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Detection of *Batrachochytrium dendrobatidis* in Eastern New Mexico, USA

The spread of *Batrachochytrium dendrobatidis* (*Bd*) has caused a rapid decline of many amphibian species worldwide, including in the Southwestern United States (e.g., Berger et al. 1998; Bradley et al. 2002; Muths et al. 2003; Voyles 2015). Ryan et al. (2014) speculated that the fungal disease might be a factor contributing to mass mortalities in Chiricahua Leopard Frog (*Lithobates chiricahuensis*), Northern Leopard Frog (*Lithobates pipiens*), Lowland Leopard Frog (*Lithobates yavapaiensis*), and Western Toad (*Anaxyrus boreas*) in New Mexico, USA because of a massive amphibian die-off caused by chytridiomycosis in neighboring states. Although survey efforts have been made to determine the prevalence of *Bd* in association with the amphibian declines, information on *Bd* in New Mexico remains scarce. With our best effort to compile the currently available data, the assessments of *Bd* to date have only been reported from surveys and reports in northern, western, and part of eastern New Mexico (Cummer et al. 2005; Lannoo et al. 2011; Ryan et al. 2014; Voyles 2015).

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Twenty-six species of amphibians (3 salamander species and 23 anuran species) are native to New Mexico (Painter et al. 2017). Of those, four species are listed as endangered (Jemez Mountains Salamander, *Plethodon neomexicanus*; Lowland Leopard Frog, *Lithobates yavapaiensis*; Boreal Toad, *Anaxyrus boreas*; Western Narrow-mouthed Toad, *Gastrophyne olivacea*), and two species are considered threatened (Sacramento Mountain Salamander, *Aneides hardii*; Sonoran Desert Toad, *Incilius alvarius*) by the state of New Mexico (NMDGF 2018). Assessing the full extent of *Bd* prevalence among amphibians is crucial to better understand the threat *Bd* may pose and to advance the conservation of endangered and threatened species in New Mexico. Here, we report the results of opportunistic amphibian surveys and subsequent assessment of *Bd* in three counties of eastern New Mexico.

We conducted a total of 20 sampling events in nine locations across Roosevelt, Curry, and Eddy counties from April to August of 2016 and 2017 (Table 1). Most of our surveys occurred on nights after heavy-rain events when amphibians were active. The

Table 1. Results of laboratory analyses (positive detections: +; no detections: -) of amphibians sampled for *Batrachochytrium dendrobatidis* (*Bd*) at nine sites in Curry, Eddy, and Roosevelt counties, New Mexico, USA, during 2016 and 2017. Confidence intervals were calculated using Jeffrey's method with proportional data.

Site	County	Location	Sample date (mo/yr)	Species	No. sampled	No. *Bd*+	No. *Bd*-	Prevalence (95% CI)
1	Curry	34.381898°N, 103.19371°W	07/2016	*Anaxyrus cognatus*	1	0	1	0 (0.0–0.85)
			04/2017	*Anaxyrus woodhousii*	1	0	1	0 (0.0–0.85)
2	Eddy	32.22549°N, 104.21616°W	06/2016; 07/2016; 05/2017	*Acris blanchardi*	17	4	13	0.24 (0.09–0.47)
			05/2017	*Lithobates berlandieri*	1	0	1	0 (0.0–0.85)
3		32.206496°N, 104.247302°W	05/2017; 08/2017	*A. blanchardi*	7	4	3	0.57 (0.26–0.86)
			08/2017	*Scaphiopus couchii*	12	2	10	0.17 (0.4–0.44)
			08/2017	*Anaxyrus speciosus*	4	1	3	0.25 (0.03–0.72)
4		32.115471°N, 104.456342°W	07/2016	*Lithobates catebeianus*	1	0	1	0 (0.0–0.85)
5	Roosevelt	34.164481°N, 103.357861°W	06/2016; 04/2017	*A. woodhousii*	23	2	21	0.09 (0.02–0.25)
6		34.126061°N, 103.526684°W	07/2017	*Spea bombifrons*	9	2	7	0.22 (0.6–0.55)
			07/2017	*A. cognatus*	11	1	10	0.09 (0.01–0.35)
			07/2017	*S. couchii*	3	0	3	0 (0.0–0.54)
			07/2017	*Anaxyrus debilis*	1	0	1	0 (0.0–0.85)
			07/2017	*A. woodhousii*	2	0	2	0 (0.0–0.67)
7		34.153255°N, 103.325991°W	05/2017	*A. cognatus*	4	0	4	0 (0.0–0.44)
			05/2017	*A. debilis*	4	2	2	0.5 (0.12–0.88)
			05/2017	*A. woodhousii*	2	1	1	0.5 (0.6–0.94)
8		34.195843°N, 103.337171°W	04/2017	*A. woodhousii*	1	1	0	1 (0.15–1)
9		34.190677°N, 103.336638°W	04/2017	*A. woodhousii*	3	1	2	0.33 (0.4–0.82)

sampled landscapes included highway roads, neighborhoods, ponds in urban areas, and riparian areas. Amphibians were captured by hand and sampled by swabbing abdomen, thighs, hind feet, and forefeet using sterile cotton-tip swabs as described by Hyatt et al. (2007). Swabs were stored in a -20°C freezer. To avoid cross contamination, surveyors wore new pairs of disposable Nitrile latex-free gloves for each capture (Phillott et al. 2010). After processing, captures were immediately released at the capture locations.

DNA extractions were completed at Texas State University, San Marcos, Texas, USA using standard protocols from the Prepman Ultra (Applied Biosystems) kit. To detect the presence of *Bd*, we used a real time Taqman qPCR assay described by Boyle et al. (2004). The probe ChytrMGB2 was used with two species-specific primers ITS1-3 Chytr and 5.8S Chytr (Boyle et al. 2004; Garland et al. 2010). We analyzed each sample in duplicate with a consecutive 10-fold dilution of known five standards of *Bd* DNA as positive controls and nuclease-free water as a negative control. The results for presence or absence of *Bd* DNA in the samples were then compared to the standards. Since each sample was run in duplicate, samples that resulted in both positive and negative for *Bd* were repeated before confirming the presence of *Bd*.

We collected swabs from 107 individuals of 9 amphibian species across Curry (N = 2), Eddy (N = 42), and Roosevelt counties (N = 63; Table 1). The sample size per species ranged from 1 to 31. All specimens appeared to be healthy and phenotypically unremarkable, except one Woodhouse's Toad (*Anaxyrus woodhousii*) from Roosevelt County that presented redness on the abdomen and also subsequently resulted in a positive amplification for *Bd*. However, clinical signs alone may not be a good indicator of chytridiomycosis infection as *Bd* is also known to co-occur with other pathogens, such as ranavirus (Kik et al. 2012; Blackburn et al. 2015; Watters et al. 2018). *Bd* was detected in all sampled counties except Curry County (Table 1); the absence of *Bd* from Curry County is likely simply due to insufficient sample size (N = 2). The most common species in our study was *A. woodhousii* (N = 31), followed by *Acris blanchardi* (N = 24) and *Anaxyrus cognatus* (N = 16). We detected *Bd* in 21 individuals (19.6% overall prevalence) from 7 species. The species with the highest *Bd* prevalence (33.33%) was *A. blanchardi* caught along the Black River in Eddy County (Table 1). Based on 10,000 zoospore equivalents (Vredenburg et al. 2010; Kinney et al. 2011), infection intensity varied within our sampled locations and species ranging from 1 zoospore to 7,612 zoospores. Out of all infected specimens, 18 exhibited zoospore equivalents of < 100; the only three individuals with infection intensity of more than 1,000 zoospores were two *A. blanchardi* from Eddy County and one *A. woodhousii* from Roosevelt County. However, we would note that ongoing work would indicate that the copy number (often presumed to be 1 genomic copy per spore) is likely to be greater than one genomic copy per spore making these estimates for comparability not a measure of zoospore quantity.

Our study expanded the *Bd* survey efforts in New Mexico particularly the eastern portion of the state. Prior to this study, *Bd* has been surveyed in 10 of 33 counties; however, this information is not sufficient to understand the nature of *Bd* in New Mexico as there are still substantial assessment gaps. Although *Bd* has been previously reported in eastern New Mexico (Curry County; Lannoo et al. 2011), our study has extended the survey of *Bd* into Roosevelt and Eddy counties. Further surveys are still needed in central and southern New Mexico to bridge the distribution gap of the fungus. New Mexico Department of Game and Fish is collaborating with researchers to continue assessments of *Bd* in New Mexico, but to our knowledge the updated reports have not yet been published.

Here we report positive *Bd* results in six amphibian species from two counties in New Mexico: Blanchard's Cricket Frog (*Acris blanchardi*), Great Plains Toad (*Anaxyrus cognatus*), Couch's Spadefoot Toad (*Scaphiopus couchii*), Texas Toad (*Anaxyrus speciosus*), Plains Spadefoot Toad (*Spea bombifrons*), and Green Toad (*Anaxyrus debilis*) from Eddy and Roosevelt counties. We also point out that although *Lithobates catesbeianus* and *L. berlandieri* were *Bd*-negative in this study, they had tested positive for *Bd* in earlier studies and are considered potential reservoir species for *Bd* (Daszak et al. 2004; Lovich et al. 2008; Lannoo et al. 2011). Our inability to detect *Bd* for *Lithobates* species could be due to the small sample size.

Although species sampled in this study are not the Species of Greatest Conservation Need (SGCN) and we observed no apparent signs of infection in any specimens, with the exception of one *A. woodhousii*, it is possible that they are acting as reservoirs for the pathogen and could represent a potential risk to more vulnerable or threatened species. It is also noteworthy that some of the *Bd*-positive species co-occur with other SGCN. The detection of *Bd* in *A. blanchardi* is of concern due to the occurrence of this species in close proximity to the Barking Frog (*Craugastor augusti*). In the United States, the distribution of *C. augusti* is limited to the proximity of Roswell and Carlsbad in southeastern New Mexico and adjacent west-central Texas (Dodd 2013; Ryan et al. 2015). The current status and potential threats for the species are still unknown, but a recent study detected Barking Frogs in only 6 of 23 historical sites (Ryan et al. 2015). Our findings cannot be used to draw conclusions regarding the decline of *C. augusti* but emphasize the need for assessment of the disease in this taxon. Further investigation is needed to evaluate the occurrence of *Bd*, species vulnerability, and potential reservoir species in New Mexico.

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A Survey for the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* in the Mexican States of México, Morelos, Oaxaca, and Puebla

The chytridiomycete fungus *Batrachochytrium dendrobatidis* (*Bd*) is implicated in having an important role in amphibian population declines (Berger et al. 1998; Wake and Vredenburg 2008). This fungus has been detected in populations of frogs on all continents where they exist. In Mexico, its presence has long been documented (Hale et al. 2005; Luja et al. 2012). In a survey of tadpoles and adult anurans in Oaxaca, Mexico, Köhler et al. (2016) found evidence of *Bd* presence in most species assessed from streams in the southern Sierra Madre del Sur. Despite the presence of this pathogen for at least a 15-year time span (Lips et al. 2004), these anurans clearly had persisted and at least some species were abundant at the study sites, with hundreds of tadpoles and dozens of adults seen during a single night survey. Yet, Köhler et al. (2016) documented various stages of bleaching of the keratineous mouth parts (beak and keradonts) in tadpoles of several species, a symptom of chytridiomycosis (Fellers et al. 2001; Rachowicz and Vredenburg 2004). Herein we report on screening for the presence of *Bd* of anurans from several localities in the Mexican states of México, Morelos, Oaxaca, and Puebla, including an updated survey of the sites in the southern Sierra Madre del Sur sampled by Köhler et al. (2016).

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During opportunistic amphibian surveys in Mexico during March 2013, and May and June 2017, *Bd* screening was carried out at lowland and montane sites (690–3325 m elevation; Fig. 1). All collected specimens were screened for *Bd* infection and later deposited in the herpetology collections of the Instituto de Biología, Universidad Nacional Autónoma de México, México D.F., Mexico, and in the herpetological collection of the Senckenberg Research Institute Frankfurt, Germany.

Infection status was determined through a standardized swabbing protocol (Hyatt et al. 2007). Using a synthetic cotton swab, each individual was swabbed with 30 strokes (five times between the toes on each hind foot, five times on each thigh, and five times on each side of the ventral abdomen). Swabs were automatically (using a robot, model Microlab Star, Hamilton, Bonaduz, Switzerland) extracted using the NucleoSpin 8 virus