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Batrachochytrium dendrobatidis in Venezuela

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Venezuela is among the ten most biodiverse countries in the world, particularly in terms of amphibians (IUCN et al. 2006). However, 18 amphibian species endemic to this country are critically endangered (IUCN et al. 2006; Rodríguez and Rojas-Suárez 1995); seven of these have not been observed in their natural habitats since the early 1990s despite intensive sampling efforts during the last decade (La Marca and Lötters 1997; La Marca and Reinthaler 1991; Manzanilla and La Marca 2004). Although the causes for most of these declines are not well understood, the high prevalence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in museum specimens of species collected just before their

disappearance lead us to question whether chytridiomycosis was linked to the declines of some Venezuelan amphibians (Bonaccorso et al. 2003; Lampo et al. 2006b).

In Venezuela, the Cordillera de Mérida and the Cordillera de La Costa harbor most of the critically endangered species, including nine *Atelopus* species, a neotropical genus severely affected by declines (IUCN et al. 2006; La Marca et al. 2005; Lötters et al. 2004). As with other regions, most declines occurred at high altitudes in relatively pristine habitats. Other high-elevation pristine habitats where unusual mortalities of frogs were reported during the 1980s are the remote tepuis (tabletop mountains) of the Guianan Basin. This area is particularly interesting in light of hypotheses about human-mediated translocation of *Bd*, because human contact has been very limited on these tepuis (Lampo and Señaris 2006). Therefore, *Bd* should be absent from these remote and isolated areas.

To assess the risk that *Bd* represents to the Venezuelan amphibian biodiversity several studies have been conducted aiming to detect *Bd* in anuran species, identify hosts and reservoirs, quantify prevalence or identify variables affecting the prevalence and intensity of infection (Hanselmann et al. 2004; Lampo et al. 2006b; Lampo et al. 2006a; Lampo and Señaris 2006; Nicolás 2007; Rodríguez-Contreras et al. 2008; Sánchez et al. 2008). Based on these studies and one new host species reported here, we summarize the species and geographic distribution of *Bd* in Venezuela. Samples were taken from live wild frogs or museum specimens collected from three montane regions: the Cordillera de Mérida, the Cordillera de la Costa, and the Guianan tepuis (Table 1). In all these localities species had suffered declines (Bonaccorso et al. 2003; Lampo et al. 2006b; Lötters et al. 2004; Manzanilla and La Marca 2004) or unusual mortalities have been reported (Ayarzagüena et al. 1992; Gorzula and Señaris 1998). Detection of *Bd* from museum specimens was conducted using histology (Berger et al. 1999). Except for all infected bullfrogs reported in Hanselmann et al. (2004), all live specimens were diagnosed by conventional PCR (Annis et al. 2004) and real time PCR assays (rt-PCR) (Boyle et al. 2004) (Table 1). Standards for zoospore quantification in rt-PCR assays were provided by the Animal Health Laboratory (Australia). We adopted the species nomenclature published in the Amphibian Species of the World (Frost 2007) and listed the geographic coordinates for all sampling locations, except for that of *A. cruciger*. Whenever samples from a species included more than two locations, we provided the upper left and bottom right geographic coordinates for the distribution polygon (Table 1). Except for the two localities in the remote tepuis of the Guianan Basin, species with only negative records were not included; some were the result of small sample sizes and others had no geographic coordinates associated in the original publications.

In Venezuela, *Bd* has been detected in species that have suffered population crashes in the past (Bonaccorso et al. 2003), and also in many other common species showing no evidence of declines (Table 1). This pathogen appears to be widespread in amphibian communities of the Cordillera de Mérida between 8.50°N, 71.25°W and 8.67°N, 71.50°W, in altitudes from 120 to 2600 m (Fig. 1). Therein, *Bd* infects 17 species in six families found in ephemeral and permanent ponds, streams and terrestrial habitats of cloud forests or highly disturbed areas (Table 1) (Lampo et al. 2006b; Lampo et al. 2006a; Sánchez et al. 2008). Among infected species

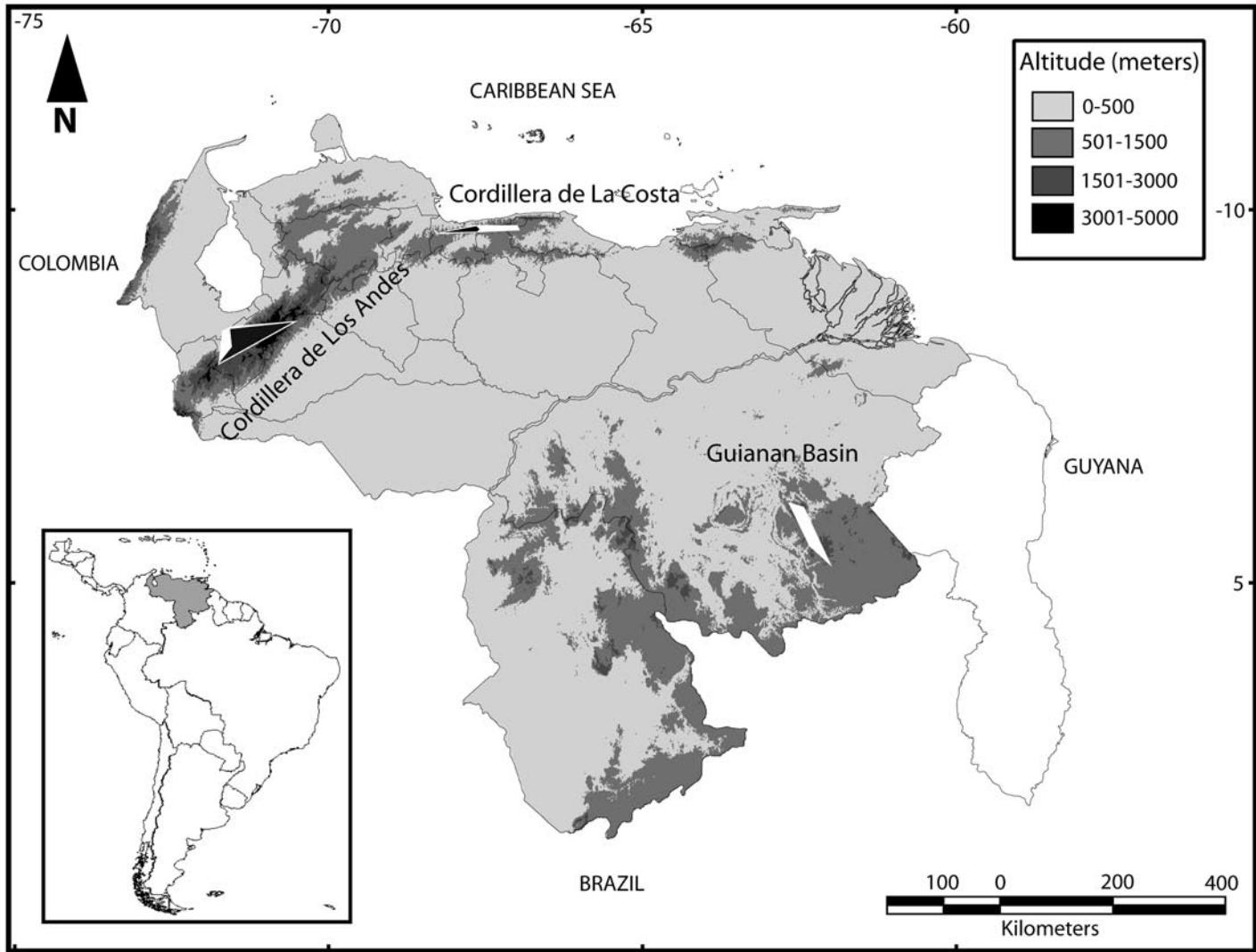


FIG. 1. Geographic distribution of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) in Venezuela. White polygons contain all sampling locations (positive and negative) and represent the areas sampled for *Bd*. Black polygons are subsets of the white polygons that contain all positive samples and delimit the areas for which we have evidence of infection. Polygons were constructed using the minimum convex method adopted by the IUCN for determining extent of occurrence (http://www.iucnredlist.org/info/categories_criteria1994).

in the Cordillera de Mérida, the American Bullfrog (*Lithobates catesbeianus* [formerly *Rana catesbeiana*]) appears to be a key reservoir; it has the highest prevalence of infection (80–96%) but no apparent clinical signs (Hanselmann et al. 2004; Sánchez et al. 2008). Although *L. catesbeianus* currently occupies an area less than 50 km², its geographic distribution is expanding. Thus, the exposure of endemic species to *Bd* is likely to increase as bullfrogs invade new geographic areas.

Most critically endangered species of the Cordillera de Mérida have not been observed since the early 1990s. Nonetheless, *Bd* appears to persist in remnant populations of some of these species. For example, it was detected in the only Andean *Atelopus* frog seen since 1994 (*Atelopus mucubajensis*) (Lampo et al. 2006a), and in one *Aromobates meridensis*, a species with a few known populations (IUCN et al. 2006). Although it is not well understood why some species have suffered crashes while many others coexist with *Bd* with no apparent effect, increasing evidence suggests that species-specific responses (Blaustein et al. 2005; Carey et al. 2006; Daszak et al. 2004; Lampo et al. 2006a; Nichols et al.

2001) and environmental stressors are important in determining the relationship between infection and disease (Alford et al. 2007; Berger et al. 2004; Daszak et al. 2004; Di Rosa et al. 2007; Pounds et al. 2006).

In the Cordillera de la Costa, two sympatric frogs, *Atelopus cruciger* and *Mannophryne herminae*, tested positive for *Bd* (Nicolás, 2007; Rodríguez-Contreras et al. 2008) (Fig. 1; Table 1). *Atelopus cruciger* is a critically endangered species that occurs in this region whose disappearance for almost two decades was associated with *Bd* (Bonaccorso et al. 2003). Before 1986, its altitudinal distribution ranged from sea level to 2400 m, however, all recently discovered populations are located below 500 m (Rodríguez-Contreras et al. 2008). In these populations the prevalence and intensity of infection appears to be low. However, one adult male was found morbid with excessive sloughing and severe infection (244,484 zoospores in 1–3 mg of tissue) (Rodríguez-Contreras et al. 2008). This evidence suggests that *A. cruciger* could be recovering only in those locations where *Bd* growth is limited by high temperatures. *Mannophryne herminae*, on the contrary, is a widely distributed frog of

TABLE 1. Geographic and species distribution of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd) in Venezuela. IUCN red list categories (see http://www.iucnredlist.org/info/categories_criteria2001): CR = critically endangered; NT = Near Threatened; EN = Endangered; LC = Least Concern; VU = Vulnerable. MHNLS = Museo de Historia Natural La Salle (voucher specimen number indicated).

Region/State/ Location	Species (Red list categories)	Bd detection (No. specimens) examined	Specimens examined	Method of detection	Date of occurrence
Cordillera de la Costa, Aragua					
1. Extremely sensitive to reveal	<i>Atelopus cruciger</i> (CR)	4	24	rt-PCR	2006 (Rodríguez-Contreras et al. 2003)
2. 10.40°N, 67.74°W 10.32°N, 67.65°W	<i>Manophryne herminae</i> (NT)	27	209	rt-PCR	2006–2007 (Nicolás 2007)
Cordillera de la Costa, Carabobo					
3. 10.3°N, 68.22°W	<i>Atelopus cruciger</i> (CR)	1	59	Histology	1986 (Bonaccorso et al. 2003)
Cordillera de Los Andes, Mérida					
4. 08.62°N, 71.47°W	<i>Aromobates meridensis</i> (CR)	1	5	rt-PCR	2006 (Present study, MHNLS 18995)
5. 08.65°N, 71.39°W	<i>Atelopus carbonerensis</i> (CR)	1	18	Histology	1988 (Lampo et al. 2006b)
6. 08.85°N, 70.71°W	<i>Atelopus mucubajensis</i> (CR)	2	14	Histology	1988 (Lampo et al. 2006b)
7. 08.84°N, 70.73°W	<i>Atelopus mucubajensis</i> (CR)	1	1	rt-PCR	2004 (Lampo et al. 2006a)
8. 08.26°N, 71.72°W	<i>Atelopus sorianoi</i> (CR)	4	17	Histology	1988 (Lampo et al. 2006b)
9. 08.69°N, 71.47°W 08.57°N, 71.33°W	<i>Dendropsophus meridensis</i> (EN)	28	105	rt-PCR	2003–2004 (Sánchez et al., <i>in press</i>)
10. 08.82°N, 71.46°W	<i>Engystomops pustulosus</i> (LC)	2	3	rt-PCR	2003, 2005 (Sánchez et al., <i>in press</i>)
11. 08.70°N, 71.44°W	<i>Gastrotheca nicefori</i> (LC)	1	1	rt-PCR	2004 (Sánchez et al., <i>in press</i>)
12. 08.69°N, 71.42°W	<i>Gastrotheca nicefori</i> (LC)	1	1	rt-PCR	2005 (Sánchez et al., <i>in press</i>)
13. 08.58°N, 71.33°W	<i>Hyloscirtus platyactylus</i> (VU)	1	1	rt-PCR	2005 (Sánchez et al., <i>in press</i>)
14. 08.58°N, 71.37°W 08.51°N, 71.31°W	<i>Hypsiboas crepitans</i> (LC)	1	20	rt-PCR	2003–2006 (Sánchez et al., <i>in press</i>)

TABLE 1. Continued.

Region/State/ Location	Species (Red list categories)	Bd detection (No. specimens)	Specimens examined	Method of detection	Date of occurrence
15. 08.49°N, 71.53°W	<i>Leptodactylus</i> sp.	1	1	Histology	1996
16. 08.60°N, 71.36°W	<i>Lithobates catesbeianus</i> (NT)	44	48	Histology	(Lampo et al. 2006b)
17. 8.65°N, 71.38°W 8.58°N, 71.31°W	<i>Lithobates catesbeianus</i> (NT)	279	408	rt-PCR/PCR	(Hanselmann et al. 2004)
18. 8.50°N, 71.55°W	<i>Manophryne collaris</i> (EN)	1	2	rt-PCR	2003–2005 (Sánchez et al., <i>in press</i>)
19. 08.88°N, 70.64°W	<i>Manophryne cordilleriana</i> (VU)	1	1	Histology	2005 (Sánchez et al., <i>in press</i>)
20. 8.78°N, 71.55°W	<i>Pseudis paradoxa</i> (LC)	1	1	rt-PCR	2002 (Lampo et al. 2006b)
21. 8.61°N, 71.62°W 8.51°N, 71.31°W	<i>Rhinella marina</i> (LC)	2	20	rt-PCR	2005 (Sánchez et al., <i>in press</i>)
22. 8.78°N, 71.58°W 8.51°N, 71.31°W	<i>Scarthyla vigilans</i> (LC)	4	35	rt-PCR	2003–2004; 2006 (Sánchez et al., <i>in press</i>)
Guianan Basin, Bolívar (Auyán-tepui Massif)	<i>Tepuihyla edelcae</i> (LC)	0	13	Histology	2004–2005 (Sánchez et al., <i>in press</i>)
23. 6.03°N, 62.67°W 5.95°N, 62.42°W	<i>Tepuihyla edelcae</i> (LC)	0	13	Histology	1983–1984 (Lampo and Señaris 2006)
Guianan Basin, Bolívar (Chimantá Massif)	<i>Tepuihyla edelcae</i> (LC)	0	24	Histology	1984–1986 (Lampo and Señaris 2006)]

the Cordillera de La Costa whose habitat overlaps extensively with that of *A. cruciger*. The presence of *Bd* in this species could affect the dynamics of chytridiomycosis in *A. cruciger*, depending on the relative magnitude of interspecific transmission (Nicolás 2007). Therefore, *in situ* conservation efforts for *A. cruciger* must necessarily include the monitoring of *Bd* infection in *M. herminae*.

The remote highlands of the Guianan tepuis is the only area in Venezuela where unusual mortalities were reported between 1984 and 1986, but no evidence of *Bd* was found in 37 museum specimens collected during those years (Fig. 1) (Lampo and Señaris 2006). No detection of *Bd* from museum specimens from this area do not necessarily indicate the absence of the pathogen. However, the accumulation of evidence from this and other secluded areas will be important in evaluating the hypothesis of human-mediated translocation of *Bd* (Lampo and Señaris 2006).

Conserving endangered amphibian species is a major environmental challenge. Rates of extinction in the Neotropics are among the highest in the world, yet data to inform conservation efforts remain scarce. Although several factors likely contribute alone and synergistically to amphibian declines in the Neotropics (La Marca et al. 2005; La Marca and Reithaler 1991; Lampo et al. 2006b; Pounds and Puschendorf 2004; Pounds et al. 1999; Pounds et al. 2006; Pounds and Crump 2004), amphibian chytridiomycosis appears to be a key factor (Bonaccorso et al. 2003; La Marca et al. 2005; Lampo et al. 2006b; Lips et al. 2003; Lips et al. 2005a; Lips et al. 2005b; Lips et al. 2006; Pounds et al. 2006; Pounds and Crump 2004; Puschendorf et al. 2006a; Rodríguez-Contreras et al. 2008). *Bd* also seems to be widespread among species for which there is no evidence of declines (Beard and O'Neill 2005; Kriger and Hero 2007; Nicolás, 2007; Ouellet et al. 2005; Puschendorf et al. 2006b; Sánchez et al. 2008; Woodhams and Alford 2005). A comprehensive map of pathogen distribution in relation to species declines as well as a thorough understanding of the relationship between infection and disease under different ecological and climatic scenarios are necessary before we can assess the risk associated with this emerging disease.

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NATURAL HISTORY NOTES

Instructions for contributors to Natural History Notes appear in Volume 39, Number 1 (March 2008).

CAUDATA – SALAMANDERS

ANEIDES AENEUS (Green Salamander) **DIGIT MORPHOLOGY.** Green Salamanders are known to have unique digits that are thought to be an adaptation to a climbing lifestyle on rock outcrops and trees (Petránka 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington D.C. 587 pp.). Although the complete osteology of the genus *Aneides* shows that *A. aeneus* has a curved terminal phalanx (Wake 1963. *J. Morphol.* 113:77–118), no studies have been done to determine

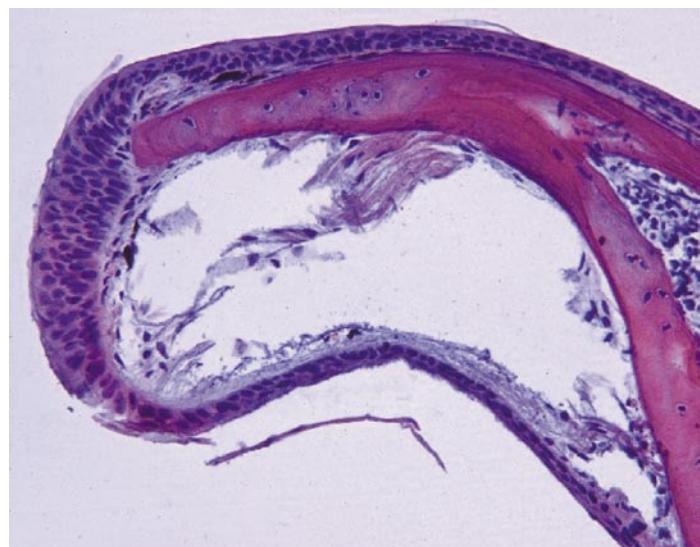


FIG. 1. Sagittal section showing terminal phalanx (TP), squamous tissue layer (Sq), and ventral position (V). 200x.

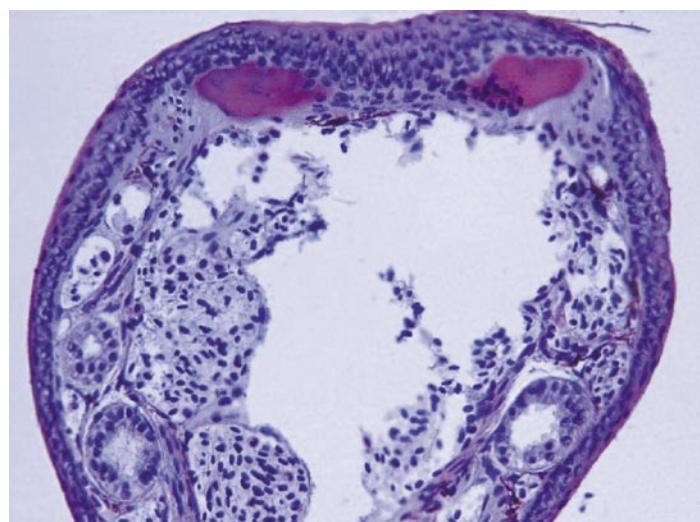


FIG. 2. Horizontal section showing doublet tips of terminal phalanx (TP), glandular cells (G), squamous tissue layer (Sq), and distal (Di) orientation. 200x.