

Short Communication

Chytridiomycosis and Amphibian Population Declines Continue to Spread Eastward in Panama

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Abstract: Chytridiomycosis is a globally emerging disease of amphibians and the leading cause of population declines and extirpations at species-diverse montane sites in Central America. We continued long-term monitoring efforts for the presence of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) and for amphibian populations at two sites in western Panama, and we began monitoring at three new sites to the east. Population declines associated with chytridiomycosis emergence were detected at Altos de Campana National Park. We also detected *Bd* in three species east of the Panama Canal at Soberanía National Park, and prevalence data suggests that *Bd* may be enzootic in the lowlands of the park. However, no infected frogs were found further east at Tortí (prevalence <7.5% with 95% confidence). Our results suggest that Panama's diverse and not fully described amphibian communities east of the canal are at risk. Precise predictions of future disease emergence events are not possible until factors underlying disease emergence, such as dispersal, are understood. However, if the fungal pathogen spreads in a pattern consistent with previous disease events in Panama, then detection of *Bd* at Tortí and other areas east of the Panama Canal is imminent. Therefore, development of new management strategies and increased precautions for tourism, recreation, and biology are urgently needed.

Keywords: amphibian, *Batrachochytrium dendrobatidis*, chytridiomycosis, emerging disease, Panama, population declines

The emerging disease chytridiomycosis, caused by the skin fungus *Batrachochytrium dendrobatidis* (*Bd*), has resulted in population declines and possible extinctions of hundreds of

amphibian species and caused subsequent ecosystem alterations (Lips et al., 2006; Whiles et al., 2006; Finlay and Vredenburg, 2007; Gascon et al., 2007; Skerratt et al., 2007; Verburg et al., 2007). The disease has spread in a predictable pattern southeastward throughout the Cordillera

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Central mountain range in Central America (Lips et al., 2006; Gagliardo et al., 2008). We continued long-term monitoring of two sites in western Panama: Fortuna Forest Reserve (Fortuna) and General de División Omar Torrijos Herrera National Park (Omar Torrijos) (Lips, 1999; Lips et al., 2006). We began monitoring three additional sites to the east predicted to be affected by chytridiomycosis (Altos de Campana, Soberanía, and Torti) (Lips et al., 2008).

Altos de Campana National Park (Campana, area: 4925 ha; elevational range: 140–1020 m) is a high elevation site just west of the Panama Canal and separated from the Cordillera Central by approximately 35 km of lowlands that include a mosaic of heavily modified land cover. Bordering the canal to the east, Soberanía National Park (Soberanía, area: 19,541 ha; elevational range: 26–332 m) is 30 km north of Panama City. These parks contain a relatively high diversity of amphibian species; 65 species have been recorded at Campana (59 anuran, 4 salamander, and 2 caecilian species) and 56 at Soberanía (53 anuran, 2 salamander, and 1 caecilian species). Generalized drastic amphibian population declines have not previously been reported from these sites, the lowlands along the canal, or anywhere east of the canal in Panama (Condit et al., 2001; Ibáñez et al., 2002), and the fungal pathogen was not previously detected.

We surveyed amphibian assemblages for diversity and disease at five sites ranging across Panama (Fig. 1). Each survey included two people searching along forest and stream transects for approximately 10 hours over 2–3 nights. Amphibians were captured by hand in new plastic bags and swabbed using sterile technique 10 times each on the ventral surface, thighs, and feet according to Hyatt et al. (2007). Diagnosis of *Bd* infection status was performed by Taqman real-time PCR assay according to Boyle et al. (2004). All samples were analyzed in triplicate (except samples from Campana, June–August 2006, run once) and compared with Australian Animal Health Laboratory zoospore standards.

The mean prevalence of infection and 95% confidence interval based on a binomial distribution are recorded for each site and survey period (Table 1). All site-survey periods were then designated into one of four disease categories: naïve, emerging, epizootic, or enzootic (Table 1). We defined “naïve” as any amphibian assemblage in which *Bd* was never detected and population declines had not been observed. The “emerging” disease category indicates that *Bd* infections were recently detected (the first instance) and no signs of chytridiomycosis or population declines were observed. The “epizootic” disease category indicates that *Bd* infections were detected and associated with disease and population declines.

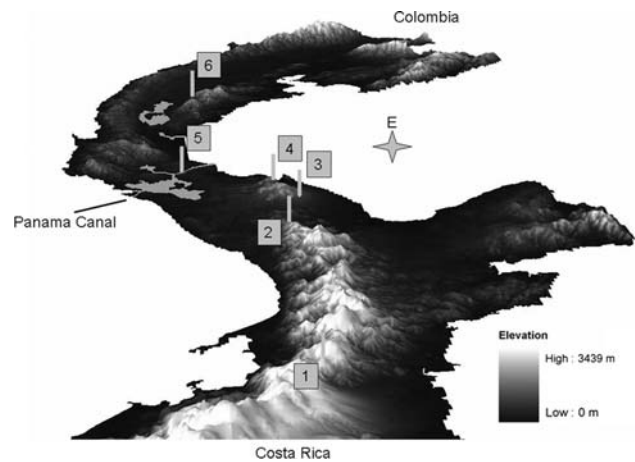


Figure 1. Elevation map projection of Panama displaying sites and dates of first detection of *B. dendrobatidis* in amphibian populations. Sites are described in Table 2. Produced by Matthew Becker using ArcGIS Version 9.2, Environmental Systems Research Institute, Inc., Redlands, CA with data available from the United States Geological Survey, EROS Data Center, Sioux Falls, SD based on the World Geodetic coordinate System of 1984 (WGS84).

The “enzootic” disease category indicates that *Bd* infections were present after population declines.

Amphibian declines associated with *Bd* were well documented at three sites where amphibian diversity (richness and abundance) remains low: Fortuna, Omar Torrijos, and El Valle (Lips, 1999; Lips et al., 2006; Gagliardo et al., 2008). Our sampling revealed that *Bd* persists at Fortuna 11 years after disease emergence, and is now enzootic at these sites (Table 1). At Campana, we monitored amphibian populations for 3 years. Our results showed sharp population declines occurred after the detection of *Bd* in June 2006, and sometime between August 2006 and January 2007 (Fig. 2A). A decline in species richness after the epizootic was also evident (Appendix). Koch’s postulates were fulfilled for the common rocket frog *Colostethus panamensis* sampled from Campana (Lips et al., 2006). Thus, the timing of disease emergence and associated population declines at Campana is on a similar scale to that shown for Omar Torrijos (Lips et al., 2006).

We detected infections on three species at Soberanía in a January 2007 survey. These species persisted at the site and we did not detect a population decline upon repeat survey in February 2008. Earlier surveys for disease at Soberanía are not known, but comparisons to infection prevalence and disease category of other sites suggests that *Bd* may already be enzootic at Soberanía (Fig. 2B). *Bd* was not detected (actual prevalence <7.5% with 95% confidence) at Parque Natural San Francisco (area: 1500 ha;

Table 1. Prevalence of Infection with *Batrachochytrium dendrobatidis* (*Bd*) in Panama^a

Location	Date	<i>N</i> species sampled	Infection prevalence (%), (<i>N</i> infected/ <i>N</i> swabbed)	95% Confidence interval	Disease category
Fortuna	1993–1995	35	0 ^{b,c}		Naïve
	Dec 1996	10	^{c,d}		Epizootic
	Jan 2006	3	18.2 (2/11)	2.3–51.8	Enzootic
	Jan 2007	2	42.9 (3/7)	9.9–81.6	Enzootic
Omar Torrijos	Jan 2004	9	0 (0/61)	0–5.9	Naïve
	2000–Sept 2004	59	0 (0/1566) ^e	0–0.0024	Naïve
	Oct–Dec 2004	48	49.2 (432/879) ^e	45.8–52.5	Epizootic
	Jan 2005	6	23.8 (5/21)	8.2–47.2	Enzootic
	Jan 2006	3	0 (0/3)	0–70.8	Enzootic
	Jan 2007	1	0 (0/10)	0–30.9	Enzootic
	Aug 2007	11	5.1 (3/59)	1.1–14.2	Enzootic
Barrigón–Omar Torrijos	Jan 2005	1	100.0 (12/12)	73.5–100	Epizootic
Rio Blanco–Omar Torrijos	Jan 2007	1	60.0 (6/10)	26.2–87.8	Epizootic
El Valle	Jan 2005	2	25.0 (1 ^f /4)	0.6–80.6	Naïve
	Apr 2006		^g		Epizootic
Chica/Campana	Jan 2005	1	0 (0/32)	0–10.9	Naïve
Campana	Jan 2005	14	0 (0/145)	0–2.5	Naïve
	Jan 2006	4	0 (0/27)	0–12.8	Naïve
	June–Aug 2006	25	12.8 (34/266)	9.0–17.4	Emerging
	Jan 2007	4	47.1 (8/17)	23.0–72.2	Epizootic
	Aug 2007	3	5.0 (1/20)	0.1–24.9	Enzootic
Soberanía	Jan 2007	3	30.0 (9/30)	14.7–49.4	Possibly enzootic
Tortí	Aug 2007	8	0 (0/49)	0–7.25	Naïve

^aSites are categorized as naïve, emerging, epizootic, or enzootic based on *Bd* presence, disease occurrence, and population declines (see text).

^bNo indications of *Bd*.

^cLips, 1999.

^dBerger et al., 1998.

^eLips et al., 2006.

^fSuspicious positive.

^gGagliardo et al., 2008.

elevational range: 120–415 m) near Tortí (Tortí), bordering the Darién Province as of August 2007 (Table 1).

Based on the sequential geographic pattern of *Bd* detection and amphibian declines, we calculated the putative rate of pathogen spread between adjacent sites (Table 2). By our most conservative estimate, amphibian declines are not predicted to occur at Tortí until September 2012. However, it is equally plausible that *Bd* is already present at Tortí if it disperses at a rate equivalent to that calculated for Campana. Without knowing the dispersal mechanism or other factors underlying disease emergence, it is not possible to make more precise predictions.

Chytridiomycosis epizootics caused by *Bd* are continuing to spread among Central American amphibians

resulting in population declines and extirpations at montane sites. Amphibian species extirpations and reduced population sizes can have cascading effects on both aquatic and terrestrial ecosystems (Whiles et al., 2006; Finlay and Vredenburg, 2007; Verburg et al., 2007). Chytridiomycosis is clearly an emerging disease of amphibians, but many questions remain (McCallum, 2005). Is chytridiomycosis caused by novel introductions and subsequently spread in epizootic waves (Lips et al., 2006, 2008), or are changing environmental conditions or other cofactors causing *Bd* to emerge as an amphibian pathogen (Pounds et al., 2006)? Our data suggest that, at montane sites, *Bd* may emerge as a novel pathogen, cause population collapse of multiple species, and then become locally enzootic. This transition

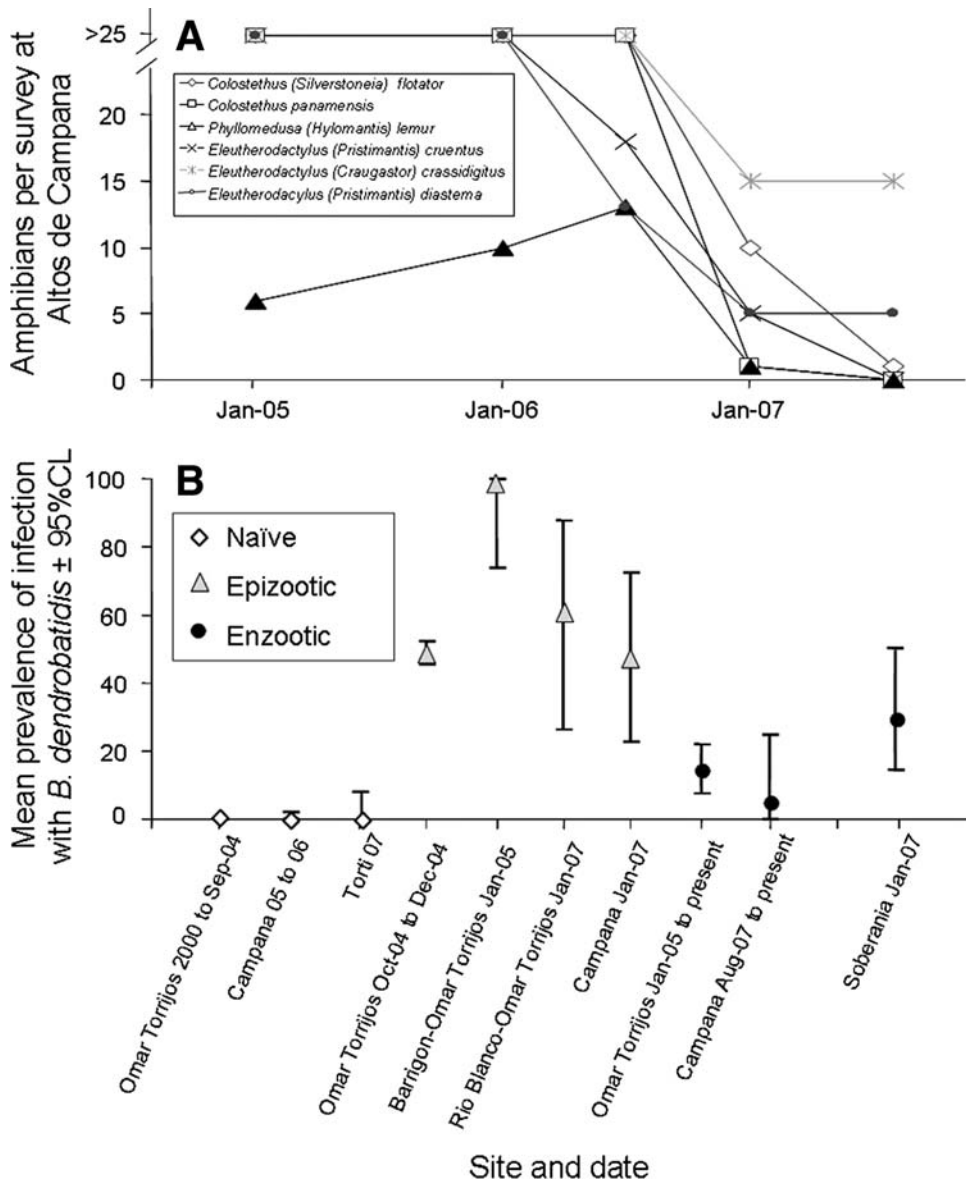


Figure 2. (A) Population trends of six amphibian species at Altos de Campana National Park. Infection of amphibians with *B. dendrobatidis* was first detected at this site in June 2006. A survey includes two people \times 2–3 nights search effort. (B) Mean prevalence of infection with *B. dendrobatidis* across sites categorized as naïve, emerging, or enzootic (see Table 1). Infection prevalence at Soberanía in January 2007 is displayed for comparison. Contributing to the variation shown here, overall infection prevalence may be influenced by species, elevation, and season sampled.

in disease category is consistent with a study attributing a chytridiomycosis outbreak in *Rana muscosa* to possibly a single *Bd* genotype (Morgan et al., 2007). At tropical lowland sites, chytridiomycosis may not be as severe (e.g., Woodhams and Alford, 2005; Puschendorf et al., 2006; Whitfield et al., 2007). Thus, we suspect that amphibians in the lowlands along the Panama Canal may carry *Bd* without developing clinical signs of chytridiomycosis.

We detected *Bd* at Soberanía, a lowland site east of the Panama Canal. Because this site was not previously surveyed for *Bd*, it is difficult to determine when or how it may have arrived. Evidence including infection prevalence (Fig. 2B) and repeat surveys indicates that *Bd* at Soberanía is possibly enzootic. Tropical lowland sites in which populations remain relatively stable may be reservoirs for *Bd*.

Puschendorf et al. (2006) showed that *Bd* was present in lowlands and highlands and appears to be enzootic throughout most of Costa Rica, but population declines occurred most frequently in the highlands. In South America, detection of chytridiomycosis associated population declines was most common at high elevations (Lips et al., 2008). Similarly, the emergence of chytridiomycosis in the Australian wet tropics may have impacted amphibian diversity at highland sites more severely than in the lowlands (McDonald and Alford, 1999). Consistent access to high temperatures or behavioral modification may inhibit infections from developing into chytridiomycosis in tropical lowlands (Woodhams et al., 2003).

For Campana, we demonstrated that *Bd* was probably not present before detection in June 2006 (0 of 204 swabs

Table 2. Theoretical Rates of Spread of *B. dendrobatidis* in Panama Assuming That the Pathogen Is Introduced from Adjacent Sites^a

Site	Name (date <i>B. dendrobatidis</i> first detected)	Latitude	Longitude	Distance from previous site (km)	Months before next epizootic	Rate of spread (km/month)
1	Fortuna (Dec 1996)	N08°43'30.0	W082°14'0.0			2.75 ^b
2	Omar Torrijos, El Copé (Sept 2004)	N08°40'12.8	W080°35'36.1	182	94	1.9
3	El Valle (Apr 2006)	N08°34'48.0	W080°10'12.0	48	19	2.5
4	Altos de Campana (June 2006)	N08°40'30.1	W079°55'39.2	29	2	14.5
5	Soberanía (Jan 2007)	N09°40'30.7	W079°39'32.4	54	7	7.7
6	Parque Natural San Francisco, Tortí (not detected)	N08°56'20.0	W078°27'44.2	134	9–69 ^c	1.9–14.5

^aDate that *Bd* was first detected is an estimate for *Bd* appearance in amphibians at a given site; pre-decline data on the prevalence of *Bd* is not available from Fortuna.

^bPrevious amphibian population declines caused by chytridiomycosis were reported from 1993 at Las Tablas, Puntarenas Province, Costa Rica (8°55'N, 82°44'W) (Lips, 1998; Lips et al., 2008).

^cAmphibian population declines are predicted to occur at Parque Natural San Francisco upon arrival of *B. dendrobatidis*. Depending upon the rate of spread, an epizootic may occur as late as September 2012 or as early as September 2007.

were positive) (Fig. 2B). *Bd* may have been present before our surveys at Soberanía, and examination of museum specimens would be informative. The theoretical rate of spread (Table 2) to Soberanía and Campana when calculated from adjacent epizootic sites was nearly an order of magnitude higher than that to Omar Torrijos and El Valle. This may indicate a separate introduction (e.g., Lips et al., 2008) rather than spread from a single source. Alternatively, changing environmental conditions may be partly responsible for chytridiomycosis emergence at high elevations (Pounds et al., 2006), although this has not been investigated in Panama. The proximity of Soberanía and Campana to Panama City (human population approximately 1 million) and the many tourists visiting the parks suggests human introduction is a strong possibility. Although the mechanism of dispersal between sites is not known, infection can be transmitted between amphibians by direct contact or exposure to water or substrates with infectious zoospores. Movement patterns of several species of amphibians, mainly forest floor and understory frogs, are related to seasonal rainfall in central Panama. Seasonal drying conditions of the forest floor and vegetation induce frogs to move in search of moist places, resulting in frog congregations along stream margins and drying pools (Toft et al., 1982; Ibáñez et al., 1995 [1997]; Ibáñez et al., 2002). If chytridiomycosis emergence is density dependent, these concentrations of individuals of various species of amphibians in moist areas may increase the probability of *Bd* transmission, and could partly explain a rapid rate of spread of *Bd* in central Panama. The mature sporangia

stage can attach and grow on sterile moist soil and bird feathers (Johnson and Speare, 2005). Reptile and fish scales or invertebrates have not been ruled out as possible reservoirs. Zoospores can survive for hours to days depending on temperature, and a sudden drop in temperature can induce zoospore release (Woodhams et al., 2008).

Lips et al. (2008) hypothesized that certain habitats may slow the spread of *Bd* or prevent invasion. Because the fungus dies at temperatures exceeding 30°C and in salt water (Longcore et al., 1999; Johnson et al., 2003), hot deforested lowlands, the freshwater Panama Canal, and the marine coastal environment may be physical barriers that could slow the spread of *Bd* in Panama. We did not test these barriers to dispersal specifically, but suggest that human movement of the pathogen would easily overcome these barriers.

The significance of our results is that amphibian population declines associated with chytridiomycosis are continuing in Panama. Amphibian populations, species, and by extension ecosystems naïve to *Bd*, particularly at high elevation sites east of the Panama Canal, may be at risk. In addition, the detection of *Bd* in Colombian museum specimens (Ruiz and Rueda-Almonacid, 2008) raises the potential for disease spread from the south (Lips et al., 2008). Future studies should focus greater sampling effort in eastern Panama.

A range of management options should be considered. Captive breeding programs such as at the El Valle Amphibian Conservation Center in Panama and at zoos associated with the amphibian ark project (<http://www.amphibianark.org>, March 5, 2008) have already been successful at preserving some species in captivity

(Gagliardo et al., 2008). However, repatriation is complicated by enzootic *Bd* at all sites with a history of disease. Thus, development of local conservation management options is urgently needed to preserve a significant portion of Panama's large and not fully described amphibian diversity and functions within their natural ecosystems. Presently, biosecurity should be increased for scientists and ecotourism, including bleaching boots and cleaning field gear between sites, and providing information at eco-lodges about the spread of epizootics.

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APPENDIX

Amphibian Species Encountered at Campana before (Six Surveys) and after the Chytridiomycosis Epizootic^a

Species	Present before epizootic	Present January 2006	Present August 2007
<i>Anotheca spinosa</i>	x	x	x
<i>Bufo (Rhinella) marinus</i>	x	x	
<i>Smilisca sila</i>	x	x	
<i>Centrolene ilex</i>	x		
<i>Colostethus (Silverstoneia) flotator</i>	x	x	x
<i>Colostethus (Silverstoneia) nubicola</i>	x	x	
<i>Colostethus panamensis</i>	x	x	
<i>Colostethus pratti</i>	x		
<i>Colostethus (Allobates) talamancae</i>	x		
<i>Cochranella euknemos</i>	x	x	
<i>Cochranella albomaculata</i>	x	x	
<i>Dendrobates (Ranitomeya) minutus</i>	x		
<i>Phyllomedusa (Hylomantis) lemur</i>	x	x	
<i>Eleutherodactylus (Limnophys) bufoniformis</i>	x		
<i>Eleutherodactylus (Pristimantis) pardalis</i>	x	x	
<i>Eleutherodactylus (Craugastor) gollmeri</i>	x	x	x
<i>Eleutherodactylus (Pristimantis) cruentus</i>	x	x	
<i>Eleutherodactylus (Pristimantis) caryophyllaceus</i>	x	x	
<i>Eleutherodactylus (Craugastor) crassidigitus</i>	x	x	x
<i>Eleutherodactylus (Pristimantis) taeniatus</i>	x	x	
<i>Eleutherodactylus (Pristimantis) diastema</i>	x	x	x
<i>Eleutherodactylus (Pristimantis) gaigeae</i>	x		
<i>Eleutherodactylus (Craugastor) megacephalus</i>	x		
<i>Eleutherodactylus (Craugastor) punctariolus</i>	x		
<i>Eleutherodactylus (Pristimantis) ridens</i>	x		
<i>Oedipina</i> sp.	x		

^aMore in depth post-epizootic amphibian surveys are needed to fully assess the loss of species richness at this site; columns do not represent equal sampling effort. Scientific names preserve name recognition according to Smith and Chiszar (2006) and include taxonomically reorganized subgenus in parentheses (Frost et al., 2006).

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