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Absence of invasive Chytrid fungus (*Batrachochytrium dendrobatidis*) in native Fijian ground frog (*Platymantis vitiana*) populations on Viwa-Tailevu, Fiji Islands

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Abstract. We report on the first survey of chytridiomycosis (*Batrachochytrium dendrobatidis- Bd*) in the endangered Fijian ground frog (*Platymantis vitiana*) population on Viwa-Tailevu, Fiji Islands. This fungal pathogen has been implicated as the primary cause of amphibian declines worldwide. Few cases have been reported from tropical Asia however it was recently documented in 4 species of frogs in Indonesia. Two hundred individual frogs were swabbed from 5 different sites on Viwa Island. Swabs were tested to quantify the number of *Bd* zoospore equivalents using real-time Polymerase Chain Reaction (qPCR) technique. We found zero (%) prevalence of *Bd* in ground frogs. The lack of *Bd* may be due to 1) hot weather all year round inhibiting the spread of *Bd*, 2) *Bd* may be absent from Viwa Island due to a lack of amphibian introductions (not introduced or importation of exotic frogs such as *Rana catesbeiana*, or *Xenopus* spp or pet trade spp) or 3) the lack of introduction by human vectors due to the geographic isolation, and low visitation of non-local people into the island. While it is difficult to test these hypotheses, a precautionary approach would suggest an effective quarantine is required to protect Fiji's endemic frogs from future disease outbreak. Conservation effort and research is needed at international level to assist the Fiji government in monitoring and protecting their unique endemic amphibians from outbreaks of *B. dendrobatidis*.

Keywords. *Platymantis vitiana*, chytridiomycosis, infection, conservation, biosecurity, *Bd*.

Chytridiomycosis is the only explanation, for which supporting evidence is available, for the global "enigmatic" declines and disappearances of frog populations and species (Skerratt et al., 2007; Fig 1: *Bd* Global Mapping project www.bd-maps.net). The high prevalence of *Bd* fungus amongst native frog species in Australia and New Zealand (Skerratt

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et al., 2007; Bishop et al., 2009) in all habitats suggest that it could be widespread in the Pacific Islands region.

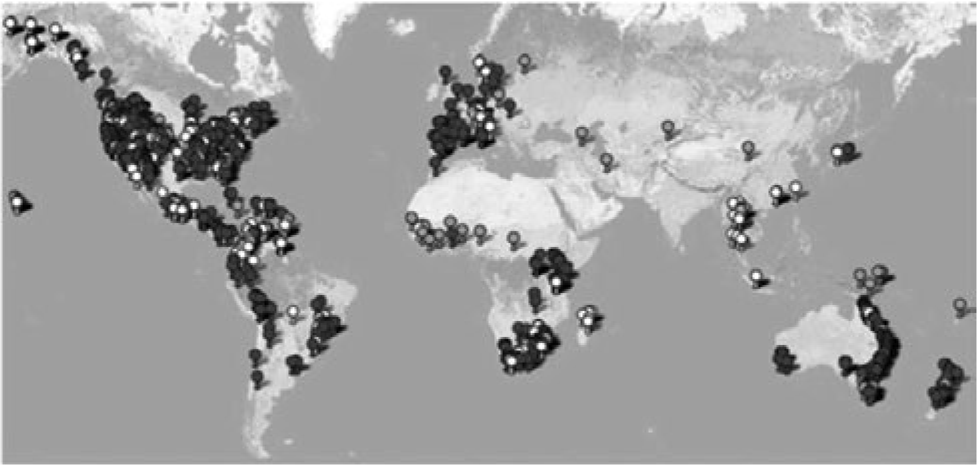

Fig. 1. Worldwide distribution of *Batrachochytrium dendrobatidis* (Bd), the amphibian chytrid fungus. The image is a screenshot from www.spatialepidemiology.net/bd-maps/. Viwa Island is located on latitude (-18.0986) and longitude (178.6615).

Of the currently recognized 29 families of frogs in the world, 10 are located in the Pacific Islands. Fiji Island has eastern most distribution of frogs in the southwest Pacific, with two extant native frog species belonging to the genus *Platymantis* (Fijian ground frog, *P. vitiana* and Fijian tree frog, *P. vitiensis*). The Platymantines belong to the largest anuran family (Ceratobatrachidae), and are endemic to the Philippines, Papua New Guinea, Palau, the Moluccas (eastern Indonesia), New Britain and Admiralty Island, the Solomon Islands, and Fiji.

The Fijian ground frog has disappeared from natural habitats on several islands in Fiji, and the discovery of *Bd* fungus in native frog populations in Fiji Islands could provide the major piece of the conservation puzzle solving the cause of these declines. Thus the aim of the current study was to determine the prevalence of *Bd* fungus amongst a native frog population on Viwa (Fiji) Island.

The Fiji Islands (latitude 18°00’ south and longitude 175°00’ east) are a biodiversity hot spot of the South Pacific archipelago that historically included 59 species of breeding land birds (46% endemic), 20 breeding seabird species, 3 species of amphibians, 26 species of reptiles (40% endemic), and six species of bats (Pernetta and Watling, 1978). The Fijian island of Viwa-Tailevu is being proposed as a potential biodiversity hotspot and tourist destination because much of its flora and fauna, including many endemic species, remains intact. Recent conservation action on this island included eradication of small mammalian introduced predators including the Polynesian rats *Rattus exulans* and pigs *Sus scrofa*, and Europeans introduced cats *Felis catus* and dogs *Canis familiaris* (Morley, 2006). Community based conservation actions for increasing the reproductive success of the ground frogs were also achieved (see: ruffordsmallgrants.org/rsg/projects/edward_narayan). Viwa Island is also free of the invasive small Indian mongoose *Herpestes javanicus*.

Viwa, a 60 ha island situated 900 m east off the coast of Fiji's mainland, Tailevu, Viti Levu, is the smallest of the five islands in Fiji with extant populations of the Fijian ground frog.

The Fijian ground frog (Fig. 2) is currently listed as endangered (EN B1 + 2c) under the current IUCN criteria (Zug et al., 2004). The Fijian ground frog has a small population surviving within remaining forested habitats as well as agricultural sites on Viwa. The Fijian ground frog mainly faces threats from habitat destruction and predation from non-native cane toad (*Rhinella marina*). There is no previous report of *Bd* fungus within wild Fijian frog populations. Furthermore, if this small island frog population is free of *Bd* then this naïve population could be used as a reserve population for future captive breeding programmes without risk of translocating diseased animals into captivity.

Fig. 2. Adult female Fijian ground frog (snout-vent length = 75 mm) from Viwa (Fiji) Islands.

Field sampling was conducted near ponds within, forested landscapes and agricultural areas on Viwa Island (60 ha island present 900 m east off the coast of mainland Viti Levu. Frogs along 100 m transects (n = 5) within each habitat and 20 frogs per transect were caught. Field work was done during the coldest months in Fiji (April to July) because of high intensity of *Bd* first reported elsewhere during cold periods (Kriger and Hero, 2007a). Frogs were captured using clean, unused 20 - 25 cm plastic bags. Each caught frog was sampled for *Bd* by firmly running a cotton swab (Medical Wire & Equipment, MW 100-100; 10 times over each of the following locations: (i) the frog's dorsal surface; (ii) the frog's sides, from groin to armpit; (iii) the ventral surface; and (iv) the undersides of the thighs. Additionally, five outward strokes of the swab were employed on the undersides of each frog's feet, for a total of 70 strokes. Swabbing was done based on methods of Kriger et al. (2006c). Swabs were replaced in their original container (a plastic tube), stored on ice in a cooler until return from the field, and frozen at -20°C. To ensure that no frogs are inadvertently sampled twice, sampling of frogs will not commence until all frogs at given section of each transect were caught, and no further sampling took place at that section of transect after frogs were released.

Swabs were analysed for the presence of *Bd* using quantitative (real-time) polymerase chain reaction techniques (qPCR) described by Boyle et al. (2004), and employing the changes described by Kriger et al. (2006c). Thus, all samples that tested positive in the initial singlicate qPCR assay were re-analysed using a triplicate assay and a full set of *Bd* standards, in order to confirm the initial result and accurately quantify the number of *Bd* zoospores present.

Results of real time PRC analysis of *Bd* swabs from 200 ground frogs were all negative.

Baseline data for islands, even when negative, is highly valuable. The baseline study such as is described here showed no presence of *Bd* (Garcia et al., 2007) until the pathogen emerged on Montserrat Island in 2009, extirpating the mountain chicken frog (*Leptodactylus fallax*), the largest Caribbean amphibian species. In February 2009, dead and dying frogs were found at several sites on Montserrat, prompting an evacuation of 40 apparently healthy *L. fallax* to three different institutions for establishment of a captive breeding program (García et al., 2009).

Several hypotheses could explain the lack of *Bd* in Fijian ground frogs on Viwa Island:

1. that *Bd* fungus has possibly not yet reached or emerged on the Fiji Islands;
2. that *Bd* fungus has been introduced to Fiji but the high temperatures all year round inhibit the likelihood of *Bd* surviving and infecting amphibians (such as thermal tolerance, Kriger and Hero, 2007a);
3. Viwa island is physically isolated from the mainland and *Bd* has not been able to colonise the island;
4. the FGF's on Viwa have been exposed to *Bd* but the entirely terrestrial breeding nature of ground frogs may enable these frogs to reduced exposure and hence susceptibility to this aquatic pathogen. Low prevalence has been recorded in some terrestrial breeding species such as Microhylids (Kriger and Hero, 2006a) and the Pouched Frog (*Assa darlingtoni*) record in Kriger and Hero (2007b) but not others i.e. the Eleutherodactylids in Central and South America (Beard and O'Neill, 2005);
5. the FGF on Viwa have been exposed to *Bd* but they have skin defences that prevent *Bd* infection (Woodhams et al. 2003; Woodhams and Alford, 2005). This is unlikely as most species that are resilient still have *Bd* positive individuals within the population – however no clinical sign of disease (Parker et al., 2002). Many other anuran species are resilient to the pathogen and either do not become susceptible to it or have some level of population-level resistance (e.g. *Rana catesbeiana*, *Xenopus leavis*) [Fisher et al. 2009], *L. wilcoxi* (Kriger and Hero, 2006b). Species that are non-susceptible to *Bd* are presumably either protected by poor abiotic growing conditions for *Bd* (Piotrowski et

al., 2004), immunological response (Ramsey et al., 2010) low virulence of *Bd* strain, or bacterial epibiotic symbionts (Harris et al. 2009). Furthermore, the Pacific Islands are within the ecological niche occupied by *Bd*. Therefore, it is unlikely that these environments are too hot for *Bd* to persist (Lotters et al., 2010).

In summary, the results suggests that *Bd* has either not yet emerged on Viwa, Fiji Islands or that some other ecological factor is preventing *Bd* from fully invading Fijian ground frogs. More data is needed from frogs living on other islands around Fiji to fully assess the threat of *Bd* to native Fijian frogs. Although *Bd* appears widely distributed throughout Australia and New Zealand, the evidence from this study suggests that *Bd* has not yet reached amphibian populations in Pacific Islands. Thus far, no positive *Bd* results have been reported from Fijian tree frogs and native frogs from neighboring islands such as Solomon Islands and Papua New Guinea (Dahl et al., unpublished data), but that may change as the disease emerges or as conditions change (e.g., climate change, general biodiversity loss). Funds are urgently required to assist long-term amphibian population monitoring throughout Pacific Islands in order to detect any future amphibian population declines.

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