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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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TABLE 1. List of species tested for *Batrachochytrium dendrobatidis* infection from Lien Hua Chih Forestry Station, Nantou County, Taiwan. * indicates endemic species. Ecological data from Chou and Lin (1997).

Family	Species	No. Infected / No. Tested	Breeding Habitat	Elevation Range (m)
Bufonidae	*Bufo bankorensis**	0 / 2	ponds, ditches	0–2700
	Duttaphrynus melanostictus	0 / 1	ponds	0–1500
Dicroglossidae	*Limnonectes kuhlii*	0 / 2	streams, ditches	300–600
Ranidae	*Babina adenopleura*	0 / 1	ponds	0–2000
	Huia swinhoana	0 / 2	streams	150–1700
	Pelophylax plancyi	0 / 2	ponds	0–1000
	Rana sauteri	0 / 3	streams	170–2600
	Staurois latouchii	0 / 2	ponds, ditches	0–1000
Rhacophoridae	*Buergeria japonica*	0 / 2	ditches, streams	175–1200
	*Buergeria robustus**	0 / 1	streams	175–800
	*Kurixalus idootocus**	0 / 1	ponds, ditches	50–1200
	*Rhacophorus moltrechi**	0 / 1	ponds, cisterns	150–2500
Total		0 / 20		

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