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Extensive occurrence of the amphibian chytrid fungus in the Albertine Rift, a Central African amphibian hotspot

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Recent surveys for the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, *Bd*) in Africa have documented the infectious disease in frogs and caecilians from forested habitats in multiple areas of Central and East Africa. We tested 166 frogs for the presence of *Bd* from 45 localities representing a diverse array of habitats and elevations (793–2852 m.a.s.l.) in the Albertine Rift (AR) region of eastern Democratic Republic of the Congo during four field seasons from 2008–2011. Fifty-eight of these frogs were positive, for an overall *Bd*-prevalence of 34.9%. Three genera of frogs (*Callixalus*, *Chrysobatrachus* and *Nectophryne*) are reported to be *Bd* positive for the first time. Behavioural observations of *Bd*-positive frogs calling for mates and basking suggest the AR amphibian fauna was not severely affected by chytridiomycosis during the survey. Given the enormous levels of endemism and conservation value of the AR amphibian fauna, additional studies of *Bd* should focus on the region.

Key words: anuran, Democratic Republic of the Congo, endemism, Infectious disease

INTRODUCTION

Recent studies of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, *Bd*) have documented devastating effects of chytridiomycosis on amphibian communities in several areas of the world (e.g., Wake & Vredenburg, 2008; Collins & Crump, 2009). Wave-like movements of the fungus into Mexico and Central America supported the novel-pathogen hypothesis, which posits that the fungus was introduced to habitats with amphibians that had not been exposed to the disease previously, with resulting catastrophic mortality and extinction (Lips et al., 2006, 2008). Although population declines have been associated with the chytrid fungus in at least one African amphibian (*Nectophrynoides asperginis*, Weldon & du Preez, 2004; Channing et al., 2006), baseline data on the distribution of the fungus in Africa are lacking in many areas.

The origin of *Bd* remains problematic, but many studies have pointed to Africa as the most likely source of the pathogen. Soto-Azat et al. (2010) focused on historical specimens from multiple African forested countries, and identified a *Bd*-infected *Xenopus* frog from Cameroon in 1933 (a second species of *Xenopus* was detected from specimens collected in Uganda in 1934), supporting a previous hypothesis that *Bd* originated in Africa (Weldon et al., 2004). Vredenburg et al. (2013) also detected chytrid infections in *Xenopus* collected from Kenya in 1934. Weldon et al. (2004, 2007) suggested *Bd*-positive *Xenopus* frogs facilitated the spread of the fungus across the world via international trade for pregnancy assays, and by its use as a model organism for biomedical teaching and research (see also Reed et al., 2000). This African origin hypothesis was not supported by James et al. (2009), who provided data showing relatively low genetic diversity in African chytrid isolates compared to those from North America. However, the latter authors stated that additional African isolates were needed to test the "out of Africa" hypothesis for the origin of the *Bd* pandemic. Farrer et al. (2011) noted that the sampling of James et al. (2009) was heavily biased towards North American samples, and the former authors hypothesised that the "anthropogenic mixing" (via recombination and/or hybridisation) of allopatric *Bd* lineages engendered the hypervirulent *Bd*GPL strain, which is mainly responsible for global amphibian declines. Because *Bd* represents a morphologically and genetically diverse conglomeration of lineages, Farrer et al. (2011) postulated that the parental genotypes and geographic origin of the hypervirulent *Bd*GPL strain will probably remain unclear until much more extensive sampling is possible. Recently, Rosenblum et al. (2013) examined the genomics of *Bd* isolates from several areas of the world, and reported a surprising amount of evolutionary complexity and

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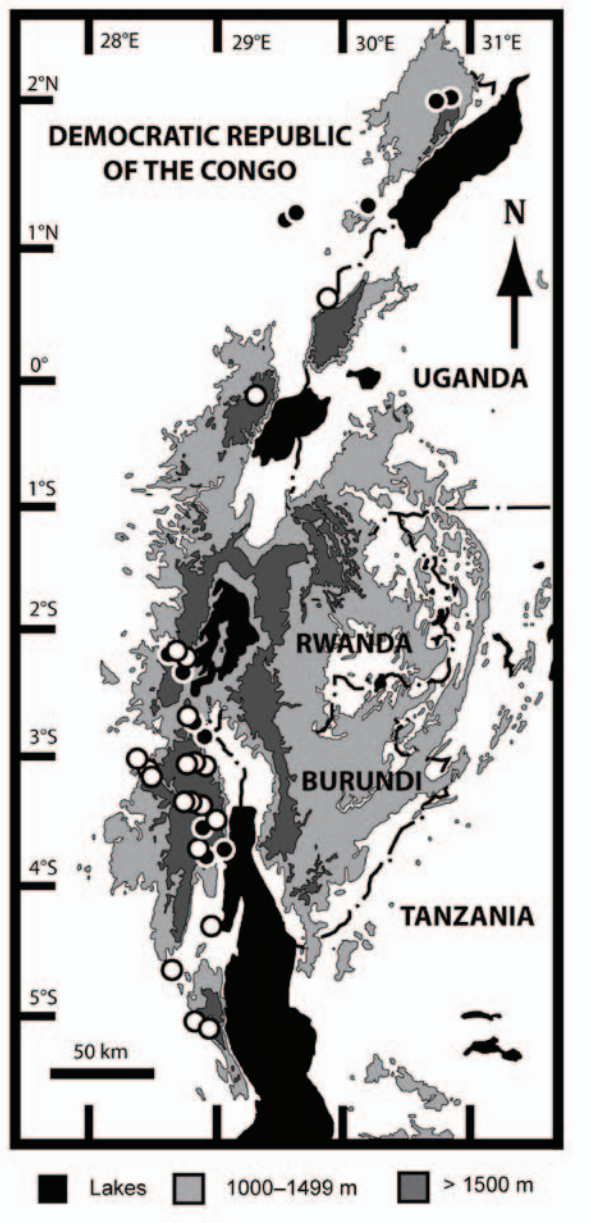

Fig. 1. Map of the Albertine Rift showing sampling localities for this study. Open circles represent localities with *Bd*-positive samples, closed circles are localities where *Bd* was undetected.

phylogenetic diversity that predate recent global amphibian declines linked to the fungus. Brazilian *Bd* isolates were recovered in a basal position in the latter study's phylogenetic analyses, but sampling from Africa was limited, and the authors cautioned that it would be premature to assign a geographic origin to *Bd*.

Until recently, no studies examined the presence of the fungus in forested regions of Africa, which contain the majority of amphibian species in continental Africa (Stuart et al., 2004). Goldberg et al. (2007) provided the first record of the fungus in six species of frogs from Kibale National Park in Uganda. Greenbaum et al. (2008) reported the fungus in the Central African treefrogs *Hyperolius langi* (erroneously listed by these authors as *H. kuligae*) and *H. kivuensis* in forests of eastern Democratic Republic of the Congo (DRC). Additional *Bd*-positive records soon followed from forested regions in Kenya (Kielgast et al., 2010), DRC (Roelke et al., 2011), Gabon (Bell et al., 2011), Nigeria (Imasuen et al., 2009, 2011; Reeder et al., 2011), Malawi (Conradie et al., 2011a), Ethiopia (Gower et al., 2012), Cameroon (Baláž et al., 2012; Doherty-Bone et al., 2013; Gower et al., 2013), Uganda (Viertel et al., 2012), São Tomé (Hydeman et al., 2013), and Tanzania (Weldon & du Preez, 2004; Gower et al., 2013; Zancolli et al., 2013). Intriguingly, several studies noted the complete absence of *Bd*-infected amphibians in forested regions of Cameroon (Doherty-Bone et al., 2008, but see Doherty-Bone et al., 2013), Gabon (Daversa et al., 2011; Gratwicke et al., 2011; Zimkus & Larson, 2013), and the entirety of Madagascar (Weldon et al., 2008; Crottini et al., 2011, 2014; Vredenburg et al., 2012) and West Africa (Penner et al., 2013). In an analysis of global patterns, Olson et al. (2013) argued that *Bd* has not yet reached a global spatial equilibrium, which could partially explain its absence from parts of Africa.

Herein, we focus on the Albertine Rift (AR) of DRC from its northernmost limit in the Lendu Plateau (west of Lake Albert) to the Kabobo Plateau at the South Kivu/Katanga Province border west of Lake Tanganyika. Long recognised as a key component of the Eastern Afromontane hotspot (Mittermeier et al., 2004), the AR is renowned as one of the most species-rich areas for vertebrates in continental Africa (Plumptre et al., 2007). The AR includes relatively large numbers of endemic and threatened species of amphibians, and recent studies have increased these numbers (e.g., Evans et al., 2008, 2011; Greenbaum et al., 2013; Portillo & Greenbaum, 2014 a,b). Preliminary molecular data suggest the amphibian diversity of the AR is substantially underestimated (EG, unpubl. data). Despite the widely recognised conservation value of the AR, extensive areas of the rift are either unprotected or weakly protected from threats ranging from deforestation to mining activities (Greenbaum & Kusamba, 2012).

Because of the exceptional diversity and enormous conservation challenges associated with the AR amphibian fauna, an assessment of the potential threat from chytridiomycosis is urgently needed. Moreover, Rödder et al. (2009: Fig. 2) and Penner et al. (2013: Fig. 1) used Maxent models to demonstrate that extensive areas of the AR are highly susceptible to chytrid infection of amphibian species. With the exception of the relatively limited chytrid screenings conducted in and around Kahuzi-Biega National Park in DRC (Greenbaum et al., 2008) and Kibale National Park in Uganda (Goldberg et al., 2007), the exact distribution and extent of chytrid fungal infections in the AR remains unknown. We tested for the presence of chytrid fungus infections in multiple genera and species of AR anurans from numerous localities across the AR from 2008–2011.

METHODS

Forty-five localities in the AR representing a diverse array of habitats and elevational gradients (793–2852 m.a.s.l.) were visited during field surveys from May–July in 2008 and 2009, and December–January in 2010 and 2011. Most of these surveyed habitats were secondary montane forest, but the team also visited savannahs,

Table 1. Results of *Bd* screening for amphibians in the Albertine Rift of Democratic Republic of the Congo from 2008–2011.

Genus	Species	Sample Size					*Bd* Positive					Prevalence of *Bd* 2008–2011 (95% CI)
		2008	2009	2010	2011	Total	2008	2009	2010	2011	Total	
Arthroleptis	*adolfifriederici*	0	1	0	0	1	—	0	—	—	0	0
Arthroleptis	*pyrrhoscelis*	0	1	0	1	2	—	0	—	0	0	0
Arthroleptis	*sylvaticus*	0	0	1	0	1	—	—	0	—	0	0
Arthroleptis	*variabilis*	0	0	1	0	1	—	—	0	—	0	0
Arthroleptis	*xenochirus*	0	1	0	0	1	—	0	—	—	0	0
Arthroleptis	sp. (Kabobo Plateau)	0	1	0	0	1	—	1	—	—	1	1
Leptopelis	*anebos*	0	0	0	2	2	—	—	—	0	0	0
Leptopelis	cf. *calcaratus*	0	0	2	0	2	—	—	1	—	1	0.50 (0–1.19)
Leptopelis	cf. *cynnamomeus*	0	0	2	0	2	—	—	0	—	0	0
Leptopelis	*kivuensis*	1	1	0	0	2	1	0	—	—	1	0.50 (0–1.19)
Leptopelis	cf. *mackayi*	1	0	0	0	1	0	—	—	—	0	0
Leptopelis	*christyi*	1	0	0	0	1	0	—	—	—	0	0
Leptopelis	*karissimbensis*	7	4	1	0	12	4	2	1	—	7	0.58 (0.30–0.86)
Leptopelis	*mtoewaate*	1	0	3	0	4	1	—	3	—	4	1
Leptopelis	sp. 1 (Mt. Tshiaberimu)	1	0	0	0	1	1	—	—	—	1	1
Amietophrynus	cf. *camerunensis*	1	0	0	0	1	0	—	—	—	0	0
Amietophrynus	*channingi*	0	0	1	0	1	—	—	0	—	0	0
Nectophryne	cf. *batesii*	0	0	1	0	1	—	—	1	—	1	1
Afrixalus	cf. *laevis*	0	1	1	0	2	—	1	0	—	1	0.50 (0–1.19)
Afrixalus	cf. *osorioi*	0	0	2	0	2	—	—	0	—	0	0
Afrixalus	cf. *quadrivittatus* 1	2	0	1	0	3	1	—	0	—	1	0.33 (0–0.87)
Afrixalus	cf. *quadrivittatus* 2	3	1	0	0	4	1	0	—	—	1	0.25 (0–0.67)
Callixalus	*pictus*	1	0	0	0	1	1	—	—	—	1	1
Chrysobatrachus	*cupreonitens*	0	2	0	2	4	—	2	—	2	4	1
Hyperolius	*castaneus*	4	6	0	0	10	1	0	—	—	1	0.10 (0–0.29)
Hyperolius	cf. *cinnamomeoventris*	0	1	0	0	1	—	0	—	—	0	0
Hyperolius	cf. *langi*	4	1	2	0	7	1	0	0	—	1	0.14 (0–0.40)
Hyperolius	cf. *nasicus*	2	0	0	0	2	0	—	—	—	0	0
Hyperolius	cf. *tuberculatus*	3	0	0	0	3	2	—	—	—	2	0.67 (0.14–1.20)
Hyperolius	*constellatus*	0	9	0	1	10	—	4	—	0	4	0.40 (0.10–0.70)
Hyperolius	*discodactylus*	0	1	0	0	1	—	0	—	—	0	0
Hyperolius	*kivuensis*	2	4	1	0	7	1	3	—	—	4	0.57 (0.20–0.94)
Hyperolius	*langi*	0	1	0	0	1	—	0	—	—	0	0
Hyperolius	*lateralis pleurospilus*	2	0	0	0	2	0	—	—	—	0	0
Hyperolius	*leucotaenius*	0	0	1	2	3	—	—	0	0	0	0
Hyperolius	*quinquevittatus*	0	0	3	0	3	—	—	0	—	0	0
Hyperolius	*rwandae*	3	1	0	0	4	0	0	—	—	0	0
Hyperolius	sp. 1 (Force Bendera)	0	1	0	0	1	—	0	—	—	0	0

Table 1. *Continued*.

Genus	Species	Sample Size					*Bd* Positive					Prevalence of *Bd* 2008–2011 (95% CI)
		2008	2009	2010	2011	Total	2008	2009	2010	2011	Total	
Hyperolius	sp. 2 (Kamango)	1	0	0	0	1	0	—	—	—	0	0
Hyperolius	sp. 3 (Lendu Plateau)	0	1	0	0	1	—	0	—	—	0	0
Hyperolius	sp. 4 (Mwenga)	1	0	1	0	2	0	—	0	—	0	0
Hyperolius	sp. 5 (Albertine Rift)	3	1	0	0	4	1	0	—	—	1	0.25 (0–0.67)
Hyperolius	*viridiflavus schubotzi*	0	1	0	0	1	—	0	—	—	0	0
Kassina	cf. *senegalensis*	1	0	0	0	1	0	—	—	—	0	0
Phlyctimantis	*verrucosus*	2	0	0	0	2	2	—	—	—	2	1
Phrynobatrachus	*acutirostris*	1	0	0	1	2	1	—	—	0	1	0.50 (0–1.19)
Phrynobatrachus	*asper*	0	2	0	0	2	—	1	—	—	1	0.50 (0–1.19)
Phrynobatrachus	cf. *graueri*	2	0	0	0	2	1	—	—	—	1	0.50 (0–1.19)
Phrynobatrachus	cf. *keniensis*	1	0	0	0	1	0	—	—	—	0	0
Phrynobatrachus	cf. *perpalmatus*	1	4	1	0	6	1	2	0	—	3	0.50 (0.10–0.90)
Phrynobatrachus	cf. *natalensis* 1	0	2	1	0	3	—	1	1	—	2	0.67 (0.14–1.20)
Phrynobatrachus	cf. *natalensis* 2	2	1	0	0	3	0	0	—	—	0	0
Phrynobatrachus	cf. *parvulus*	0	0	1	0	1	—	—	0	—	0	0
Phrynobatrachus	cf. *petropedetoides*	0	0	1	0	1	—	—	0	—	0	0
Phrynobatrachus	cf. *versicolor*	0	0	0	1	1	—	—	—	0	0	0
Phrynobatrachus	*dendrobates*	0	0	4	0	4	—	—	3	—	3	0.75 (0.33–1.17)
Phrynobatrachus	*graueri*	0	2	0	1	3	—	0	—	0	0	0
Phrynobatrachus	*versicolor*	1	0	0	0	1	1	—	—	—	1	1
Ptychadena	cf. *mascareniensis* C	1	0	0	0	1	0	—	—	—	0	0
Ptychadena	cf. *mascareniensis* D	1	0	0	0	1	0	—	—	—	0	0
Ptychadena	*cf. nilotica*	0	3	0	0	3	—	0	—	—	0	0
Ptychadena	cf. *tellinii*	1	0	0	0	1	0	—	—	—	0	0
Xenopus	*pygmaeus*	0	0	1	0	1	—	—	0	—	0	0
Amietia	cf. *amieti*	0	0	1	0	1	—	—	0	—	0	0
Amietia	sp. 1 (Force Bendera)	0	2	0	0	2	—	2	—	—	2	1
Amietia	sp. 2 (Itombwe)	1	4	0	1	6	1	4	—	0	5	0.83 (0.53–1.13)
Total		59	61	34	12	166	23	23	10	2	58	0.35 (0.28–0.42)

woodlands, marshes, lowland rainforest, transitional forest, bamboo forest and agricultural areas and villages (Online Appendix Table A1). A total of 166 frogs were swabbed during the study, representing seven families, 14 genera, and 66 species (Online Appendix Table A2). When considered by year, a total of 59 frogs representing 10 genera and 32 species were sampled opportunistically in 2008, 61 in 2009 (representing eight genera and 29 species), 34 in 2010 (representing nine genera and 23 species), and 12 in 2011 (representing six genera and nine species).

Frogs were collected by hand (without gloves) or dipnet following audio recording of male advertisement calls (in the case of calling males), or during opportunistic visual encounters. To assist with specimen organisation and subsequent photographing, frogs were deposited into well-ventilated and labeled plastic containers immediately after capture. Frogs were usually captured individually, but rarely, individuals of the same species (up to six individuals) were kept in the same container. Plastic containers were disinfected with a 10% bleach solution after each collection event. A subset of individuals, mostly post-metamorphic, were swabbed in their containers for approximately 20 seconds on the ventral surfaces of the abdomen, throat, hands and feet. Frogs were swabbed with sterile swabs (MW100;

Medical Wire & Equipment Co, Crosham, UK), which were stored individually and dry in shaded containers away from direct sunlight and heat. Swabs were stored at 4°C upon arrival to the laboratory. DNA was extracted with the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) using the manufacturer's tissue protocol. Real-time PCR was performed on a LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA) according to the protocol of Boyle et al. (2004), with *Bd* primers specific to the ITS-1/5.8S region of the ribosomal gene. DNA extracted from a *Bd*-positive culture was used as a positive control and amplicons from two positive frog samples were sequenced to confirm the specificity of the amplification products. Samples were considered positive if they showed distinct sigmoidal amplification in the real-time PCR, whereas negative samples and negative controls showed no such amplification (Soto-Azat et al., 2010). Confidence intervals (CI) for prevalence were calculated with the methods of Thrusfield (2007).

RESULTS

A total of 166 frogs were swabbed during this study, and 58 of these individuals were positive, for an overall *Bd*-prevalence of 34.9% (Fig. 1, Table 1). When considered by year, 23 individuals were positive in 2008 (39.0% prevalence), 23 in 2009 (37.7% prevalence), 10 in 2010 (29.4% prevalence) and two in 2011 (16.7% prevalence). With the exception of the poorly sampled genera *Amietophrynus* (1 specimen), *Kassina* (1 specimen), *Ptychadena* (5 specimens in 3 species), and *Xenopus* (1 specimen), at least one specimen of each of the remaining ten genera tested positive for *Bd* infection. Considering the number of *Bd*-positive species for each of the remaining genera, *Callixalus*, *Chrysobatrachus*, *Phlyctimantis* and *Nectophryne* had one positive species each (100% prevalence), *Amietia* was positive for two of three species (66.7% prevalence), *Afrixalus* was positive for three of four species (75% prevalence), *Leptopelis* was positive for five of nine species (55.5% prevalence), *Phrynobatrachus* was positive for seven of 13 species (53.8% prevalence), *Hyperolius* was positive for six of 19 species (31.6% prevalence), and *Arthroleptis* was positive for one of six species (16.7% prevalence). Most of the species for which *Bd* was not detected were poorly sampled - only one individual was sampled for 26 of these species, two individuals were sampled for seven of the *Bd*-undetected species, and three individuals were sampled for the remaining six *Bd*-undetected species. Among the species for which more than one specimen was sampled, the highest *Bd* prevalence was a four-way tie between *L. mtoewaate*, *C. cupreonitens*, *P. verrucosus*, and *Amietia* sp. 1 (100% prevalence); high values were also found for *Amietia* sp. 2 (83% prevalence), *P. dendrobates* (75% prevalence), *P*. cf. *natalensis* 1 and *H*. cf. *tuberculatus* (67% prevalence each), and *L. karissimbensis* (58% prevalence). The lowest prevalence values for well-sampled species occurred in *H. constellatus* (40% prevalence), and *P.* cf. *perpalmatus* (50% prevalence).

Among the 45 localities visited during the study, 28 contained *Bd*-positive frogs, for an overall site occupancy of 62.2%. Several of the sites where *Bd* was undetected were poorly sampled - nine localities were sampled for only one frog; four localities were sampled for two frogs, and one locality was sampled for three frogs. Among the localities for which more than two frogs were tested, the highest *Bd*-prevalence occurred at Miki, Itombwe Plateau (75% prevalence), Mugaba, Kahuzi-Biega National Park (71% prevalence), Force Bendera (67% prevalence), Mitamba, Itombwe Plateau (60% prevalance), and Komesha and Kizuka, both in the Itombwe Plateau (50% prevalence). The lowest prevalence values for well-sampled localities (three or more tested frogs) was a four-way tie between Bitale, road near N'Komo River, and Milembwe and Tumungu, both in the Itombwe Plateau (0% prevalence). Other localities with low *Bd*-infection rates included Kamango (18% prevalence), Nyakasanza (13%), and Lulimba and Tshibati (both 25% prevalence). During fieldwork activity, no dead or morbid frogs were encountered.

DISCUSSION

Several studies have documented the tendency of *Bd* infections to be more severe in relatively cool, mountainous areas that are similar to those in the Albertine Rift (e.g., Stuart et al., 2004; Pounds et al., 2006; Brem & Lips, 2008; Gower et al., 2012; but see Olson et al., 2013). Bielby et al. (2008) and Rödder et al. (2009) used models to infer that amphibian species with *Bd* infections or susceptibility to infections have low fecundity, an aquatic life stage, occur at high elevations and have small geographic distributions; the Albertine Rift was emphasised in global maps of areas with high likelihoods for *Bd* infection. Becker & Zamudio (2011) recently argued that relatively pristine environments in the Australian tropics and Neotropics, regardless of elevation, seem to be correlated with a higher risk of *Bd* infection. These observations are likely explained by the *Bd* optimal growth requirements of mild temperature (17–25°C) and high humidity (Piotrowski et al., 2004; Bustamante et al., 2010), but some variation in these requirements have been noted in isolates from Australia (Stevenson et al., 2013). In deforested habitats, lower amphibian host species richness (see also Olson et al., 2013) and potentially suboptimal microclimates from edge effects are correlated with lower *Bd* infection rates (Becker & Zamudio, 2011). Previous studies might have underestimated the effect of habitat loss on *Bd* infection, because of deforestation biases related to topography and elevation (Becker & Zamudio, 2011).

Because our data set from the Albertine Rift includes localities from a wide array of habitats and elevations, we were able to search for trends that might be consistent with the predictions of Becker & Zamudio (2011). When focused on well-sampled localities (four or more sampled frogs) that included primary forest, we observed relatively high *Bd*-infection rates in the montane forests at Miki (75% prevalence, Itombwe Plateau) and Mugaba (71.4% prevalence, Kahuzi-Biega National Park), but low infection rates were seen in the primary transitional forest at Bizombo (30% prevalence), and no infections at

all in the montane forest at Tumungu (Itombwe Plateau), which seemed to be the most pristine forest the team visited. Although local taboos prohibiting deforestation were in place at Tumungu during our visit in 2011, we cannot exclude the possibility of some environmental damage from mining operations that were conducted during the colonial era in the mid-20th century.

Trends in *Bd*-infection rates at well-sampled localities (six or more sampled frogs) with other habitat types were generally moderate to low. Infection rates at the secondary forest/village habitats at Kiandjo were moderate (44.4% prevalence, Itombwe Plateau), and the marsh at the edge of secondary forest at Nyakasanza was low (12.5%). The montane marshes in the forest/savannah mosaic sampled at Mt. Tshiaberimu was moderate (42.9% prevalence, Virunga National Park), and a similar habitat at lower elevations at Kamango had low *Bd* infection rates (18.2% prevalence, Virunga National Park). Marshes in montane savannah at Komesha (Itombwe Plateau) had 50% prevalence. Other habitats were not sampled well enough to document infection rate trends. Additional sampling is needed to assess whether the trends noted by Becker & Zamudio (2011) occur in the Albertine Rift. North of 1° N latitude, sample sizes for these localities were small, and it would be premature to conclude that *Bd* is truly absent from this portion of the AR.

The low prevalence (16.7%) of *Bd* infection in the direct-developing genus *Arthroleptis* is consistent with previous studies that noted low prevalence in this genus elsewhere in Africa (Bell et al., 2011; Conradie et al., 2011a; Doherty-Bone et al., 2013; Olson et al., 2013) and the presumably direct-developing genus *Balebreviceps* in Ethiopia (Gower et al., 2012). The high rates of *Bd* infection seen in the genus *Amietia* (66.7% prevalence) in our study are mostly consistent with previous findings in other parts of tropical Africa (Kielgast et al., 2010; Conradie et al., 2011a; Viertel et al., 2012; Zancolli et al., 2013), but Conradie et al. (2011b) documented seasonal variation in relatively low infection rates of larval *A. angolensis*. Three other genera (*Callixalus*, *Chrysobatrachus* and *Nectophryne*) of frogs are reported by us to be positive for *Bd* for the first time. The high infection rates seen in *Chrysobatrachus* and *Amietia* sp. 2 are of special concern, because these taxa have limited distributions in montane savannah, a biome known to have high probability of *Bd* infection in other parts of the world (Olson et al., 2013). As a consequence, other anurans that occur in montane savannahs of the AR must be monitored carefully, especially in the Itombwe and Kabobo plateaus, where multiple additional conservation concerns have been identified (Greenbaum & Kusamba, 2012).

In addition to the complete lack of morbid or dying frogs at our field sites, several other behavioural observations suggest the AR amphibian fauna are likely not severely affected by *Bd* infection. As discussed originally by Roelke et al. (2011) for the genus *Leptopelis*, we recorded the male advertisement call for several *Bd*-positive frogs, including *Afrixalus* cf. *quadrivittatus* 2, *Hyperolius kivuensis*, *H.* sp. 5, *Leptopelis kivuensis*, *L. karissimbensis*, and *Phrynobatrachus* cf. *perpalmatus*. The parasite load of these calling males is unknown, but presumably, these individuals would not be healthy enough to spend the energy required for male advertisement calls if the chytrid infection had severely weakened them. *Hyperolius langi* juveniles that were positive for *Bd* in the lowland rainforest at Irangi, DRC (Greenbaum et al., 2008) were observed perched on branches of a small tree about two metres above a stream in direct sunlight, apparently basking (EG, pers. obs.). Because elevated body temperatures have been shown to cure *Bd*-infected frogs (Woodhams et al., 2003), we hypothesise that these hyperoliids were undergoing behavioural thermoregulation to induce a so-called "behavioural fever" as an adaptive response to *Bd* infection. Such behavioural fevers have been documented in wild populations of the Panamanian golden frog (*Atelopus zeteki*) following an epidemic of chytrid fungus in lowland rainforest (290 m.a.s.l.) in western Panama (Richards-Zawacki, 2010). Rowley & Alford (2013) did not document strong evidence for behavioural fevers in three species of lowland rainforest (20–200 m.a.s.l.) *Litoria* treefrogs in Australia, but their results suggested individual probabilities of *Bd* infection decreased with increasing percentage of body temperatures above 25°C in these wild populations.

More field observations of *Bd*-positive frogs are needed to understand the negative effects of *Bd* infection on the AR amphibian fauna, but our results (coupled with the above behavioural observations) suggest the effects may be minor in comparison to most populations outside of Africa. Considered together with many previous studies of *Bd* in African amphibians, our study supports the hypothesis that the African amphibian fauna likely coevolved with the enzootic pathogen and formed a commensal relationship over a long period of time (Weldon et al., 2004; Kielgast et al., 2010; Soto-Azat et al., 2010; Baláž et al., 2012; Tarrant et al., 2013). Although this hypothesis is consistent with an African origin for *Bd*, more data from *Bd* isolates from around the world are needed to understand the definitive origin of the pandemic.

We acknowledge that our results could have been affected by the lack of gloves during capture of frogs in the field, or by housing individuals of the same species together. However, because there are scores of cases in our data set where *Bd*-negative frogs were collected after *Bd*-positive frogs during a given collection event, the possibility of cross contamination during capture is likely minimal. Our study is also limited by the lack of zoospore genomic equivalent estimates, which does not allow for comparisons of parasite loads to previous studies.

Despite the relatively large number of *Bd*-positive frogs in this study, our results (and likely those of many previous studies - see Zancolli et al., 2013) might have been affected by unavoidable exposure of stored swabs to high temperatures during transportation between field sites, which could have subsequently decreased the ability to detect the fungus (and associated parasite loads) in the laboratory (Van Sluys et al., 2008). Doherty-Bone et al. (2013) recently demonstrated that bovine serum albumin can also be crucial to detecting *Bd*-

positive amphibians. Kriger and Hero (2007) noted fluctuations in *Bd* prevalence associated with seasonality in Australia (i.e. a negative relationship between mean air temperature and disease prevalence), and because our sampling spanned different times of year (rainy and dry periods varied by time of year and locality relative to the equator) across habitats in multiple elevations (Online Appendix Table A1), ambient temperature variation could have affected our results. Conradie et al. (2011b) demonstrated such seasonal variation in *Bd* prevalence in South African *Amietia* frogs. Because our sample sizes and life-stage sampling for each species and locality were relatively limited, it is likely that *Bd*-positive frogs were not detected if their parasite loads were low (Skerratt et al., 2008). At least two studies of Central African *Bd* infection in frogs from forested regions of Cameroon (Doherty-Bone et al., 2013) and Nigeria (Reeder et al., 2011) reported low parasite loads. Future studies will need to consider these methodological limitations and other related sources of error (Olson et al., 2013) to maximise the probability of detecting *Bd*-positive amphibians. Future collections could benefit from protocols that: (i) prevent or greatly reduce the chance of cross-contamination of specimens; (ii) standardise swabbing so that detection of *Bd* can be quantified with improved precision; and (iii) include sample preservation for culture isolation to facilitate more in-depth genetic comparisons and *Bd* evolution studies.

In a model-based, global assessment of *Bd*-infection risk to amphibians, Rödder et al. (2009) identified 379 species considered to be at the highest level of risk, but only six of these were from the Albertine Rift (*Arthroleptis schubotzi*, *A. vercammeni*, *H. xenorhinus*, *L. karissimbensis*, *P. dalcqi* and *P. rouxi*), of which only one (*L. karissimbensis*) was examined by us and found to be positive for chytrid infection. Given the relatively high rates of infection in other AR species with limited geographic distributions (Table 2), we suggest that the risk of *Bd*-infection to the AR amphibian fauna is substantially higher than currently recognised. Several of the *Bd*-positive frogs from our study are considered threatened by the IUCN Red List criteria (IUCN, 2013), including *L. karissimbensis* (endangered), *Callixalus pictus*, *H. castaneus*, *P. acutirostris* and *P. versicolor*. The latter four species are currently classified as vulnerable, but Greenbaum et al. (2013) resurrected *H. contellatus* from the synonymy of *H. castaneus*, and as a result, both of these taxa are likely threatened. *Chrysobatrachus cupreonitens* and *P. asper* are currently considered to be data deficient, but given their limited geographic distribution and ongoing deforestation in the Itombwe Plateau (Greenbaum & Kusamba, 2012), these species are clearly threatened. Scores of species and possibly distinct subspecific taxa in the AR with limited geographic distributions have not been recorded for half a century or more (EG, pers. obs.), and are likely to be susceptible to chytrid infection. Moreover, 37 of the taxa we studied (labeled as "cf." or "sp." in Table 1) are genetically distinct from their closest named relatives (EG, unpubl. data) and are likely to be threatened as well. By comparison, only two of the 12 least-concern species (*H. kivuensis* and *P. dendrobates*) were positive for *Bd* in our study. Given the enormous levels of endemism and conservation value of vertebrates in the AR in general (Plumptre et al., 2007; Greenbaum & Kusamba, 2012) and the ongoing discovery of new AR amphibian species (Evans et al., 2011; Portillo & Greenbaum, 2014 a,b), additional *Bd* studies are needed to inform conservation measures in the 21st century. Specifically, factorial infection experiments, long-term monitoring in the field, and screening of historical specimens will provide more precise data on the impact of this pathogen on AR amphibians.

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