

First Record of *Batrachochytrium dendrobatidis* in the Arizona Toad (*Anaxyrus microscaphus*) in Southwestern New Mexico, USA

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis, is geographically widespread across the United States (Lannoo et al. 2013; Olson et al. 2013). Chytridiomycosis has caused the decline of some North American species (e.g., Southern Mountain Yellow-legged Frog, *Rana muscosa*; Briggs et al. 2010; Vredenburg et al. 2010), yet other species seem relatively unaffected by *Bd* infection (e.g., American Toad, *Anaxyrus americanus*; Longcore et al. 2007). Many arid and desert-adapted amphibian species in the

southwest U.S. such as *Rana chiricahuensis*, (Chiricahua Leopard Frog) have experienced declines and are now considered to be high conservation priorities (Bradley et al. 2002; Schlaepfer et al. 2007; Olson et al. 2013). The role of chytridiomycosis in these declines has not been determined but is important to resolve in order to identify vulnerable species that require conservation intervention.

Within New Mexico, four amphibian species have declined to critically low levels: *R. chiricahuensis*, *R. pipiens* (Northern Leopard Frog), *R. yavapaiensis* (Lowland Leopard Frog), and *Anaxyrus boreas* (Western Toad). In neighboring states where these species occur, declines have been attributed to chytridiomycosis (Bradley et al. 2002; Muths et al. 2003; Lannoo et al. 2011); hence it is suspected that *Bd* is contributing to the New Mexico declines as well. Recent investigations have found that 53% (9 of 17) of tested species of New Mexican amphibians have tested positive for *Bd* (Painter et al., unpubl.), but there is still not a clear understanding of the distribution and prevalence of *Bd* in New Mexican amphibians (Olson et al. 2013; Painter et al., unpubl.).

The Arizona Toad, *Anaxyrus microscaphus* (Fig. 1), is a riparian species that occurs in scattered populations in Arizona, Nevada, Utah, and New Mexico (Schwaner and Sullivan 2005). Previous studies have indicated that declines of *A. microscaphus* may be due to hybridization between *A. microscaphus* and *A. woodhousii* (Woodhouse's Toad) in Arizona, Nevada, and Utah (Sullivan 1995; Schwaner and Sullivan 2009) and altered water

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FIG. 1. An adult *Anaxyrus microscaphus* calling along Little Creek, New Mexico, USA on 29 March 2013.

flow regimes from dam construction and habitat modification (Bradford 2002; Hammerson and Schwaner 2004). To date, *Bd* has not been detected in *A. microscaphus*, and it is unknown if *Bd* poses a yet unrecognized threat to this species.

During a 2013 study of *A. microscaphus* in southwestern New Mexico, we sampled 25 *A. microscaphus* for *Bd* from four sites in the Gila National Forest (Fig. 2): Hells Hole (33.787°N, 108.694°W; 1915 m elev.; Catron Co., nine samples), San Francisco River (33.344°N, 108.902°W; 1461 m elev.; Catron Co., two samples), Little Creek (33.194°N, 108.220°W; 1724 m elev.; Grant Co., 12 samples), and the upper Mimbres River (33.040°N, 107.980°W; 2081 m elev.; Grant Co., three samples) (Fig. 2). In addition, we monitored *A. microscaphus* at Little Creek weekly from 28 March to 15 May 2013. Previously, 11 years earlier, on 3 June 2002, following a mass die-off of the threatened species *R. chiricahuensis*, two *A. microscaphus* individuals were sampled for *Bd* at the die-off location in the vicinity of Six-shooter Tank (33.518°N, 108.699°W; 2430 m elev.). Following standardized field protocols, we collected and stored *Bd* swab samples in 95% ethanol and a -80°C freezer until processing (Boyle et al. 2004; Hyatt et al. 2007). Samples were submitted in batches of six to Pisces Molecular Laboratory (Boulder, Colorado, USA) for pooled analyses of *Bd* DNA using qPCR.

Bd was detected from one of two batch samples of *A. microscaphus* collected from Little Creek (collected on 29 March 2013). Samples from Hells Hole, upper Mimbres River, and San Francisco River were *Bd*-negative. One *Bd*-positive *A. microscaphus* individual was found at the site of the 2002 die-off of *R. chiricahuensis*. During the March to May 2013 field observations of *A. microscaphus* at Little Creek site, we observed breeding activity among a maximum of 21 toads (15 males, 6 females), including male calling, and pairs in amplexus and laying eggs. We did not observe sick or dead *A. microscaphus* during this monitoring, or during handling for swab collection and measurements at any location.

These *Bd*-positive results from New Mexico are the first records of *Bd* in *A. microscaphus*. Both records are from the Gila National Forest, which encompasses much of the range of *A. microscaphus* in New Mexico (Degenhardt et al. 1995). *Bd* was previously detected on amphibians in the Mogollon Rim of Arizona and New Mexico (Painter et al., unpubl.).

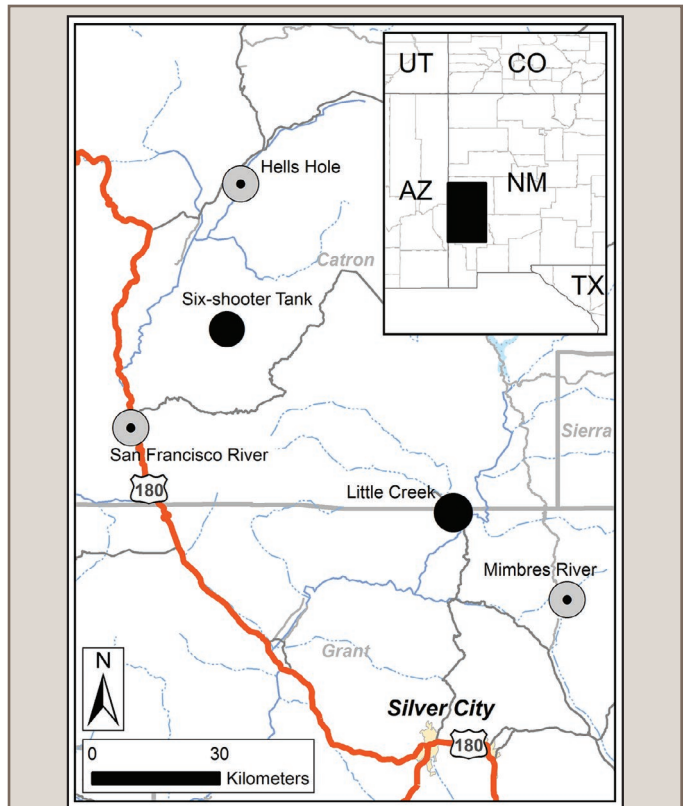


FIG. 2. Distribution of localities in two counties of southwestern New Mexico from which Arizona Toads (*Anaxyrus microscaphus*) were examined for *Batrachochytrium dendrobatidis* (*Bd*). Filled circles indicate sites that tested positive, whereas open circles with a dot indicate sites where *Bd* was not detected.

Although *Bd* has been detected in its range, the role of *Bd* in population declines of *A. microscaphus* remains unknown. Populations of *A. microscaphus* have declined in areas of New Mexico where hybridization and altered stream flows from water impoundments are not a threat (Ryan et al., unpubl.), and remain enigmatic. To our knowledge, no mass die-offs characteristic of a *Bd* outbreak have been observed in *A. microscaphus* in Arizona and New Mexico (Bradley et al. 2002), but the possibility of *Bd* as a threat cannot be ruled out. Further studies utilizing museum specimens and reviews of unpublished data and reports may fill in gaps of possible die-offs and the prevalence of *Bd* in *A. microscaphus*.

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