


Microhabitat Temperatures and Prevalence of the Pathogenic Fungus *Batrachochytrium dendrobatidis* in Lowland Amazonian Frogs

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Abstract

Until recently, it was assumed that the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) was not widely distributed in warm ecosystems such as lowland tropical rainforests because high environmental temperatures limit its growth. However, several studies have documented *Bd* infection in lowland rainforest amphibians over the past decade. In addition, a recent study focusing on museum-stored specimens showed that *Bd* has been present in the lowland Amazon for more than 80 years. These findings lent support to the idea that some lowland rainforest habitats offer suitable environmental conditions for *Bd* growth, even though most lowland areas may contain suboptimal conditions limiting the pathogen spread and growth. Here, we surveyed four sites in southeast Peru to examine the prevalence and the intensity of infection of *Bd* in lowland Amazonian amphibians and to fill a gap between two areas where *Bd* has been present for more than a decade. In one of these “hotspots” of *Bd* infection, the upper slopes of Manu National Park, several species experienced population declines attributed to *Bd* epizootics over the past 15 years. We also examined the thermal profile of the main microhabitats used by lowland Amazonian frogs to infer whether these microhabitats offer suitable thermal conditions for *Bd* growth. We detected *Bd* in nine lowland frog species and variation in prevalence of infection across years. Our findings suggest that the temperatures in the leaf litter and understory vegetation of some habitats offer suitable conditions for *Bd* growth.

Keywords

Chytridiomycosis, emerging infectious disease, frogs, HOBO temperature data loggers, iButton, lowland tropical rainforest

Introduction

Until recently, it was assumed that the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) was not widely distributed in warm ecosystems such as lowland tropical rainforests because maximum environmental temperatures limit its growth (Ron, 2005; Rödder et al., 2009). This assumption received general support because laboratory studies showed that the optimal temperature for growth of *Bd* in the lab is 15°C to 25°C, whereas temperatures above 28°C resulted in spore death (Johnson, Berger, Phillips, & Speare, 2003; Piotrowski, Annis, & Longcore, 2004; Roznik, Sapsford, Pike, Schwarzkopf, & Alford, 2015; Stevenson et al., 2013; Stevenson, Roznik, Alford, & Pike, 2014). However, several studies have documented *Bd* infection in lowland rainforest amphibians over the past decade (Flechas et al., 2012; Kosch, Morales, & Summers, 2012; McCracken, Gaertner, Forstner, & Hahn, 2009; Whitfield, Kerby, Gentry, & Donnelly, 2012). These findings lent support

to the idea that some lowland tropical forest habitats offer suitable environmental conditions for *Bd* growth, even though most lowland areas may contain suboptimal

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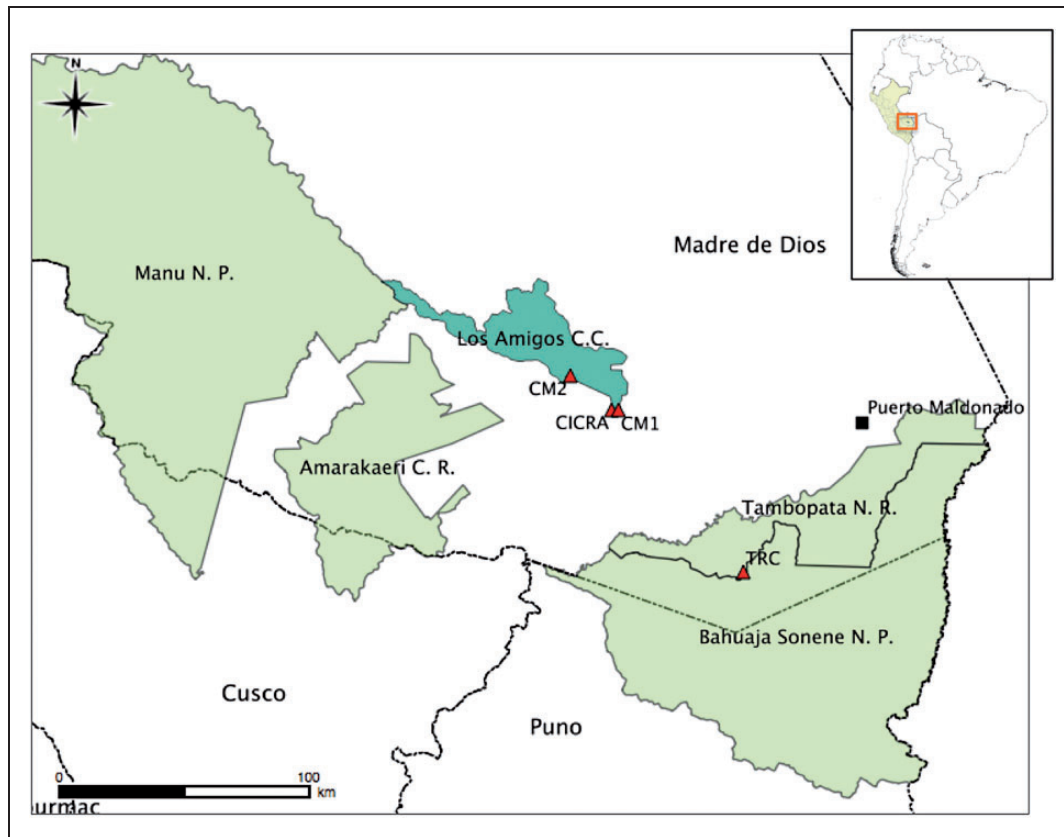


Figure 1. Map showing the location of the study sites (red triangles) and the main natural protected areas in southeast Peru. The inset shows the location of the study area in South America (orange polygon), and Peru highlighted in yellow. Map by Rudolf von May made with Quantum GIS (<https://qgis.org/en/site/>). CICRA = Los Amigos Biological Station; CMI = Centro de Monitoreo 1; CM2 = Centro de Monitoreo 2.

conditions limiting the pathogen spread and growth (Flechas, Vredenburg, & Amézquita, 2015). A recent study focusing on museum-stored specimens of species in the genus *Leptodactylus* (Anura: Leptodactylidae) showed that *Bd* has been present in the lowland Amazon for more than 80 years (Becker, Rodriguez, Lambertini, Toledo, & Haddad, 2016). In contrast, studies using species distribution models suggested that lowland Amazonian rainforest might not be suitable for *Bd* because high environmental temperatures prevent its growth (James et al., 2015).

Here, we surveyed four sites in southeast Peru to examine the prevalence and the intensity of infection of *Bd* in lowland Amazonian amphibians and to fill a gap between two areas where *Bd* has been present for more than a decade, namely, the montane forests in Manu National Park, Cusco (Catenazzi, Lehr, Rodriguez, & Vredenburg, 2011; Catenazzi, Lehr, & von May, 2013) and the lowland forests in Acre, Brazil (Becker et al., 2016). In one of these “hotspots” of *Bd* infection, the upper slopes of Manu National Park, more than 20 montane amphibian species have experienced population declines attributed to *Bd*

epizootics over the past 15 years (Catenazzi et al., 2011; Catenazzi, Lehr, & Vredenburg, 2014). We also examined the thermal profile of the main microhabitats used by lowland Amazonian frogs to infer whether these microhabitats offer suitable thermal conditions for *Bd* growth.

Methods

We conducted this study at four lowland sites in the Madre de Dios region of southeastern Peru (Figure 1): Los Amigos Biological Station (CICRA is the Spanish acronym), 12°34′07″ S, 70°05′57″ W, 270 m elevation; Centro de Monitoreo 1, 12°34′17″ S, 70°04′29″ W, approximately 250 m elevation; Centro de Monitoreo 2, 12°26′57″ S, 70°15′06″ W, 260 m elevation; and Tambopata Research Center, 13°08′30″ S, 69°36′24″ W, 350 m elevation. The first three sites are relatively close to each other (3.5–25 km) and the fourth site (Tambopata Research Center) is 80 to 105 km away from the other three sites. A general overview of the amphibian fauna, the habitats, and the local climate at these sites was provided by von May et al. (2009), von

Table 1. Species Infected by *Batrachochytrium dendrobatidis* (Bd) in Madre de Dios, Peru.

Family	Species	Site	Year	Habitat (forest type)	Breeding microhabitat	Postbreed microhabitat
Dendrobatidae	<i>Ameerega hahneli</i>	CICRA	2012	PS, FP, TF, B	Lotic	Terrestrial
Hylidae	<i>Dendropsophus koehlini</i>	CICRA	2012	FP	Lentic	Arboreal
Hylidae	<i>Scinax garbei</i>	CICRA	2012	FP, PS	Lentic	Arboreal
Leptodactylidae	<i>Adenomera andreae</i>	CICRA	2014	TF, FP, B, PS	Terrestrial	Terrestrial
Leptodactylidae	<i>Leptodactylus petersii</i>	CMI	2008 ^a	PS, FP, TF, B	Lentic	Semi-aquatic
Microhylidae	<i>Hamptophryne boliviana</i>	CICRA	2012	FP, TF, B, PS	Lentic	Terrestrial
Microhylidae	<i>Chiasmocleis royi</i>	CICRA	2014	FP, B, TF, PS	Lentic	Terrestrial
Strabomantidae	<i>Pristimantis carvalhoi</i>	CICRA	2014	FP, B	Terrestrial	Arboreal
Strabomantidae	<i>Pristimantis reichlei</i>	CICRA	2014	TF, B, FP, PS	Terrestrial	Arboreal

Note. Site and year where Bd-positive individuals were detected (CICRA = Los Amigos Biological Station; CMI = Centro de Monitoreo I), and habitat data (FP = floodplain forest; TF = terra firme forest; B = bamboo forest; PS = palm swamp) listed in decreasing order of importance (i.e., habitat where a species was more common to less common; von May, Jacobs, Santa-Cruz, et al., 2010; von May et al., 2009).

^aRecord of Bd-positive individual from Kosch et al. (2012).

May et al. (2010) and von May, Jacobs, Santa-Cruz et al. (2010).

We took noninvasive skin swab samples from 282 individuals encountered in the field during the wet seasons of 2008 ($N=138$), 2012 ($N=55$), and 2014 ($N=89$). Swabs collected in 2008 were analyzed using laboratory protocols described in Kosch et al. (2012). In 2012 and 2014, we used an MW113-Advantage Bundling sterile synthetic cotton swabs to sample the abdomen, thighs, and hind limbs from each specimen and stored dried in 1.5 ml tubes. We quantified *Bd* infection by quantitative polymerase chain reaction following the procedures of Boyle, Boyle, Olsen, Morgan, and Hyatt (2004) and Hyatt et al. (2007). We captured frogs by hand and stored them in separate plastic bags in the field. Occurrence of *Bd* was determined by estimating the quantity of zoospores on each animal (Zswab, zoospore equivalents; Briggs, Knapp, & Vredenburg, 2010; Vredenburg, Knapp, Tunstall, & Briggs, 2010), which in turn is calculated by multiplying the genomic equivalent obtained during quantitative polymerase chain reaction by the dilution factor (genomic equivalents \times dilution factor = zoospore equivalents). We calibrated sample results with a qPCR standard curve. Swabs were categorized as *Bd*-positive when the equivalent of zoospores was >0 and *Bd*-negative when the equivalent of zoospores was $=0$ (Vredenburg et al., 2010). *Bd* prevalence was calculated by dividing the number of infected amphibians by the total number of individual swab samples. We considered two categories for the intensity of infection: low infection when Zswab was between 1 and 10,000 and high infection when Zswab was greater than 10,000 (Voyles et al., 2012). These threshold values are equivalent to those estimated as critical in the mortality (10,000 zoospores) of frog species reported in the previous studies (Vredenburg et al., 2010).

We used data loggers to obtain empirical data on microhabitat temperatures from lowland Amazonian habitats. First, we used iButton data loggers (Maxim Integrated Products, Sunnyvale, CA) placed in two forest microhabitats, leaf-litter (3 cm above the ground) and understory vegetation (180 cm above the ground), used by frogs across four forest types. Daily temperatures were recorded in floodplain forest, terra firme forest, bamboo forest, and palm swamp during part of the wet season of 2008 (44 days). In addition, we used HOBO data loggers (Onset Computer Corporation, Pocasset, MA) placed in the leaf litter of the floodplain and the terra firme forests to record the temperature from November 16, 2016 to December 5, 2017 (385 days). We compared these data with air temperatures measured at the local weather station over the same time period.

Results

We obtained *Bd* prevalence and infection intensity data from 51 species sampled in 2008, 27 species sampled in 2012, and 29 species sampled in 2014 (Appendix A). Nine species of frogs were infected by *Bd* (Table 1), and the prevalence of infection varied across years: *Bd* prevalence was 0.7% in 2008, 7.3% in 2012, and 4.5% in 2014. The infected species belong to five families that are found in different forest types and use different microhabitats (Table 1). Most infected species use aquatic microhabitats for breeding and larval development, as their tadpoles use either lotic or lentic bodies of water. These included a poison frog (Dendrobatidae), two tree frogs (Hylidae), one leptodactylid frog (Leptodactylidae), and two narrow-mouthed frogs (Microhylidae). In addition, one infected species (*Adenomera andreae*) lays foam nests on land and

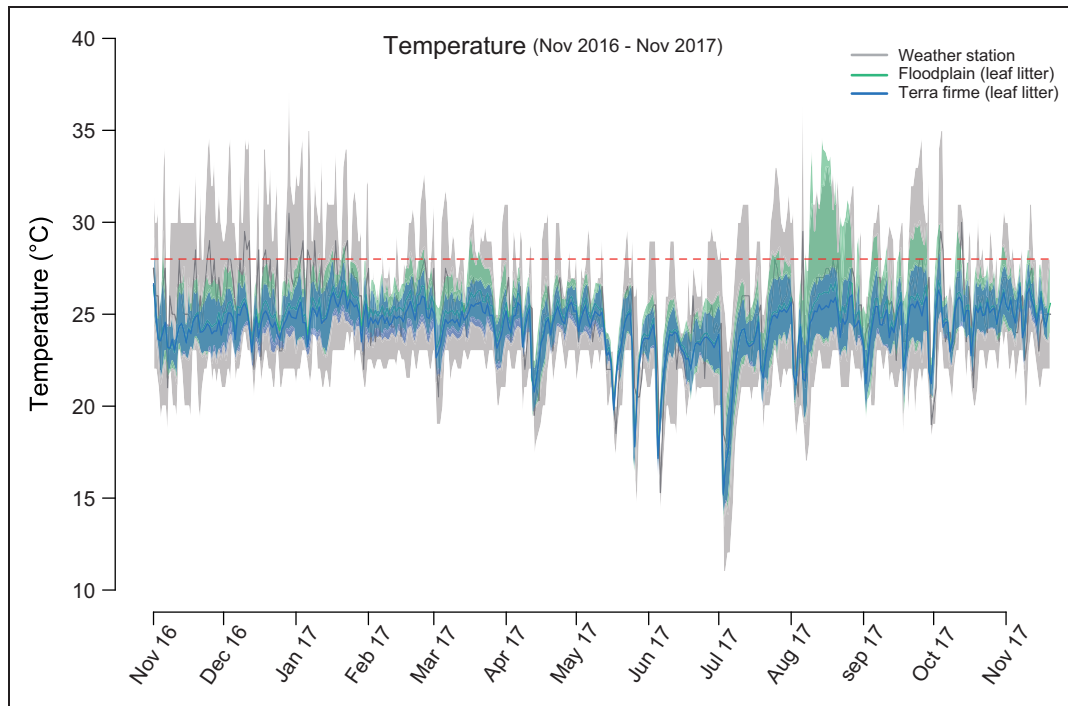


Figure 2. Daily temperatures recorded from November 16, 2016 to December 5, 2017 (385 days) in two forest types at Los Amigos Biological Station, Peru. Weather station data are shown in gray; temperature data collected in the leaf litter are shown in green (floodplain forest) and blue (terra firme forest). In each case, the maximum and minimum temperatures delimited the polygons, and the line in the middle represents the mean temperature. The maximum temperature measured in the leaf litter rarely exceeds 28°C (red line).

larvae complete their development inside the foam, while two species belong to the diverse terrestrial breeding frogs (Strabomantidae) and have direct development (i.e., there are no free-living tadpoles).

Daily leaf litter temperatures of the two main forest types at Los Amigos Biological Station were very similar throughout the year (Figure 2), with greater fluctuations in the dry season (between May and October). During this period, low temperatures were associated with cold fronts. These cold surges occur during the Austral winter and are characterized by a rapid drop in air temperature associated with incursions of cold air masses originating in the Antarctic region and southern South America (Marengo, 1984; Marengo, Cornejo, Satyamurty, Nobre, & Sea, 1997). Animal activity in lowland Amazonia is affected during these cold surges. The ranges of daily minimum temperatures in both habitats were similar in the floodplain (13.4°C–25.7°C) and the terra firme (13.9°C–25.3°C). Likewise, the ranges of daily mean temperatures in both habitats were similar in the floodplain (15.1°C–26.9°C) and the terra firme (15.2°C–26.7°C). We also observed similar daily maximum temperatures throughout most of the year, with the exception of some periods of higher maximum

temperature in the floodplain in August, September, and October 2017 (Figure 2). Furthermore, during most of the year, the maximum temperature measured in the leaf litter rarely exceeded 28°C (red line in Figure 2).

Daily temperatures measured in the leaf litter and the understory vegetation of the four main forest types at Los Amigos show that two habitats, floodplain forest and terra firme forest, experience lower temperature fluctuations than the bamboo forest and the palm swamp (Figure 3). In addition to the distribution of *Bd*-positive records provided by Becker et al. (2016), we updated the known distribution of *Bd*-positive records in the lowland Amazon (<300 m elevation) to include records by McCracken et al. (2009), Kosch et al. (2012), and this study (Figure 4).

Discussion

Our findings suggest that the environmental temperatures in the leaf litter and understory vegetation of some lowland Amazonian forests offer suitable conditions for *Bd* growth. In particular, the leaf litter and understory vegetation of the floodplain forest and terra

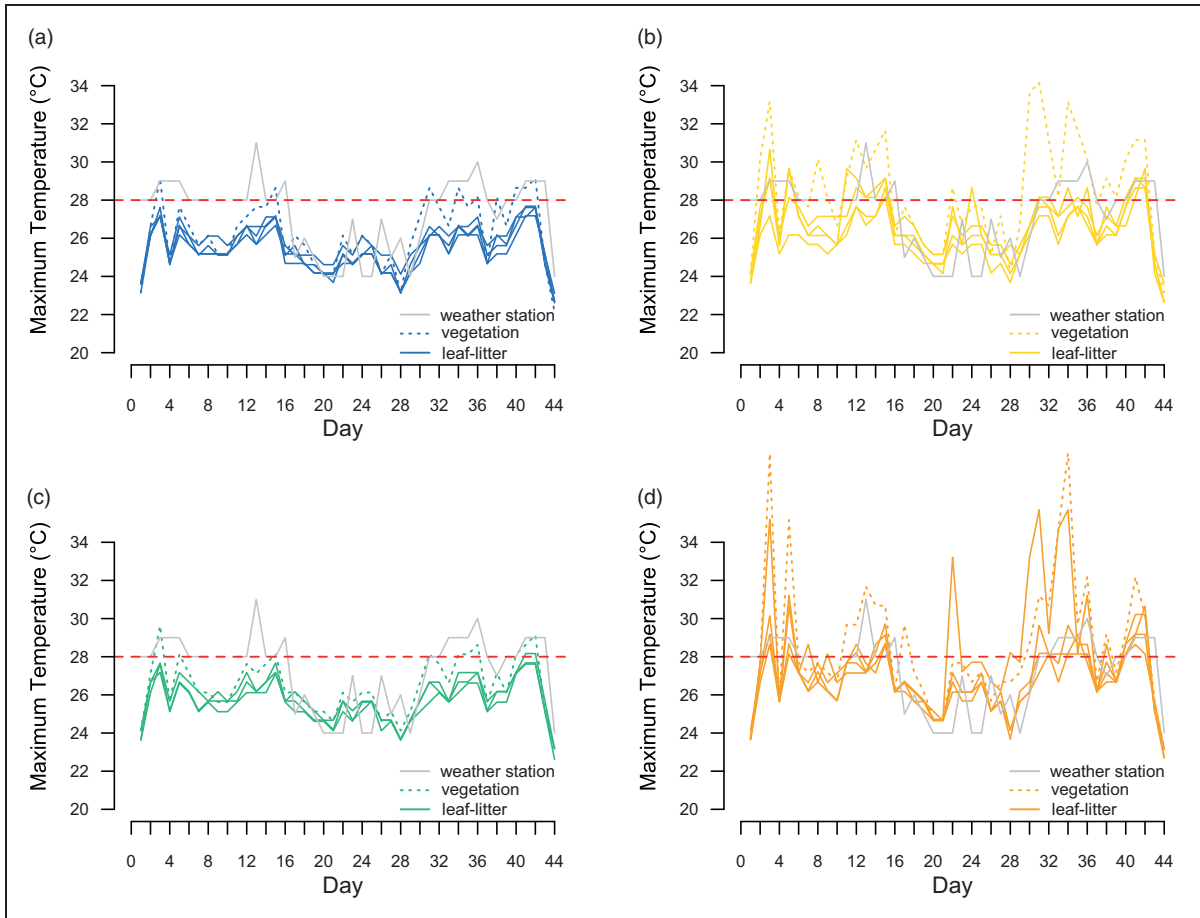


Figure 3. Daily maximum temperatures recorded during the wet season in four habitats around Los Amigos Biological Station, Peru. These data were collected using iButton data loggers between February 14, 2008, and March 30, 2008. (a) Terra firme, (b) bamboo, (c) floodplain, and (d) palm swamp.

firme forest experience lower temperature fluctuations and maximum temperatures than bamboo and palm swamp habitats (Figure 3). Considering these findings, we hypothesize that *Bd* prevalence and infection will be less common in bamboo and palm swamp habitats because the leaf litter and understory vegetation of these forest types experience higher temperatures and broader temperature fluctuations. These conditions are known to inhibit growth of *Bd* in the lab and likely will inhibit its growth on hosts that primarily live in these forest types.

Many studies have predicted that lowland Amazonian forests do not offer suitable conditions for *Bd* growth (Ron, 2005, Rödder et al., 2009), and some researchers have argued that including lowland records of *Bd* infection in nonendangered amphibians will not be useful for understanding the climatic conditions of the pathogen (Menéndez-Guerrero & Graham, 2013). Although these studies recognized that *Bd* has a broad

environmental niche, they suggested that lowland amphibian populations are not experiencing declines caused by chytridiomycosis. Nevertheless, given that a number of amphibian species are known to be infected by *Bd* in lowland Amazonia (<300 m elevation; Becker et al., 2016; McCracken et al., 2009; this study), and considering that at least one lowland species (*Pristimantis toftae*, Strabomantidae) is highly susceptible to chytridiomycosis (Catenazzi et al., 2017), we think that all *Bd*-positive records should be considered when making predictions about the pathogen's distribution and potential impact on lowland rainforest species.

James et al. (2015) emphasized that questions relating to prevalence and distribution of *Bd* should be reframed, given that *Bd* might have been present historically at “cold spots”—areas where the pathogen may be present but offer suboptimal conditions for *Bd* growth—such as lowland tropical forests. Our findings support the idea

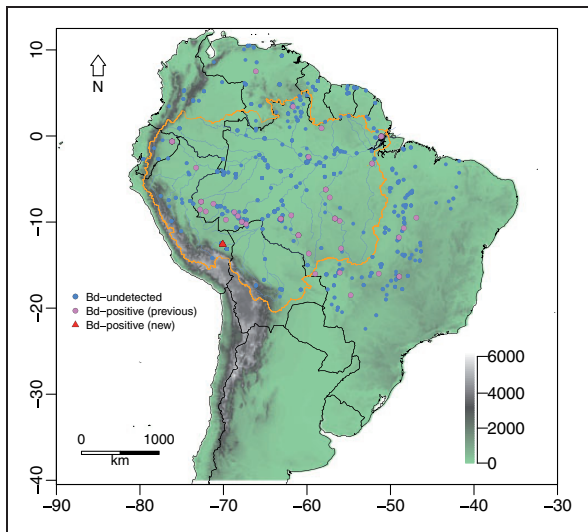


Figure 4. Map showing the location of previous *Bd*-positive records (violet circles) in lowland sites (<300 m elevation; Becker et al., 2016; Kosch et al., 2012; McCracken et al., 2009) and new records from Madre de Dios, Peru (red triangle). The area outlined in orange indicates the boundary of the Amazon Basin, and the major river network is indicated in blue. *Bd* = *Batrachochytrium dendrobatidis*.

that these “cold spots” might be widespread, especially in old-growth habitats that contain environmental conditions within the pathogen’s tolerance window. Another important consideration is that amphibian immunity (especially adaptive) generally improves with temperature (Raffel, Rohr, Kiesecker, & Hudson, 2006; Raffel et al. 2012) so at warm temperatures, the frog’s immune system might be more effective at deterring *Bd* infection. A possible test of this idea would be to track the progress of *Bd* infections throughout the year, and examine if prevalence of infection decreases at warmer temperatures (e.g., Kriger & Hero 2007).

We hypothesized that 28°C is the critical maximum temperature limiting *Bd* growth in lowland Amazonia, assuming it has the same thermal niche of other *Bd* lineages. However, this assumption needs to be tested through experimental studies and special attention should be placed on measuring microhabitat characteristics that may differ from general habitat characteristics. In the meantime, it is reasonable to assume that many lowland Amazonian rainforest habitats offer suitable conditions for *Bd* growth. In turn, *Bd* growth is limited by occasional pulses of high temperatures that exceed 30°C as suggested by our iButton temperature data, and we propose that some *Bd* lineages may be able to adapt to the local conditions. Recent experimental studies focusing on Australian rainforest frogs (*Litoria spenceri*) support this idea, given that frequent

exposure to temperatures exceeding optimum *Bd* growth values result in lower infection (Greenspan et al., 2017). Furthermore, *Bd* infections were cleared in animals that were exposed to daily heat pulses of 29°C lasting 4 h (Greenspan et al., 2017). At our main study site in Peru (Los Amigos Biological Station), our iButton data showed that some microhabitats experience heat pulses exceeding the hypothesized critical temperature of 28°C. Thus, these daily temperature fluctuations and occasional heat pulses might protect lowland Amazonian frogs from more severe infections caused by *Bd*.

Two lineages of *Bd* have been reported in South America, *Bd*-Brazil and the globally dispersed pandemic lineage (O’Hanlon et al., 2018). Further experimental work is required to test whether critical maximum temperature of these lineages is similar to that reported for other tropical isolates (Stevenson et al., 2013). If the *Bd* lineage from Madre de Dios is more similar to the *Bd*-Brazil lineage, then studies on critical temperature might require controlled laboratory experiments that include both *Bd*-Brazil and *Bd*-globally dispersed pandemic lineage.

Implications for Conservation

Over the past decade, many studies have examined the prevalence of *Bd* on amphibian populations in montane areas such as the eastern slopes of the Andes (e.g., Catenazzi et al., 2011; Seimon et al., 2017), and researchers highlighted that protecting the continuity and connectivity of natural habitats is essential for species’ long-term survival in the face of pathogen infections and environmental change (Seimon et al., 2017; von May et al., 2008). Fewer studies examined the incidence of *Bd* in lowland tropical amphibians because none of these sites appeared to have suffered species loss attributable to chytridiomycosis. Nevertheless, it is essential to continue monitoring the prevalence of chytridiomycosis in lowland Amazonia and other lowland tropical rainforests (e.g., Indonesia, the Philippines, Equatorial Africa) to better understand what limits the pathogen’s distribution. Field studies in Amazonia will need to track the progress of *Bd* infections throughout the year, and especially during the dry season when cold fronts cause sudden drops in temperature that could potentially make frogs more susceptible to disease. Further studies focusing on lowland rainforest frogs are needed to assess whether *Bd* is enzootic in Amazonian amphibians and the role of “cold spots” for long-term disease dynamics. These studies will allow researchers to examine how many species have evolved defenses (Catenazzi et al., 2017) and identify those that are more vulnerable in order to prioritize conservation actions.

Appendix A

Table A1. Species Sampled in 2008, 2012, and 2014 and Data on Bd Prevalence and Intensity of Infection.

Sample no.	Sample ID	Bd load	Bd pres.	Record	Species	Latitude	Longitude	State province	Year collected
I227	I227	NA	1	Previous	<i>Leptodactylus petersii</i>	-12.57139	-70.07472	Madre de Dios	2008
H48	H48	1.288	1	New	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2012
H7	H7	1.416	1	New	<i>Dendropsophus koechlini</i>	-12.56861	-70.09917	Madre de Dios	2012
H20	H20	0.5512	1	New	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2012
H47	H47	138.4	1	New	<i>Scinax garbei</i>	-12.56861	-70.09917	Madre de Dios	2012
CH_019	RVM112	8080	1	New	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_039	RVM132	7.536	1	New	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_090	RVM183	21.8	1	New	<i>Pristimantis carvalhoi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_020	RVM113	5.34	1	New	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
I222	I222	0	0	Negative	<i>Adenomera sp.</i>	-12.44916	-70.25167	Madre de Dios	2008
I237	I237	0	0	Negative	<i>Adenomera sp.</i>	-12.56861	-70.09917	Madre de Dios	2008
I242	I242	0	0	Negative	<i>Adenomera sp.</i>	-12.56861	-70.09917	Madre de Dios	2008
I289	I289	0	0	Negative	<i>Adenomera sp.</i>	-13.13333	-69.60000	Madre de Dios	2008
I330	I330	0	0	Negative	<i>Adenomera sp.</i>	-12.56861	-70.09917	Madre de Dios	2008
I248	I248	0	0	Negative	<i>Allobates conspicuus</i>	-12.56861	-70.09917	Madre de Dios	2008
I265	I265	0	0	Negative	<i>Allobates femoralis</i>	-13.13333	-69.60000	Madre de Dios	2008
I314	I314	0	0	Negative	<i>Amazophrynella javierbustamantei</i>	-13.13333	-69.60000	Madre de Dios	2008
I316	I316	0	0	Negative	<i>Amazophrynella javierbustamantei</i>	-13.13333	-69.60000	Madre de Dios	2008
I317	I317	0	0	Negative	<i>Amazophrynella javierbustamantei</i>	-12.56861	-70.09917	Madre de Dios	2008
I349	I349	0	0	Negative	<i>Amazophrynella javierbustamantei</i>	-12.44916	-70.25167	Madre de Dios	2008
I353	I353	0	0	Negative	<i>Amazophrynella javierbustamantei</i>	-12.44916	-70.25167	Madre de Dios	2008
I256	I256	0	0	Negative	<i>Ameerega hahneli</i>	-13.13333	-69.60000	Madre de Dios	2008
I325	I325	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2008
I326	I326	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2008
I343	I343	0	0	Negative	<i>Ameerega hahneli</i>	-12.44916	-70.25167	Madre de Dios	2008
I348	I348	0	0	Negative	<i>Ameerega hahneli</i>	-12.44916	-70.25167	Madre de Dios	2008
I351	I351	0	0	Negative	<i>Ameerega hahneli</i>	-12.44916	-70.25167	Madre de Dios	2008
I245	I245	0	0	Negative	<i>Ameerega trivittata</i>	-12.56861	-70.09917	Madre de Dios	2008
I352	I352	0	0	Negative	<i>Ameerega trivittata</i>	-12.44916	-70.25167	Madre de Dios	2008
I282	I282	0	0	Negative	<i>Bolitoglossa altamazonica</i>	-13.13333	-69.60000	Madre de Dios	2008
I312	I312	0	0	Negative	<i>Ceratophrys cornuta</i>	-13.13333	-69.60000	Madre de Dios	2008
I229	I229	0	0	Negative	<i>Chiasmocleis royi</i>	-12.57139	-70.07472	Madre de Dios	2008
I254	I254	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2008
I301	I301	0	0	Negative	<i>Chiasmocleis royi</i>	-13.13333	-69.60000	Madre de Dios	2008
I310	I310	0	0	Negative	<i>Chiasmocleis royi</i>	-13.13333	-69.60000	Madre de Dios	2008
I338	I338	0	0	Negative	<i>Chiasmocleis royi</i>	-12.44916	-70.25167	Madre de Dios	2008
I230	I230	0	0	Negative	<i>Ctenophryne geayi</i>	-12.57139	-70.07472	Madre de Dios	2008
I336	I336	0	0	Negative	<i>Ctenophryne geayi</i>	-12.44916	-70.25167	Madre de Dios	2008
I347	I347	0	0	Negative	<i>Ctenophryne geayi</i>	-12.44916	-70.25167	Madre de Dios	2008
I286	I286	0	0	Negative	<i>Dendropsophus minutus</i>	-13.13333	-69.60000	Madre de Dios	2008
I298	I298	0	0	Negative	<i>Dendropsophus acreanus</i>	-13.13333	-69.60000	Madre de Dios	2008
I305	I305	0	0	Negative	<i>Dendropsophus rhodopeplus</i>	-13.13333	-69.60000	Madre de Dios	2008
I337	I337	0	0	Negative	<i>Dendropsophus rhodopeplus</i>	-12.44916	-70.25167	Madre de Dios	2008
I221	I221	0	0	Negative	<i>Dendropsophus koechlini</i>	-12.56861	-70.09917	Madre de Dios	2008
I258	I258	0	0	Negative	<i>Engystomops freibergi</i>	-13.13333	-69.60000	Madre de Dios	2008
I260	I260	0	0	Negative	<i>Engystomops freibergi</i>	-13.13333	-69.60000	Madre de Dios	2008
I263	I263	0	0	Negative	<i>Engystomops freibergi</i>	-13.13333	-69.60000	Madre de Dios	2008
I266	I266	0	0	Negative	<i>Engystomops freibergi</i>	-13.13333	-69.60000	Madre de Dios	2008
I274	I274	0	0	Negative	<i>Engystomops freibergi</i>	-13.13333	-69.60000	Madre de Dios	2008
I297	I297	0	0	Negative	<i>Engystomops freibergi</i>	-13.13333	-69.60000	Madre de Dios	2008
I226	I226	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.57139	-70.07472	Madre de Dios	2008
I233	I233	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2008
I235	I235	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2008
I238	I238	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2008

(continued)

Table A1. Continued

Sample no.	Sample ID	Bd load	Bd pres.	Record	Species	Latitude	Longitude	State province	Year collected
1240	1240	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2008
1264	1264	0	0	Negative	<i>Hamptophryne boliviana</i>	-13.13333	-69.60000	Madre de Dios	2008
1270	1270	0	0	Negative	<i>Hamptophryne boliviana</i>	-13.13333	-69.60000	Madre de Dios	2008
1295	1295	0	0	Negative	<i>Hamptophryne boliviana</i>	-13.13333	-69.60000	Madre de Dios	2008
1340	1340	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.44916	-70.25167	Madre de Dios	2008
1333	1333	0	0	Negative	<i>Hemiphractus helioi</i>	-12.44916	-70.25167	Madre de Dios	2008
1231	1231	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2008
1236	1236	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2008
1268	1268	0	0	Negative	<i>Boana</i> sp. Clade G	-13.13333	-69.60000	Madre de Dios	2008
1272	1272	0	0	Negative	<i>Boana</i> sp. Clade G	-13.13333	-69.60000	Madre de Dios	2008
1292	1292	0	0	Negative	<i>Boana</i> sp. Clade G	-13.13333	-69.60000	Madre de Dios	2008
1293	1293	0	0	Negative	<i>Boana</i> sp. Clade G	-13.13333	-69.60000	Madre de Dios	2008
1309	1309	0	0	Negative	<i>Boana</i> sp. Clade G	-13.13333	-69.60000	Madre de Dios	2008
1345	1345	0	0	Negative	<i>Boana</i> sp. Clade G	-12.44916	-70.25167	Madre de Dios	2008
1322	1322	0	0	Negative	<i>Boana cinerascens</i>	-12.56861	-70.09917	Madre de Dios	2008
1332	1332	0	0	Negative	<i>Boana cinerascens</i>	-12.56861	-70.09917	Madre de Dios	2008
1356	1356	0	0	Negative	<i>Boana cinerascens</i>	-12.44916	-70.25167	Madre de Dios	2008
1328	1328	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2008
1321	1321	0	0	Negative	<i>Boana geographica</i>	-12.56861	-70.09917	Madre de Dios	2008
1350	1350	0	0	Negative	<i>Boana geographica</i>	-12.44916	-70.25167	Madre de Dios	2008
1250	1250	0	0	Negative	<i>Boana lanciformis</i>	-12.56861	-70.09917	Madre de Dios	2008
1279	1279	0	0	Negative	<i>Boana lanciformis</i>	-13.13333	-69.60000	Madre de Dios	2008
1223	1223	0	0	Negative	<i>Leptodactylus cf petersii</i>	-12.57139	-70.07472	Madre de Dios	2008
1290	1290	0	0	Negative	<i>Leptodactylus didymus</i>	-13.13333	-69.60000	Madre de Dios	2008
1259	1259	0	0	Negative	<i>Leptodactylus knudseni</i>	-13.13333	-69.60000	Madre de Dios	2008
1304	1304	0	0	Negative	<i>Lithodytes lineatus</i>	-13.13333	-69.60000	Madre de Dios	2008
1308	1308	0	0	Negative	<i>Lithodytes lineatus</i>	-13.13333	-69.60000	Madre de Dios	2008
1228	1228	0	0	Negative	<i>Leptodactylus petersii</i>	-12.57139	-70.07472	Madre de Dios	2008
1232	1232	0	0	Negative	<i>Leptodactylus petersii</i>	-12.56861	-70.09917	Madre de Dios	2008
1244	1244	0	0	Negative	<i>Leptodactylus petersii</i>	-12.56861	-70.09917	Madre de Dios	2008
1307	1307	0	0	Negative	<i>Leptodactylus petersii</i>	-13.13333	-69.60000	Madre de Dios	2008
1327	1327	0	0	Negative	<i>Leptodactylus petersii</i>	-12.56861	-70.09917	Madre de Dios	2008
1331	1331	0	0	Negative	<i>Leptodactylus petersii</i>	-12.56861	-70.09917	Madre de Dios	2008
1306	1306	0	0	Negative	<i>Leptodactylus rhodomystax</i>	-13.13333	-69.60000	Madre de Dios	2008
1273	1273	0	0	Negative	<i>Noblella myrmecoides</i>	-13.13333	-69.60000	Madre de Dios	2008
1313	1313	0	0	Negative	<i>Noblella myrmecoides</i>	-13.13333	-69.60000	Madre de Dios	2008
1269	1269	0	0	Negative	<i>Oreobates cruralis</i>	-13.13333	-69.60000	Madre de Dios	2008
1276	1276	0	0	Negative	<i>Oreobates cruralis</i>	-13.13333	-69.60000	Madre de Dios	2008
1323	1323	0	0	Negative	<i>Osteocephalus buckleyi</i>	-12.56861	-70.09917	Madre de Dios	2008
1329	1329	0	0	Negative	<i>Osteocephalus buckleyi</i>	-12.56861	-70.09917	Madre de Dios	2008
1243	1243	0	0	Negative	<i>Osteocephalus cf leprieurii</i>	-12.56861	-70.09917	Madre de Dios	2008
1285	1285	0	0	Negative	<i>Osteocephalus cf leprieurii</i>	-13.13333	-69.60000	Madre de Dios	2008
1277	1277	0	0	Negative	<i>Osteocephalus taurinus</i>	-13.13333	-69.60000	Madre de Dios	2008
1288	1288	0	0	Negative	<i>Osteocephalus taurinus</i>	-13.13333	-69.60000	Madre de Dios	2008
1311	1311	0	0	Negative	<i>Osteocephalus taurinus</i>	-13.13333	-69.60000	Madre de Dios	2008
1357	1357	0	0	Negative	<i>Osteocephalus taurinus</i>	-12.44916	-70.25167	Madre de Dios	2008
1299	1299	0	0	Negative	<i>Phyllomedusa camba</i>	-13.13333	-69.60000	Madre de Dios	2008
1339	1339	0	0	Negative	<i>Phyllomedusa camba</i>	-12.44916	-70.25167	Madre de Dios	2008
1291	1291	0	0	Negative	<i>Phyllomedusa tomopterna</i>	-13.13333	-69.60000	Madre de Dios	2008
1267	1267	0	0	Negative	<i>Phyllomedusa vaillantii</i>	-13.13333	-69.60000	Madre de Dios	2008
1284	1284	0	0	Negative	<i>Phyllomedusa vaillantii</i>	-13.13333	-69.60000	Madre de Dios	2008
1234	1234	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2008
1251	1251	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2008
1247	1247	0	0	Negative	<i>Pristimantis cruralis</i>	-12.56861	-70.09917	Madre de Dios	2008
1275	1275	0	0	Negative	<i>Pristimantis fenestratus</i>	-13.13333	-69.60000	Madre de Dios	2008
1249	1249	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2008
1252	1252	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2008
1253	1253	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2008
1255	1255	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2008

(continued)

Table A1. Continued

Sample no.	Sample ID	Bd load	Bd pres.	Record	Species	Latitude	Longitude	State province	Year collected
I283	I283	0	0	Negative	<i>Pristimantis reichlei</i>	-13.13333	-69.60000	Madre de Dios	2008
I287	I287	0	0	Negative	<i>Pristimantis reichlei</i>	-13.13333	-69.60000	Madre de Dios	2008
I302	I302	0	0	Negative	<i>Pristimantis reichlei</i>	-13.13333	-69.60000	Madre de Dios	2008
I303	I303	0	0	Negative	<i>Pristimantis reichlei</i>	-13.13333	-69.60000	Madre de Dios	2008
I319	I319	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2008
I341	I341	0	0	Negative	<i>Pristimantis reichlei</i>	-12.44916	-70.25167	Madre de Dios	2008
I261	I261	0	0	Negative	<i>Pristimantis skydmainos</i>	-13.13333	-69.60000	Madre de Dios	2008
I271	I271	0	0	Negative	<i>Pristimantis skydmainos</i>	-13.13333	-69.60000	Madre de Dios	2008
I220	I220	0	0	Negative	<i>Pristimantis</i> sp.	-12.56861	-70.09917	Madre de Dios	2008
I315	I315	0	0	Negative	<i>Pristimantis</i> sp.	-13.13333	-69.60000	Madre de Dios	2008
I257	I257	0	0	Negative	<i>Pristimantis toftae</i>	-13.13333	-69.60000	Madre de Dios	2008
I324	I324	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2008
I344	I344	0	0	Negative	<i>Pristimantis toftae</i>	-12.44916	-70.25167	Madre de Dios	2008
I278	I278	0	0	Negative	<i>Ranitomeya sirensis</i>	-13.13333	-69.60000	Madre de Dios	2008
I246	I246	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2008
I280	I280	0	0	Negative	<i>Rhinella margaritifera</i>	-13.13333	-69.60000	Madre de Dios	2008
I281	I281	0	0	Negative	<i>Rhinella margaritifera</i>	-13.13333	-69.60000	Madre de Dios	2008
I296	I296	0	0	Negative	<i>Rhinella margaritifera</i>	-13.13333	-69.60000	Madre de Dios	2008
I342	I342	0	0	Negative	<i>Rhinella margaritifera</i>	-12.44916	-70.25167	Madre de Dios	2008
I346	I346	0	0	Negative	<i>Rhinella margaritifera</i>	-12.44916	-70.25167	Madre de Dios	2008
I355	I355	0	0	Negative	<i>Rhinella marina</i>	-12.44916	-70.25167	Madre de Dios	2008
I318	I318	0	0	Negative	<i>Scarthyla goinorum</i>	-12.56861	-70.09917	Madre de Dios	2008
I320	I320	0	0	Negative	<i>Scarthyla goinorum</i>	-12.56861	-70.09917	Madre de Dios	2008
I354	I354	0	0	Negative	<i>Scinax garbei</i>	-12.44916	-70.25167	Madre de Dios	2008
I239	I239	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2008
I262	I262	0	0	Negative	<i>Scinax ictericus</i>	-13.13333	-69.60000	Madre de Dios	2008
I294	I294	0	0	Negative	<i>Scinax ictericus</i>	-13.13333	-69.60000	Madre de Dios	2008
I300	I300	0	0	Negative	<i>Scinax ictericus</i>	-13.13333	-69.60000	Madre de Dios	2008
I334	I334	0	0	Negative	<i>Scinax ictericus</i>	-12.44916	-70.25167	Madre de Dios	2008
I241	I241	0	0	Negative	<i>Scinax pedromedinae</i>	-12.56861	-70.09917	Madre de Dios	2008
I224	I224	0	0	Negative	<i>Syncope antenori</i>	-12.57139	-70.07472	Madre de Dios	2008
I225	I225	0	0	Negative	<i>Syncope antenori</i>	-12.57139	-70.07472	Madre de Dios	2008
I335	I335	0	0	Negative	<i>Syncope antenori</i>	-12.44916	-70.25167	Madre de Dios	2008
H42	H42	0	0	Negative	<i>Adenomera</i> sp.	-12.56861	-70.09917	Madre de Dios	2012
H8	H8	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2012
H27	H27	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2012
H35	H35	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2012
H5	H5	0	0	Negative	<i>Ameerega trivittata</i>	-12.56861	-70.09917	Madre de Dios	2012
H39	H39	0	0	Negative	<i>Ameerega trivittata</i>	-12.56861	-70.09917	Madre de Dios	2012
H43	H43	0	0	Negative	<i>Ameerega trivittata</i>	-12.56861	-70.09917	Madre de Dios	2012
H38	H38	0	0	Negative	<i>Bolitoglossa altamazonica</i>	-12.56861	-70.09917	Madre de Dios	2012
H21	H21	0	0	Negative	<i>Ceratophrys cornuta</i>	-12.56861	-70.09917	Madre de Dios	2012
H23	H23	0	0	Negative	<i>Ceratophrys cornuta</i>	-12.56861	-70.09917	Madre de Dios	2012
H11	H11	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2012
H15	H15	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2012
H31	H31	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2012
H36	H36	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2012
H13	H13	0	0	Negative	<i>Dendropsophus koechlini</i>	-12.56861	-70.09917	Madre de Dios	2012
H14	H14	0	0	Negative	<i>Dendropsophus schubarti</i>	-12.56861	-70.09917	Madre de Dios	2012
H18	H18	0	0	Negative	<i>Dendropsophus schubarti</i>	-12.56861	-70.09917	Madre de Dios	2012
H19	H19	0	0	Negative	<i>Edalorhina perezi</i>	-12.56861	-70.09917	Madre de Dios	2012
H40	H40	0	0	Negative	<i>Engystomops freibergi</i>	-12.56861	-70.09917	Madre de Dios	2012
H41	H41	0	0	Negative	<i>Engystomops freibergi</i>	-12.56861	-70.09917	Madre de Dios	2012
H58	H58	0	0	Negative	<i>Engystomops freibergi</i>	-12.56861	-70.09917	Madre de Dios	2012
H22	H22	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2012
H56	H56	0	0	Negative	<i>Boana geographica</i>	-12.56861	-70.09917	Madre de Dios	2012
H2	H2	0	0	Negative	<i>Boana cinerascens</i>	-12.56861	-70.09917	Madre de Dios	2012
H3	H3	0	0	Negative	<i>Boana cinerascens</i>	-12.56861	-70.09917	Madre de Dios	2012
H6	H6	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2012

(continued)

Table A1. Continued

Sample no.	Sample ID	Bd load	Bd pres.	Record	Species	Latitude	Longitude	State province	Year collected
H32	H32	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2012
H49	H49	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2012
H1	H1	0	0	Negative	<i>Boana lanciformis</i>	-12.56861	-70.09917	Madre de Dios	2012
H26	H26	0	0	Negative	<i>Leptodactylus didymus</i>	-12.56861	-70.09917	Madre de Dios	2012
H50	H50	0	0	Negative	<i>Noblella myrmecoides</i>	-12.56861	-70.09917	Madre de Dios	2012
H34	H34	0	0	Negative	<i>Phyllomedusa tomopterna</i>	-12.56861	-70.09917	Madre de Dios	2012
H37	H37	0	0	Negative	<i>Pristimantis altamazonicus</i>	-12.56861	-70.09917	Madre de Dios	2012
H9	H9	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2012
H33	H33	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2012
H43A	H43A	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2012
H44	H44	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2012
H45	H45	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2012
H63	H63	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2012
H46	H46	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2012
H29	H29	0	0	Negative	<i>Rhaebo guttatus</i>	-12.56861	-70.09917	Madre de Dios	2012
H12	H12	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2012
H16	H16	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2012
H17	H17	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2012
H28	H28	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2012
H30	H30	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2012
H51	H51	0	0	Negative	<i>Scinax ictericus</i>	-12.56861	-70.09917	Madre de Dios	2012
H10	H10	0	0	Negative	<i>Scinax pedromedinae</i>	-12.56861	-70.09917	Madre de Dios	2012
H24	H24	0	0	Negative	<i>Scinax pedromedinae</i>	-12.56861	-70.09917	Madre de Dios	2012
H25	H25	0	0	Negative	<i>Scinax pedromedinae</i>	-12.56861	-70.09917	Madre de Dios	2012
CH_016	RVM109	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_021	RVM114	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_024	RVM117	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_027	RVM120	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_028	RVM121	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_031	RVM124	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_035	RVM128	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_053	RVM146	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_055	RVM148	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_057	RVM150	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_074	RVM167	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_088	RVM181	0	0	Negative	<i>Adenomera andreae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_023	RVM116	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_025	RVM118	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_038	RVM131	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_052	RVM145	0	0	Negative	<i>Allobates trilineatus</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_043	RVM136	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_045	RVM138	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_062	RVM155	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_073	RVM166	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_082	RVM175	0	0	Negative	<i>Ameerega hahneli</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_051	RVM144	0	0	Negative	<i>Chiasmocleis bassleri</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_042	RVM135	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_046	RVM139	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_047	RVM140	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_060	RVM153	0	0	Negative	<i>Chiasmocleis royi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_048	RVM141	0	0	Negative	<i>Ctenophryne geayi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_075	RVM168	0	0	Negative	<i>Ctenophryne geayi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_076	RVM169	0	0	Negative	<i>Ctenophryne geayi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_089	RVM182	0	0	Negative	<i>Ctenophryne geayi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_040	RVM133	0	0	Negative	<i>Amazophrynella javierbustamantei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_067	RVM160	0	0	Negative	<i>Dendropsophus acreeanus</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_072	RVM165	0	0	Negative	<i>Dendropsophus parviceps</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_063	RVM156	0	0	Negative	<i>Dendropsophus schubarti</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_068	RVM161	0	0	Negative	<i>Dendropsophus schubarti</i>	-12.56861	-70.09917	Madre de Dios	2014

(continued)

Table A1. Continued

Sample no.	Sample ID	Bd load	Bd pres.	Record	Species	Latitude	Longitude	State province	Year collected
CH_070	RVM163	0	0	Negative	<i>Dendropsophus triangulum</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_083	RVM176	0	0	Negative	<i>Edalorhina perezii</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_015	RVM108	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_059	RVM152	0	0	Negative	<i>Hamptophryne boliviana</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_037	RVM130	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2014
CH_065	RVM158	0	0	Negative	<i>Boana</i> sp. Clade G	-12.56861	-70.09917	Madre de Dios	2014
CH_077	RVM170	0	0	Negative	<i>Leptodactylus cf-petersii</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_081	RVM174	0	0	Negative	<i>Leptodactylus cf-petersii</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_041	RVM134	0	0	Negative	<i>Noblella myrmecoides</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_005	RVM98	0	0	Negative	<i>Oreobates cruralis</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_050	RVM143	0	0	Negative	<i>Oreobates cruralis</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_058	RVM151	0	0	Negative	<i>Oreobates cruralis</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_071	RVM164	0	0	Negative	<i>Osteocephalus leprieurii</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_013	RVM106	0	0	Negative	<i>Phyllomedusa vaillanti</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_004	RVM97	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_026	RVM119	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_032	RVM125	0	0	Negative	<i>Pristimantis buccinator</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_044	RVM137	0	0	Negative	<i>Pristimantis carvalhoi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_064	RVM157	0	0	Negative	<i>Pristimantis carvalhoi</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_001	RVM94	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_002	RVM95	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_008	RVM101	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_010	RVM103	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_012	RVM105	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_014	RVM107	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_018	RVM111	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_022	RVM115	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_030	RVM123	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_034	RVM127	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_054	RVM147	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_056	RVM149	0	0	Negative	<i>Pristimantis reichlei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_006	RVM99	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_007	RVM100	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_009	RVM102	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_036	RVM129	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_066	RVM159	0	0	Negative	<i>Pristimantis toftae</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_049	RVM142	0	0	Negative	<i>Rhaebo guttatus</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_011	RVM104	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_079	RVM172	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_080	RVM173	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_084	RVM177	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_085	RVM178	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_086	RVM179	0	0	Negative	<i>Rhinella margaritifera</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_003	RVM96	0	0	Negative	<i>Rhinella marina</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_087	RVM180	0	0	Negative	<i>Rhinella marina</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_061	RVM154	0	0	Negative	<i>Scartyla goinorum</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_069	RVM162	0	0	Negative	<i>Scartyla goinorum</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_078	RVM171	0	0	Negative	<i>Scartyla goinorum</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_033	RVM126	0	0	Negative	<i>Scinax garbei</i>	-12.56861	-70.09917	Madre de Dios	2014
CH_017	RVM110	0	0	Negative	<i>Scinax pedromedinae</i>	-12.56861	-70.09917	Madre de Dios	2014

Note. NA = not applicable; Bd = Batrachochytrium dendrobatidis.

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References

- Becker, C. G., Rodriguez, D., Lambertini, C., Toledo, L. F., & Haddad, C. F. B. (2016). Historical dynamics of *Batrachochytrium dendrobatidis* in Amazonia. *Ecography*, *39*, 954–960.
- Boyle, D., Boyle, D., Olsen, V., Morgan, J., & Hyatt, A. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, *60*, 141–148.
- Briggs, C. J., Knapp, R. A., & Vredenburg, V. T. (2010). Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proceedings of the National Academy of Sciences*, *107*(21), 9695–9700.
- Catenazzi, A., Lehr, E., & May, R.V. (2013). The amphibians and reptiles of Manu National Park and its buffer zone, Amazon basin and eastern slopes of the Andes, Peru. *Biota Neotropica*, *13*(4), 269–283.
- Catenazzi, A., Lehr, E., Rodriguez, L. O., & Vredenburg, V. T. (2011). *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the Upper Manu National Park, Southeastern Peru. *Conservation Biology*, *25*, 382–391.
- Catenazzi, A., Lehr, E., & Vredenburg, V. T. (2014). Thermal physiology, disease and amphibian declines in the eastern slopes of the Andes. *Conservation Biology*, *28*, 509–517.
- Catenazzi, A., Swei, A., Finkle, J., Foreyt, E., Wyman, L., & Vredenburg, V. T. (2017). Epizootic to enzootic transition of a fungal disease in tropical Andean frogs: Are surviving species still susceptible? *PLoS One*, *12*, e0186478.
- Flechas, S. V., Sarmiento, C., Cardenas, M. E., Medina, E. M., Restrepo, S., & Amezcuita, A. (2012). Surviving chytridiomycosis: Differential anti-*Batrachochytrium dendrobatidis* activity in bacterial isolates from three lowland species of *Atelopus*. *PLoS One*, *7*, e44832.
- Flechas, S. V., Vredenburg, V. T., & Amézquita, A. (2015). Infection prevalence in three lowland species of harlequin toads from the threatened genus *Atelopus*. *Herpetological Review*, *46*(4), 528–532.
- Greenspan, S. E., Bower, D. S., Webb, R. J., Roznik, E. A., Stevenson, L. A., Berger, L., . . . Alford, R. A. (2017). Realistic heat pulses protect frogs from disease under simulated rainforest frog thermal regimes. *Functional Ecology*, *31*(12), 2274–2286.
- Hyatt, A., Boyle, D., Olsen, V., Boyle, D., Berger, L., Obendorf, D., . . . Colling, A. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *73*, 175–192.
- James, T. Y., Toledo, L. F., Rödder, D., Leite, D. S., Belasen, A. M., Betancourt-Román, C. M., . . . Longcore, J. E. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: Lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution*, *5*(18), 4079–4097.
- Johnson, M. L., Berger, L., Phillips, L., & Speare, R. (2003). Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, *57*, 255–260.
- Kosch, T., Morales, V., & Summers, K. (2012). *Batrachochytrium dendrobatidis* in Peru. *Herpetological Review*, *43*(2), 150–159.
- Kruger, K.M. & Hero, J.-M. (2007). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology*, *271*, 352–359.
- Marengo, J. (1984). Estudio sinóptico-climático de los Friajes en la Amazonia peruana. *Revista Forestal del Perú*, *12*, 55–80.
- Marengo, J., Cornejo, A., Satyamurty, P., Nobre, C., & Sea, W. (1997). Cold surges in tropical and extratropical South America: The strong event in June 1994. *Monthly Weather Review*, *125*(11), 2759–2786.
- McCracken, S., Gaertner, J. P., Forstner, M. R. J., & Hahn, D. (2009). Detection of *Batrachochytrium dendrobatidis* in amphibians from the forest floor to the upper canopy of an Ecuadorian Amazon lowland rainforest. *Herpetological Review*, *40*(2), 190–195.
- Menéndez-Guerrero, P. A., & Graham, C. H. (2013). Evaluating multiple causes of amphibian declines of Ecuador using geographical quantitative analyses. *Ecography*, *36*(7), 756–769.

- O'Hanlon, S. J., Rieux, A., Farrer, R. A., Rosa, G. M., Waldman, B., Bataille, A., . . . Martin, M. D. (2018). Recent Asian origin of chytrid fungi causing global amphibian declines. *Science*, *360*(6389), 621–627.
- Piotrowski, J. S., Annis, S. L., & Longcore, J. E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*, *96*, 9–15.
- Raffel, T. R., Rohr, J. R., Kiesecker, J. M. & Hudson, P. J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology*, *20*, 819–828.
- Raffel, T. R., Romansic, J. M., Halstead, N. T., McMahon, T. A., Venesky, M. D. & Rohr, J.R. (2012). Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, *3*, 146–151.
- Rödger, D., Kielgast, J., Bielby, J., Schmidlein, S., Bosch, J., Garner, T. W. J., . . . S. Lötters. (2009). Global amphibian extinction risk assessment for the panzootic chytrid fungus. *Diversity*, *1*, 52–66.
- Ron, S. R. (2005). Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. *Biotropica*, *37*, 209–221.
- Roznik, E. A., Sapsford, S. J., Pike, D. A., Schwarzkopf, L., & Alford, R. A. (2015). Natural disturbance reduces disease risk in endangered rainforest frog populations. *Scientific Reports*, *5*, 13472.
- Seimon, T. A., Seimon, A., Yager, K., Reider, K., Delgado, A., Sowell, P., . . . Halloy, S. (2017). Long-term monitoring of tropical alpine habitat change, *Andean anurans*, and chytrid fungus in the Cordillera Vilcanota, Peru: Results from a decade of study. *Ecology and Evolution*, *7*, 1527–1540.
- Stevenson, L. A., Alford, R. A., Bell, S. C., Roznik, E. A., Berger, L., & Pike, D. A. (2013). Variation in thermal performance of a widespread pathogen, the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *PLoS One*, *8*, e73830.
- Stevenson, L. A., Roznik, E. A., Alford, R. A., & Pike, D. A. (2014). Host-specific thermal profiles affect fitness of a widespread pathogen. *Ecology and Evolution*, *4*, 4053–4064.
- von May, R., Catenazzi, A., Angulo, A., Brown, J., Carrillo, J., Chávez, G., . . . Twomey, E. (2008). Current state of conservation knowledge on threatened amphibian species in Peru. *Tropical Conservation Science*, *1*, 376–396.
- von May, R., Jacobs, J. M., Jennings, R. D., Catenazzi, A., & Rodríguez, L. O. (2010). Anfíbios de los Amigos, Manu y Tambopata, Perú. *Rapid Color Guide*, *236*, 12.
- von May, R., Jacobs, J. M., Santa-Cruz, R., Valdivia, J., Huamán, J., & Donnelly, M. A. (2010). Amphibian community structure as a function of forest type in Amazonian Peru. *Journal of Tropical Ecology*, *26*, 509–519.
- von May, R., Siu-Ting, K., Jacobs, J. M., Medina-Müller, M., Gagliardi, G., Rodríguez, L. O., & Donnelly, M. A. (2009). Species diversity and conservation status of amphibians in Madre de Dios, Perú. *Herpetological Conservation and Biology*, *4*, 14–29.
- Vredenburg, V. T., Knapp, R. A., Tunstall, T. S., & Briggs, C. J. (2010). Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences*, *107*, 9689–9694.
- Voyles, J., Vredenburg, V. T., Tunstall, T. S., Parker, J. M., Briggs, C. J. & Rosenblum, E. B. (2012) Pathophysiology in Mountain Yellow-Legged Frogs (*Rana muscosa*) during a Chytridiomycosis Outbreak. *PLoS ONE*, *7*(4), e35374.
- Whitfield, S. M., Kerby, J., Gentry, L. R., & Donnelly, M. A. (2012). Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. *Biotropica*, *44*(6), 779–784.