

**Distribution of the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* (Bd) in New Mexico.**

**Draft report to:**

**New Mexico Department of Game and Fish**

**Share with Wildlife Program**

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**25 April 2018**

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## **Abstract**

The aquatic amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*) was first detected in New Mexico in 2001 on a Chiricahua Leopard Frog (*Lithobates chiricahuensis*) tadpole collected in 1984, using histological sectioning techniques. Since 2003, PCR genetic techniques have been employed to understand which species and geographic areas of New Mexico are affected by *Bd*. Swabs were collected opportunistically from 2003 to 2018. We report here on 3,190 individuals sampled representing 21 of the 27 amphibian species known to occur in New Mexico. *Bd* was detected in 12 of the 21 species tested. Salamanders (three species) appeared to exhibit lower detection rates than did anurans (nine species). All six ecoregions and all five major river drainages (HUC 2 hydrologic units) possessed amphibians that tested positive for *Bd*.

## **Objective**

The objectives of this project were to compile all records, reports, and publications dealing with the chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) in New Mexico and assemble a data file of known positive and negative samples with dates and locality information in order to assess the spatial distribution of *Bd* across the state and its prevalence within the state's amphibian species.

## Introduction

### History of Amphibian and Southwestern Ranid Frog Decline

During the 1980s, herpetologists around the world began to notice that amphibians appeared to be declining in abundance and distribution (Barinaga 1990, Phillips 1990, Wake and Morowitz 1990). By the early 1990s, most herpetologists agreed that the suspected pattern of decline was real and widespread (Barinaga 1990, Phillips 1990, but see Pechmann et al. 1991) but did not affect all areas equally. Amphibians were thought to be highly sensitive indicators of environmental quality (Wake and Morowitz 1990). Amphibian skin is moist, allowing the absorption of air- and water-borne pollutants. The life cycles of many amphibians include aquatic and terrestrial life stages (Duellman and Trueb 1985), permitting degradation in both types of habitats to affect population viability and fitness. Amphibians exhibit a broad variety of reproductive strategies (Duellman and Trueb 1985), allowing different demographic responses to short- and long-term environmental changes. The sensitivity of amphibians, and the apparent widespread nature of their declines, were thought to reflect global environmental degradation (Wake and Morowitz 1990).

In the western United States, a variety of hypotheses have been proposed to explain declines in amphibian populations. These include: 1) habitat alteration, 2) the introduction of non-native bullfrogs, crayfish, and/or predatory fishes, 3) acid precipitation, 4) the introduction of toxicants and pollutants, 5) the introduction of novel parasites and pathogens (disease), 6) catastrophic events (droughts, floods, etc.), 7) natural population fluctuations, or 8) some combination of the above (see reviews by Hayes and Jennings 1986, Pechmann et al. 1991, Carey et al. 2003). The Tiger Salamander (*Ambystoma mavortium*) may be adversely affected by episodic acidification

following snowmelt (Harte and Hoffman 1989, Corn et al. 1989), outbreaks of viral infection (also impacts Sonora Tiger Salamander [*Ambystoma mavortium stebbinsi*]; Jancovich et al. 1997, Collins et al. 2003), and infection by chytrid fungus (Davidson et al. 2003). The Western Toad (*Anaxyrus boreas*) has disappeared from much of its former range in New Mexico and Colorado (Corn et al. 1989, C. W. Painter, pers. comm.), apparently succumbing to chytrid fungus (Carey et al. 2003). Many members of the Northern Leopard Frog (*Lithobates pipiens*) complex (Applegarth 1983, Platz 1984, Hayes and Jennings 1986, Hale and Jarchow 1988, Clarkson and Rorabaugh 1989, Jennings and Scott 1991, Fernandez and Rosen 1998) and members of the Foothill Yellow-legged (*Rana boylii*) group of true frogs (Hayes and Jennings 1986, Miller 1989, McAllister and Leonard 1990) may have suffered from competition and predation by bullfrogs or predation by introduced fishes and crayfish (see review in Hayes and Jennings 1986). Populations of Tarahumara Frog (*Lithobates tarahumarae*) probably have been extirpated from the United States. Those in northern Mexico are perhaps succumbing to chytridiomycosis, but the fungal infection may be exacerbated by high levels of cadmium, a result of nearby copper smelter activity (Hale and Jarchow 1988).

### **History of Chytrid Fungus in New Mexico**

The chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) was described in 1999 (Longcore et al.) and its discovery shortly followed biologists' initial documentation of declines in frog populations around the world. Declines in ranid frog populations, specifically the Chiricahua Leopard Frog (*Lithobates chiricahuensis*) in New Mexico and Arizona, were documented in the western US from 1970's to 1990's. Declines resulted in federal listing of Chiricahua Leopard Frog as threatened (USFWS 2002). Our earliest documented records of *Bd* in New Mexico are from histologically sectioned Chiricahua Leopard Frog tadpole mouthparts from 1984, with other

early records from 1985, 1987, and 1988. Histological sections (performed from 2000 to 2001) focused on examining keratinized tadpole mouthparts for the presence of thalli or zoosporangia of *Bd* and predated the PCR techniques that predominated later. In the fall of 2002, we documented a significant die-off of Chiricahua Leopard Frog on the Deep Creek Divide of the Gila National Forest within the San Francisco River drainage, which resulted in the total loss of at least four robust populations. In approximately 2002, a PCR test was developed to amplify the DNA of *Bd*, allowing a non-lethal method of *Bd* detection. In 2005, Cummer et al. documented *Bd* in Jemez Mountains Salamanders (*Plethodon neomexicanus*). Due to the threat of *Bd* and other factors (climate change and stand-replacing wildfire), the Jemez Mountains Salamander was listed as federally endangered (USFWS 2013).

## Methods

Sampling for *Bd*, as reflected in the records compiled for this project, was based on two different methods. The first method entailed visually examining histological sections of the keratinized mouthparts of preserved tadpoles to look for the presence of zoosporangia. This technique allowed us to sample preserved tadpoles in regional collections dating as far back as 1948. This technique possesses the possibility for false negative results once all the keratin in the mouthparts has been lost. The second method entailed the detection of *Bd* DNA on the integument of amphibians using Polymerase Chain Reaction (PCR) techniques. Using a cotton-tipped swab, we rubbed the belly, inguina, the inside of the thigh, and feet and webbing of individuals three to five times (Fig. 1). The swab was stored in a tube with 70 or 95% ETOH following techniques outlined in Livo (2004) and Hyatt et al. (2007), then sent to a lab for

genetic analysis. Most samples were analyzed by Pisces Molecular, LLC (Boulder Colorado, USA) using a DNA PCR assay specific to *Bd* that is modified from Annis et al (2004). Some



Figure 1. Swabbing inguina of Chiricahua Leopard Frog for *Bd*.

samples were analyzed using Real Time Taqman PCR (Boyle et al. 2004). Some swabs were air dried, placed into dry tubes, and sent to San Diego Zoo's Amphibian Disease Lab (Escondido California, USA), also for PCR analysis. Samples occasionally were pooled to reduce costs.

Preserved tadpoles examined histologically were collected by authors or were from the Museum of Southwestern Biology, University of New Mexico. Samples analyzed using PCR were collected opportunistically when amphibians were encountered during surveys for sensitive species; herpetological fieldwork associated with Charlie W. Painter, NMDGF Herpetologist; or



were part of more dedicated projects addressing more specific questions (see Acknowledgements for contributors).

## **Results and Discussion**

We tested 3,190 individuals representing 21 of the 27 amphibian species known to occur in New Mexico and found 12 of those species to be positive for *Bd* (Table 1). Seventy-seven samples were histological sections of larval mouthparts, while the remainder used one of the PCR approaches described above to detect *Bd*. Of the 308 individuals that tested positively for *Bd*, 17 (of 77 tested, 22.1%) were from histological sections. There were 291 (of 3113 tested, 9.3%) *Bd* positive samples that resulted from PCR of swabs. Our sampling covered 27 of New Mexico's 33 counties, all six ecoregions, and all five HUC2 hydrological units and represented 190 distinct localities (Fig. 2).

### **Diversity**

We tested 21 of the 27 amphibian species known from New Mexico [sampled species in the following families: Ambystomatidae (N=1 of 1 species), Plethodontidae (N=2 of 2), Bufonidae (N=6 of 8), Hylidae (N=4 of 5), Ranidae (N=5 of 6), and Scaphiopodidae (N=3 of 3). We did not sample species in the following families: Craugastoridae (N=1 species) and Microhylidae (N=1)]. We found evidence of *Bd* in 12 species (Table 1). Samples tested per species varied greatly from 1 to 1190.

Table 1. Detection of *Batrachochytrium dendrobatidis* (*Bd*) in 12 (red highlights) of 21 species in New Mexico sampled from 2002 to 2018. The number of *Bd* positive detections (+) and the total number of samples collected (N) are summarized for each species across all sites and individuals.

Species	Sites		Individuals	
	+ / N	<i>Bd</i> Positive (%)	+/N	<i>Bd</i> Positive (%)
<i>Ambystoma mavortium</i>	4 / 52	7.7	9 / 248	3.6
<i>Aneides hardii</i>	0 / 4	0.0	0 / 5	0.0
<i>Plethodon neomexicanus</i>	2 / 34	5.9	2 / 155	1.3
<i>Scaphiopus couchii</i>	0 / 8	0.0	0 / 111	0.0
<i>Spea bombifrons</i>	0 / 3	0.0	0 / 16	0.0
<i>Spea multiplicata</i>	1 / 6	0.0	1 / 86	0.0
<i>Anaxyrus boreas</i>	1 / 1	100.0	49 / 99	49.4
<i>Anaxyrus cognatus</i>	0 / 4	0.0	0 / 29	0.0
<i>Anaxyrus debilis</i>	0 / 5	0.0	0 / 124	0.0
<i>Anaxyrus microscaphus</i>	3 / 17	17.6	5 / 154	3.2
<i>Anaxyrus punctatus</i>	0 / 8	0.0	0 / 32	0.0
<i>Anaxyrus woodhousii</i>	3 / 14	21.4	6 / 130	4.6
<i>Acris blanchardi</i>	1 / 5	20.0	1 / 7	14.3
<i>Hyla arenicolor</i>	0 / 30	0.0	0 / 55	0.0
<i>Hyla wrightorum</i>	0 / 1	0.0	0 / 1	0.0
<i>Pseudacris maculata</i>	13 / 44	29.2	66 / 264	25.8
<i>Lithobates berlandieri</i>	0 / 6	0.0	0 / 10	0.0
<i>Lithobates blairi</i>	9 / 21	42.8	22 / 187	11.8
<i>Lithobates catesbeiana</i>	10 / 27	37.0	11 / 79	13.9
<i>Lithobates chiricahuensis</i>	30 / 69	43.5	103 / 1190	8.6
<i>Lithobates pipiens</i>	13 / 42	30.9	33 / 146	22.6

Initially *Bd* sampling focused on Chiricahua Leopard Frog, for which we sampled the greatest number of individuals (N = 1190) and localities (N = 103). Thirty localities for Chiricahua Leopard Frog tested positive for *Bd* (Fig. 3). These localities were exclusively in the Lower Colorado and Rio Grande hydrologic units (HUC2) and included our earliest dates of detected *Bd* infection in the state (1984, 1985, 1987, and 1988). Several species were sampled more heavily than others including Boreal Chorus Frog (*Pseudacris maculata*, N = 264), Tiger Salamander (*Ambystoma mavortium*, N = 248), Plains Leopard Frog (*Lithobates blairi*, N = 187),

Jemez Mountains Salamander (*Plethodon neomexicanus*, N = 155), Arizona Toad (*Anaxyrus microscaphus*, N = 154), Northern Leopard Frog (N = 146), Woodhouse's Toad (*Anaxyrus woodhousii*, N = 130), Green Toad (*Anaxyrus debilis*, N=124), Couch's Spadefoot (*Scaphiopus couchii* N = 111), Boreal Toad (*Anaxyrus boreas*, N = 99), Mexican Spadefoot (*Spea multiplicata* N = 86), and American Bullfrog (*Lithobates catesbeiana*, N = 79). The remaining eight species had from 1 to 55 samples: Canyon Treefrog (*Hyla arenicolor*, N = 55), Red-spotted Toad (*Anaxyrus punctatus*, N = 32), Great Plains Toad (*Anaxyrus cognatus*, N = 29), Plains Spadefoot (*Spea bombifrons* N = 16), Rio Grande Leopard Frog (*Lithobates berlandieri*, N = 10), Blanchard's Cricket Frog (*Acris blanchardi*, N = 7), Sacramento Mountain Salamander (*Aneides hardii*, N = 5), and Arizona Treefrog (*Hyla wrightorum*, N = 1) (Table 1).

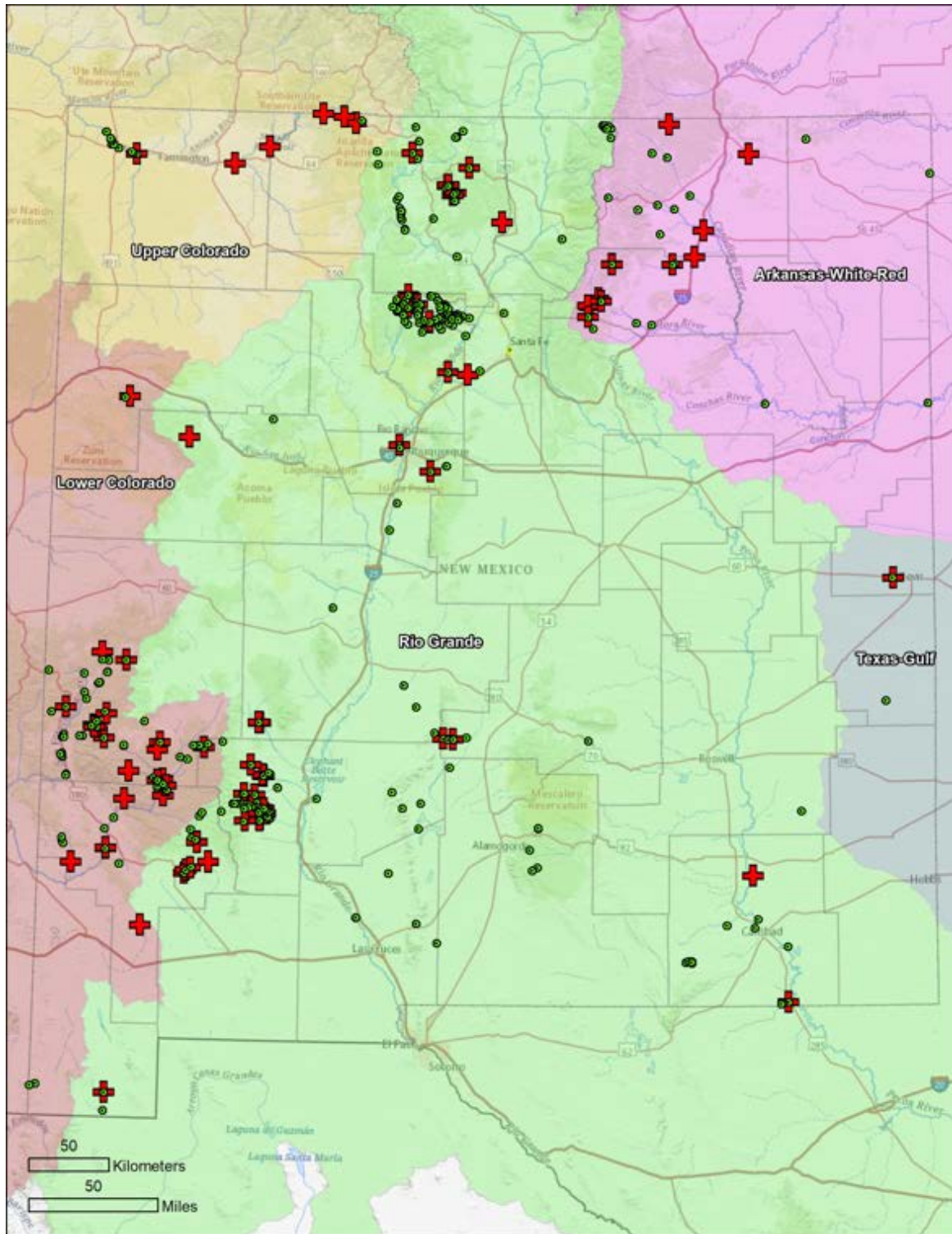


Figure 2. Sites sampled for *Batrachochytrium dendrobatidis* (Bd) in New Mexico for all species. Red plus signs = Bd detected, green dots = Bd not detected. Colored shading with labels indicates HUC 2 hydrologic units.



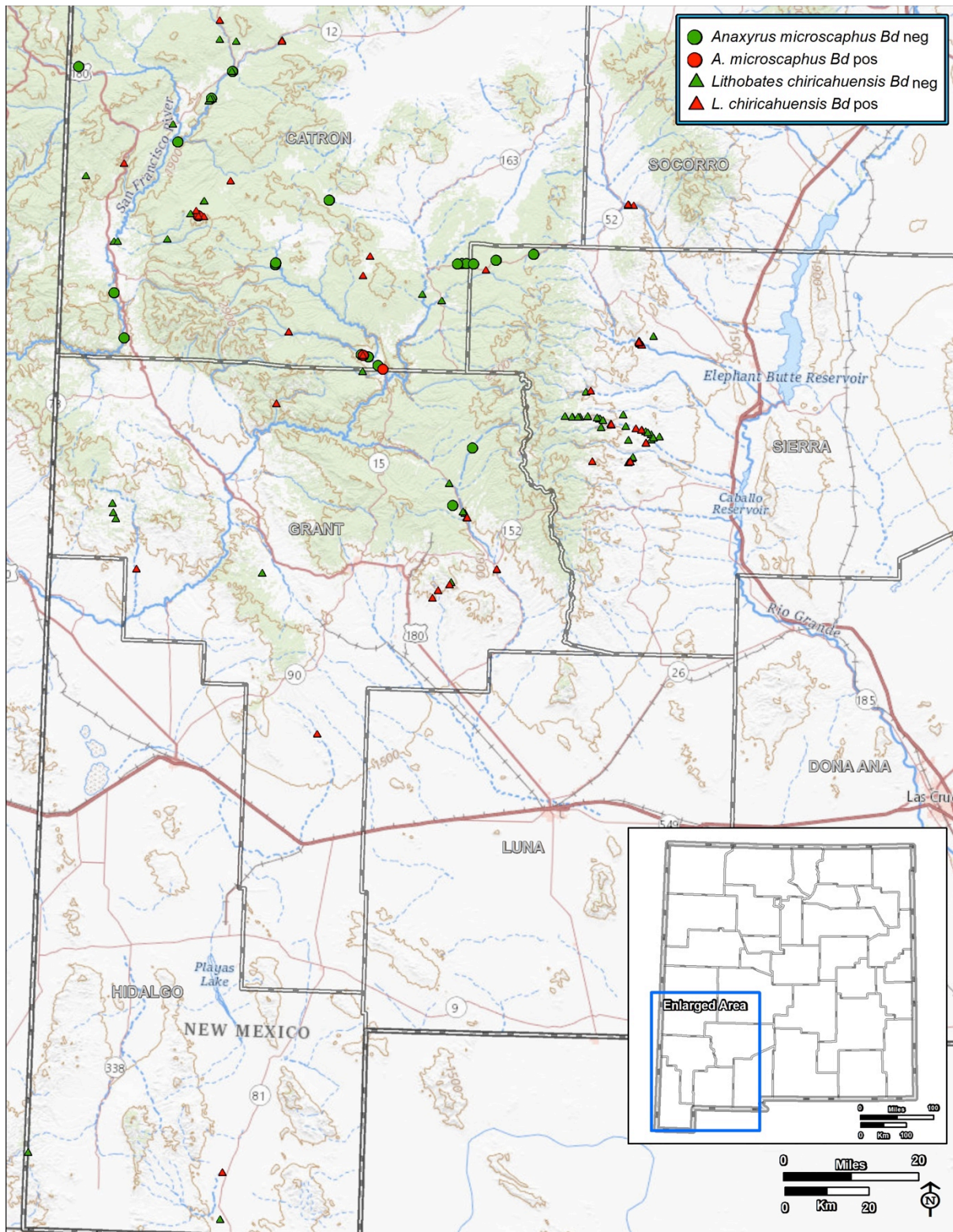


Figure 3. *Batrachochytrium dendrobatidis* (Bd) positive (red) and negative (green) sampling locations for Arizona Toad (circles) and Chiricahua Leopard Frogs (triangles) in southwestern New Mexico.

Three salamander species from two families in New Mexico exhibited low incidence rates of *Bd*.

### **Ambystomatidae**

***Ambystoma mavortium*, Tiger Salamander.** We tested 248 specimens of this species from 52 localities (Fig. 4). Nine individuals (3.6%) from five sites tested positive for *Bd*.

### **Plethodontidae**

***Aneides hardii*, Sacramento Mountain Salamander.** We tested five specimens from 4 localities. No specimens tested positive for *Bd*.

***Plethodon neomexicanus*, Jemez Mountains Salamander.** We tested 155 specimens from 34 localities (Fig. 4). Two specimens (1.29 %) from two sites, Oat Canyon and along FR 527, Jemez Mountains, Sandoval County, tested positive for *Bd* (Cummer *et al.* 2005).

Species of the three frog families exhibited greater variability and higher incidence rates for *Bd* than did New Mexico's salamanders. Differences in breeding phenology, behavior, physiology, seasonality, or hydrologic cycles among New Mexico's anuran species may be factors driving the observed variability in *Bd* susceptibility, prevalence, and intensity.

### **Bufonidae**

***Anaxyrus boreas*, Western Toad.** We tested 99 individuals of this species from a single locality that was the focus of Western Toad reintroduction efforts in New Mexico (i.e., Trout Lakes, Rio Arriba County, NM). Forty-four specimens (49.49 %) from that site tested positive for *Bd*.

***Anaxyrus cognatus*, Great Plains Toad.** We tested 29 individuals from four localities. All individuals were negative for the presence of *Bd*.

***Anaxyrus debilis*, Green Toad.** We tested 124 individuals from five sites, all which tested negative for *Bd*.

***Anaxyrus microscaphus*, Arizona Toad.** We tested 154 individuals from 17 localities. Five individuals (3.22 %) from two sites, Little Creek near the Heart Bar Wildlife Management Area and Six-Shooter Tank, Deep Creek Divide, Catron County, tested positive for *Bd* (Ryan *et al.* 2014).

***Anaxyrus punctatus*, Red-spotted Toad.** We tested 32 individuals from 8 localities. All individuals were negative for the presence of *Bd*.

***Anaxyrus woodhousii*, Woodhouse's Toad.** We tested 130 individuals from 14 localities in seven counties. Six specimens (4.6 %) from three sites, Stubblefield Canyon, Colfax County, Canon AFB, and Kirtland AFB tested positive for *Bd*.

### **Hylidae**

***Acris blanchardi*, Blanchard's Cricket Frog.** We tested seven individuals of this species from five localities. One individual (14.3 %) from one site, Delaware River, upstream US 285 bridge, Eddy County, tested positive for *Bd*.

***Hyla arenicolor*, Canyon Treefrog.** We tested 55 individuals from 28 localities in six counties. None of those individuals tested positive for the presence of *Bd*.

***Hyla wrightorum*, Mountain Treefrog.** We tested one individual from one locality, Bill Lewis Cienaga, Catron County, NM. That individuals tested negative for the presence of *Bd*.



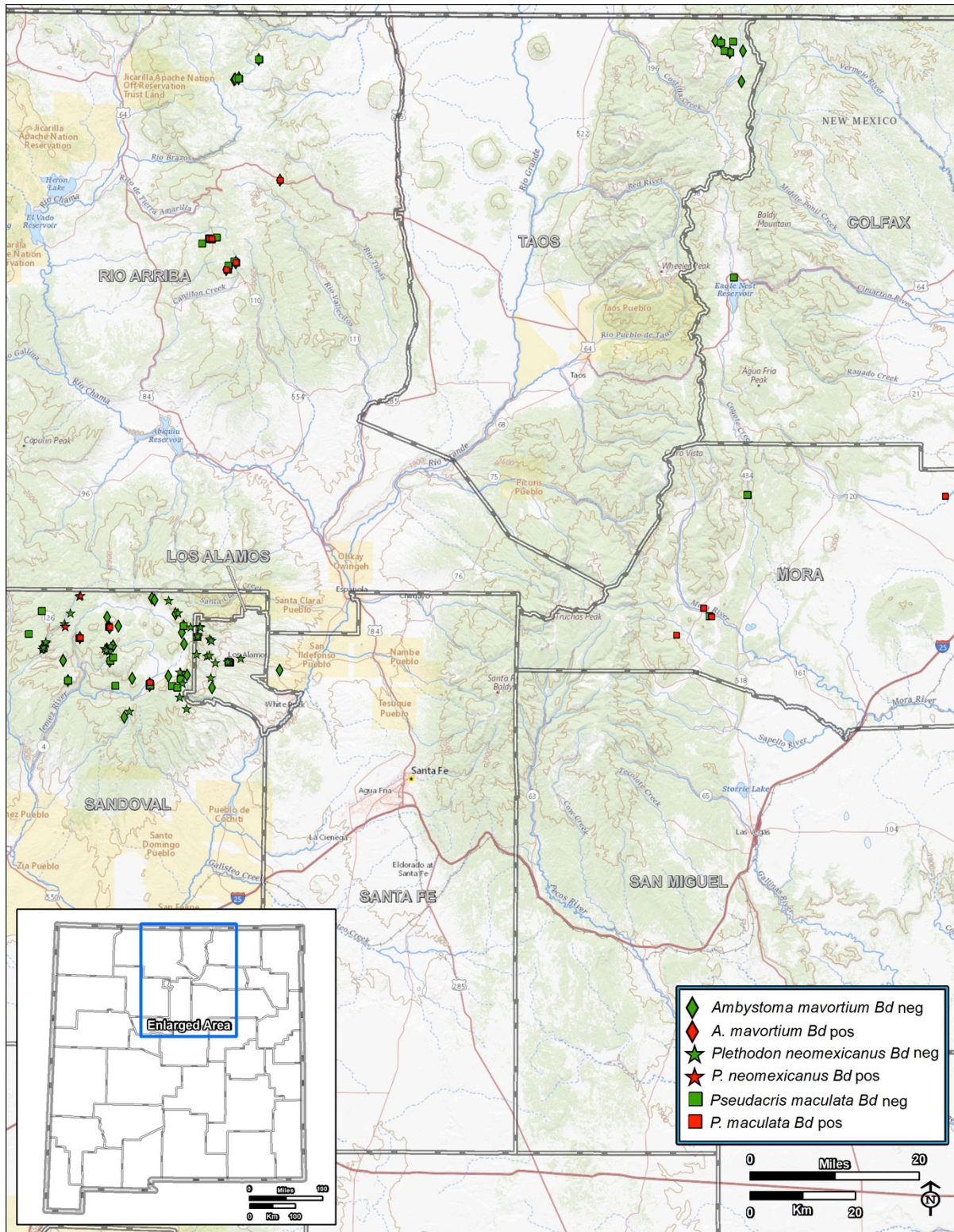


Figure 4. *Batrachochytrium dendrobatidis* (Bd) positive (red) and negative (green) sampling locations for Tiger Salamander (diamonds), Jemez Mountains Salamander (stars), and Boreal Chorus Frog (squares) in north central New Mexico.



***Pseudacris maculata*, Boreal Chorus Frog.** We tested 264 individuals from 44 localities in nine counties. Sixty-six individuals (25.8 %) from 14 sites in six counties, Catron, Cibola, McKinley, Mora, Rio Arriba, and Sandoval, NM (Fig. 4), tested positive for *Bd*.

### **Ranidae**

***Lithobates berlandieri*, Rio Grande Leopard Frog.** We tested 10 individuals of this species from 7 localities in Eddy County. All 10 individuals tested negative for the presence of *Bd*.

***Lithobates blairi*, Plains Leopard Frog.** We tested 187 individuals from 21 localities in 11 counties. Twenty-two specimens (11.8 %) from five sites in Colfax, Eddy, Mora, and Sierra counties tested positive for *Bd*.

***Lithobates catesbeiana*, American Bullfrog.** We tested 79 individuals from 25 localities in nine counties. Eleven individuals (13.9 %) from 10 sites in Catron, Grant, Rio Arriba, Sandoval, San Juan, and Sierra counties tested positive for *Bd*.

***Lithobates chiricahuensis*, Chiricahua Leopard Frog.** We tested 1190 individuals from 69 localities. One hundred-three individuals (8.6 %) from 30 sites in Catron, Grant, Hidalgo, Socorro, and Sierra counties tested positive for *Bd*. These counties include all the counties in which this federally threatened species occurs in New Mexico (Degenhardt et al. 1996).

***Lithobates pipiens*, Northern Leopard Frog.** We tested 146 individuals from 42 localities in nine counties. Thirty-three individuals (22.6 %) from 13 sites in Mora, Rio Arriba, Sandoval, San Juan, and Santa Fe counties tested positive for *Bd*.

### **Scaphiopodidae**

***Scaphiopus couchii*, Couch's Spadefoot.** We tested 111 individuals of this species from eight localities in four counties. All samples tested negative for *Bd*.

***Spea bombifrons*, Plains Spadefoot.** We tested 16 individuals from three localities in three counties, which tested negative for *Bd*.

***Spea multiplicata*, Plains Spadefoot.** We tested 86 individuals from six localities in six counties. One sample tested positive for *Bd* from Red Tank in Lincoln County, where Tiger Salamanders have also tested positive.

Several species have been suggested as potential “reservoir species”: Tiger Salamander (Davidson et al. 2003), Northern Leopard Frog (Woodhams et al. 2008), and American Bullfrog (Daszak et al. 2004). Our data suggest that species’ susceptibility to chytridiomycosis varies greatly and other species may also serve as potential “reservoir species” (e.g., Boreal Chorus Frog) which seem to persist at *Bd* positive sites.

## Geography

Samples were collected in 27 of New Mexico’s 33 counties (Table 2, Fig. 5) and all six ecoregions (Fig. 6). Seventeen of the 27 counties sampled were positive for *Bd*. Most counties possessed two to three species that tested *Bd* positive. The statewide infection rate for *Bd* was 9.6%. Geographically, the majority of sampling was done in the middle elevations of the more mountainous regions of the state: Gila, Jemez, San Juan and Sangre De Cristo mountains (Figs. 2 and 5). Large portions of New Mexico remain unsampled for *Bd*. Future research should focus on these areas and the species that occur there. The High Plains and Tablelands stands out as the least sampled ecoregion in the state (Fig. 6).

### Rio Grande Drainage (HUC 2=13)

We sampled 1392 (of 3190 total samples) amphibians, of 20 species (excluding Mountain Treefrog), from the Rio Grande Region (Fig. 2). In New Mexico, the Rio Grande drainage also

includes the Pecos River and its tributaries, as well as tributaries to the mainstem of the Rio Grande (Fig. 7). Twenty-four counties in New Mexico are contained in part or in total

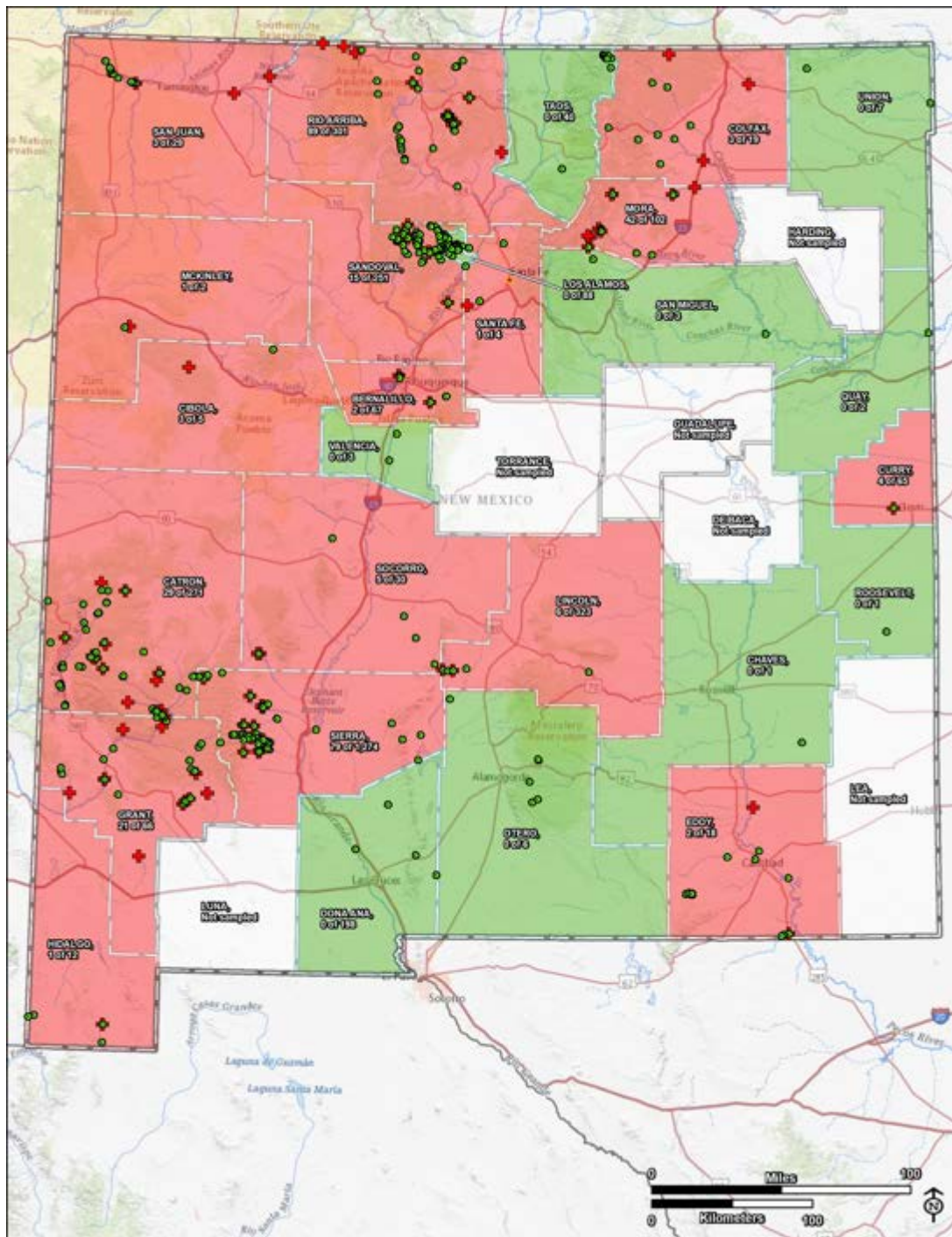


Figure 5. Counties sampled for *Batrachochytrium dendrobatidis* (*Bd*) in New Mexico, all species. Red plus signs = *Bd* detected, green dots = *Bd* not detected. Red shaded counties = *Bd* positive, green shaded = *Bd* negative, white = unsampled.

Table 2. New Mexico counties sampled for *Bd*. +/N indicates the number of individuals of all species that tested positive (+) over the total number of individuals tested (N). *Bd* Positive indicates the percentage of individuals that tested positive for all species. + Species / N Species indicates the number of species that tested positive (+ Species) over the total number of species tested (N Species). Counties highlighted in red were *Bd* positive.

County	+ / N	<i>Bd</i> Positive (%)	+ Species / N Species
Bernalillo	2 / 69	0.0	2 / 5
Catron	29 / 271	10.7	4 / 7
Chaves	0 / 1	0.0	0 / 1
Cibola	3 / 5	60.0	1 / 2
Colfax	3 / 19	15.8	2 / 5
Curry	4 / 65	6.2	1
De Baca	0 / 0	0.0	0
Dona Ana	0 / 1	0.0	1
Eddy	2 / 18	11.0	2 / 3
Grant	21 / 66	31.8	2 / 5
Guadalupe	0 / 0	0.0	0
Harding	0 / 0	0.0	0
Hidalgo	1 / 12	8.0	1 / 2
Lea	0 / 0	0.0	0
Lincoln	6 / 323	1.9	2 / 6
Los Alamos	0 / 88	0.0	0 / 5
Luna	0 / 0	0.0	0
McKinley	1 / 2	50.0	1 / 1
Mora	42 / 102	41.2	3 / 4
Otero	0 / 6	0.0	0 / 2
Quay	0 / 2	0.0	0 / 1
Rio Arriba	89 / 301	30.0	4 / 6
Roosevelt	0 / 1	0.0	0 / 1
San Juan	3 / 29	10.3	2 / 2
San Miguel	0 / 3	0.0	0 / 2
Santa Fe	1 / 4	25.0	1 / 4
Sandoval	15 / 251	6.0	5 / 5
Sierra	81 / 1274	6.4	4 / 11
Socorro	5 / 30	23.8	1 / 8
Taos	0 / 40	0.0	0 / 3
Torrance	0 / 0	0.0	0
Union	0 / 7	0.0	0 / 1
Valencia	0 / 3	0.0	0 / 1

within this HUC 2 region. The western border of this region corresponds to the continental divide, while the eastern border extends almost to the New Mexico-Texas border. It represents the largest HUC 2 region in New Mexico.

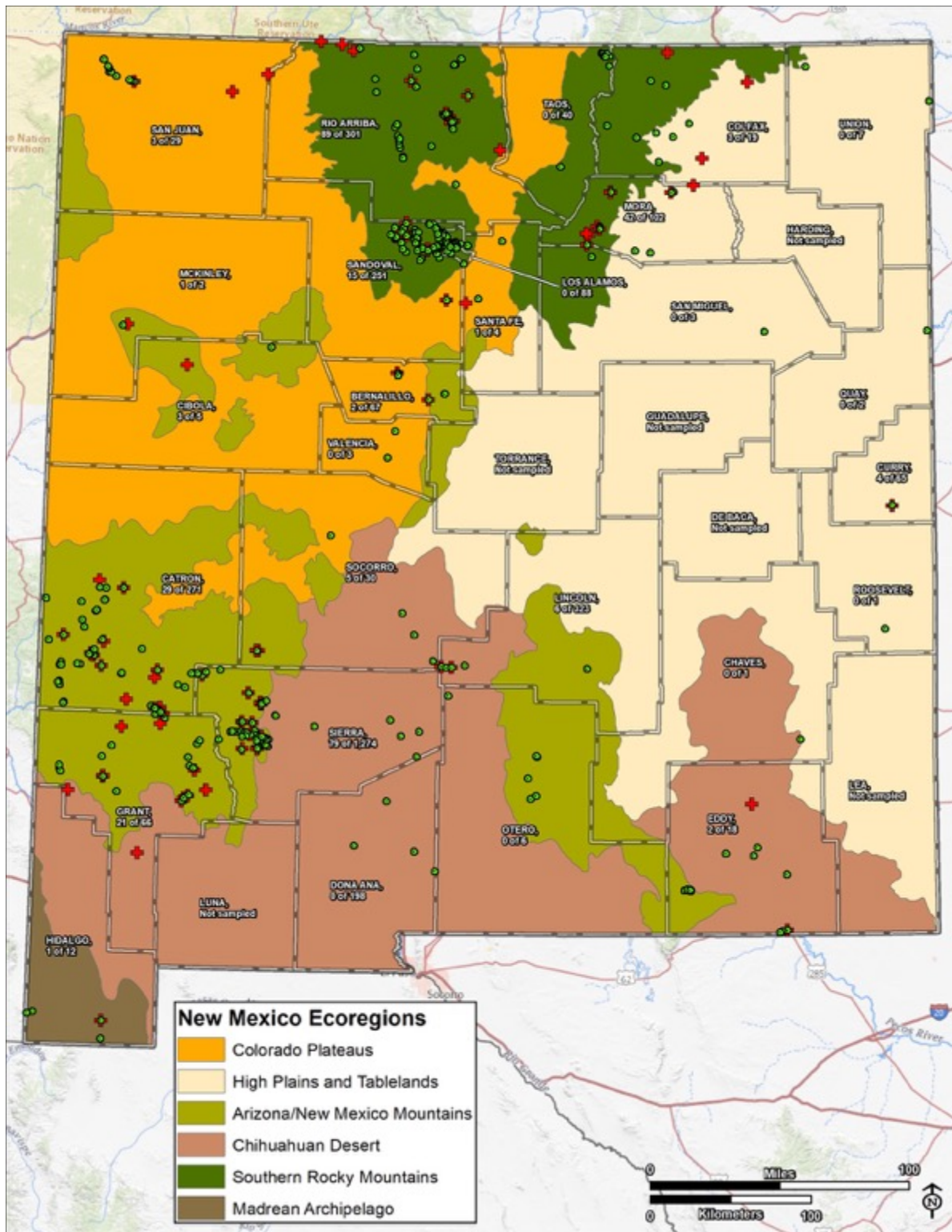


Figure 6. New Mexico's six ecoregions with *Bd* results by county.



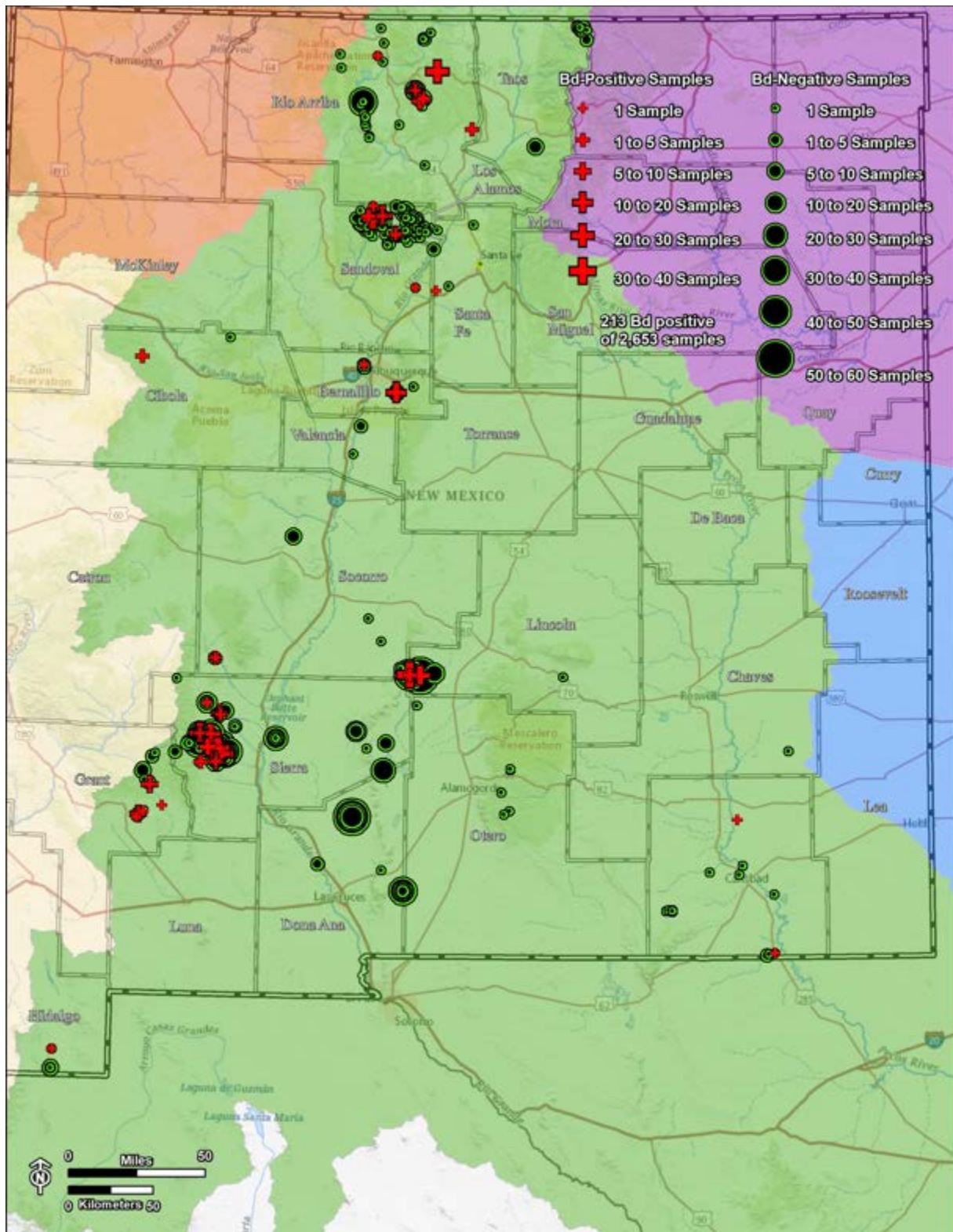


Figure 7. *Batrachochytrium dendrobatidis* (Bd) detections in the Rio Grande drainage. Green shows the HUC 2 boundary.

Most samples were collected at intermediate elevations within montane portions of the Rio Grande drainage, including the San Mateo Mountains, Magdalena Mountains, eastern slope of the Black Range, Guadalupe Mountains, Mt. Taylor area, Jemez Mountains, west slopes of the Sangre de Cristo Mountains, southern San Juan Mountains, and the Chama area. Two hundred thirteen samples (8.0 %) from the Rio Grande drainage tested positive for *Bd*. Most of the samples testing positive were collected at higher elevations within this region. We detected at least one *Bd* positive sample in most tributaries within the Rio Grande drainage (HUC 2 = 13) in New Mexico.

#### **Arkansas, White, Red River Drainage (HUC 2=11)**

We sampled 133 (of 3190 total samples) amphibians representing six species, including American Bullfrog, Boreal Chorus Frog, Northern Leopard Frog, Plains Leopard Frog, Woodhouse's Toad, and an unidentified *Spea* spadefoot, from the Arkansas, White, Red River drainage. In New Mexico, this region includes the Cimarron, Canadian, Mora, Conchas, and Vermejo rivers and their tributaries (Fig. 8). Seven counties in New Mexico are contained in part or in total within this HUC 2 region. The western border of this region corresponds to the divide along the spine of the Sangre de Cristo Mountains, while the eastern border extends well east of the eastern New Mexico border. It represents the second largest HUC 2 region in New Mexico. Most samples were collected in intermediate to higher elevations of the Arkansas, White, Red River drainage including the Cimarron Mountains, eastern slope of the Sangre de Cristo Mountains, and the High Plains of northeastern New Mexico. Forty-five samples (33.8 %) from the Arkansas, White, Red River drainage tested positive for *Bd*. Most of the samples testing positive were collected in higher elevations within this region. For most tributaries within this HUC2

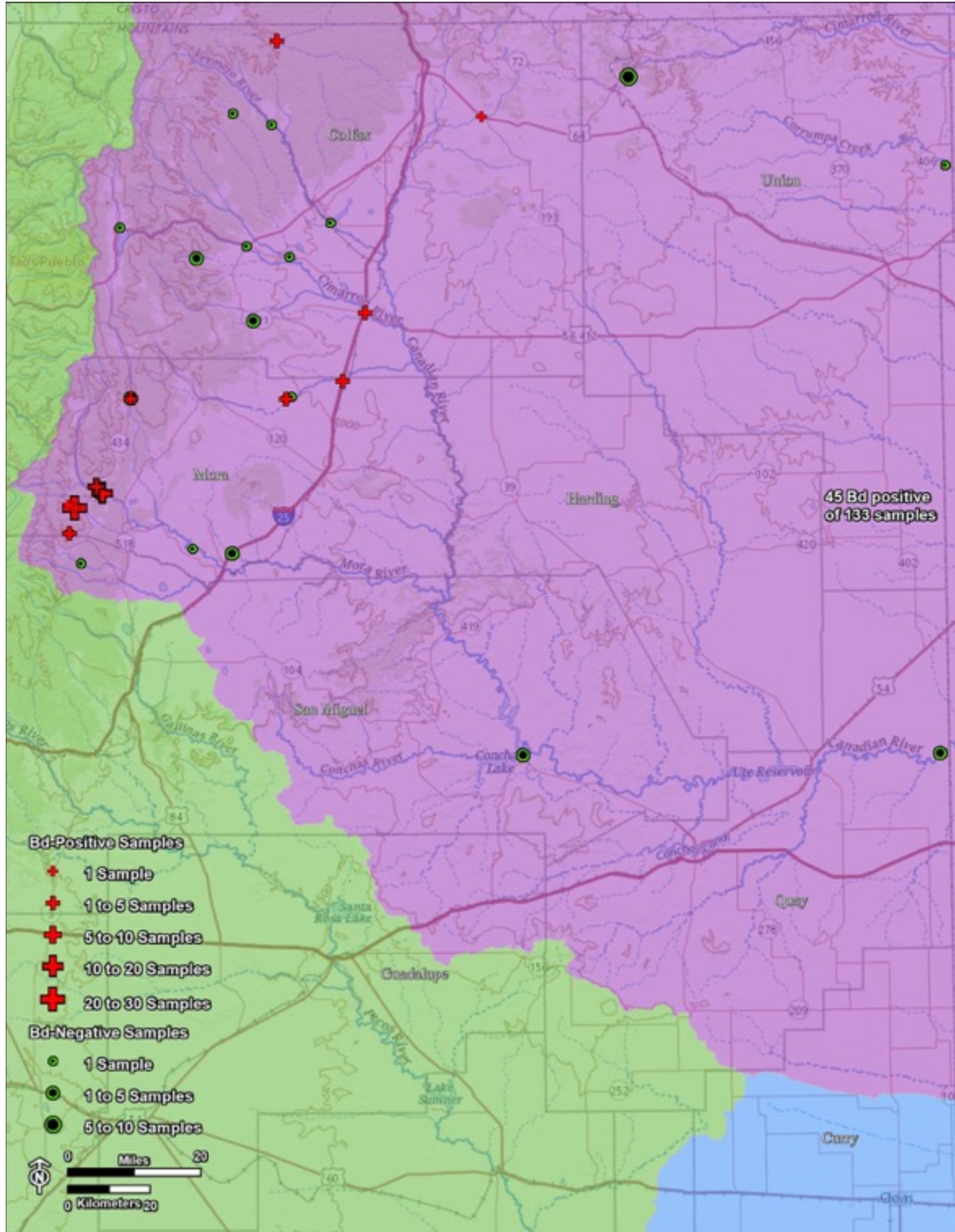


Figure 8. *Batrachochytrium dendrobatidis* (Bd) detections in the Arkansas-White-Red River drainage. Purple shows the HUC 2 boundary.



region, positive test results were obtained for at least one sample collected along each tributary, including the Mora, Vermejo, and Canadian rivers and Coyote Creek.

### **Lower Colorado River Drainage (HUC 2=15)**

We sampled 301 (of 3190 total samples) amphibians, of eight species including American Bullfrog, Arizona Toad, Boreal Chorus Frog, Canyon Treefrog, Chiricahua Leopard Frog, Great Plains Toad, Mountain Treefrog, and Tiger Salamander, from the Lower Colorado River drainage. In New Mexico, this region includes the Gila, San Francisco, and Zuni rivers, the Rio Nutria, and all tributaries of these four rivers (Fig. 9). Eight counties in New Mexico are contained in part within this HUC 2 region. The western border of this region extends well west of the western New Mexico border, while the eastern border corresponds with the Continental Divide. The northern border extends just north of the I-40 corridor. It is intermediate in size when compared to other New Mexico HUC 2 regions. Most samples were collected within intermediate to higher elevations of the Lower Colorado River drainage, including mountains associated with eastern portions of the Mogollon Rim and the western slope of the Black Range. Thirty-eight samples (12.6 %) from the Lower Colorado River drainage tested positive for *Bd*. Most of the samples testing positive were collected in intermediate to higher elevations within this region. Positive test results were obtained for at least one of the samples collected along all major tributaries within this HUC 2 region, including the Rio Nutria, Gila, and San Francisco rivers.

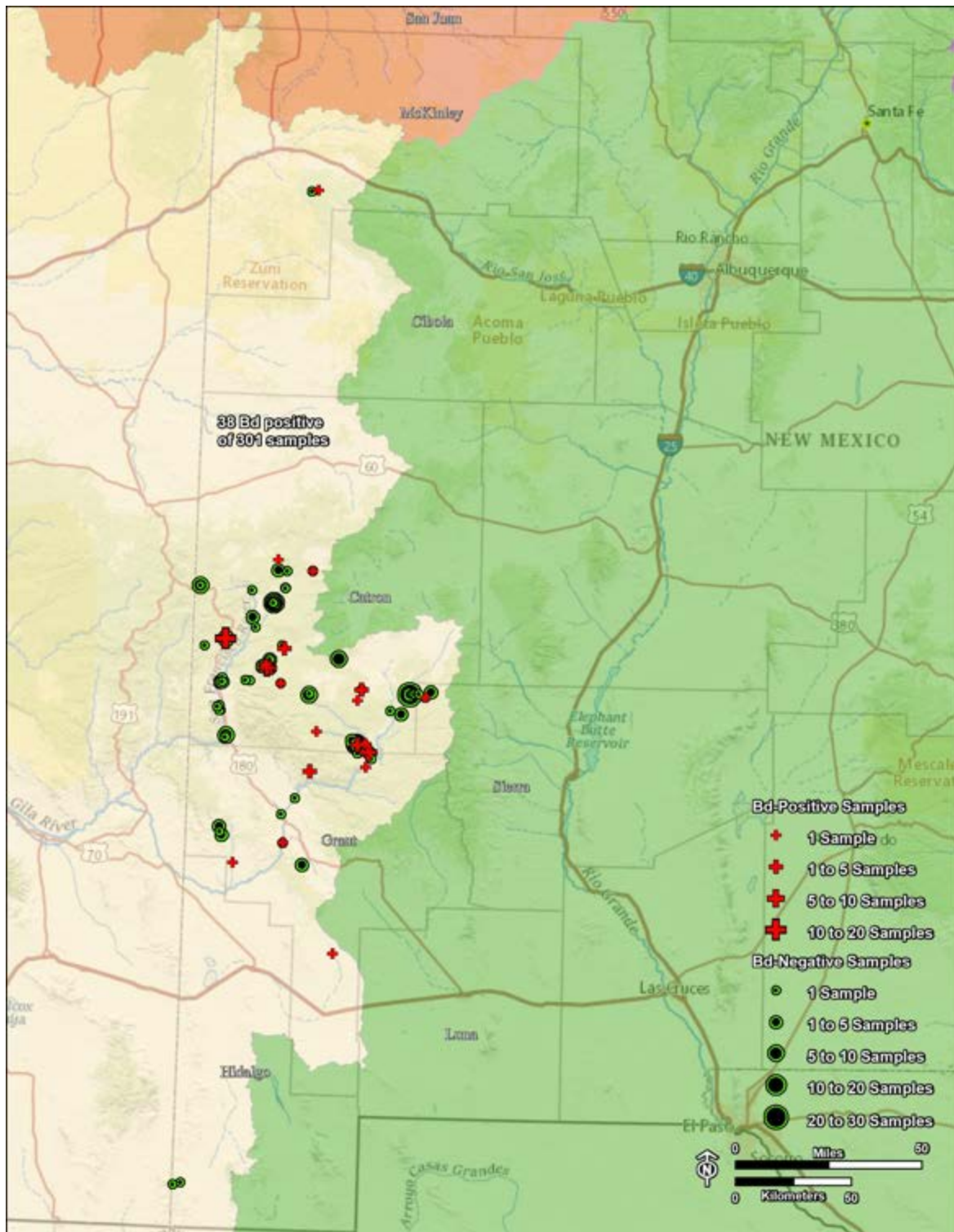


Figure 9. *Batrachochytrium dendrobatidis* (Bd) detections in the Lower Colorado River drainage. Cream shows HUC 2 boundary.

### **Upper Colorado River Drainage (HUC 2=14)**

We sampled 35 (of 3190 total samples) amphibians, of two species including American Bullfrog and Northern Leopard Frog, from the Upper Colorado River drainage. In New Mexico, this region includes the Animas, San Juan, Navajo, and La Plata rivers and their tributaries (Fig. 10). Four counties in New Mexico are contained in part within this HUC 2 region. The western border of this region extends well west of the western New Mexico border, while the eastern border corresponds with the Continental Divide, just north of the I-40 corridor, to the New Mexico-Colorado Border. It is a small HUC 2 region within New Mexico. Most samples collected in this drainage were collected along two of the major river courses (i.e., the San Juan and Navajo rivers). Eight samples (22.8%) from the Upper Colorado River drainage tested positive for *Bd*. Most of the samples testing positive were collected along the San Juan River and within the Navajo River drainage. Positive test results were obtained for all major tributaries (except the Animas) within this HUC 2 region from which samples were collected. Further sampling is needed in this region.

### **Texas Gulf Region (HUC 2=12)**

We sampled 66 (of 3190 total samples) amphibians, of two species including Plains Leopard Frog and Woodhouse's Toad, from the Texas Gulf Region. In New Mexico, this region includes no major streams or rivers (Fig. 11). Four counties in New Mexico are contained in part within this HUC 2 region. The western border of this region corresponds with the Caprock in southeastern New Mexico, while the eastern border extends well east of the eastern New Mexico border. It is the smallest HUC 2 region within New Mexico. Samples were collected on the Milnesand Prairie Preserve near Dora, and at Canon AFB near Clovis. This was the only HUC 2 region with only one *Bd* positive locality. More sampling is needed in this region.

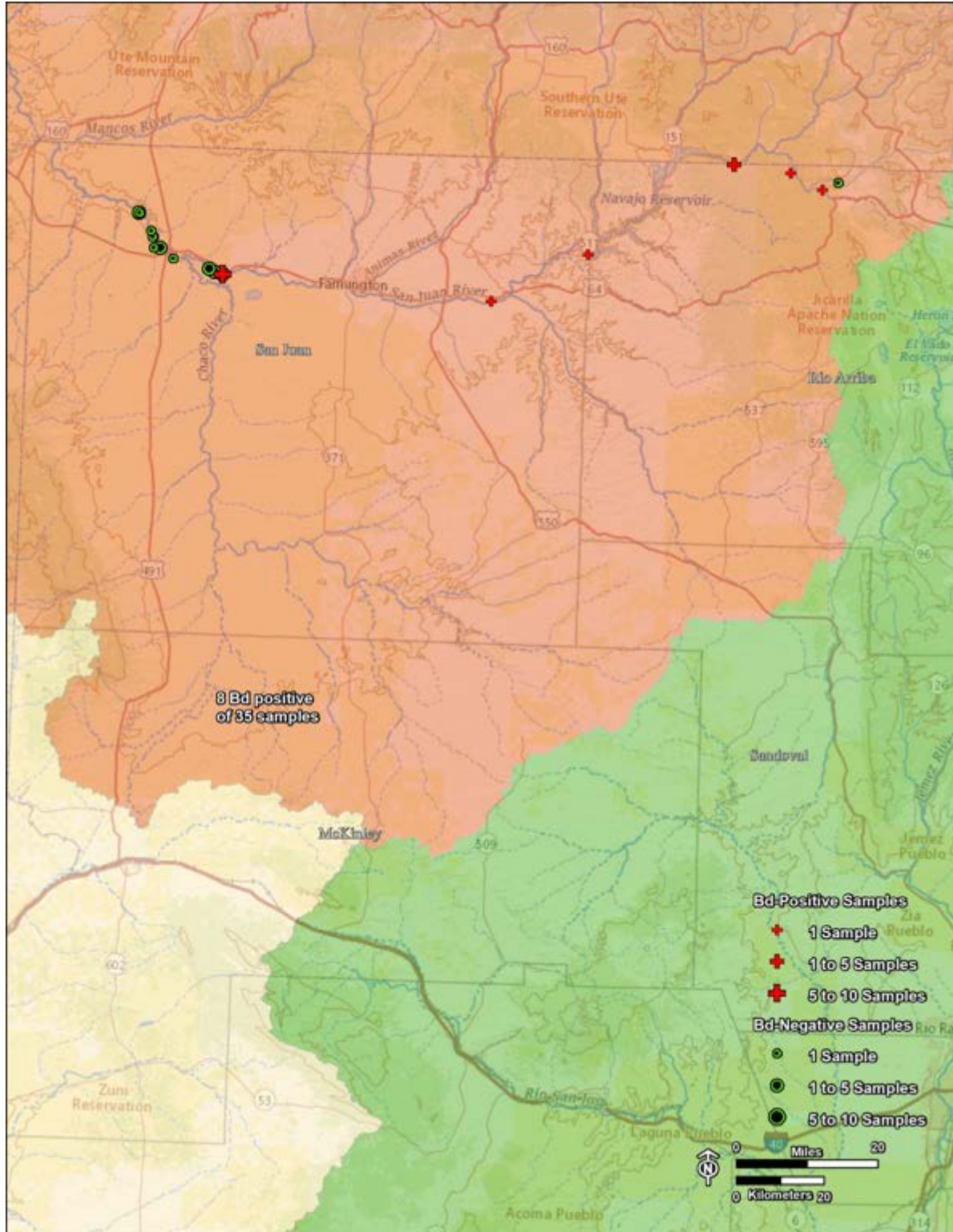


Figure 10. *Batrachochytrium dendrobatidis* (Bd) detections in the Upper Colorado River drainage. Orange shows HUC 2 boundary.



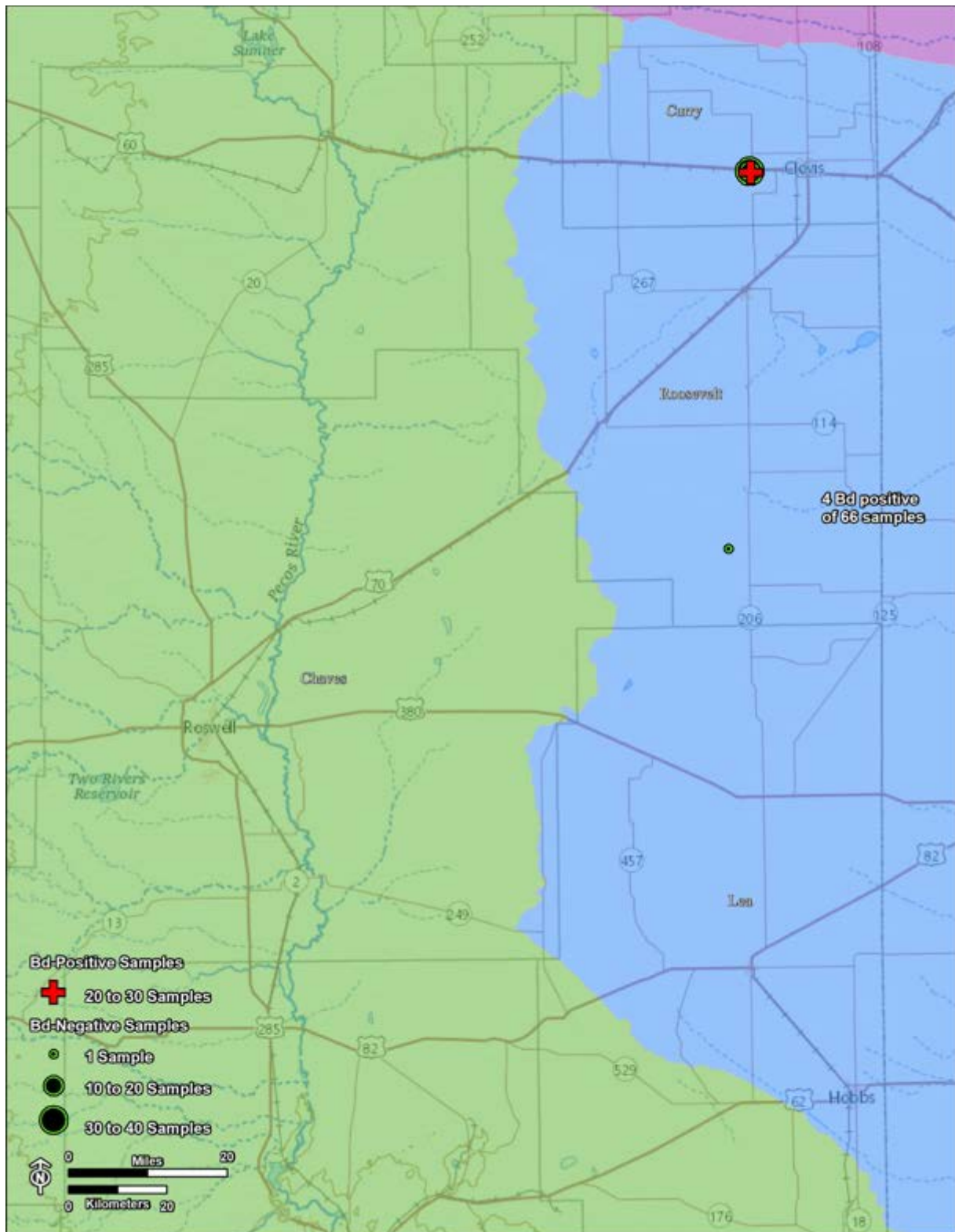


Figure 11. *Batrachochytrium dendrobatidis* (Bd) detections in the Texas Gulf Region drainage. Blue shows HUC 2 boundary.

## State-Wide Summary

*Bd* has been detected throughout New Mexico, including in all major drainages where adequate sampling effort has been employed. Among the 8-digit HUCs (HUC 8s) where samples were collected, there were 15 for which no *Bd* was detected (Fig. 12). For six of these 15 HUC 8s, only a single sample was collected. Seven of these 15 had nine or fewer samples collected. Detectability of *Bd* within an individual HUC 8 is quite variable (1/1 up to 6/455), which demonstrates that considerable effort may be needed across seasons and species to detect *Bd*. For example; 84 samples had been collected within one HUC 8 for which no *Bd* was detected (i.e., the Upper Rio Grande HUC, located primarily in Taos, Rio Arriba, Santa Fe, and Los Alamos counties). Many of these samples were from the Jemez Mountains Salamander, a terrestrial salamander that exhibits a low detection rate of *Bd*, or the Tiger Salamander, a mole salamander that also has low *Bd* detection rates. These observations support the notion that *Bd* was not adequately surveyed in these 15 HUCs. Several more HUC 8s have not been sampled within New Mexico. These under-sampled and unsampled HUCs should be the focus of future efforts to refine our understanding of the distribution of *Bd* in New Mexico. Furthermore, all aquatic systems in New Mexico should be treated as if they are *Bd* positive until the distribution and phenology of *Bd* is better understood. Any work conducted in aquatic systems should require following a standardized disinfection protocol.

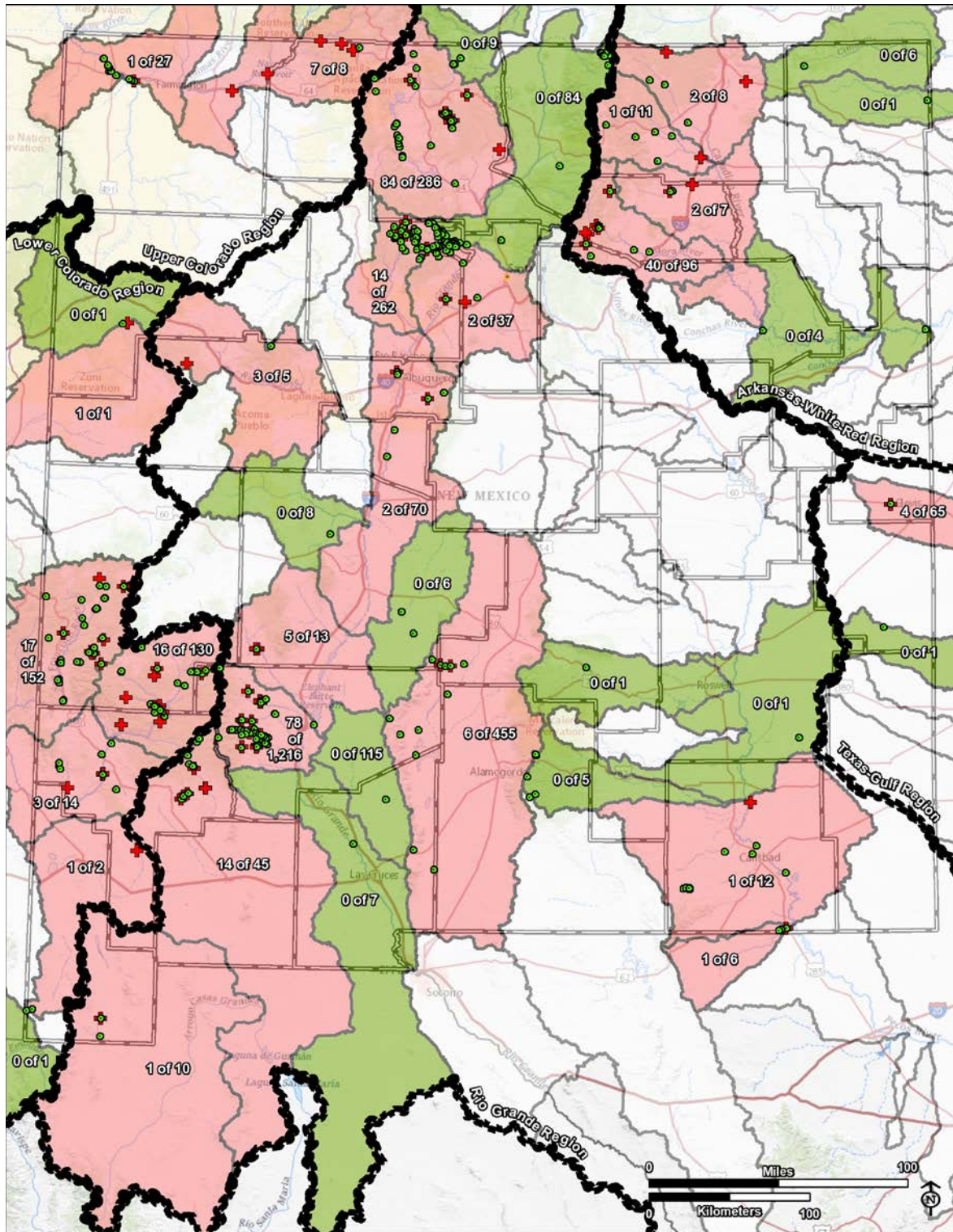


Figure 12. *Batrachochytrium dendrobatidis* (Bd) detections across the state. Bd was not detected in 15 HUC 8s (green) that were sampled. White HUC 8s were not sampled. Pink HUC 8s were sampled and Bd was detected. Numbers within HUCs represent detections / sample sizes. Thick black lines delineate HUC 2 boundaries.

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## **Acknowledgements**

This report represents many years of data collection and collaboration with many individuals and agencies. Assistance with sample collection and data sharing: M. Hill, J. Vencill, L. Mc Innis, J. Borgmeyer, M. Christman, C. Painter, A. Faca, A. Lamb, C. Crowder, L. Pierce, J. Stuart, C. Hathcock, M. Wright, B. Barker, E. Gilbert, S. Hitt, J. Holms, K. Narum, A. Fitzgerald, L. Cole, C. Wolf, H. Johnson, H. Small, C. Coobos, J. Schofer, A. Gehrt, J. Barnitz, D. Burkett, I. Latella, T. Giermakowski, M. Watson, M. Ryan, K. Elder, Dan Shaw, G. Rios-Sotelo, H. Snell, M. Lannoo, R. Lovich, C. Petersen, P. Nanjappa, C. Phillips, J. Mitchell, I. Macallister, R. Stiles, S. Corey-Rivas, J. Zebrowski, and A. Miller.

GIS mapping of data: M. Hill.

Analysis of samples: P. Fernandez (Glenwood Community College), J. Wood (Pisces Molecular), A. Pressier (San Diego Zoo), J. Voyles (University of Nevada, Reno), and M. Keller (USFWS).

Institutional Assistance and Funding: New Mexico Department of Game and Fish, Bureau of Land Management, U. S. Forest Service, U. S. Fish and Wildlife Service (Ecological Services NM Field Office, Maxwell and Rio Mora National Wildlife Refuges), Bandelier National Monument, Valles Caldera National Preserve, Los Alamos National Lab, Turner Endangered

Species Fund, T and E Inc., Museum of Southwest Biology, UNM, Gila Natural History Collection, and WNMU.

Access to private land: Turner Ranch Properties, Bill and Mary Zedike, AT and Cinda Cole.

Funding for compilation of these data was provided by the Share with Wildlife Program at New Mexico Department of Game and State Wildlife Grant T-32-5, #5.