The first assessment of *Batrachochytrium dendrobatidis* in amphibian populations in the Kanuku Mountains Protected Area of Guyana

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ABSTRACT - *Batrachochytrium dendrobatidis (Bd)* is a fungal pathogen threatening hundreds of amphibian species with extinction across the globe, especially in Latin America. Extensive investigations have revealed the presence of *Bd* in many South American countries, but there has been a lack of such research conducted in Guyana. We assessed the presence of *Bd* in the amphibian populations of the Kanuku Mountains Protected Area, in the south-west of the country. We swabbed two hundred and fifty anurans and processed the samples using standard Polymerase Chain Reaction analysis to identify cutaneous presence of *Bd*, making this the most comprehensive investigation into the existence of *Bd* in Guyana. All samples were negative for the presence of *Bd* DNA. Given the presence of *Bd* in countries neighbouring Guyana, and the severe declines it has caused in amphibian populations, we consider Guyana to be under severe threat. We advocate further surveillance in Guyana to fully determine the presence or absence of *Bd*, and we emphasise the importance of biosecurity and monitoring in mitigating a potential outbreak of this fungal pathogen.

INTRODUCTION

Across the globe an array of amphibian populations are experiencing severe declines, with threats attributed to habitat loss and exploitation, but many declines are described as "enigmatic" (Stuart et al., 2004). Research suggests that many of these enigmatic declines are caused by infectious disease, such as chytridiomycosis (Lips et al., 2006, Lötters et al., 2009). Chytridiomycosis has contributed to the extinction of species such as the golden toad (*Incilius periglenes*) and both species of gastric brooding frogs (*Rheobatrachus silus* and *R. vitellinus*; Hero et al., 2004; Meyer et al., 2004). Chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis (Bd)* and results in high morbidity and mortality in susceptible species and individuals. The fungus infects the permeable skin of amphibians causing hyperkeratosis (Van Rooij et al., 2015), which disrupts respiration, osmoregulation and electrolyte exchange across the skin, ultimately leading to cardiac arrest (Campbell et al., 2012). This fungal pathogen can be spread by direct contact between individuals or by contact with water sources infected with waterborne fungal zoospores (Van Rooij et al., 2015).

Chytridiomycosis is associated with declines in at least 43 species in South America, a region which harbours half of the World's amphibian species richness (Young et al., 2001; Lips et al., 2005; Lips et al., 2006). Numerous reports from countries including French Guiana (Curtois et al., 2012; Curtois et al., 2015), Venezuela (Hanselmann et al., 2004; Sánchez et al., 2008), Brazil (Valencia-Aguilar et al., 2015; Jenkinson et al., 2016) and Peru (Catenazzi et al., 2011) find evidence of *Bd* in local amphibian populations, demonstrating the extent to which this pathogen has spread across the continent. However, the presence of *Bd* in Guyana remains undetermined (Olson et al., 2013; Olson & Ronnenberg, 2014). There are 148 documented amphibian species in Guyana, 27 % of which are endemic (Cole et al., 2013) with more species likely to be added to this inventory in the future (Gower et al., 2010; Kok et al., 2006; MacCulloch & Lathrop, 2002). The majority of species in Guyana are under-studied (Jarvis, 2018) with many species known from only a handful of specimens (Cole et al., 2013). This lack of knowledge leads to uncertainty about the threats facing these species. There have been only two previous *Bd* surveys including data from Guyana, and both found no evidence of the fungus (Gower et al., 2013; Luger et al., 2008). However, considering the devastating effects *Bd* has had on endemic populations in countries neighbouring Guyana, it is essential to further investigate *Bd* in this region's amphibian fauna.

There is an increasing body of evidence indicating the complexity of *Bd* epidemiology; a population's susceptibility is variable as some species are not necessarily threatened by the presence of *Bd*, and may even act as a disease reservoir for others (Searle et al., 2011; Gervasi et al., 2017; Reeder et al., 2012). In addition, Hanselmann et al. (2004) describe the absence of significant clinical disease or mortality, despite high prevalence of infection in *Lithobates catesbeianus* (formerly *Rana catesbeiana*) in an introduced population in Venezuela. Therefore, the threat of *Bd* to amphibian populations is not fully understood. Conducting surveys for *Bd* in Guyana will shed light on the local disease status, broaden the epidemiological knowledge of this pathogen and potentially aid mitigation efforts (Rödder et al., 2009).

The aim of this work was to carry out a community-wide

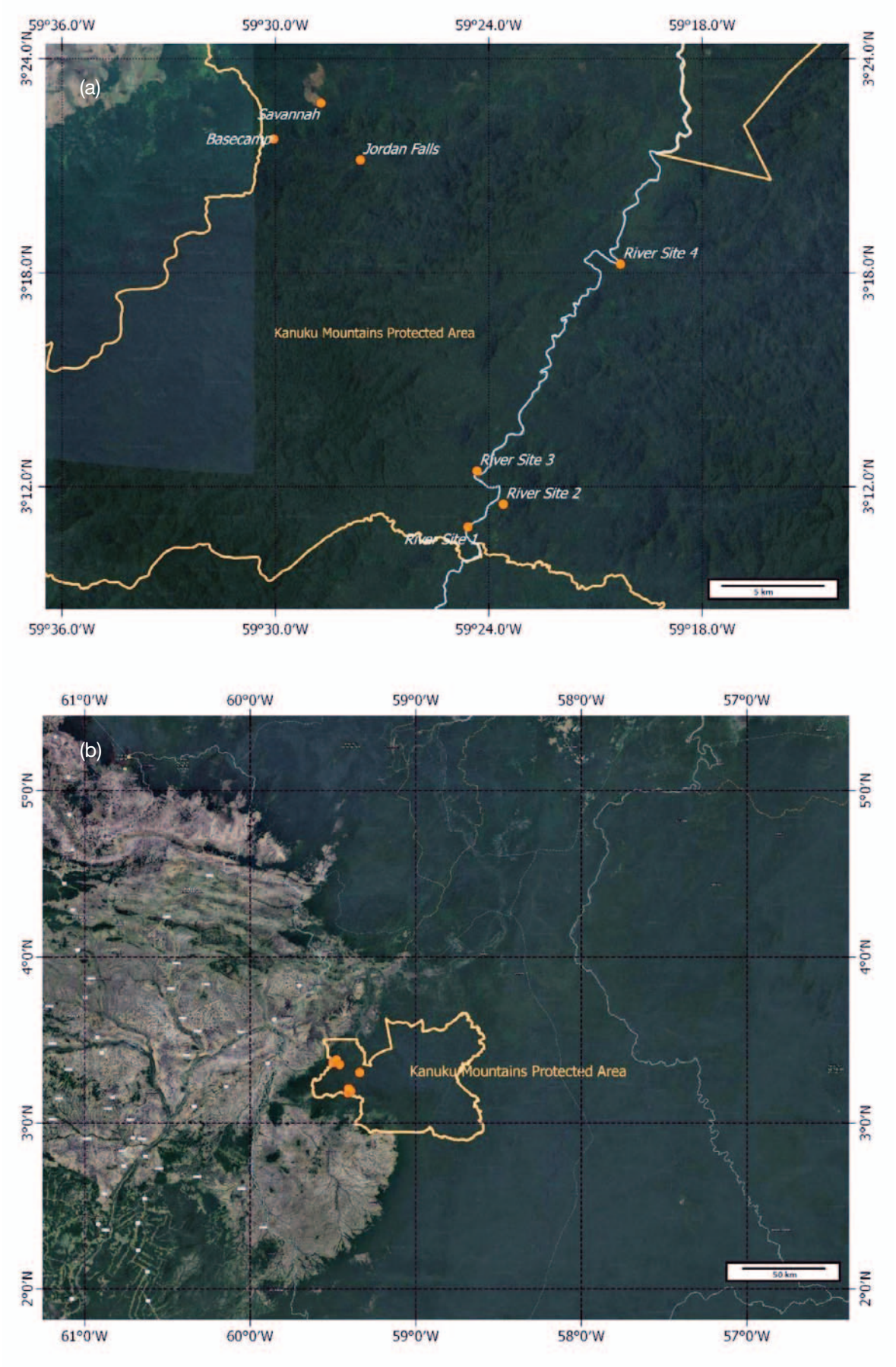

Figure 1. (a) Map of locations in the KMPA where the amphibians were sampled. Orange points represent main areas in which sampling occured; the boundary of the KMPA is marked in yellow. **(b)** View of the KMPA within south-west Guyana.

survey to investigate the presence of *Bd* in the amphibian population of the western Kanuku Mountains Protected Area (KMPA), a remote area of rainforest in the south of the country. We see this as a first step towards *Bd* surveillance and monitoring in the Protected Area, as well as an important contribution to the limited body of evidence regarding *Bd* in the Guiana Shield.

METHODS

The KMPA is a national protected area, established in 2011, located in the Rupununi region of south-western Guyana, and one of five protected areas in the country. The KMPA is divided into its east and west ranges by the Rupununi River, and the predominant ecosystem of the KMPA is pristine rainforest, considered the most ecologically diverse in Guyana (Conservation International, 2016). The biodiversity of the KMPA is understudied, and the most recent rapid assessment of this area did not include an investigation of the amphibian community (Montambault & Missa, 2002). Our amphibian survey in the KMPA was conducted over four weeks in July - August 2017. From 14-July to 31-July surveys (n=26) took place at the Nappi base camp (3°22'9.57" N, 59°29'56.74" W, Fig. 1a), and the nearby sites of "Jordan falls" (3°21'9.68"N, 59°27'36.55"W) and "Savannah" (3°22'41.19"N, 59°28'46.41"W). From 4-Aug to 8-Aug we surveyed at sites along the Rupununi River (River sites 1-4, surveys n=8; Fig. 1a). Surveys consisted of diurnal and nocturnal searches for amphibians in the surrounding area. A visual search technique, aided by auditory signals, was used to locate amphibians in leaf litter, shrubbery and understorey, stream sides and swamp habitats. Each location was visited maximally twice, once at night and once during the day, to minimise recapture given that no marking protocol was followed. On most occasions, amphibians from the nocturnal search were released after the diurnal search the next morning, avoiding recapture.

Amphibians were captured by hand in clean inside-out sealable bags, to avoid potential pathogen transmission between specimens and surveyors. Once caught, Ziploc bags were reversed, inflated and closed. Amphibians were brought back to camp for identification and swabbing. Surveyors used clean gloves for every amphibian swabbed. Individuals were swabbed following standard procedure guidelines (Brem et al., 2007), using clinical grade sterile Deltalab single-packed swabs directed to the ventral surfaces of the individuals. Ziploc bags and gloves were used up to three times and disinfected and dried between surveys using 0.5 % sodium hypochlorite bleach solution. Individuals were identified to species level where possible (Table 1), using Cole et al. (2013). Individuals were released back to the site in which they were initially found within 12 hours of capture. Swabs were stored dry in eppendorf tubes and kept at ambient temperature in the field until return to the UK, after which they were stored at -20 °C until analysis.

DNA extraction and Polymerase Chain Reaction analysis

DNA was extracted from swabs using phenol-chloroform extraction (Sambrook & Russell, 2001). The presence or absence of *Bd* in each sample was identified using a standardised Polymerase Chain Reaction (PCR), with ITS/5.8S primers (Boyle et al., 2004; ITS Chytr 5' CCTTGATATAATACAGTGTGCCATATGTC-3' and 5.8S Chytr 5'-AGCCAAGAGATCCGTTGTCAAA-3'). Each sample of extracted DNA was subjected to replicate PCR analysis (25 µl reaction volume), with a positive control (Greener et al., 2017; Shepherd et al., 2016). As in previous studies (Greener et al., 2017; Shepherd et al., 2016) an additional control, to demonstrate that PCR quality DNA had been obtained from the extraction, a PCR was performed using universal 16S rDNA primers (Palumbi, 1996), on 12 randomly selected samples.

Table 1. Amphibians swabbed for *Batrachochytrium dendrobatidis*, ranked by frequency of encounter

Rank	Identification	No. individuals
1	*Leptodactylus mystaceus*	62
2	*Leptodactylus sp*	29
3	*Adenomera hylaedactyla*	28
4	*Bufonidae*	23
5	*Rhinella martyi*	21
6	*Physalaemus cuvieri*	13
7-8	*Allobates spumaponens*	12
7-8	*Unidentified*	12
9-10	*Allophryne ruthveni*	7
9-10	*Ameerega trivittata*	7
11	*Anomaloglossus kaiei*	6
12	*Aromabatidae*	9
13-14	*Leptodactylus guianensis*	4
13-14	*Leptodactylus knudseni*	4
15	*Ceuthomantidae/Craugastoridae/ Eleutherodactylidae*	3
16-18	*Adenomera andreae*	2
16-18	*Hylidae*	2
16-18	*Boana xerophylla*	2
19-22	*Boana boans*	1
19-22	*Leptodactylus leptodactyloides*	1
19-22	*Leptodactylus petersii*	1
19-22	*Pristimantis sp*	1
	Total	250

RESULTS

A total of 250 anurans were swabbed successfully, of 22 taxa (15 confirmed nominal species with seven taxa to generic or family level; Table 1). No individuals of Gymnophiona or Caudata were encountered. No anurans observed displayed obvious disease. Of the 247 swabs for which DNA was successfully extracted (three swabs were discarded due to labelling ambiguity), all were negative for *Bd* DNA.

DISCUSSION

This study is the first survey for *Bd* in the KMPA, and one of only three *Bd* surveys, to our knowledge, to have been conducted on amphibians in Guyana. The first survey consisted of 22 caecilians swabbed in the Iwokrama Forest Reserve, over 130 km from the KMPA. All caecilians samples were negative for *Bd* (Gower et al., 2013). Luger et al. (2008) sampled 202 harlequin frogs (*Atelopus hoogmoedi*) for *Bd* in the Mabura Hill Forest Reserve, more than 200 km from the KMPA, and found negative results. Our sample size was considerably larger than in the aforementioned studies and we sampled the broad amphibian community rather than a specific taxonomic group. Thus, our survey provides further evidence for the current absence of *Bd* from southern Guyana. The KMPA is over 500 km from the closest *Bd* surveys in Surinam (Luger et al., 2008) and over 700 km from the study sites of Courtois et al. (2012) in French Guiana.

The methodology established for swabbing amphibians was based upon previous field studies, and investigations on the presence of *Bd* in Trinidad and Tobago utilised a similar methodology for PCR analysis by the same laboratory (Strathclyde University, Shepherd et al., 2016; Thomson et al., 2018). Although conventional PCR assay has been considered less sensitive compared to real time assays for the detection of *Bd* DNA (Boyle et al., 2004; Hyatt et al., 2007), an experimental study comparing both methods of detection for *Bd* DNA found conventional PCR and gel-based detection to be as sensitive, and a cost-effective alternative, to real time assays when performing prevalence studies (Garland et al., 2011). Confidence in the *Bd*-negative status of the KMPA is additionally supported by the lack of any overt cutaneous lesions or behaviours associated with chytridiomycosis observed in the field. These include: excessive exfoliation of the skin, ulceration, erythema, abnormal posture, absence of flight response and neurological signs (Van Rooij et al., 2015). It should be noted, however, that chytridiomycosis can be asymptomatic and cause sudden death, and basing disease status on clinical findings alone is an insensitive approach to disease surveillance. Additionally, there is likely to be temporal variability in the infection status of populations, and some populations have the ability to recover from infection (Shepherd et al., 2016; Thomson et al., 2018; Alemu et al., 2008; Alemu et al., 2013). Based on Thursfield (1995), with a sample size of 247, we are 95 % confident of detecting *Bd* in the amphibian population given a prevalence of infection >2 % (as found in South American rainforests by Courtois et al., 2015; James et al., 2015; von May et al., 2018; Mccracken & Forstner, 2009). A larger sample size would be required to detect *Bd* if prevalence is <2 %. Such low levels of infection should be considered as an explanation for why this survey did not identify the presence of *Bd*. Therefore, continued surveillance in the KMPA should be directed to new areas as well as previously monitored sites, and include large sample sizes, to maintain an up-to-date knowledge on the *Bd*- status of the area. Additionally, we would greatly encourage future researchers and the PAC to address why *Bd* has not reached the KMPA, or if it is existing at very low levels of infection, as this could shed light on important epidemiological characteristics of this infectious disease.

Species dispersal may be limited by geographical barriers such as mountain ranges or oceans. To the west between the KMPA and the nearest forests of Brazil (*Bd*-positive) lies a vast expanse of savannah (Fig. 1b). The average daily temperatures in the savannahs of south-western Guyana and northern Brazil are >25 °C, and in the dry seasons ≥ 27 °C (Bovolo et al., 2012). *Bd* survival is limited by environmental variables; temperatures above 25 °C limit growth of *Bd* in culture, and sustained periods above 30 °C are incompatible with survival (Piotrowski et al., 2004). There is evidence to suggest amphibians adapted to higher thermal environment can clear *Bd* infections (Woodhams et al., 2003). Therefore, the climate of the savannahs may have a barrier effect on the range expansion of *Bd* from Brazilian rainforest into south-western Guyana. Other reasons behind the lack of *Bd* in the KMPA could be the absence of potential non-amphibian hosts from the area, and the minimal human movement in and out of the KMPA. While further research is necessary to understand non-amphibian hosts and their role in transmission of *Bd* (McMahon et al., 2013), it is reasonable to hypothesize upon their influence on the distribution of *Bd*. Human movement and the pet trade have resulted in *Bd* becoming a global pandemic since its emergence during the early 20th century in the Korean Peninsula (O'Hanlon et al., 2018). Human movement is considered one of the ongoing routes of transmission of *Bd* to new areas (Kriger & Hero, 2009). The low level of human movement in southern Guyana should be considered as a potential limiting factor to the spread of *Bd* into the KMPA. Further investigations should focus on the risk of anthropogenic introduction of *Bd* into the KMPA.

Constant surveillance and strictly implemented biosecurity measures are essential to mitigate the alarmingly rapid spread of *Bd* across Latin America. Human movement and activity has played a key role in the rapid geographical spread of *Bd* (Kriger & Hero, 2009; O'Hanlon et al., 2018), and therefore we hope that the PAC may act as a platform for raising awareness of this risk and promoting good hygiene during field research. A priority should be developing Standard Operating Procedures for the rangers, visiting researchers and field technicians, to include specific instructions regarding hygiene and biosecurity during amphibian work. Basic bio-exclusion principles could be extrapolated from other areas of research such as food production systems (Leibler et al., 2009), human populations (Meyerson et al., 2002) or emerging wildlife diseases (Msami, 2008), though not all possibilities are appropriate for chytridiomycosis; for instance, the use of antifungals in the event of a *Bd* incursion would not be viable in the long term. Phillott et al. (2010) provide a comprehensive review of biosecurity measures recommended for *Bd*.

The combination of high diversity and endemism of amphibians in Guyana make this area a priority for

conservation. Our survey showing the absence of evidence for *Bd* in the KMPA gives an opportunity for the pre-emptive conservation of amphibians in the pristine rainforest of southern Guyana, but it remains true that an outbreak of chytridiomycosis in this area could be catastrophic. PAC personnel, KMPA rangers and local field guides have gained a new awareness of chytridiomycosis as a result of this field work. This survey serves as a first step towards continued monitoring and surveillance of *Bd* in Guyana to encourage implementation of measures to prevent an outbreak. It is recommended that the prevention of chytridiomycosis should be paramount in the conservation agenda of Guyana's Protected Areas Commission.

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REFERENCES

Alemu, J.B., Cazabon, M.N., Dempewolf, L., Hailey, A., Lehtinen, R.M., Mannette, R.P., Naranjit, K.T. & Roach, A.C. (2008). Presence of the chytrid fungus *Batrachochytrium dendrobatidis* in populations of the critically endangered frog Mannophryne olmonae in Tobago, West Indies. *EcoHealth* 5: 34-39.

Alemu, J.B., Cazabon-Mannette, M.N., Cunningham, A.A., Dempewolf, L., Hailey, A., Mannette, R.P., Naranjit, K.T., Perkins, M.W. & Schmidt-Roach, A.C. (2013). Presence of the chytrid fungus *Batrachochytrium dendrobatidis* in a Vulnerable frog in Trinidad, West Indies. *Endangered Species Research* 20: 131-136.

Boyle, D.G, Boyle, D.B., Olsen, V., Morgan, J.A.T. & Hyatt, A.D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60: 141–148.

Brem, F., Mendelson III, J. R., & Lips, K. R. (2007). Field-sampling protocol for *Batrachochytrium dendrobatidis* from living amphibians, using alcohol preserved swabs. Version, 1, 18.

Bovolo, C.I., Pereira, R., Parkin, G., Kilsby, C. & Wagner, T. (2012). Fine-scale regional climate patterns in the Guianas, tropical South America, based on observations and reanalysis data. *International Journal of Climatology* 32: 1665-1689.

Campbell, C.R., Voyles, J., Cook, D.I. & Dinudom, A. (2012). Frog skin epithelium: electrolyte transport and chytridiomycosis. *The International Journal of Biochemistry & Cell Biology* 44: 431-434.

Catenazzi, A., Lehr, E., Rodriguez, L.O. & Vredenburg, V.T. (2011). *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the upper Manu National Park, south-eastern Peru. *Conservation Biology* 25: 382-391.

Cole, C.J., Townsend, C.R., Reynolds, R.P., MacCulloch, R.D. & Lathrop, A. (2013). Amphibians and reptiles of Guyana, South America: illustrated keys, annotated species accounts, and a biogeographic synopsis. *Proceedings of the Biological Society of Washington* 125: 317-578.

Conservation International, (2016). Kanuku Mountains. [Online] Available at: https://www.conservation.org/global/guyana/explore/Pages/Kanuku-Mountains.aspx [Accessed: 26th April 2018]

Courtois, E.A., Pineau, K., Villette, B., Schmeller, D.S. & Gaucher, P. (2012). Population estimates of *Dendrobates tinctorius* (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection. *Phyllomedusa: Journal of Herpetology* 11: 63-70.

Courtois, E.A., Gaucher, P., Chave, J. & Schmeller, D.S. (2015). Widespread occurrence of Bd in French Guiana, South America. *PloS one* 10(4), p.e0125128.

Frost, Darrel R. (2018). Amphibian Species of the World: an Online Reference. [Online] Version 6.0. American Museum of Natural History, New York, USA. Electronic Database accessible at: http://research.amnh.org/herpetology/amphibia/index.html.

Garland, S., Wood, J. & Skerratt, L. F. (2011). Comparison of sensitivity between real-time detection of a TaqMan assay for *Batrachochytrium dendrobatidis* and conventional detection. *Diseases of Aquatic Organisms* 94: 101-105.

Gervasi, S.S., Stephens, P.R., Hua, J., Searle, C.L., Xie, G.Y., Urbina, J., Olson, D.H., Bancroft, B.A., Weis, V., Hammond, J.I. & Relyea, R.A. (2017). Linking ecology and epidemiology to understand predictors of multi-host responses to an emerging pathogen, the amphibian chytrid fungus. *PloS one* 12: e0167882.

Gower, D.J., Doherty-Bone, T., Loader, S.P., Wilkinson, M., Kouete, M.T., Tapley, B., Orton, F., Daniel, O.Z., Wynne, F., Flach, E. & Müller, H. (2013). *Batrachochytrium dendrobatidis* infection and lethal chytridiomycosis in caecilian amphibians (Gymnophiona). *EcoHealth* 10: 173-183.

Gower, D.J., Wilkinson, M., Sherratt, E. & Kok, P.J. (2010). A new species of *Rhinatrema* Duméril & Bibron (Amphibia: Gymnophiona: Rhinatrematidae) from Guyana. *Zootaxa* 2391: 47-60.

Greener, M.S., Shepherd, R., Hoskisson, P.A., Asmath, H. & Downie, J.R. (2017). How many Trinidad stream frogs (*Mannophryne trinitatis*) are there, and should they be regarded as vulnerable to extinction? *Herpetological*

Journal 27: 5-11.

Hanselmann, R., Rodrıguez, A., Lampo, M., Fajardo-Ramos, L., Aguirre, A.A., Kilpatrick, A.M., Rodrıguez, J.P. & Daszak, P. (2004). Presence of an emerging pathogen of amphibians in introduced bullfrogs *Rana catesbeiana* in Venezuela. *Biological Conservation* 120: 115-119.

Hero, J-M., McDonald, K., Alford, R., Cunningham, M. & Retallick, R. (2004). *Rheobatrachus vitellinus*. [Online] The IUCN Red List of Threatened Species 2004. Available at: e.T19476A8897826. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T19476A8897826.en.

Hyatt, A. D., Boyle, D. G., Olsen, V., Boyle, D. B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F. & Colling, A. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73: 175-192.

James, T.Y., Toledo, L.F., Rödder, D., da Silva Leite, D., Belasen, A.M., Betancourt-Román, C.M., Jenkinson, T.S., Soto-Azat, C., Lambertini, C., Longo, A.V. & Ruggeri, J. (2015). Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution* 5: 4079-4097.

Jarvis, L.E. (2018) Guiana Shield amphibian assessment, 2017. Unpublished final report, Froglife, Peterborough, UK.

Jenkinson, T.S., Román, B., Lambertini, C., Valencia-Aguilar, A., Rodriguez, D., Nunes-de-Almeida, C.H.L., Ruggeri, J., Belasen, A.M., Silva Leite, D., Zamudio, K.R. & Longcore, J.E. (2016). Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. *Molecular Ecology* 25: 2978-2996.

Kok, P.J.R., Sambhu, H., Roopsind, I., Lenglet., G.L., & Bourne, G.R. (2006). A new species of *Colostethus* (Anura: Dendrobatidae) with maternal care from Kaieteur National Park, Guyana. *Zootaxa* 1238: 35-61.

Kriger, K.M. & Hero, J.M. (2009). Chytridiomycosis, amphibian extinctions, and lessons for the prevention of future panzootics. *EcoHealth* 6: 6.

Leibler, J.H., Otte, J., Roland-Holst, D., Pfeiffer, D.U., Magalhaes, R.S., Rushton, J., Graham, J.P. & Silbergeld, E.K. (2009). Industrial food animal production and global health risks: exploring the ecosystems and economics of avian influenza. *Ecohealth* 6: 58-70.

Lips, K.R., Burrowes, P.A., Mendelson III, J.R. and Parra-Olea, G. (2005). Amphibian Declines in Latin America: Widespread Population Declines, Extinctions, and Impacts 1. Biotropica: *The Journal of Biology and Conservation* 37: 163-165.

Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R. A., Voyles, J., Carey, C., Livo, L., Pessier, A.P. & Collins, J.P. (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences* 103: 3165-3170.

Lötters, S., Kielgast, J., Bielby, J., Schmidtlein, S., Bosch, J., Veith, M., Walker, S.F., Fisher, M.C. & Rödder, D. (2009). The link between rapid enigmatic amphibian decline and the globally emerging chytrid fungus. *EcoHealth* 6: 358-372.

Luger, M., Garner, T.W., Ernst, R., Hödl, W. and Lötters, S. (2008). No evidence for precipitous declines of harlequin frogs (Atelopus) in the Guyanas. *Studies on Neotropical Fauna and Environment* 43: 177-180.

MacCulloch, R.D. & Lathrop, A. (2002). Exceptional diversity of Stefania (Anura: Hylidae) on Mount Ayanganna, Guyana: three new species and new distribution records. *Herpetologica* 58: 327-346.

McCracken, S., Gaertner, J.P., Forstner, M.R. and Hahn, D. (2009). Detection of *Batrachochytrium dendrobatidis* in amphibians from the forest floor to the upper canopy of an Ecuadorian Amazon lowland rainforest. *Herpetological Review* 40: 190.

McMahon, T.A., Brannelly, L.A., Chatfield, M.W., Johnson, P.T., Joseph, M.B., McKenzie, V.J., Richards-Zawacki, C.L., Venesky, M.D. & Rohr, J.R. (2013). Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences* 110: 210-215.

Meyer, E., Newell, D., Hines, H., May, S., Hero, J-M., Clarke, J. & Lemckert, F. (2004). *Rheobatrachus silus*. [Online] The IUCN Red List of Threatened Species 2004. Available at: e.T19475A8896430. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T19475A8896430.en.

Meyerson, L.A. & Reaser, J.K. (2002). Biosecurity: Moving toward a Comprehensive Approach: A comprehensive approach to biosecurity is necessary to minimize the risk of harm caused by non-native organisms to agriculture, the economy, the environment, and human health. *BioScience* 52: 593-600.

Montambault, J.R. & Missa, O. (2002). A biodiversity assessment of the eastern Kanuku Mountains, lower Kwitaro River, Guyana. *Rapid Assessment Program Bulletin of Biological Assessment, Washington* 26: 1-88.

Msami, H. (2008). Good biosecurity practices in non-integrated commercial and in scavenging production systems in Tanzania. Food and Agriculture Organisation of the United Nations: Strategies for the Prevention and Control of Infectious Diseases in Eastern Africa. [online] http://www.fao.org/docrep/013/al839e/al839e00.pdf

O'Hanlon, S., Rieux, A., Farrer, R., Rosa, G., Waldman, B., Bataille, A., Kosch, T., Murray, K., Brankovics, B., Fumagalli, M., Martin, M., Wales, N., Alvarado-Rybak, M., Bates, K., Berger, L., Böll, S., Brookes, L., Clare, F., Courtois, E., Cunningham, A., Doherty-Bone, T., Ghosh, P., Gower, D., Hintz, W., Höglund, J., Jenkinson, T., Lin, C., Laurila, A., Loyau, A., Martel, A., Meurling, S., Miaud, C., Minting, P., Pasmans, F., Schmeller, D., Schmidt, B., Shelton, J., Skerratt, L., Smith, F., Soto-Azat, C., Spagnoletti, M., Tessa, G., Toledo, L.,

Valenzuela-Sánchez, A., Verster, R., Vörös, J., Webb, R., Wierzbicki, C., Wombwell, E., Zamudio, K., Aanensen, D., James, T., Gilbert, M., Weldon, C., Bosch, J., Balloux, F., Garner, T. & Fisher, M. (2018). Recent Asian origin of chytrid fungi causing global amphibian declines. *Science* 360: 621-627.

Olson, D.H., Aanensen, D.M., Ronnenberg, K.L., Powell, C.I., Walker, S.F., Bielby, J., Garner, T.W., Weaver, G. & Fisher, M.C. (2013). Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. *PloS one* 8: e56802.

Olson, D.H. & Ronnenberg, K.L. (2014). Global *Bd* mapping project: 2014 update. *FrogLog* 22: 17-21.

Palumbi, S. (1996). Nucleic acids II: polymerase chain reaction. In Hills, D.M., Moritz, C. & Mable, B.K. (Eds.). *Molecular Systematics*, Sinauer Associates, Sunderland, MA. 205–247

Phillott, A.D., Speare, R., Hines, H.B., Skerratt, L.F., Meyer, E., McDonald, K.R., Cashins, S.D., Mendez, D. & Berger, L. (2010). Minimising exposure of amphibians to pathogens during field studies. *Diseases of Aquatic Organisms* 92: 175-185.

Piotrowski, J.S., Annis, S.L. & Longcore, J.E. (2004). Physiology of Batrachochytrium dendrobatidis, a chytrid pathogen of amphibians. Mycologia 96: 9-15.

Reeder, N.M., Pessier, A.P. & Vredenburg, V.T. (2012). A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. *PLoS One* 7: e33567.

Rödder, D., Kielgast, J., Bielby, J., Schmidtlein, S., Bosch, J., Garner, T.W., Veith, M., Walker, S., Fisher, M.C. & Lötters, S. (2009). Global amphibian extinction risk assessment for the panzootic chytrid fungus. *Diversity* 1: 52-66.

Sambrook, J. & Russell, D.W. (2001). *Molecular cloning: a laboratory manual*, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Sánchez, D., Chacón-Ortiz, A., León, F., Han, B.A. & Lampo, M. (2008). Widespread occurrence of an emerging pathogen in amphibian communities of the Venezuelan Andes. *Biological Conservation* 141: 2898-2905.

Searle, C.L., Gervasi, S.S., Hua, J., Hammond, J.I., Relyea, R.A., Olson, D.H. & Blaustein, A.R. (2011). Differential host susceptibility to *Batrachochytrium dendrobatidis*, an emerging amphibian pathogen. *Conservation Biology* 25: 965-974.

Shepherd, R., Hoskisson, P.A. & Downie, J.R. (2016). Apparent absence of chytrid infection in Trinidad's frogs. *Living World, Journal of the Trinidad and Tobago Field Naturalists' Club*: 19-22.

Skerratt, L., Speare, R. & Berger, L. (2011). Mitigating the impact of diseases affecting biodiversity-retrospective on the outbreak investigation for chytridiomycosis. *Ecohealth* 7: 26.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S., Fischman, D.L. & Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783-1786.

Thomson, R., Hoskisson, P.A., Brozio, S. & Downie, J.R. (2018). Apparent lack of chytrid infection in north-east Tobago's frogs. *Living World, Journal of the Trinidad and Tobago Field Naturalists' Club*: 82-86

Thrusfield, M. (1995). Veterinary epidemiology, 2nd edition. *Oxford: Blackwell Science:* 187–191

Valencia-Aguilar, A., Ruano-Fajardo, G., Lambertini, C., a Silva Leite, D., Toledo, L.F. & Mott, T. (2015). Chytrid fungus acts as a generalist pathogen infecting species-rich amphibian families in Brazilian rainforests. *Diseases of Aquatic Organisms* 114: 61-67.

Van Rooij, P., Martel, A., Haesebrouck, F. & Pasmans, F. (2015). Amphibian chytridiomycosis: a review with focus on fungus-host interactions. *Veterinary Research* 46: 137.

von May, R., Catenazzi, A., Santa-Cruz, R., Kosch, T.A. & Vredenburg, V.T. (2018). Microhabitat temperatures and prevalence of the pathogenic fungus *Batrachochytrium dendrobatidis* in Lowland Amazonian Frogs. *Tropical Conservation Science* 11: p.1940082918797057.

Woodhams, D.C., Alford, R.A. & Marantelli, G. (2003). Emerging disease of amphibians cured by elevated body temperature. *Diseases of Aquatic Organisms* 55: 65-67.

Young, B.E., Lips, K.R., Reaser, J.K., Ibáñez, R., Salas, A.W., Cedeño, J.R., Coloma, L.A., Ron, S., La Marca, E., Meyer, J.R. and Muñoz, A. (2001). Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15: 1213-1223.

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