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## First record of *Batrachochytrium dendrobatidis* in Nicaragua

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The infectious disease chytridiomycosis caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is considered one of the main culprits causing major amphibian declines and extinctions worldwide. It is known to occur on every continent except Antarctica and is particularly damaging to amphibian tropical populations. We studied 18 different amphibian species from three different localities in Nicaragua. Our results confirm the presence of *Bd* for first time in Nicaragua and involve ten amphibian species corresponding to Bufonidae, Craugastoridae, Hylidae and Ranidae. We additionally record *Bd* for the first time in four amphibian species: *Craugastor lauraster*, *Dendropsophus microcephalus*, *Smilisca baudinii* and *Lithobates brownorum*.

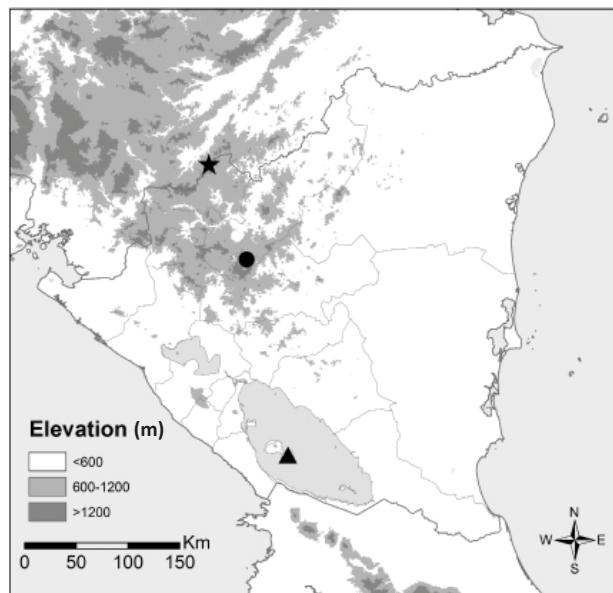
**Key words:** *Bd*, Chytridiomycosis, Datanlí-El Diablo, infectious disease, Isla Ometepe, Cerro Jesús, Volcán Maderas

Chytridiomycosis is considered one of the most threatening emerging infectious diseases faced by amphibian natural populations (Fisher, 2012). It is caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and is considered one of the major driving factors behind the mass mortality and subsequent decline of over 200 amphibian species worldwide (Berger et al., 1998; Skerratt et al., 2007). This disease is considered to have a high range of distribution and is present in at least five continents (Berger et al., 1998; Bosch et al., 2001; Green et al., 2002; Lips et al., 2006; Goldberg et al., 2007). In order to aid management and conservation, more surveillance efforts are needed worldwide. In Central America, *Bd* has been recorded in all countries with the exception of Nicaragua and Belize. Although Nicaragua is the largest Central American country, its amphibian diversity is relatively small (74 species, seven of which are endemic to the country: Sunyer & Köhler 2010; Sunyer et al., 2010, 2011, 2012). Nicaragua's historical political instability and pronounced economic constraints have hindered much needed biological investigations and surveillance efforts when compared

to neighbouring Central American countries where the presence of *Bd* has been recorded for decades (Crump et al., 1992; Lips, 1998, 1999; Puschendorf et al., 2006; Frías-Álvarez et al., 2008). The goals of this study are to determine the presence of *Bd* in Nicaraguan amphibians, and to identify the species affected by this introduced pathogen.

Field surveys were conducted during July and November 2011. Species were identified using dichotomous keys provided in Köhler (1999, 2001, 2011), Savage (2002), McCranie & Wilson (2002) and McCranie & Castañeda (2007). During field work, no individuals showed signs of infection and no dead specimens were found. We sampled for chytrid by swabbing the skin of the sampled amphibians according to standard protocol described in Hyatt et al. (2007).

The survey spanned an altitudinal gradient between 810–1470 m and was conducted at three localities in



**Fig. 1.** Map of Nicaragua showing all sampled localities: Cerro Jesús (star), El Gobiado (circle), and Volcán Maderas (triangle). Water surfaces are coloured light grey.

a north-to-south transect along Nicaragua's central highlands (Fig. 1): 1 - Cerro Jesús (13.984° N, 86.190° W, 1080–1470 m elevation), Dept. Nueva Segovia; 2 - El Gobiado (13.158° N, 85.873° W, 1080–1250 m elevation),

Reserva Natural Datanlí-El Diablo, Dept. Jinotega, and 3 - Reserva Natural Volcán Maderas (11.442° N, 85.512° W, 810–1250 m elevation), Reserva de la Biosfera Isla de Ometepe, Dept. Rivas. The crater lake at Volcán

**Table 1.** List of Nicaraguan *Bd*-positive and/or *Bd*-negative amphibian species sampled in three localities (Cerro Jesús, El Gobiado and Volcán Maderas). GE stands for Genomic zoospores equivalent (including positive and negative samples) of *Bd*.

Taxon	Locality	Positive samples/ Total samples	GE (Mean-Range)
<b>GYMNOPHIONA</b>			
Caeciliidae			
<i>Gymnopis multiplicata</i>	Cerro Jesús	0/1	-
<b>CAUDATA</b>			
Plethodontidae			
<i>Bolitoglossa striatula</i>	Cerro Jesús	0/1	-
<b>ANURA</b>			
Bufonidae			
<i>Incilius coccifer</i>	El Gobiado	0/1	-
<i>Incilius valliceps</i>	Cerro Jesús	2/4	5.28(0–20)
<i>Rhinella marina</i>	Cerro Jesús	0/1	-
	El Gobiado	1/4	1.27(0–6.39)
Centrolenidae			
<i>Espadarena prosoblepon</i>	Cerro Jesús	0/1	-
<i>Hyalinobatrachium fleischmanni</i>	Cerro Jesús	0/1	-
Craugastoridae			
<i>Craugastor fitzingeri</i>	Volcán Maderas	1/2	0.35(0–0.71)
<i>Craugastor lauraster</i>	Cerro Jesús	0/1	-
	Volcán Maderas	1/1	110
Eleutherodactylidae			
<i>Diasporus diastema</i>	El Gobiado	0/1	-
Hylidae			
<i>Agalychnis callidryas</i>	Cerro Jesús	1/2	0.6(0–0.13)
	El Gobiado	0/2	-
	Volcán Maderas	3/4	3.70(0–7.84)
<i>Dendropsophus microcephalus</i>	Cerro Jesús	2/5	8.22(0–40)
	Volcán Maderas	3/4	60.22(0–200)
<i>Ptychohyla hypomykter</i>	Cerro Jesús	0/2	-
	El Gobiado	0/6	-
<i>Smilisca baudinii</i>	Cerro Jesús	0/1	-
	El Gobiado	1/2	2.58(0–5.16)
<i>Smilisca phaeota</i>	El Gobiado	1/2	0.07(0–0.14)
Ranidae			
<i>Lithobates brownorum</i>	Cerro Jesús	1/2	0.27(0–0.55)
	El Gobiado	0/3	-
<i>Lithobates maculatus</i>	Cerro Jesús	1/2	0.05(0–0.11)
	El Gobiado	0/1	-
Strabomantidae			
<i>Pristimantis ridens</i>	Cerro Jesús	0/3	-
	El Gobiado	0/5	-

Maderas is the only locality in Nicaragua with numerous anuran death records (Froglog, 1997). All three localities correspond to pre-montane moist forests (600–1200 m elevation and lower montane moist forests (>1200 m elevation; Holdridge, 1967), surrounded (Locality 1 and 3) or invaded (Locality 2) by coffee farms. We took one swab sample for each anuran and one independent swab from each side of the sampled caecilian. Geocoordinates are based on datum WGS84. For chytrid detection and DNA quantification, samples were analysed using real-time PCR (RT-PCR) following Boyle et al. (2004). DNA was extracted with PrepMan Ultra and amplified using a CFX96™ Real-Time PCR Detection System, BIO-RAD. Each 96-well assay plate included a negative control and four different standards containing DNA from 100, 10, 1 and 0.1 *Bd* genome equivalents. All the samples, as well as the negative control and the standards were tested in duplicate.

We collected a total of 67 swab samples from 18 amphibian species corresponding to nine families and three orders (Table 1), for the first time reporting the presence of *Bd* in Nicaragua and filling a gap in the distribution of chytrid in Central America. Ten anuran species belonging to the families Bufonidae, Craugastoridae, Hylidae and Ranidae tested positive (Table 1). Of special relevance are the *Bd*-positive samples on *Dendropsophus microcephalus*, *Craugastor lauraster*, *Smilisca baudinii*, and *Lithobates brownorum*, for which *Bd* had not been previously confirmed. Several species with confirmed infection in other countries tested negative in our study (*Diasporus diastema*, *Espadarena prosoblepon* and the endangered *Ptychohyla hypomykter*, Picco & Collins, 2007; Saenz et al., 2009).

We recorded *Bd* in 27% of the total samples and confirmed its presence in all three sampled localities: Volcán Maderas had the highest prevalence of infection (72%) followed by Cerro Jesús (25%) and El Gobiado (11%). The high prevalence of *Bd* in Volcán Maderas urges the need for further research in this particular area isolated from the mainland, together with wider surveys to understand the distribution, spread and potential impact of *Bd* on amphibian populations in Nicaragua.

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