolerate (79). Given the various disadvantages associated with all currently described *B. dendrobatidis* treatment methods, itraconazole remains the most widely applied and successful treatment. However, Woodhams et al. (9) expressed concern that its wide use might encourage *B. dendrobatidis* to develop resistance to itraconazole over time.

Amphibian: temperature and desiccation

Since antifungal chemotherapies can produce harmful side effects in certain species and life stages, there has been interest in seeking nonchemotherapeutic treatment to aid survival of infected amphibians. It has long been known that *B. dendrobatidis* is vulnerable to elevated temperatures and desiccation. Extended continuous exposure to temperatures of at least 27°C and above, for varying amounts of time, has cleared *B. dendrobatidis* infection on frogs in captivity (83, 84). While this treatment is relatively cost-effective and easy to provide, its effectiveness varies by species. Many amphibians are adapted to cool environments, and the elevated temperatures necessary to kill *B. dendrobatidis* may likewise harm or kill the infected animals. Recent work also explored manipulation of humidity as another potential mode of controlling *B. dendrobatidis* on infected frogs, since complete drying kills *B. dendrobatidis*. Unfortunately, the experiments found that a drying regime provided to the southern corroboree frog (*Pseudophryne corroboree*) neither increased survival nor reduced infection loads (85). Similar to the species-specific variable effectiveness of heat treatment, it remains plausible that other species tested might likewise respond differently if exposed to reduced levels of humidity. Therefore, heat treatment remains the only proven nonchemotherapeutic treatment for *B. dendrobatidis* infection, but it is limited to heat-tolerant species.

Amphibian: vaccination

Infection with *B. dendrobatidis* suppresses the immune response of most amphibian hosts, except for the minority of experimentally tested species that possess a measurable degree of innate resistance. For the less-fortunate majority of species, methods to abate disease outside of captivity are urgently needed to assist survival of wild animals living in *B. dendrobatidis*–established habitats. Two major attempts to investigate whether

amphibians can acquire resistance through vaccination have met with mixed results. One study performed in Australia found that vaccination in the form of prior *B. dendrobatidis* infection, treatment with itraconazole to clear the infection, and then re-exposure had no effect on survival or infection intensities in booroolong frogs (*Litoria booroolongensis*) (86). Meanwhile, a study in the United States exposed Cuban tree frogs to *B. dendrobatidis*, treated them with heat to clear infections, and observed a 20% increase in frog survival over the next five months (87). The latter study provided hope that an effective vaccination-type treatment might someday be developed, but it appears that pre-exposure to *B. dendrobatidis* does not alone trigger enough of an adaptive immune response to protect populations from infection in the wild. Still, like the species-specific variable responses to itraconazole and heat treatment, it is also likely that vaccination might elicit a greater adaptive immune response in some species than others.

Amphibian: probiotics

A diversity of microorganisms inhabit the layer of mucus coating an amphibian's skin, many of which are bacteria. The species composition of these bacterial communities varies between amphibian species and sometimes includes symbiotic bacteria that possess anti–*B. dendrobatidis* properties. When isolated and cultured, these "anti–*B. dendrobatidis*" bacteria can inhibit the growth of *B. dendrobatidis* in the laboratory. The most well-studied bacterial species with such properties is *Janthinobacterium lividum*, which is isolated from the red-backed salamander (*Plethodon cinereus*) (88, 89). The exact mechanism by which *B. dendrobatidis* is inhibited remains unknown but likely involves the production of fungicidal compounds that either interrupt *B. dendrobatidis* reproduction or directly kill *B. dendrobatidis*. Efforts are underway to isolate and culture this and additional bacterial species found to demonstrate similar properties for eventual application as a probiotic treatment. Such treatment would involve a bacterial bath to provide bioaugmentation to amphibian species not normally colonized by anti–*B. dendrobatidis* bacteria or those that carry low levels insufficient to manifest *B. dendrobatidis* resistance. This treatment was tested on the mountain yellow-legged frog (*R. muscosa*), a species that is highly susceptible to chytridiomycosis, and reduced mortality was observed (89). Probiotic treatment does appear to provide susceptible amphibians with some additional defense against disease, but it remains unknown how long these bacteria will continue to remain on the skin of "new" amphibian species. The duration of bacterial persistence will dictate how long amphibians will retain boosted resistance to *B. dendrobatidis*, and this remains to be investigated over the long term in wild frog populations under natural conditions.

Amphibian: selective breeding for disease resistance

In circumstances where amphibian species are threatened with extinction in the wild due to *B. dendrobatidis*, animals are sometimes collected and brought into captivity to establish captive assurance populations. The end goal of these efforts is to breed animals and eventually reintroduce their offspring back into the wild to supplement the remaining dwindling populations. Although several such breeding operations are in progress, few have reintroduced animals due to the challenges posed by the presence of only *B. dendrobatidis*–contaminated habitats within a species' range. The incorporation of selective breeding into these operations, or assisted evolution, is one possible method that might reduce the risk of disease to animals placed back into an affected habitat. While no attempts to selectively breed amphibians for resistance to *B. dendrobatidis* have been reported in the literature, preliminary evidence from field experiments does suggest this might be possible. In field reintroduction and mark-recapture surveys of the alpine tree frog (*Litoria verreauxii alpina*) in Australia, it was recently observed that susceptibility to chytridiomycosis varied significantly among clutches of offspring, despite being a highly susceptible species (90). Although this represents a potential long-term solution for some species, it remains unknown whether the genetic traits that provide *B. dendrobatidis* resistance can be identified, selected for, and consistently inherited by the offspring.

Habitat-Level Mitigation: Eradication versus Management

Eradication of a newly introduced pathogen is desirable to halt an epidemic and prevent pathogen establishment in a new location. Unfortunately, this has not yet been considered feasible with respect to *B. dendrobatidis*. By the time *B. dendrobatidis* was discovered in 1999, it had already spread to dozens of countries. The global reach of *B. dendrobatidis* soon became apparent, and focus shifted toward identifying ways to abate *B. dendrobatidis* abundance and mitigate the impact of its presence in amphibian habitats rather than eradicate the pathogen entirely.

Habitat: site-level treatment

Although *B. dendrobatidis* eradication has not been approached as a primary management target, it does warrant mention. There are still regions that might not have been exposed to *B. dendrobatidis*, where early intervention to prevent establishment may be possible to protect the area's amphibians. Few concerted efforts to abolish *B. dendrobatidis* from a location have been seriously considered, and fewer still have been attempted in the natural environment. While antifungal compounds can be introduced to a water body in an effort to kill *B. dendrobatidis* (91), none are specific to *B. dendrobatidis* and thus will cause unintended damage to additional aquatic life, the scope of which is unknown and difficult to predict. Therefore, site-level chemical treatment is not generally embraced as a viable option for *B. dendrobatidis* eradication.

In lieu of chemical application, other more dramatic eradication attempts could include the drainage of entire wetland systems to fight *B. dendrobatidis* with desiccation. Although this may seem extreme, it is often employed to control mosquito vectors of human disease and might likewise be effective to combat *B. dendrobatidis* (9). Pond-level drainage was performed on *B. dendrobatidis*–infected populations of the Majorcan midwife toad (*Alytes muletensis*) inhabiting livestock water cisterns in Europe (92). All tadpoles were removed, held in a laboratory where they were treated with itraconazole to clear *B. dendrobatidis* infections, and the cisterns were completely drained and allowed to dry. When the cisterns naturally filled again with rainwater the following season, the *B. dendrobatidis*–negative tadpoles were reintroduced. Unfortunately, soon after reintroduction, *B. dendrobatidis* reappeared in these animals, demonstrating the importance of our ability to predict and mitigate *B. dendrobatidis* dispersal pathways, which still remain relatively poorly understood.

Another complicating factor in any possible attempt to eradicate *B. dendrobatidis* is a lack of understanding about precisely where it occurs when outside the amphibian host. While we know that *B. dendrobatidis* zoospores are shed into the water, it is uncertain where they are most commonly found: do they remain near the surface of the water column exposed to a potential chemical or physical treatment, or do they settle to the bottom where they may become embedded in mud or layers of dead vegetation, largely shielded from assault? The reality likely straddles both, which would jeopardize the chances for success of any eradication attempt.

Although rapid response and eradication of *B. dendrobatidis* from a newly invaded location has never been attempted, the recent discovery of *B. dendrobatidis* in Madagascar (93–95) might warrant such action. Following nearly a decade of surveillance with only negative detection, *B. dendrobatidis* was detected in amphibians exported to the U.S. pet trade and shortly thereafter in wild amphibian populations within the country. A true eradication effort would require swift and decisive action as quickly as possible following the arrival of *B. dendrobatidis*. Although additional research is needed to identify which strain of *B. dendrobatidis* is present and whether or not it threatens Malagasy amphibians, eradication might still be feasible, if justified, although the window of opportunity is now shrinking.

Habitat: biological control

Although it is a formidable predator with respect to amphibians, *B. dendrobatidis* itself becomes subject to predation and competition for resources with other aquatic organisms when present outside the host. *Daphnia* and other freshwater zooplankton that graze on organisms in the water column may consume *B. dendrobatidis* zoospores and reduce pathogen density (96). In laboratory experiments, predation by *Daphnia* reduced the number of zoospores present in the water sample. In turn, exposure to this water then resulted in a lower rate of *B. dendrobatidis* transmission to tadpoles versus that in which *Daphnia* had not been introduced. Also, the presence and amount of algae in the water containing *B. dendrobatidis* sometimes reduced its abundance, perhaps due to competition for resources if *B. dendrobatidis* was acting as a saprobe by feeding on nonliving organic matter. Therefore, manipulation of the zooplankton community in a contaminated habitat might help mitigate the impact of *B. dendrobatidis* on amphibians. Despite these laboratory results, however, it is uncertain whether similar phenomena would occur in the natural environment and whether the abundance of zooplankton could be manipulated on such a grand scale as to yield the desired effect.

Habitat: physical modification

Many factors affect the presence and survival of *B. dendrobatidis* at a particular location, but temperature and moisture are especially important. Scheele at al. (97) described potential methods of *in situ B. dendrobatidis* mitigation by manipulating habitat structure to modify microclimates; for instance, by selectively pruning vegetation to control for the amount of direct sunlight

exposure, it might be possible to push temperatures of amphibian basking sites and standing bodies of water slightly beyond conditions optimal for *B. dendrobatidis* growth. Since *B. dendrobatidis* can survive extended exposure to neither elevated temperatures nor drying, this manner of intervention may provide a way to reduce *B. dendrobatidis* densities in natural habitats. By mitigating the presence of *B. dendrobatidis*, this could then help assist an amphibian's own immune response by reducing pathogen burden. This would also help reduce the likelihood of *B. dendrobatidis* survival on the surface of riparian vegetation where infected amphibians shed zoospores as they emerge from the water (66).

A recent field study in Queensland, Australia, found that severe tropical cyclone Yasi reduced *B. dendrobatidis* infection risk at sites that suffered considerable habitat disturbance (98). This cyclone damaged the forest structure at some locations where powerful winds snapped trees and stripped foliage, reducing the canopy cover at certain stream habitats, some of which were part of a long-term *B. dendrobatidis* infection survey. Comparing damaged versus primarily intact survey sites, the amount of canopy cover was inversely related to both temperature and evaporative water loss, suggesting that amphibians at disturbed locations were exposed to conditions less favorable to *B. dendrobatidis* survival than at intact sites, where temperatures remained lower and greater humidity persisted. Accordingly, endangered rainforest frogs (*Litoria rheocola*) sampled at these disturbed sites demonstrated significantly lower risk of *B. dendrobatidis* infection than those at intact sites with greater moisture retention and lower temperatures. These data are encouraging because they suggest that *B. dendrobatidis* management via habitat modification may help reduce pathogen burden at some locations (97, 98).

REMAINING QUESTIONS

Despite past and present efforts, certain aspects of *B. dendrobatidis* ecology and chytridiomycosis remain enigmatic and challenge our ability to effectively mitigate the impact of disease. Although framed within the context of *B. dendrobatidis*, the essence of these questions and uncertainties is equally relevant to any wildlife emerging infectious disease that we have not yet been able to control. These lingering questions include but are not limited to:

- **Persistence of *B. dendrobatidis* outside the host.** *B. dendrobatidis* zoospores are frequently shed from an infected host into the environment, but how long do these zoospores typically survive? Few published studies are available for reference: one found that *B. dendrobatidis* generally became inactive after 48 h in distilled water (99); another detected the presence of infectious zoospores for 7 weeks in autoclaved pond water (100), and a third detected *B. dendrobatidis* survival for three months in sterile, moist river sand without the addition of nutrients (57).
- **Virulence of different *B. dendrobatidis* strains.** What causes certain strains of *B. dendrobatidis* to express greater virulence than others?
- **Variable innate resistance to *B. dendrobatidis* and disease.** Why do some frogs (within a species) tolerate *B. dendrobatidis* infection while others succumb?
- **Long-term global presence but recent emergence of disease.** *B. dendrobatidis* has been spreading globally for over 100 years, so why does chytridiomycosis appear to be a novel phenomenon?
- **History of emergence.** Where did *B. dendrobatidis* originate, and when did it first emerge?
- **Abundance and diversity of nonpathogenic *B. dendrobatidis* strains.** What proportion of *B. dendrobatidis* strains are pathogenic, or does each express virulence when placed in a certain context of exposure (amphibian species exposed, dose of *B. dendrobatidis* inoculum, environmental influences, etc.)?
- **Host spectrum.** Is *B. dendrobatidis* correctly referred to as an amphibian pathogen, or does it affect yet additional classes of organisms?

ADDITIONAL EMERGING INFECTIOUS DISEASES OF WILDLIFE

Although *B. dendrobatidis* is the first emerging infectious disease of wildlife to become pandemic, it will certainly not be the last. The pace of globalization is racing ahead more quickly than our ability to discover and prevent the spread of diseases, and especially those affecting wildlife. Over the past decade, several additional disease events have emerged in the United States that are also now causing dramatic uncontrollable declines in wildlife populations. This includes bat white nose syndrome, spread by the fungus *Pseudogymnoascus destructans*, which infects skin of the muzzle, ears, and wings of hibernating bats. White nose syndrome has caused sudden

and widespread mortality, precipitating the death of millions of bats in recent years (101), which has been said to be analogous to chytridiomycosis for amphibians. More recently, the emergence of snake fungal disease has been described, spread by the fungus *Ophidiomyces ophiodiicola*, which infects the skin and causes high rates of mortality and is said to be analogous to bat white nose syndrome in many respects (102). Like *B. dendrobatidis*, it remains uncertain what catalyzed the emergence of these disease events, although the international movement of pathogen-contaminated material is suspected, whether by the trade in live animals or fomites. It is reasonable to assume that additional wildlife pathogens not yet described are already circulating within the international wildlife trade and spillover events may have occurred without our knowledge. The accelerated global spread of *B. dendrobatidis* by the international wildlife trade proceeded unabated for decades before a series of obvious mortality events led to the discovery of *B. dendrobatidis* existed and that our actions had been facilitating a pandemic.

SALAMANDER CHYTRID FUNGUS: THE NEXT AMPHIBIAN "PLAGUE"?

The recent near-extinction of fire salamanders (*Salamandra salamandra*) in The Netherlands led to a surprising and alarming discovery—that a second species of amphibian chytrid fungus exists which specifically attacks salamanders, and it is soon expected to ignite a wave of salamander extinctions in the United States unless immediate intervention occurs (103, 104). This species of "salamander-eating" chytrid fungus (*Batrachochytrium salamandrivorans*) is believed to have originated in Asia, where it appears to have existed for nearly 30 million years until the exportation of infected salamanders by the pet trade recently introduced this pathogen to Europe (104, 105). Like *B. dendrobatidis*, *B. salamandrivorans* is easily transmitted through skin contact with infected salamanders or by exposure to contaminated materials, such as water, soil, and shipping containers (104). A disease outbreak in The Netherlands resulted in the near extirpation of fire salamanders, which raised the alarm and led to the discovery of this pathogen (103). Initially unaware of the true cause of this mortality phenomenon and then unprepared to quickly mitigate this novel disease event, European scientists already report *B. salamandrivorans* to be spreading uncontrollably in Western Europe, where it has recently been detected in The Netherlands, Belgium, and the United Kingdom (103–105).

Fortunately, recent surveys in the United States have not yet detected the presence of *B. salamandrivorans* (104), but with the importation of nearly 200,000 salamanders from Asia annually (USFWS amphibian import records provided to J. Kolby) and without any required disease screening, an outbreak in the United States similar to that in The Netherlands appears inevitable. Although *B. salamandrivorans*–infected salamanders have not yet been detected in the wild in the United States, it is likely that *B. salamandrivorans*–infected salamanders have been and continue to be imported from Asia. It is now only a matter of time before spillover occurs, precipitating a disease-driven decline in forest biomass and species diversity.

This is the first time advance warning of an impending wildlife disease outbreak in the United States existed prior to discovery of the pathogen within the country. The recent near-extinction of fire salamanders in The Netherlands caused by *B. salamandrivorans* exposure from Asian salamanders in the pet trade has provided a clear call to arms. A rapid proactive response is necessary to prevent similar salamander declines in the United States, the "salamander capital of the world." Research shows that *B. salamandrivorans* is highly lethal to North American salamanders, including the eastern newt (*Notophthalmus viridescens*), striped newt (*Notophthalmus perstriatus*), black-spotted newt (*Notophthalmus meridionalis*), rough skinned newt (*Taricha granulosa*), red-bellied newt (*Taricha rivularis*), and California newt (*Taricha torosa*), and likely additional species not yet tested in the laboratory (106). The USFWS is currently considering regulatory actions to mitigate the spread of *B. salamandrivorans* through the trade in salamanders and is expected to announce their approach in the coming months.

CONCLUSIONS

Scientists first became aware of *B. dendrobatidis* nearly 15 years ago, a fungal pathogen associated with global frog declines, mass mortality events, and extinctions (3, 18). Nearly a decade of exhaustive research to find a silver bullet solution and gain control over this pandemic has been largely unsuccessful (9), leaving the long-term survival of thousands of species in jeopardy. While many questions still surround this disease event, the most straightforward explanation for the apparent recent global emergence of chytridiomycosis is that of pathogen pollution driven by rapid globalization in the absence of wildlife health screening and regulatory intervention. Since the protection of global biodiversity

is often valued below that of human and agricultural health, mitigation of wildlife disease is rarely viewed as a national priority unless it is closely linked to short-term economic consequences of inaction. The slow global response to the emergence of *B. dendrobatidis* and the absence of a coordinated international mitigation attempt helped to facilitate the continued spread of this pathogen to dozens of countries and hundreds of amphibian species worldwide.

Legislative barriers continue to provide an impediment to mounting a rapid response to emerging wildlife diseases in the United States, one of the greatest consumers of the international wildlife trade. The majority of laws and regulations administered by the USFWS to regulate the international wildlife trade were developed long before pathogen pollution and the threat of wildlife disease was realized, and thus the legislative toolbox available to intervene in such events is virtually empty. The only potentially applicable existing legislation is the Lacey Act, under which authority a species may be banned from importation and interstate transport if listed as injurious, but this act only allows species of mammals, birds, fish, amphibians, reptiles, mollusks, and crustacea to be considered for listing. This language excludes authority for the listing of microorganisms such as pathogens. Although the animal vector of a pathogen can potentially be listed as injurious as a way to work around this policy gap, this has only ever been approved once, to protect salmonid fish from the importation of fish diseases (18 U.S.C. 42: 50 CFR §16.13).

The relative lack of interest and concern in responding to emerging wildlife diseases is problematic and threatens not only animals, but also human health (107). The majority of recent emerging infectious diseases affecting humans were in fact zoonotic, at an earlier point only affecting wildlife. Some examples include hantavirus in rodents and Marburg and Ebola viruses in nonhuman primates. In a world of rapidly increasing globalization, human and animal health are becoming increasingly connected as wildlife habitats shrink, human–wildlife contact increases, and global commerce carries pathogens past historical boundaries. Therefore, while the investment of greater resources toward mitigation and prevention of wildlife disease events may appear to benefit only wildlife health, it actually contributes toward the longer-term protection of environmental and human health. Despite current and future amphibian declines as a result of chytridiomycosis, there remains much to learn from this disease event. As a case study, *B. dendrobatidis* can offer insight into how to better address the next wildlife disease event that emerges, hopefully more rapidly and with greater international coordination.

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