Apparent absence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in frogs in Malaita Province, Solomon Islands

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Abstract. A major driver of global biodiversity loss is disease. One of the most devastating wildlife diseases known is chytridiomycosis, which is caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis*, and is implicated in population declines in over 500 frog species. Thought to originate in Asia, *B. dendrobatidis* now has a global distribution, likely due to human movement and trade. The pathogen has yet to be detected in Melanesia, but there have been few surveys for *B. dendrobatidis* in the region, and none in the Solomon Islands archipelago, a biogeographic region with a unique and culturally important frog fauna. We swabbed 200 frogs of eight species in three genera in lowland and highland sites in East Kwaio on the island of Malaita in the Solomon Islands. All frogs tested negative for the pathogen but it is possible that the pathogen is present despite non-detection, so further surveys for the pathogen are needed throughout the country. Despite this, it is safest to take a precautionary approach and assume that *B. dendrobatidis* has not yet been introduced to the Solomon Islands, and that naïve native amphibian populations may be at risk of decline if the pathogen is introduced. Protocols are needed to prevent the accidental import of infected frogs via tourism or in logging or mining equipment. Monitoring of frog populations near areas of high risk such as ports is also recommended. The frogs of the Solomon Islands archipelago are biologically unique and culturally significant, and protecting them from the potentially devastating impacts of *B. dendrobatidis* is vital.

Keywords: amphibian, bacteria, biodiversity loss, biosecurity, chytridiomycosis, *Cornufer guentheri*, *Cornufer guppyi*, *Cornufer hedigeri*, *Cornufer solomonis*, *Cornufer vertebralis*, East Kwaio, frog, fungus, *Litoria lutea*, *Litoria thesaurensis*, *Papurana krefftii*, pathogen, Solomon Islands, wildlife disease.

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Introduction

A significant driver of global biodiversity loss is disease, and the most devastating wildlife disease known to date is chytridiomycosis (Fisher et al. 2012; Scheele et al. 2019). Caused by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) (Berger et al. 1998; Longcore et al. 1999), chytridiomycosis is responsible for the greatest recorded loss of biodiversity attributable to a disease, driving declines in over 500 species (Scheele et al. 2019). *Batrachochytrium dendrobatidis* is thought to have originated in Asia (O’hanlon et al. 2018) but is now distributed across the globe (Scheele et al. 2019). Human movements and commercial trade have been linked to international movement of
B. dendrobatidis (Fisher and Garner 2007; Schloegel et al. 2009; Farrer et al. 2011). Despite its near-ubiquitous distribution, B. dendrobatidis has yet to be detected in Melanesia, with surveys of frogs in Papua New Guinea (Swei et al. 2011; Dahl et al. 2012; Bower et al. 2020) and Fiji (Narayan et al. 2011) failing to detect the pathogen.

The Solomon Islands is a country comprised of six major islands and over 900 smaller islands east of Papua New Guinea. The Solomon Islands and the biogeographically aligned Bougainville and Buka Islands of Papua New Guinea (collectively known as the Solomon Islands archipelago) has a unique frog fauna, with 16 of the 19 known species endemic to them (Pikacha et al. 2008). The second largest (and most heavily populated) island in the country is Malaita, covering 4300 km². Frogs are known to be culturally significant on Malaita, and different species may be used as food, traditional medicine and as totem animals (Pollard et al. 2015). For the Kwaio community, particularly those living away from the coast, frogs are an important source of protein and are strongly incorporated into cultural beliefs. As part of collaborative biodiversity research led by the Kwaio community (Alabai et al. 2019; Callaghan et al. 2019; Lavery et al. 2018), we conducted a survey for B. dendrobatidis in East Kwaio on Malaita, the first published survey for the pathogen in the Solomon Islands and in the Solomon Islands archipelago.

**Methods**

From 20 to 25 July 2019 we surveyed for frogs along streams and in rainforest at four sites from 285 to 1155 m elevation in East Kwaio, Malaita (Fig. 1): Kwainaa’asi (–8.946, 161.011, 920 m elevation), Alalau Fulanitofe (–8.976, 161.037, 1155 m elevation), Aifasu (–8.995, 160.984, 285 m elevation) and Kafurumu (–8.958, 160.987, 460 m elevation). Lowland sites were a mosaic of gardens and rainforest, while highland sites were rainforest and montane cloud forest. The region has a wet equatorial tropical environment, with high rainfall and relatively constant temperatures. The average temperature is 27°C near the coast (Bradbury et al. 2017).

During nocturnal fieldwork, each frog was captured by hand and placed in plastic bags, before swabbing a total of 30 times (five strokes along each side of the abdominal area, five strokes along the underside of each thigh, and five strokes along the underside of each foot including the digits) using a MW100 swab (Medical Wire and Equipment, Corsham, UK). Samples were transferred to –20°C within 14 days before testing with diagnostic qPCR using Taqman chemistry (Boyle et al. 2004; Hyatt et al. 2007).

The tip of each swab was removed and added to a 2-mL Sarstedt screw-capped tube containing 40 mg of 0.5 mm diameter Zirconium/silica beads (Qiagen) and 100-µL PrepMan™ Ultra Sample Preparation Reagent (Applied Biosystems). Samples were vortexed for 10 s then homogenised in a Qiagen TissueLyser 11 (2 × 45 s @ 30 cycles/s). Samples were then centrifuged (1 min @ 18407g) and incubated at 100°C for 10 min, then 20 µL of supernatant was extracted from each sample tube and placed into a new sterile 1.5 mL microcentrifuge tube. Samples were diluted 1/10 and the aliquots stored at –20°C before thermal cycling (modified from Hyatt et al. 2007).
Each swab was analysed in singlicate. The qPCR reaction contained 1 × SensiFAST Probe Lo-ROX Mix (Bioline Australia), 200 nm ITS1–3 Fwd primer (CCT TGA TAT ACA GTG TGC CAT ATG TC), 200 nm 5.8S Rev. primer (AGC CAA GAG ATC CGT TGT CAA A) (Boyle et al. 2004), 1 unit of TaqMan™ ChytrMGB2 FAM probe (Applied Biosystems), 1 × TaqMan™ Exogenous Internal Positive Control DNA (IPC-Vic. probe) and 1 × Exogenous IPC reagent mix (Applied Biosystems). The Exogenous IPC control was used to monitor PCR reaction inhibition. Triplicate No Amplification Controls (NAC) were included using 1 × TaqMan™ IPC PCR blocking reagent. Triplicate No Template Controls (NTC) were used to monitor probe degradation.

A synthetic ITS-1 gBlocks® gene fragment from Integrated DNA Technologies Inc. (IDT) was used to generate the qPCR standards (Rebollar et al. 2017; J. Kerby, unpubl. data). Five log10 dilutions ranging from 10⁹ to 10¹ copies were set up in triplicate for generation of the ITS-1 Standard curve used for quantification of ITS-1 copy numbers. The qPCR reactions were run on an ABI Quantstudio3 qPCR Machine and analysed using QuantStudio Design and Analysis Software (ver. 1.4.1), with a sample considered Bd positive if the number of ITS-1 copies amplified was greater than zero (Briggs et al. 2010; DiRenzo et al. 2018).

A subset of specimens swabbed were taken as voucher specimens and identified to species via morphological and molecular analysis. For swabs from individuals that were not collected, 38 were identified to genus only as the Kwaio names are not differentiated in Kwaio (family Ceratobatrachidae). Species in this family of direct-developing species are documented in frogs with similar life-histories elsewhere, and direct-developing species may actually be more susceptible to the pathogen (Mesquita et al. 2017). We also swabbed frogs in the genera Litoria and Papurana. In Australia, many species in the genus Litoria have been reported with B. dendrobatidis infection. Twenty-five have experienced population declines attributed to the pathogen, with declines in three species being greater than 90% (Scheele et al. 2019). As a result, if B. dendrobatidis is truly absent from the Solomon Islands archipelago, its arrival may pose a particular threat to species in the genus Litoria. However, the true susceptibility of the frog fauna of the Solomon Islands to B. dendrobatidis remains unknown, and is likely to be influenced by various factors including evolutionary history, and differences in life-histories, behaviour, innate immunity (including skin microbiome antimicrobial peptides), and environmental conditions (Rowley and Alford 2009).

Our failure to detect B. dendrobatidis during our surveys may have several explanations. The pathogen may be truly absent from the Solomon Islands archipelago, it may be patchily distributed or otherwise absent from our study sites, yet present elsewhere, or it may be present at our study sites, but at such low infection intensities and/or prevalence that it remained undetected. Further surveys for the pathogen are necessary across the Solomon Islands archipelago, but a precautionary approach

### Table 1. Samples tested for the amphibian chytrid fungus (Batrachochytrium dendrobatidis)

<table>
<thead>
<tr>
<th>Species</th>
<th>Number positive</th>
<th>Number tested</th>
<th>Upper 95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornufer guentheri</td>
<td>0</td>
<td>16</td>
<td>20.6</td>
</tr>
<tr>
<td>Cornufer guppyi</td>
<td>0</td>
<td>54</td>
<td>6.6</td>
</tr>
<tr>
<td>Cornufer hedigeri</td>
<td>0</td>
<td>18</td>
<td>18.5</td>
</tr>
<tr>
<td>Cornufer solomonis</td>
<td>0</td>
<td>28</td>
<td>12.3</td>
</tr>
<tr>
<td>Cornufer vertebralis</td>
<td>0</td>
<td>5</td>
<td>52.2</td>
</tr>
<tr>
<td>Litoria lutea</td>
<td>0</td>
<td>3</td>
<td>70.1</td>
</tr>
<tr>
<td>Litoria thesaurensis</td>
<td>0</td>
<td>15</td>
<td>21.8</td>
</tr>
<tr>
<td>Papurana krefti</td>
<td>0</td>
<td>11</td>
<td>28.5</td>
</tr>
<tr>
<td>Cornufer/Papurana spp. A</td>
<td>0</td>
<td>18</td>
<td>18.5</td>
</tr>
<tr>
<td>Litoria spp. A</td>
<td>0</td>
<td>16</td>
<td>10.3</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>200</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Samples not identified to species (see ‘Materials and methods’).
should be taken – management strategies and disease surveillance protocols that assume *B. dendrobatidis* has not yet been introduced to the Solomon Islands archipelago and that native amphibians may be at risk of impact if the fungus is introduced, should be implemented.

We echo recent calls for action for an international, multidisciplinary approach to reduce the chances of the pathogen being imported into Melanesia, and limit its impact if it is (Bower et al. 2019, 2020). Strategies should focus on preventing its importation, including via tourism, logging, and mining activities. The development of protocols to prevent escape or release of any imported frogs into the wild, including any stowaways on imported cargo (*sensu* Pili et al. 2019), and testing frog populations near areas of high risk (*i.e.* ports) for *B. dendrobatidis* is recommended.

The Solomon Islands archipelago is home to a unique frog fauna. Although there is limited western scientific knowledge of this fauna, there is extensive traditional knowledge that demonstrates the importance amphibians have in traditional livelihoods and customs. Protecting this fauna from the devastating impacts of disease is vital.

**Conflicts of interest**

The authors declare no conflicts of interest.

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**References**


NHMRC (2013). Australian code for the care and use of animals for scientific purposes, 8th edn. (Canberra: National Health and Medical Research Council.)


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