Short notes

Batrachochytrium dendrobatidis not found in rainforest frogs along an altitudinal gradient of Papua New Guinea

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Batrachochytrium dendrobatidis (*Bd*) is a fungal pathogen often responsible for amphibian declines worldwide. We report here survey on *Bd* in Papua New Guinea (PNG). The survey for *Bd* was conducted along a rainforest altitudinal gradient from Madang (50 m a.s.l.) to Mt. Wilhelm (3700 m a.s.l.). We swabbed 249 frogs of 63 native species at nine sites to quantify the number of *Bd* zoospore equivalents using real-time Syber Green Polymerase Chain Reaction (qPCR). We found no evidence for *Bd*. The lack of *Bd* may be due to 1) hot climate all year round inhibiting the spread of *Bd* in the entire lowland areas of PNG, 2) low number of non-native amphibian introductions to PNG such as *Lithobates catesbeianus* or *Xenopus* spp. or 3) the lack of invasive introductions by humans due to geographic isolation. While it is difficult to discern between these hypotheses, an effective quarantine should be devised to protect PNG from future disease outbreak. International assistance is needed in conservation education and research to assist the local scientists in monitoring and protecting these rich fauna from future *Bd* outbreaks.

Key words: altitude, amphibians, *Batrachochytrium dendrobatidis*, chytridiomycosis, Papua New Guinea, rainforest

Papua New Guinea (PNG), situated on the eastern half of the island of New Guinea, harbours approximately 5% of the world's biodiversity (Mittermeier et al., 1998). Frogs contribute greatly to the rich faunal diversity, with 325 species described so far (Frost 2011). To date, information available on frog ecology and distribution in PNG is limited, and no long-term monitoring has been established to track the status of frog populations in the wild (Richards 2002; Dahl et al., 2009). Reports on global warming, forest degradation and arrival of foreign pathogens are the primary factors implicated for amphibian declines in highly diverse tropical regions worldwide (Berger et al., 1998, Lips et al., 2006), and may also play an important role in PNG.

Batrachochytrium dendrobatidis (*Bd*) is a fungal pathogen implicated in the decline of many frog species and has threatened many species with extinction (Ron 2005; Kusrini et al., 2008; Kriger & Hero 2008). In recent years, reports on population decline in frog species have received wide coverage in Europe, North America, Central America, South America, Australia and more recently Asia (Fisher et al., 2009). It was predicted that *Bd* might occur on the mountain tops of Indonesia and PNG because of favourable temperatures (Bielby et al., 2008; Kusrini et al., 2008). Recently, *Bd* was reported from Indonesia (Kusrini et al. 2008). Australia, a neighbouring country, has a history of severe chytridiomycosis outbreaks (e.g. Woodhams & Alford 2005), providing a possible point of entry for *Bd*. Our aim was to collect chytrid swab samples from frog populations along an altitudinal gradient in PNG, in order to assess whether *Bd* exists in the areas surveyed.

A total of nine primary rainforest sites were surveyed from the lowland area near Madang at 50 metres above sea level (a.s.l) to the slopes of Mt. Wilhelm up the alpine zone at 3,700 m a.s.l. The study transects comprised mixed lowland evergreen hill forest, lower and upper montane cloud forests and alpine grassland meadows (Fig. 1, McAlpine et al., 1983).

Each site was surveyed over a two week period between May 2009 and January 2010. We used the swab technique described by Kriger et al. (2006). Frog species for swabbing were selected randomly from our samples; the common species were sampled more often. Each body part of a frog's ventral sides, the groin, armpit, thigh and foot was swabbed 4 to 6 times for *Bd* with a cotton swab (Medical Wire & Equipment, MW 100-100). Rubber gloves were worn when taking swabs to avoid the risk of transferring *Bd* zoospores between frogs.

The swabbed samples were stored in a freezer at -20°C after returning from the field. In total, swabs from 249 individual frogs were analyzed at the Center for Conservation and Evolutionary Genetics (Smithsonian Institution, USA) using the real-time Syber Green Polymerase Chain Reaction (qPCR) following the procedures outlined by Boyle et al. (2004). DNA was extracted in 96-well plates on a Qiagen BioSprint 96 following Qiagen's buccal swab protocol. Each plate contained up to 93 samples along with a negative "extraction blank" control and two known positive *Bd* controls. In addition, a 400–500 bp DNA fragment of amphibian 16S rRNA was amplified for each individual using primers 16SA-L and 16SB-H (Vences et al., 2004)

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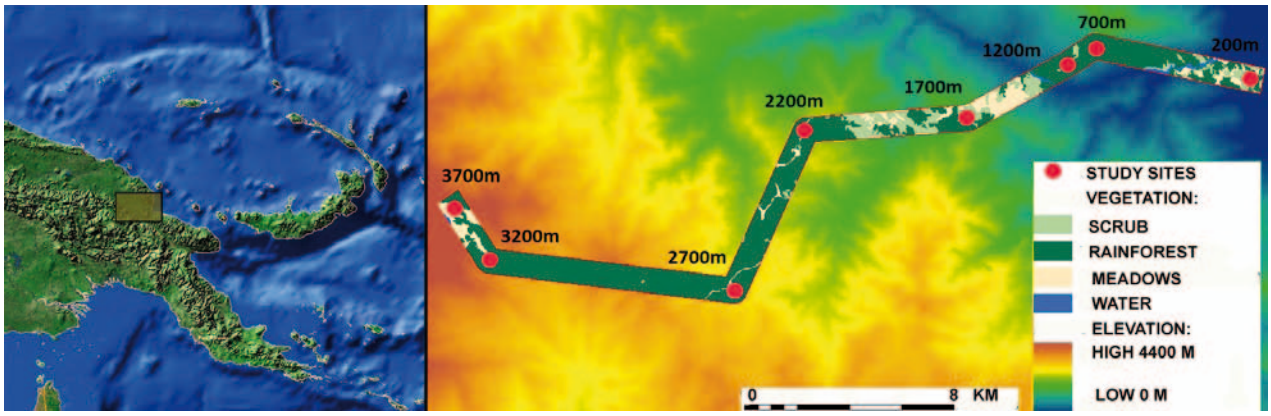

Fig. 1. Map of Papua New Guinea and study transect sites from low to the high altitudes along primary rain forest.

as a DNA quality control. A subset of individual samples was amplified using normal PCR with *Bd* primers and run on an agarose gel.

We took swab samples from 249 adult frogs (63 species, 15 genera) in all four native families (Hylidae, Microhylidae, Myobatrachidae and Ranidae, see the electronic Appendix). The species sampled represented approximately 20% of the amphibian species richness of PNG, and 83% of the species found along the surveyed sites (Dahl, C., unpublished data).

We did not detect *Bd* DNA using the real-time qPCR in our samples or the negative controls. The *Bd* positive controls amplified consistently in all assays, indicating that our method was capable of amplifying *Bd*. In addition, our 16S amphibian gene assays amplified 97.4% of samples, suggesting that our DNA extraction protocol was successful and the DNA was of sufficient quality for amplification of *Bd*. For the subset of individuals amplified using normal PCR methods, no bands indicating positive *Bd* infection were obtained. Thus we conclude that there was no evidence for *Bd* infection in these 249 frogs.

Bd introduction has threatened many of the world's pristine ecosystems (Kriger & Hero 2007). *Bd* may be rated the most devastating panzootic epidemic in amphibian history because of its widespread occurrence. This is reflected by the amount of research aimed to combat the epidemic (e.g. Boyle et al., 2004; Kriger & Hero 2008; Kusrini et al., 2008; Fisher et al., 2009).

Rohr et al. (2008) hypothesized that global warming and human alteration of the environment can facilitate the introduction and spread of *Bd*. The prediction model suggested that *Bd* is likely to occur in the New Guinea's Central Cordillera because of high climate suitability which is favourable for *Bd* (Bielby et al., 2008; Swei et al., 2011). Global warming is contributing to a favourable environment for *Bd* to reach higher altitudes (Hero & Morrison 2004; Pounds et al., 2006). Our results were negative across the entire rainforest altitudinal range in PNG, i.e. including sites with potentially favourable temperature and environment for *Bd* (Bielby et al., 2008; Kusrini et al., 2008; Kriger & Hero 2008). In particular, *Bd* is commonly found along the stream dwelling amphibians within the mean air temperature of 17°C to 28°C while higher temperatures limit *Bd* growth (Kriger & Hero 2008; Fisher et al., 2009, Longcore et al., 1999; Johnson & Speare 2003). This temperature range corresponds to temperatures between 16°C and 27°C and altitudes from 0 to 2,000 m a.s.l at our study sites (Dahl, C., unpublished data). These studies shown that *Bd* prevalence increases with increasing altitude from 50 m to >1,500 m a.s.l (see Kriger & Hero 2008; Fisher et al. 2009).

Species such as *Litoria caerulea* and *L. infrafrenata* are known to carry the disease in Australia. These taxa also have a wide spread distribution in the lowland forest of PNG (Dahl et al., 2009), and both tested negative in our study. Our failure to detect the presence of *Bd* strongly suggests that this novel pathogen has not yet reached the northern provinces of PNG.

There is a high demand for amphibians in pet trade in Asian countries (Kusrini et al., 2008). *Bd* reached various continents through pet trade as reported by Kriger & Hero (2009) and Goka et al. (2009). The intensity of pet trade is low, and no precise numbers are known for PNG. The pet trade of the Papua region in Indonesia and Australia may increase the risk of introduction of *Bd* into PNG.

We suggest that the lack of *Bd* in PNG may be due to a hot climate inhibiting the spread of *Bd* in the entire lowland areas of PNG, and non-native amphibian introductions to PNG such as *Lithobates catesbeianus* or *Xenopus* spp. The cane toad (*Rhinella marina*) is the only introduced exotic in PNG and Australia (Menzies, 2006), as compared to 45 exotic frog species to Japan (Une et al., 2008) and four species in Indonesia (Kusrini et al., 2008). The lack of invasive introductions by humans due to geographic isolation (Madang and Mt. Wilhelm sites are isolated from major towns) could further contribute to the lack of evidence for *Bd*.

That we failed to detect *Bd* along the altitudinal gradient sites may be due to our small sample size (but see Swei et al. 2011) who demonstrated a low prevalence of *Bd* in 14 Asian countries). While our result is positive for the conservation of amphibians, a precautionary approach towards effective quarantine measures required to protect the highly endemic and species rich amphibian fauna of PNG from future diseases is essential. We recommend more surveys in other mountainous regions of PNG where climate is favourable for *Bd* and those areas where high levels of tourism and other human activities are likely to introduce the infectious disease.

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