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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

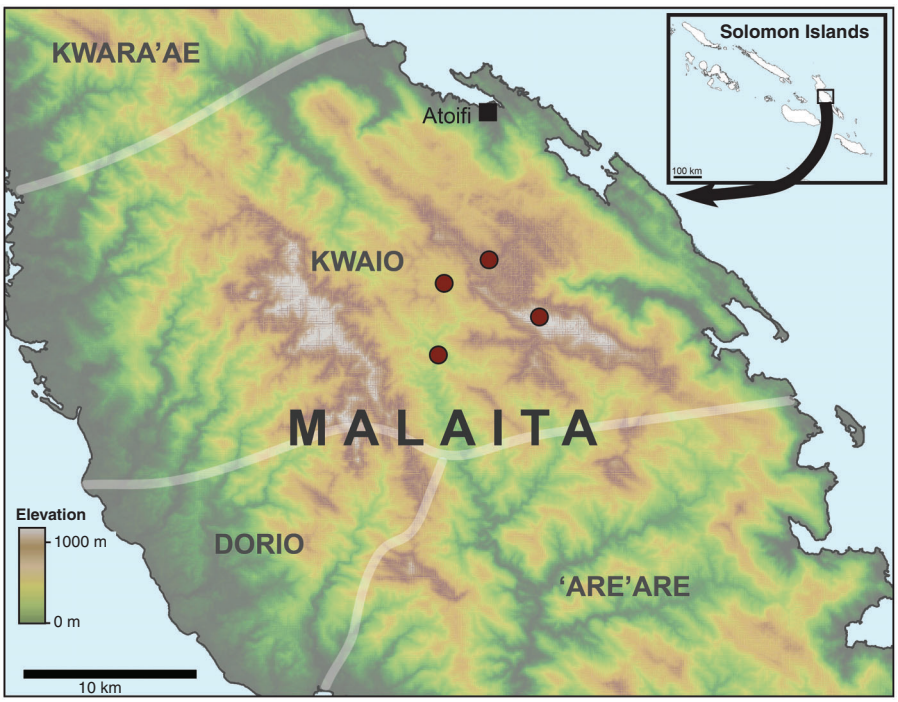

Fig. 1. Location of sites (brown circles) where frogs were sampled for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in East Kwaio, Malaita, Solomon Islands. Approximate language borders (CartoGIS Services, College of Asia and the Pacific, The Australian National University) are marked in white.

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 km^2. 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^{TM} 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 @ 18407*g*) 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 AAT 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 $\log_{10}$ dilutions ranging from 10^9 to 10^1 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 used on some swabs encompassed several species. For example, frogs in the genus *Litoria* are not differentiated in Kwaio language, and so for samples of *Litoria lutea* and *Litoria thesaurensis* taken from non-vouchered individuals, they were recorded simply as *Litoria* spp.

Exact binomial 95% confidence intervals for infection prevalence were computed using the method of Clopper and Pearson (1934).

Results

We swabbed 200 individuals of eight species belonging to three genera (Table 1), representing all native frog species observed during surveys and 36% of the known number of species in the archipelago (22 including the introduced Cane Toad *Rhinella marina*; Pikacha *et al.* 2008). All samples tested negative for *B. dendrobatidis*, giving an overall upper 95% confidence limit for prevalence of 1.8% at the time of sampling (Table 1).

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

Upper 95% confidence limit (CL) indicates the upper binomial 95% confidence limit for infection prevalence

Species	Number positive	Number tested	Upper 95% CL
Cornufer guentheri	0	16	20.6
Cornufer guppyi	0	54	6.6
Cornufer hedigeri	0	18	18.5
Cornufer solomonis	0	28	12.3
Cornufer vertebralis	0	5	52.2
Litoria lutea	0	3	70.1
Litoria thesaurensis	0	15	21.8
Papurana kreftii	0	11	28.5
Cornufer/Papurana spp.[A]	0	18	18.5
Litoria spp[A]	0	16	10.3
Total	0	200	1.8

[A]Samples not identified to species (see 'Materials and methods').

Discussion

This study reports the results of the first survey for the amphibian chytrid fungus *B. dendrobatidis* in the Solomon Islands, and is one of few published studies that has attempted to document the pathogen elsewhere in Melanesia. To date, there is no evidence of *B. dendrobatidis* infecting frog species anywhere in Melanesia.

Although we cannot rule out the presence of *B. dendrobatidis* at the survey sites in East Kwaio, Malaita, if we assume that all species are equally likely to carry the infection, the theoretical upper 95% confidence limit for prevalence is 1.8% at the time of sampling. Although susceptibility is likely to differ among species, the theoretical upper 95% confidence limits for prevalence are still very low in the most sampled species (i.e. 6.6% in *Cornufer guppyi*). In addition, our surveys included rainforest streams at high elevations (>900 m elevation), potentially suitable environmental conditions for *B. dendrobatidis* (Woodhams and Alford 2005; Bielby *et al.* 2008).

The majority of frogs swabbed were in the genus *Cornufer* (family Ceratobatrachidae). Species in this family of direct-developing frogs have yet to be reported as infected by *B. dendrobatidis*. Despite the inherently lower likelihood of exposure to *B. dendrobatidis* within direct-developing frogs, population declines and extinctions attributed to the pathogen 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

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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