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Short communication

Widespread occurrence of an emerging pathogen in amphibian communities of the Venezuelan Andes

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ARTICLE INFO

Article history:
Received 28 February 2008
Received in revised form
22 July 2008
Accepted 8 August 2008
Available online 24 September 2008

Keywords:
Chytridiomycosis
Amphibian declines
Lithobates catesbeianus
Dendropsophus meridensis
Batrachochytrium dendrobatidis
Prevalence

ABSTRACT

Many recent amphibian declines have been associated with chytridiomycosis, a cutaneous disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*, but increasing evidence suggests that this pathogen may coexist with some species without causing declines. In the Venezuelan Andes, the disappearance of three anuran species during the late eighties was attributed to *B. dendrobatidis*. Recently, this pathogen was found to be prevalent in this region on the introduced American bullfrog, *Lithobates catesbeianus*. As a first step toward assessing the risk of amphibian communities to *B. dendrobatidis* in this region, we conducted a broad survey across multiple habitat types and an altitudinal gradient spanning over 2000 m. We diagnosed 649 frogs from 17 species using real time and conventional PCR assays, and recorded relevant abiotic characteristics of host habitats. Infection was detected in 10 native species of pond, stream and terrestrial habitats from 80–2600 m, representing nine new host records. *L. catesbeianus* was the most important reservoir with 79.9% of individuals infected and an average of 2299 zoospores. Among native frogs, *Dendropsophus meridensis*, an endangered species sympatric with *L. catesbeianus*, showed the highest infection prevalence and mean zoospore load (26.7%; 2749 zoospores). We did not detect clinical signs of disease in infected hosts; however, species such as *D. meridensis* may be at risk if environmental stress exacerbates vulnerability or pathogen loads. While surveillance is an effective strategy to identify highly exposed species and habitats, we need to understand species-specific responses to *B. dendrobatidis* to stratify risk in amphibian communities.

1. Introduction

Increasing evidence suggests that several factors may be involved in the recent emergence of chytridiomycosis (Lips et al., 2006; Pounds et al., 2006, 2007; Alford et al., 2007; Di Rosa et al., 2007), a fungal disease linked to worldwide population declines and extinctions of several amphibians (Berger et al., 1998, 1999; Daszak et al., 1999; Longcore et al., 1999; Lips

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0006-3207/$ - see front matter
doi:10.1016/j.biocon.2008.08.009

et al., 2006). While some data suggests that the recent spread of the causative agent of this disease, *Batrachochytrium dendrobatidis* (*Bd*), has caused the mortality of susceptible species in naïve populations (Berger et al., 1999; Daszak et al., 1999; Lips et al., 2006; Skerratt et al., 2007), results from other studies indicate that climate shifts in recent decades have increased the vulnerability of amphibians to this disease at many highland localities (Pounds et al., 2006). Also, stressors such as crowding during dry episodes and physiological stress due to adverse climatic conditions or coinfections have also been hypothesized to promote disease development (Pounds et al., 1999; Lampo et al., 2006b; Alford et al., 2007; Di Rosa et al., 2007). However, the contribution of each of these factors in triggering chytridiomycosis outbreaks in several regions is still a matter of current debate (Alford et al., 2007; Di Rosa et al., 2007; Pounds et al., 2007; Skerratt et al., 2007; Lips et al., 2008; Pounds and Coloma, 2008).

Despite its negative impact on some frog species, *Bd* has been found in several non-declining frog species around the world (Beard and ÓNeill, 2005; Ouellet et al., 2005; Woodhams and Alford, 2005; Kriger and Hero, 2006; Lips et al., 2006; Puschendorf et al., 2006). The ability of some non-declining species to develop subclinical infection and maintain high infection prevalence (e.g., *Lithobates catesbeianus*) suggests that some host species are less vulnerable to this pathogen (Daszak et al., 2004; Hanselmann et al., 2004). Pathogens may also infect vulnerable populations without noticeable effects, if transmission rates are sufficiently low to keep mortality under recruitment rates. On the other hand, *Bd* currently persists in frog species that presumably suffered chytridiomycosis-induced declines in the past but whose populations are now recovering (e.g., *Taudactylus eungellensis* in Australia and *Atelopus cruciger* in Venezuela) (Retallick et al., 2004; Rodríguez-Contreras et al., 2008). Several explanations are possible: (1) virulence of the pathogen has decreased since the initial epidemic outbreak, (2) environmental stressors that compromised the vulnerability of frogs in the past are no longer present and (3) *Bd* transmission in recovering populations is still below the epidemic threshold. Understanding the factors affecting the prevalence and intensity of *Bd* infection among various host species, and what makes some hosts tolerant and others vulnerable, is crucial for predicting the impact this pathogen may have on amphibian communities.

In Central and South America, declines of at least 56 amphibian species have been associated with the presence of *Bd*; four of these are critically endangered species endemic to Venezuela (Berger et al., 1998; Lips, 1999; Ron and Merino-Viteri, 2000; Bonaccorso et al., 2003; Lips et al., 2003, 2004, 2006; Puschendorf, 2003; Burrowes et al., 2004; La Marca et al., 2005; Seimon et al., 2005; Carnaval et al., 2006; Lampo et al., 2006b). Although sampling of museum collections have not been extensive (431 museum specimens from 1920 to 2002 (Bonaccorso et al., 2003; Lampo et al., 2006b; Lampo and Señaris, 2006)), the earliest evidence of *Bd* in this country dates to 1986 from one *A. cruciger* from the Cordillera de la Costa (Bonaccorso et al., 2003). Also, seven specimens from three other declining *Atelopus* species collected during 1988 were found infected at the Cordillera de Mérida (Lampo et al., 2006b), a region that has 11 critically endangered species (IUCN, Conservation International and NatureServe, 2006. Global Amphibian Assessment. www.globalamphibians.org (accessed on 8th August, 2007)). It was recently proposed that an epidemic wave swept across the Cordillera de La Costa and the Cordillera de Mérida in Venezuela between 1977 and 1988 (Lips et al., 2008). However, the spatiotemporal distribution of infection in *Atelopus* frogs at the Cordillera de Mérida and at the Cordillera de la Costa, with four positive localities temporally concentrated (1986–1988) but spatially separated (70–445 km) suggests that *Bd* could have been present endemically below detectable levels, and particular climatic conditions triggered synchronized epidemics in Venezuelan during the late 1980's (Lampo et al., 2006b). While there have been no reports of massive mortalities in Venezuela, all infected *Atelopus* species disappeared for, at least, one decade (La Marca and Lötters, 1997; Manzanilla and La Marca, 2004; Lampo et al., 2006b; Rodríguez-Contreras et al., 2008). In the Venezuelan Andes, *Bd* was also detected in one *Leptodactylus* sp. and one *Mannophryne cordilleriana* collected in 1996 and 2002, respectively (Lampo et al., 2006b) and more recently, in *L. catesbeianus* populations (the American bullfrog, previously *Rana catesbeiana*), a recently introduced exotic species that carries high pathogen loads without clinical symptoms of disease (Hanselmann et al., 2004). Currently, there are no other *Bd* reports in the country.

Although most of the critically endangered species of the Cordillera de Mérida have not been observed in the last two decades, one recent rediscovery presents the possibility that some populations may be recovering to detectable levels (Barrio-Amorós, 2004). However, *Bd* will continue to threaten amphibian populations in the Andes if the climatic scenarios that promoted epidemics in the past repeat (Pounds et al., 2006). The historical and potential threat of chytridiomycosis to amphibian hosts, especially in the Andean region, and the incipient state of knowledge about *Bd* epidemiology together reinforce the need for infection surveillance in wild host populations to monitor the impact of this disease in potentially vulnerable areas (Daszak et al., 2007). As a first step towards identifying conservation priorities and assessing epidemiological risks for Andean amphibians to chytridiomycosis, we screened 17 frog species for *Bd* to (1) identify host species and key reservoirs species, (2) explore the species-specific variations in prevalence and *Bd* zoospore loads, and (3) characterize the altitudinal ranges and breeding habitats of host species.

2. Materials and methods

The study was conducted at the Cordillera de Mérida (Mérida State, Venezuela) between 8°30′N–71°37′W and 8°45′N–71°15′W, in an area that covers an altitudinal gradient between 80 and 2600 m and includes several vegetation types (cloud forest, semi-deciduous montane forest, and submontane moist forest (Ataroff and Sarmiento, 2004)), although many have been degraded due to farming activities (Fig. 1). A predominant characteristic of this area between 1800 and 2600 m of altitude is the presence of many artificial water reservoirs for cattle that are mainly used by the introduced bullfrog, *L. catesbeianus*.

We sampled 444 adult frogs from 16 species found in 22 ponds and 19 rivers or streams. Each water body was visited for a minimum of one hour three times during the study for