Predicting the potential distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in East and Southeast Asia

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ABSTRACT: *Batrachochytrium dendrobatidis* (*Bd*) is the pathogen responsible for chytridiomycosis, a disease that is associated with a worldwide amphibian population decline. In this study, we predicted the potential distribution of *Bd* in East and Southeast Asia based on limited occurrence data. Our goal was to design an effective survey area where efforts to detect the pathogen can be focused. We generated ecological niche models using the maximum-entropy approach, with alleviation of multicollinearity and spatial autocorrelation. We applied eigenvector-based spatial filters as independent variables, in addition to environmental variables, to resolve spatial autocorrelation, and compared the model's accuracy and the degree of spatial autocorrelation with those of a model estimated using only environmental variables. We were able to identify areas of high suitability for *Bd* with accuracy. Among the environmental variables, factors related to temperature and precipitation were more effective in predicting the potential distribution of *Bd* than factors related to land use and cover type. Our study successfully predicted the potential distribution of *Bd* in East and Southeast Asia. This information should now be used to prioritize survey areas and generate a surveillance program to detect the pathogen.

KEY WORDS: *Bd* · Chytrid fungus · Chytridiomycosis · Eigenvector-based spatial filtering · Species distribution model · Niche modeling · MaxEnt

INTRODUCTION

Amphibian population declines have been reported worldwide (Young et al. 2001, Stuart et al. 2004). *Batrachochytrium dendrobatidis* (*Bd*) is an aquatic fungus discovered in 1998 (Berger et al. 1998) that is responsible for the emerging infectious disease chytridiomycosis (Daszak et al. 2000). Chytridiomycosis has been identified as one of the main causes of amphibian population declines since the 1970s (Daszak et al. 1999), in addition to habitat loss, over-exploitation, and climate change (Daszak et al. 2005, Pounds et al. 2006, Kilpatrick et al. 2010). This disease is suspected to have spread globally through the international trade of *Bd* carrier amphibian species (Daszak et al. 2004, Schloegel et al. 2009, 2012). Therefore, chytridiomycosis is currently listed by the World Organization for Animal Health (OIE) as an internationally notifiable disease (Schloegel et al. 2009).

Several studies have attempted to predict the potential distribution of the invasive chytrid fungus *Bd* on a worldwide (e.g. Ron 2005, Lötters et al. 2009, Rödder et al. 2009) or regional (e.g. Puschendorf et al. 2009, Ghirardi et al. 2011, Murray et al. 2011) basis. However, statistical issues undermined the effectiveness of these studies.

The original native range of *Bd* is unknown (Kilpatrick et al. 2010), but several lineages that might be native to certain areas have been discovered (e.g. Goka et al. 2009, Farrer et al. 2011). Goka et al. (2009) and Goka (2010) suggested that Asia might be one of the geographic origins of the fungus. These authors found that Japan had the highest genetic diversity of *Bd* and that Japanese amphibians are either rarely infected or appear to be resistant to the pathogen. In addition, they found that some *Bd* haplotypes were specific to the Japanese giant salamander *Andrias japonicus*, suggesting a commensal relationship. Recent studies have reported the occurrence of *Bd* chytridiomycosis in other Asian countries, including Indonesia (Kusrini et al. 2008), Korea (Yang et al. 2009), China (Bai et al. 2010), and others (Swei et al. 2011). However, reported *Bd* infection rates vary greatly among these studies, from 2.3% (Swei et al. 2011) to 38.9% (Yang et al. 2009). Since many questions about the distribution, ecology, genetic structure, and ecological risks of *Bd* in Asia remain unanswered, it is urgent to determine the potential distribution of *Bd* as the basis for further studies on this pathogen.

Species distribution models (SDMs), which are created by means of ecological niche modeling, are useful tools for predicting the distribution of pathogens and developing strategic measures to predict and prevent the invasion of alien species (Peterson 2006). Rödder et al. (2009) created a potential *Bd* distribution map with invaded localities in North and South America, Australia, Africa, and Europe, but few in Asian countries, and none in Japan. Interestingly, Swei et al. (2011) showed that *Bd* was rarely detected in the Asian localities estimated as highly suitable areas by Rödder et al. (2009).

The existing SDMs for *Bd* have faced statistical problems in terms of multicollinearity and spatial autocorrelation that, if not considered, can lead to the misinterpretation of the results (Quinn & Keough 2002, Fortin & Dale 2005). Multicollinearity can be avoided by proper selection of the independent variables (Quinn & Keough 2002). On the other hand, effective solutions to alleviate spatial autocorrelation are limited, particularly when using SDM software such as MaxEnt (Phillips et al. 2006). Recent studies have used eigenvector-based spatial filtering (Griffith 2010); the addition of a number of spatial filters as independent variables has minimized spatial autocorrelation issues (De Marco et al. 2008, Václavík et al. 2012) and resulted in improved model fit (Blach-Overgaard et al. 2010).

In the present study, we created a potential distribution map for *Bd* based on occurrence data in East and Southeast Asia, while accounting for multicollinearity and spatial autocorrelation. This map will help researchers to prioritize survey areas for efficient collection of ecological information on *Bd*, and to understand the expansion of this pathogen around the world.

METHODS

We collected coordinates of localities positive for *Bd* occurrence and environmental data related to *Bd* prevalence while creating spatial filters to alleviate the spatial autocorrelation, to model the habitat suitability map for *Bd* in Asia (Fig. 1). The relationships
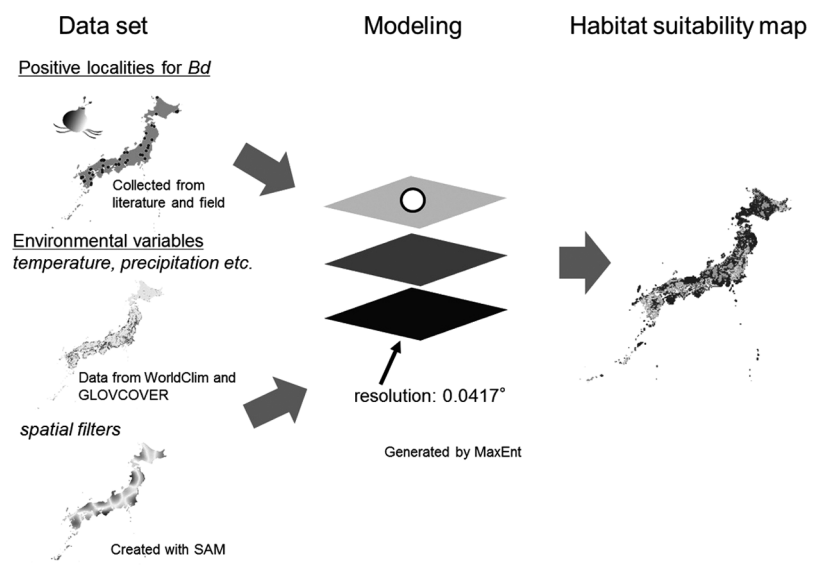

Fig. 1. Steps involved in the development of a habitat suitability map for *Batrachochytrium dendrobatidis* (*Bd*) in East and South-east Asia (using Japan as an example). We first collected coordinates of localities positive for *Bd* occurrence (white dot) and environmental data related to *Bd* prevalence, and then created spatial filters to alleviate spatial auto-correlation, in order to model the habitat suitability map for *Bd*

between habitat suitability and the variables, and between model accuracy and spatial autocorrelation of the model residuals, were evaluated. The frequency distribution of suitable indices was also compared between localities that were not used in the model and all localities, to confirm the repeatability of the model.

Environmental variables

Previous studies have revealed factors that limit the occurrence of *Bd*. One of these limiting factors is temperature; *Bd* has an optimal range of 17 to 25°C and can only persist within the range of 4 to 25°C, while temperatures greater than 30°C kill the fungus (Piotrowski et al. 2004). Moisture is also critical for the persistence of *Bd*. For instance, Johnson et al. (2003) showed that *Bd* zoospores and zoosporangia died after 3 h of desiccation.

Based on this information, we selected the following climate variables and used them to generate an SDM: annual mean temperature, seasonality of temperature (= coefficient of variation), precipitation in the driest month, and seasonality of precipitation (= coefficient of variation). These variables have been used in previous SDM studies of *Bd* (e.g. Ron 2005, Lötters et al. 2009, Puschendorf et al. 2009, Rödder et al. 2009). We created the climatic layers (with a resolution of 2.5 arc-minutes) using the WorldClim dataset (www.worldclim.org; Hijmans et al. 2005).

To avoid multicollinearity, since climatic variables were highly correlated, we combined 2 pairs of variables (annual mean temperature and seasonality of temperature; and precipitation in the driest month and seasonality of precipitation) by using principal components (PC) analysis, and used the 4 PC scores (temperature PC1, temperature PC2, precipitation PC1, and precipitation PC2) which were independent from each other as the environmental variables in the spatial distribution analyses.

Land cover (= land use and cover type) was also added to the environmental variables because habitat availability (e.g. freshwater area, farmland, forest) can affect the presence of host amphibians (Hof et al. 2011). The land cover data were created from the GLOBCOVER dataset (http://due.esrin.esa.int/globcover/; European Space Agency [ESA] and the ESA GlobCover Project, led by MEDIAS-France/POSTEL). Land cover was divided into 8 categories: forest, grassland, farmland, urban, bare, freshwater, ice, and no data area. These analyses were carried out using ArcGIS 9.3 (ESRI)

Distribution of *Bd*

We used 51 *Bd* occurrence localities in Asia in this analysis (Fig. 2, Appendix). *Bd* occurrence data were gleaned from previous studies, with samples from Malaysia (n = 2), Philippines (n = 2), South Korea (n = 7), and Japan (n = 40). The latter included 2 recent Japanese localities, as reported by Goka et al. (2009). Cluster analysis was performed with the environmental variables, and the latitude and longitude of these 51 localities (reduced to 32 to avoid using similar environmental data from nearby localities), using R-2.13.1 statistical software (R Development Core Team 2011).

Accounting for spatial autocorrelation

Eigenvector-based spatial filtering is a well known method to resolve spatial autocorrelation in SDM studies (Griffith 2010). This method embeds spatially dependent variables within independent variables to remove spatial dependence from the models.

We used the Spatial Analysis in Macroecology (SAM) 4.0 software (Rangel et al. 2010) to create spatial filters for spatial eigenvector mapping. We set the truncation distance in the geographical distance matrix for spatial eigenvector mapping to be 25% of the maximum distance. Due to computational limita-
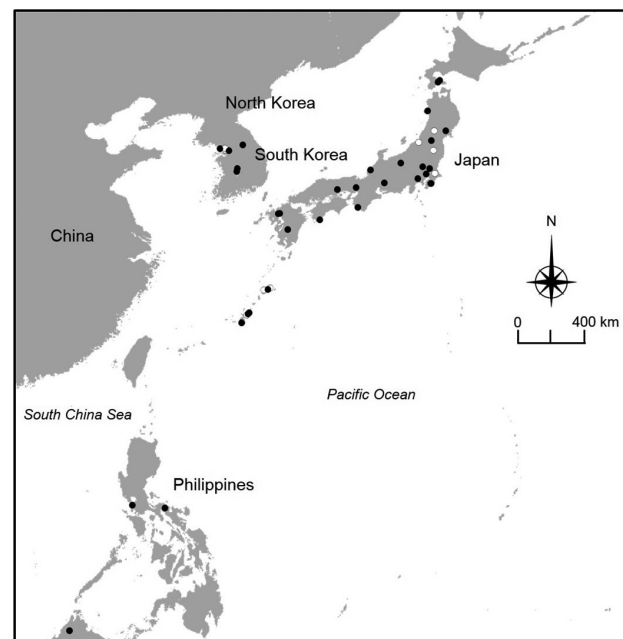

Fig. 2. Positive localities for *Batrachochytrium dendrobatidis* occurrence used in this study. Filled circles represent localities used to build the model, and open circles represent localities used to assess the model's repeatability

tions, calculations could not be performed for more than ~4000 sites. Thus, we created spatial filters with a coarse resolution of 0.3°.

Although several strategies have been proposed for the selection of spatial filters to be included as the independent variables in SDMs (e.g. Dormann et al. 2007, Blach-Overgaard et al. 2010, Griffith 2010), we adopted a method that minimizes the residuals of spatial autocorrelation (Dormann et al. 2007). Specifically, we selected spatial filters that minimized the residuals of Moran's *I* (Moran's *I* threshold = 0.01; Fortin & Dale 2005). We used the inverse distance weighted method provided by ArcGIS 9.3.1 to interpolate the spatial filters to the same resolution as the environmental variables (0.0417°).

To check for multicollinearity among independent variables, we calculated the correlation matrix for all variables. The significance levels for Pearson's product-moment correlation were adjusted with a Bonferroni correction (Quinn & Keough 2002). We eliminated 1 or more of the significantly correlated variables from the subsequent analysis. These statistical analyses were performed using R-2.13.1 software.

Species distribution modeling

We used the maximum-entropy approach (MaxEnt 3.3.3e; Phillips et al. 2006) to generate potential distribution models from presence-only data. This method performs well compared to other distribution models that use presence-only data (Elith et al. 2006), and is capable of providing highly accurate estimates even with small sample sizes (Hernandez et al. 2006). Because *Bd* infection rates fluctuate seasonally (e.g. Duncan Pullen et al. 2010, Longo et al. 2010), absence data were unreliable. We used 75% of the records to compute 10 randomly chosen replicates for model training, while the remaining 25% were used for model testing. Model runs were iterated 10 000 times, with 10 000 randomly selected background datasets. We evaluated the resulting model with receiver-operating characteristic curves to calculate the area under the curve (AUC), ranging from 0.5 (random accuracy) to 1.0 (perfect discrimination).

The suitability index values for each cell, where *Bd* was described, ranged from 0 to 1.0. We first used a model with only environmental variables, and eliminated extremely low-suitable cells (those with a suitability index <0.01) from the subsequent analyses to avoid using these sites as background points. Afterwards, we applied a model with environmental variables and spatial filters to the remaining cells.

For the comparison of the model accuracy and spatial autocorrelation, we also applied a model that only used environmental variables for the same area. We used mean AUC values to compare the models with and without spatial filters, and used median suitability indices for each cell to produce a potential distribution map. To compare the strength of the spatial autocorrelation in the 2 models, we used ArcGIS 9.3.1 to calculate global Moran's *I* coefficients for the residuals of the estimated suitability index values in each model.

We confirmed the repeatability of the model by comparing the frequency distribution of suitability indices for *Bd* occurrence localities that were not used as model data (n = 16) with that of localities where suitability indices were estimated (n = 199 062). We extracted these suitability indices from the potential distribution map.

RESULTS

From the 981 spatial filters created, we selected 11 to minimize Moran's *I* value for the residuals. For modeling, we used 7 independent variables that were not significantly correlated with each other. These 7 variables included 4 of the selected 11 spatial filters and excluded temperature PC2 and precipitation PC2.

The AUC was 0.82 ± 0.05 (mean ± SD) for the model with environmental variables, and 0.85 ± 0.0 for the model with environmental variables and spatial filters. Significant spatial autocorrelation was found in the model with only environmental variables (Moran's *I* = 0.36, p = 0.006), but autocorrelation was not significant in the model with spatial filters (Moran's *I* = −0.03, p > 0.99). This indicates that the potential distribution map estimated using the model with both environmental variables and spatial filters reveals high-suitability areas more accurately (Fig. 3).

Among the environmental variables, precipitation PC1 and temperature PC1 had higher contribution values than land cover, and were hence more effective in predicting the potential distribution of *Bd* (Table 1). We found a positive relationship between precipitation PC1 and habitat suitability for *Bd* (Fig. 4a). The suitable range (habitat suitability indices ≥0.5) for precipitation PC1 was more than 60 mm precipitation. In the case of temperature PC1, habitat suitability of *Bd* increased gradually until it reached a peak at 500, and then decreased dramatically (Fig. 4b). The most suitable range for temperature PC1 was between 400 and 500, which is equivalent to annual mean temperatures between 12 and 22°C.
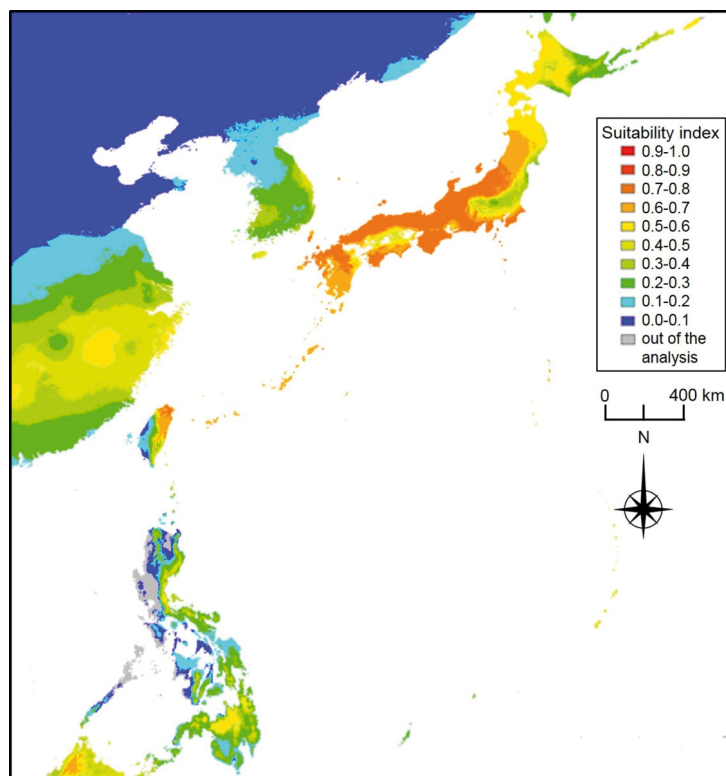
Fig. 3. Potential distribution map for *Batrachochytrium dendrobatidis* in East Asia estimated using the model with both environmental variables and spatial filters
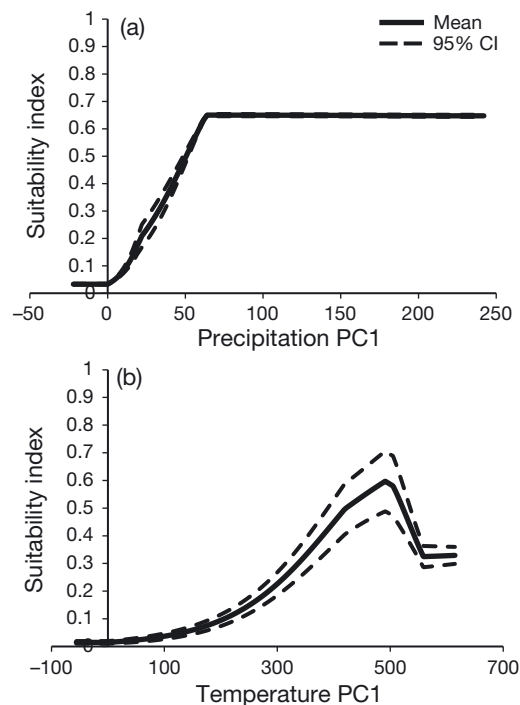

Fig. 4. Relationship between the 2 most effective environmental variables, viz. (a) minimum precipitation PC1 and (b) mean temperature PC1, and the suitability index for *Batrachochytrium dendrobatidis*. PC1 represents the first principal component integrated by principal component analysis

We tested the model's repeatability by comparing the frequency distributions of the suitability indices for unused positive localities and all localities. The distribution of the suitability indices for unused *Bd* occurrence localities tended towards higher values than that for all localities (Fig. 5).

DISCUSSION

This study provided the first potential distribution map for *Bd* in Asia, revealing that Japan is the most suitable area for *Bd* occurrence. The model that accounted for spatial autocorrelation predicted the potential suitability for *Bd* with high accuracy (AUC = 0.85). Although the resolution for creating the spatial filters was coarser than the cell size, and our data (collected from the available literature) were biased towards a few specific regions, the use of spatial filters alleviated spatial autocorrelation in the model. Swei et al. (2011) showed that the low repeatability of the potential distribution map generated by Rödder et al. (2009) resulted from predicting all sampling localities as environmentally suitable sites for *Bd* occurrence when, in fact, only 5% of these localities were positive for *Bd* presence. In this study, we achieved a higher model repeatability than did Rödder et al. (2009). This is because the positive localities in Swei et al. (2011), which were actually not used as initial data to create the distribution map, matched the areas estimated as highly suitable by our model. Therefore, our model could predict and pinpoint the potential distribution of *Bd* in Asia more accurately than previous studies (e.g. Lötters et al. 2009, Rödder et al. 2009).

Table 1. Contribution (±SD) of each independent variable and the sum of spatial filters to the model used for predicting the potential distribution of the chytrid fungus *Batrachochytrium dendrobatidis* in Asia. PC: principal component

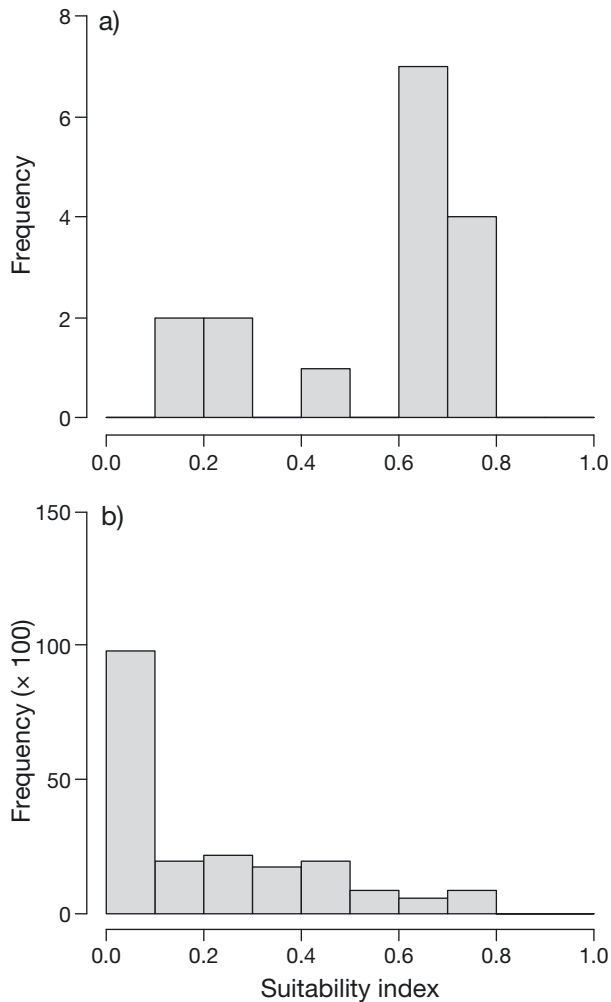| Independent variable	Contribution (%)
Precipitation PC1	77.0 ± 6.9
Temperature PC1	3.1 ± 2.2
Land cover	0.3 ± 0.5
Sum of spatial filters	19.6 ± 8.7

Fig. 5. Frequency distribution of suitability indices for (a) positive localities that were not used in the model, and (b) all localities extracted from the potential distribution map for *Batrachochytrium dendrobatidis*

There are 3 main reasons why we believe our map is superior to that of Rödder et al. (2009) in terms of the repeatability of its predictions. First, the SDM of Rödder et al. (2009) had statistical issues that resulted in overestimated suitability indices. Because this model did not adequately consider multicollinearity and spatial autocorrelation, the area of suitable habitat was distributed over a wider range than in our map.

Second, the suitability indices for Asian localities surveyed by Swei et al. (2011) were estimated by Rödder et al. (2009) using only localities invaded by the chytrid fungus in recent decades. Since initial invasions may have occurred in locations only coincidentally environmentally suitable, and because the distribution of *Bd* is still expanding, the invaded environments cannot cover all environments where the pathogen should be distributed (Roura-Pascual et al. 2006, Fitz-

patrick et al. 2007, Václavík & Meentemeyer 2012). *Bd* is regarded as an emerging infectious disease around the world; therefore, a restricted occurrence dataset is insufficient to explain habitat suitability if these data are extrapolated to other areas.

Third, *Bd* potential distribution maps estimated based on invaded localities may not be able to identify all suitable habitats in Asia since *Bd* might be native to the area, and recently invaded environments rarely include all of the native environments (Roura-Pascual et al. 2006). Goka et al. (2009) showed that the genetic variation of *Bd* in Japan was higher than that of other countries, but *Bd* prevalence was low among native amphibians in Japan (4.1 % = 87/2103), similarly to Asia in general (2.3 % = 79/3363: Swei et al. 2011). Moreover, even when native amphibians in Japan are infected by *Bd*, it seems that they do not develop disease symptoms (Goka et al. 2009). Likewise, amphibians in other Asian countries have not experienced drastic declines caused by *Bd* (Yang et al. 2009, Swei et al. 2011).

Goka et al. (2009) hypothesized that Asian native amphibians might have evolved resistance against *Bd* because of their long coexistence with the chytrid. *Bd* infection was found on all main islands of Japan, and suitable habitat areas were distributed throughout the country (Fig. 2). In particular, the southwestern islands were highly suitable for *Bd*. It has been shown that the sword-tailed newt *Cynops ensicauda*, an endemic species on these islands, had the highest prevalence of *Bd* (>60%) among Japanese native amphibians (Goka 2010) and was infected by various *Bd* strains, including the global panzootic lineage (Schloegel et al. 2012), which has caused drastic declines in amphibian populations worldwide. Similarly, prevalence was high for the Japanese tree frog *Hyla japonica* in South Korea (80%; Yang et al. 2009). Although the sword-tailed newt in the southwestern islands of Japan had high *Bd* prevalence, this species has never been reported to develop chytridiomycosis or to have experienced a rapid population decrease attributable to the pathogen (Goka et al. 2009, Yang et al. 2009). High *Bd* prevalence was also found in Japanese giant salamanders (>40%; Goka 2010) on the main islands of Japan. The salamanders were infected only by highly host-specific *Bd* strains, suggesting that *Bd* has developed a commensal relationship with these amphibians (Goka et al. 2009).

On the other hand, other native amphibians had low *Bd* prevalence, which was regarded as an inapparent infection in Japan (Goka et al. 2009, Goka 2010). It is possible that samples collected from most amphibian species were considered false negatives

or that the infection levels were so low that they remained undetected by nested PCR analysis. For example, *Bd* has been detected only in specific body parts of Japanese giant salamanders, in areas where more than 40% of individuals were infected (Goka 2010). The body parts where the infection was most easily detectable were the toe tips (Une et al. 2012). In this situation, the number of false negatives is likely to increase unless investigators swab the most frequently infected parts of the animals. Because most collectors in the study by Goka et al. (2009) were inexperienced with swab sampling of *Bd*, the sampling methods varied among the collectors, and this probably increased the number of false negatives.

The prevalence of infection by *Bd* also varies seasonally, as it is influenced by the water temperature and the life history of the host amphibian species (Longo et al. 2010, A. Tominaga unpubl. data). The *Bd* prevalence might thus have been underestimated in the study by Goka et al. (2009), since most of the samples were collected during the summer, when *Bd* is less abundant due to high water temperatures. Microclimates might also have affected *Bd* prevalence and accuracy of our model, but we were unable to account for these factors with the coarse resolution used. Although the survey conducted by Goka et al. (2009) in Japan was quite thorough, the samples were biased in terms of both the swabbed amphibian species and the sampling regions. Additional surveys using optimized methods that consider the ideal body part, species, and season to sample will be required to specify the extent of *Bd* infection.

Because *Bd* studies in Asia have just begun, we should continue to investigate the possible ecological risk of *Bd* to amphibians in this area, and verify the hypothesis that this pathogen is native to certain Asian regions. In addition, the genetic relationships among strains of *Bd* and genetic diversity should be compared between regions where *Bd* is suspected to be native, such as Japan (Goka et al. 2009, Goka 2010) and South Africa (Weldon et al. 2004), and regions where *Bd* is considered to be invasive and responsible for a drastic decline in amphibian populations, such as Latin America (e.g. Young et al. 2001, Puschendorf et al. 2009). Experimental infection of native amphibians with *Bd* could be a way of measuring the ecological risk to these species. We expect that the potential *Bd* distribution map created in the present study will provide strong guidance and improve the efficiency of future surveys. The resulting ecological information about *Bd* in Asia will also increase our understanding of the expansion of this pathogen around the world.

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Appendix. Positive localities for *Batrachochytrium dendrobatidis* used in this study. Latitudes and longitudes are in decimal degrees

Site	Country	Latitude (°N)	Longitude (°E)	Publication	Site	Country	Latitude (°N)	Longitude (°E)	Publication
1	Japan	40.0053	139.9450	Goka et al. (2009)	27	Japan	33.2814	130.1864	Goka et al. (2009)
2	Japan	38.7100	141.1158	Goka et al. (2009)	28	Japan	32.8844	132.9000	Goka et al. (2009)
3	Japan	38.7072	140.3708	Goka et al. (2009)	29	Japan	32.2381	130.7961	Goka et al. (2009)
4	Japan	38.0683	140.1847	Goka et al. (2009)	30	Japan	28.4113	129.6530	Goka et al. (2009)
5	Japan	37.9391	139.3581	Goka et al. (2009)	31	Japan	28.3308	129.5050	Goka et al. (2009)
6	Japan	37.9389	139.3578	Goka et al. (2009)	32	Japan	28.2955	129.2339	Goka et al. (2009)
7	Japan	37.4242	140.3228	Goka et al. (2009)	33	Japan	26.7998	128.2747	Goka et al. (2009)
8	Japan	36.5908	138.1883	Goka et al. (2009)	34	Japan	26.7994	128.2744	Goka et al. (2009)
9	Japan	36.3519	139.6100	Goka et al. (2009)	35	Japan	26.7250	128.2152	Goka et al. (2009)
10	Japan	36.2350	140.0769	Goka et al. (2009)	36	Japan	26.6583	128.1347	Goka et al. (2009)
11	Japan	36.1494	136.2158	Goka et al. (2009)	37	Japan	26.1758	127.8056	Goka et al. (2009)
12	Japan	35.9086	140.4033	Goka et al. (2009)	38	Japan	26.1489	127.7960	Goka et al. (2009)
13	Japan	35.8753	139.8494	Goka et al. (2009)	39	Japan	42.0022	140.7186	Present study
14	Japan	35.5792	139.2991	Goka et al. (2009)	40	Japan	41.8889	140.6131	Present study
15	Japan	35.2886	137.1122	Goka et al. (2009)	41	Malaysia	6.0086	116.5428	Swei et al. (2011)
16	Japan	35.2600	140.1547	Goka et al. (2009)	42	Malaysia	6.0090	116.5430	Swei et al. (2011)
17	Japan	35.2532	140.1459	Goka et al. (2009)	43	Philippines	14.0394	122.7866	Swei et al. (2011)
18	Japan	35.2519	140.1456	Goka et al. (2009)	44	Philippines	14.2320	120.6580	Swei et al. (2011)
19	Japan	34.9963	135.2658	Goka et al. (2009)	45	South Korea	37.3937	126.9554	Yang et al. (2009)
20	Japan	34.8547	134.0464	Goka et al. (2009)	46	South Korea	37.4441	126.7357	Yang et al. (2009)
21	Japan	34.8545	134.0497	Goka et al. (2009)	47	South Korea	37.5337	126.3729	Yang et al. (2009)
22	Japan	34.8541	134.0466	Goka et al. (2009)	48	South Korea	37.5449	126.7083	Yang et al. (2009)
23	Japan	33.6972	135.3803	Goka et al. (2009)	49	South Korea	37.7733	127.8635	Yang et al. (2009)
24	Japan	33.6952	135.3823	Goka et al. (2009)	50	South Korea	36.0515	127.4771	Swei et al. (2011)
25	Japan	33.2982	130.2766	Goka et al. (2009)	51	South Korea	36.2270	127.5050	Swei et al. (2011)
26	Japan	33.2981	130.2764	Goka et al. (2009)					