AMPHIBIAN CHYTRIDIOMYCOSIS GEOGRAPHIC DISTRIBUTION

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Infection with the Fungus *Batrachochytrium dendrobatidis* in a Non-native *Lithobates berlandieri* Below Sea Level in the Coachella Valley, California, USA

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The amphibian fungal disease chytridiomycosis caused by *Batrachochytrium dendrobatidis* (*Bd*) has been found in several parts of the world including Africa, Australia, Europe, Central, South, and North America (e.g., Skerratt et al. 2007), yet its geographic distribution is not fully known. Based on current patterns, the great majority of species and populations that have been affected have occurred in montane habitats or areas characterized by relatively moist climates (Stuart et al. 2004; Longcore et al. 2007). In North America, *Bd* has been reported from numerous species, with the earliest recorded incidence of infection dating back to 1961 (Ouellet et al. 2005; Longcore et al. 2007; AmphibiaWeb 2007). *Bd* is recorded from latitudes as far north as Alaska, USA (e.g., Adams et al. 2007; Reeves 2008), and at elevations extending from near sea-level at Point Reyes, California, USA, to 3550 m in the Sierra Nevada, California (Fellers et al. 2001; Morgan et al. 2007; Spatialepidemiology.net 2008).

Recently, *Bd* has been confirmed in native, desert frog species *Lithobates* [=*Rana*] *yavapaiensis*, *L. chircahuensis*, *L. tarahumarae* and *Hyla arenicolor* in Arizona (Bradley et al. 2002; Hale et al. 2005) and is hypothesized to be a key factor in the extirpation of populations of these species. Locally in southern California, *Bd* is known to occur in the native species *Anaxyrus* [=*Bufo*] *californicus* (Mendelsohn et al. 2004), and *Rana draytonii* (Ervin et al. 2001). Individuals from established populations of the introduced frog species *L. catesbeianus* and *L. berlandieri* in the southwestern United States have been reported to be infected with *Bd* (Sredl 2002). Consequently, the concern now is that infected individuals from these populations, and other undiagnosed populations, may serve as vectors for *Bd* to naïve populations of native frogs (Hanselmann et al. 2004, Sredl et al. 2002). *Lithobates berlandieri* is native to Texas and New Mexico but has been introduced to Arizona and California within the last 35 years (Rorabaugh et al. 2002).

On 23 January 23 2007, one of us (BC) collected a dead *L. berlandieri* in Coachella Valley, California, USA (33.5352722°N, 115.9883278°W), south of the town of Mecca, and ~1 km N of the Salton Sea. The animal was found in an agricultural ditch (~2 × 35 m) filled with 5– 20 cm of standing water and with lush emergent vegetation and a dense mat of green algae, at –58 m below sea level. The specimen was photo documented (LACM PC1457–1460), and examination revealed bright red coloration on the ventral surface of the legs that is uncharacteristic of the natural coloration for this species. Museum acronyms used follow Leviton et al. (1985). Subsequent searches of the area were made, and on 13 March 2007 one of us (MJR) collected an additional living specimen from the same agricultural ditch. Upon capture the frog was moribund and unable to right itself when placed on its back. The ventral surfaces of its legs were reddened similar to the frog that was found dead. The second frog perished within a day of capture, despite efforts to maintain it in captivity until it could be prepared as a museum specimen. It was preserved in 95% ethanol and a complete necropsy was performed on the fixed carcass. Sections of the dorsal and ventral skin, heart, lung, liver, kidney, pancreas, tongue, stomach, small and large intestine, brain, eye, skeletal muscle, bone, and bone marrow were post-fixed in 10% neutral buffered formalin and processed routinely for histology. When hematoxylin- and eosin-stained sections were examined by light microscopy, lesions were limited to the skin and other tissues were histologically normal. Histologic findings included: near diffuse mild to moderate epidermal hyperplasia with multifocal epidermal erosion; scattered single cell necrosis of keratinocytes; moderate to marked congestion of dermal capillaries: and a mild multifocal lymphohistiocytic dermatitis. Occasionally, within the superficial keratin layers (*stratum corneum*) there were small numbers of spherical fungal thalli with evidence of internal septation (colonial thalli) morphologically typical of *Bd* (Berger et al. 2005; Longcore et al. 1999). Based on these findings, death was attributed to chytridiomycosis. A swab obtained from the skin was processed following previously described methods for a Taqman® polymerase chain reaction (PCR) assay to detect *Bd* DNA (Boyle et al. 2004; Hyatt et al. 2007). PCR ^{results} were positive, confirming the morphologic identification of *Bd* on histologic examination. The frog is vouchered as SDNHM 72858.

This observation of *Bd* infection is notable for several reasons. First, it represents the first record of *Bd* in *L. berlandieri* in California. *Lithobates berlandieri* is expanding its range in the deserts of the American southwest (Rorabaugh et al. 2002), and also occurs in sympatry with other native aquatic amphibian species such as *Anaxyrus punctatus*, *A. woodhousei*, and *A. alvarius*, *Pseudacris hypochondriaca* (pers. obs.). The determination that *Bd* is now known to occur in this region highlights the need for other local native species to be monitored to investigate the impacts of this pathogen in a region otherwise characterized by extreme heat and low humidity. Second, this is the first incidence of *Bd* below sea level in one of the hottest

and driest locations in the western hemisphere (Klauber 1931). Occurrence of *Bd* in arid, desert environments is significant because desert anurans tend to be reliant on water bodies more so than species of more mesic environments resulting in higher local densities (Degenhardt et al. 1996) which may allow *Bd* to more virulently move through a population (Rowley and Alford 2007; Ryan et al. 2008). Although the prevailing environmental conditions at the sampling locations seem unsuitable to survival of *Bd*, microhabitat use (water bodies, retreat sites) and behaviors used by *L. berlandieri* (thermoregulation) could counteract the prevailing conditions by keeping the frog and thus the *Bd* at temperatures and humidities more conducive to both, because these frogs are obviously not exposing themselves to the extremes of the environment. *Lithobates berlandieri* is known to tolerate extreme salinities (McCoid 2005), similar to those conditions along the Salton Sea where the specimens were documented and collected. Interestingly, in laboratory culture *Bd* has been shown to survive, but grow poorly, at a pH of less than 6 (Piotrowski et al. 2004). The historically desert environs of the Coachella Valley have undergone urban and rural development to such an extent that extremely widespread agriculture, golf courses, and residential and private artificial landscaping have resulted in an elevated humidity and the expansion of mesic spatial and temporal conditions. Humidity is also greater along the shores of California's largest inland body of water, the Salton Sea, where the specimens were found. *Bd* is thought to be intolerant of extremely warm, dry conditions, and the occurrence of *Bd* in this type of environment is novel (Piotrowski et al. 2004).

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Preliminary Surveys for *Batrachochytrium dendrobatidis* in Taiwan

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Batrachochytrium dendrobatidis (*Bd*) is a chytrid fungal pathogen of amphibians that has been implicated in numerous amphibian extinctions and declines (Berger et al. 1998; Lips et al. 2006). The geographic distribution of this novel pathogen, however, is poorly known. While a number of reports are available from Africa (Goldberg et al. 2007; Smith et al. 2007; Weldon et al. 2004), Australia (Berger et al. 1998; Woodhams and Alford 2005), Europe (Bosch et al. 2007; Garner et al. 2005) and the Americas (Lips et al. 2006; Ouellet et al. 2005), we are aware of only two published reports from Asia. The first study (Garner et al. 2006) found no evidence of *Bd* in introduced populations of North American Bullfrogs (*Lithobates catesbeianus,* formerly *Rana*; Crother 2008; Frost et al. 2006) in Japan. The second study (Rowley et al. 2007) found no evidence of *Bd* in four native species or in frogs imported to Hong Kong, China. Herein, we provide preliminary data from surveys for *Bd* in Taiwan.

Methods.—On 3 October 2006, skin swabs were collected from 20 wild-caught adult frogs (representing 12 species in four families, taxonomy following Frost et al. 2006). Frogs were collected from ponds, streams, and roadside ditches in the vicinity of the Taiwan Forestry Research Institute's experimental forest at Lien Hua Chih Station (23.92°N, 120.87°E, Nantou County, elevation range of collection sites 600–700 m). Skin swabs were obtained by running a sterile cotton swab along the skin of the captured frog for approximately 30 seconds, focusing on the hands, feet, and pelvic region. A new pair of sterile gloves was used when capturing and handling each frog. Frogs were released immediately after the swabbing procedure. During collection, air temperatures ranged from 17–21°C. Skin swabs were preserved in 70% ethanol in 2.0 ml screw-capped microcentrifuge tubes and stored at room temperature. All samples were transported to the University of Michigan where they were tested for the presence of *Bd*.

The 20 swab samples and a total of 100 negative controls were randomized and tested for *Bd* using Taqman diagnostic quantitative PCR (q-PCR; Boyle et al. 2004). DNA was extracted from each sample and negative control following Hyatt et al. (2007) and q-PCR assays were performed in triplicate following Boyle et al. (2004). Samples containing PCR inhibitors were detected using VIC_{TM} Exogenous Internal Positive Controls (Applied Biosystems) and inhibition was overcome by dilution following Hyatt et al. (2007). Samples were considered positive if all three replicates indicated the presence of *Bd*. Samples testing positive in one or two replicates were re-assayed once.

Results.—One of the 100 negative controls tested positive for *Bd*, indicating a false positive rate for DNA extraction and *Bd* assay of 1%. All of the skin swab samples tested negative for *Bd* DNA in all three replicates except for one (from *Huia swinhoana*, Table 1). This sample initially tested positive for *Bd* in two out of three replicates. However, when this sample was re-extracted and three additional assays were performed, none of these replicates tested positive for *Bd* DNA. No dead or obviously diseased frogs were found at the study sites.

Discussion.—Taken together, the false positive rate of 1% for q-PCR and the lack of amplification in a second assay, it seems likely that the amplification of *Bd* DNA from the first assay of the *Huia swinhoana* sample was a result of cross-contamination (i.e., a false positive). While these data are from a relatively small sample in a small geographic area, they suggest that either *Bd* is absent from these sites in central Taiwan or that it occurs at such a low frequency that it was not detected.

Although we found no convincing evidence of *Bd*, it would be presumptuous to claim that it is absent from Taiwan. In fact, there have been unpublished reports of *Bd* from North American Bullfrog farms in southern Taiwan (L. Schloegel, pers. comm.). With relatively high levels of endemicity (27%; Yang 1998) and much of its land area at moderate to high elevations (where conditions for *Bd* would likely be optimal), Taiwan might be vulnerable to the kind of amphibian declines and extinctions that have been noted elsewhere. Additional surveys for *Bd* are urgently needed island-wide to assess the danger this pathogen may pose to Taiwan's amphibian fauna.

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