9-2012

High Prevalence of the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) across Multiple Taxa and Localities in the Highlands of Ethiopia

David J. Gower

Thomas M. Doherty-Bone

Roman K. Aberra

Abebe Mengistu

Silvia Schwaller

See next page for additional authors

Recommended Citation

Gower, David J., Thomas M. Doherty-Bone, Roman K. Aberra, Abebe Mengistu, Silvia Schwaller, Michele Menegon, Rafael O. de Sá, Samy A. Saber, Andrew A. Cunningham, and Simon P. Loader. "High Prevalence of the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis)* across Multiple Taxa and Localities in the Highlands of Ethiopia." *The Herpetological Journal* 22, no. 4 (October 2012): 225-3.

High prevalence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) across multiple taxa and localities in the highlands of Ethiopia

David J. Gower[1], Thomas M. Doherty-Bone[1], Roman K. Aberra[2], Abebe Mengistu[3], Silvia Schwaller[3], Michele Menegon[4], Rafael de Sá[5], Samy A. Saber[6], Andrew A. Cunningham[7] & Simon P. Loader[3]

[1]*Department of Zoology, Natural History Museum, London, SW7 5BD, UK*

[2]*Ethiopian Wildlife Conservation Authority, P.O. Box 386, Addis Ababa, Ethiopia*

[3]*University of Basel, Institute of Biogeography, Department of Environmental Sciences, Basel 4056, Switzerland*

[4]*Sezione di Zoologia dei Vertebrati, Museo Tridentino di Scienze Naturali, Via Calepina 14, I–38100 Trento, Italy*

[5]*Department of Biology, University of Richmond, Richmond VA 23173, USA*

[6]*Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia (present address: Zoology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt)*

[7]*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*

Surveys of the potentially lethal amphibian chytrid fungus (*Batrachochytrium dendrobatidis* - *Bd*) in Africa are patchy, especially in some regions of high species endemicity. We present results of the first *Bd* surveys of wild amphibians in Ethiopia, for two upland regions on either side of the Rift Valley: the Bale Mountains and the Kaffa region. Surveys were opportunistic so that robust interpretation of the data is limited. Utilizing diagnostic qPCR assays, 51 out of 120 frogs (14 species in 10 genera) tested positive for *Bd* at altitudes of 1,620–3,225 m, across all genera and species, and all but two localities. Prevalence was not significantly different between the two regions or two years (2008, 2009) sampled. Prevalence and parasite load was higher in species with aquatic tadpoles than those with terrestrial early life-history stages, but these differences were not significant. Impacts of *Bd* infection were not investigated, but no dead or dying frogs were found. This is the first report of *Bd* in Ethiopia, a country in which approximately 40% of its more than 60 species are endemic. Declines have occurred in some frog species in some localities in Ethiopia, and although habitat degradation is a likely cause in at least some places, further studies of *Bd* in Ethiopia are required to understand if it is a threat.

Key words: Africa, Bale Mountains, conservation, frogs, Harenna, Kaffa, life history

INTRODUCTION

The amphibian chytrid fungus (*Batrachochytrium dendrobatidis* - *Bd*) is a skin parasite that can cause the fatal disease amphibian chytridiomycosis (Berger et al., 1998; Lips et al., 2006). *Bd* has been implicated in rapid declines of >200 species worldwide, and has been declared a notable contributor to the global amphibian biodiversity crisis (Skerratt et al., 2007; Lötters et al., 2010). The cause of *Bd*-induced amphibian declines has been hypothesized to be: naïve host populations becoming exposed to this pathogen introduced from an endemic focus (the novel pathogen hypothesis); *Bd* being endemic in host environments and increasing its host range or virulence (the endemic pathogen hypothesis) (Rachowicz et al., 2005); or a combination of both these hypotheses (Fisher et al., 2009).

The novel pathogen hypothesis has been supported by evidence for the "wave-like" range expansions of *Bd* into regions where this pathogen has not previously been detected, followed by subsequent declines in multiple amphibian species (Lips et al., 2006; 2008; Skerratt et al., 2007). The novel pathogen hypothesis has been supported further by the oldest records for *Bd* being detected from museum specimens of African pipid frogs (genus *Xenopus*) (Weldon et al., 2004; Soto-Azat et al., 2010), anurans that have been exported widely around the world (Weldon et al., 2007). *Bd* has also been found to be widespread, occurring in most African countries sampled (Hopkins & Channing, 2003; Weldon & du Preez, 2004; Goldberg et al., 2007; Greenbaum et al., 2008; Kielgast et al., 2010; Bell et al., 2011; Reeder et al., 2011). Rapid declines of amphibians irrevocably attributed to *Bd* have not been recorded on the African continent, although many localities in Africa lack adequate baseline data to enable declines to be detected (Lawson & Klemens, 2001). The status of *Bd* as an indigenous amphibian parasite in Africa remains uncertain: sampled *Bd* isolates from Africa (although few in number and almost exclusively from South Africa) were no more heterogeneous than isolates from other continents where *Bd* has caused declines, suggesting Africa may not be the endemic focus of this pathogen (James et al., 2009).

In regions where *Bd* has become endemic post-outbreak, this pathogen undergoes seasonal fluctuations in prevalence (Retallick et al., 2004; Kriger & Hero, 2007a).

Correspondence: David J. Gower, Department of Zoology, Natural History Museum, London, SW7 5BD, UK; *E-mail:* d.gower@nhm.ac.uk

Distribution within host assemblages is predominantly in aquatic species occurring in permanent ponds and streams, with very low prevalence in anurans occurring in ephemeral wetlands and terrestrial habitats (Lips et al., 2003, 2006, Kriger & Hero, 2007b). Those species that are aquatic, have low fecundity, restricted range and occur at high elevations are more likely to decline as a result of *Bd* (Bielby et al., 2008). Predicting interspecific susceptibility to *Bd* infection in amphibians is however still uncertain, with *Bd*-related declines occurring also for terrestrial breeding species (e.g., *Leiopelma archeyi*, see Bell et al., 2004). Trends of *Bd* infection in relation to host biological traits have not been assessed in Africa, despite the continent's status as the possible endemic focus of this pathogen.

There have been calls to map the global distribution of *Bd* in order to identify sources and potential sinks for this disease, allowing biosecurity for infected and naïve amphibian populations to be managed (Skerratt et al., 2007). Bioclimatic modelling for the distribution of *Bd* has predicted that several regions hold a high suitability for the presence of this pathogen, including regions where *Bd* is either absent (e.g., Madagascar, Weldon et al., 2008) or yet to be assessed (Rödder et al., 2009). The latter includes the majority of Ethiopia, including the highland centres of diversity of its many endemic and threatened amphibians, causing concern that *Bd* may potentially negatively impact amphibian biodiversity in this country (Bielby et al., 2008; Rödder et al., 2009). The amphibian fauna of Ethiopia comprises 63 nominal

Table 1. Details of localities where frogs were swabbed for *Batrachochytrium dendrobatidis* in Ethiopia in 2008 and 2009.

			Coordinates		Dates sampled	
Region	Locality (habitats)	Altitude (m)	Latitude (N)	Longitude (E)	From	To
Bale	Magano (marsh in clearing)	1907	6.63858	39.73394	21/6/09	21/6/09
	Shawe bridge (river/ streams in forest)	1890	6.645556	39.731389	21/6/09	22/6/09
	Katcha (streams/ grassland in clearing)	2364–2370	6.716389–6.71697	39.72556–39.72583	30/7/08; 21/6/09	30/7/08; 21/6/09
	WWF (degraded woodland; stream)	2788–2830	6.750033–6.757222	39.719167–39.726389	30/7/08; 19/6/09	5/8/08; 19/6/09
	Rira (stream; degraded open woodland; village)	2880–2936	6.763056–6.773611	39.722222–39.727778	21/7/08; 19/6/09	5/8/08; 19/6/09
	Fute (streams; forest, some degraded)	3060–3165	6.755–6.763056	39.74722–39.75139	21/7/08; 21/6/09	18/8/08; 22/6/09
	Tulla Negresso (degraded forest; stream)	3225	6.776111–6.7775	39.745556–39.745833	15/7/08; 21/6/09	15/7/08; 21/6/09
	Dinsho park HQ (woodland)	3168	7.095833	39.79	15/7/08	15/7/08
Kaffa	Bonga town (small town)	1789	7.26719	36.25898	7/6/09	7/6/09
	Bonga stream (stream; farmland)	1727	7.27198	36.26	7/6/09	7/6/09
	Bonga marsh (marsh)	1734	7.24932	36.2554	7/6/09	7/6/09
	Mankira (disturbed forest; stream)	1620	7.19815	36.2854	8/6/09	8/6/09
	Koma forest stream (forest; stream)	1889	7.31803	36.07816	9/6/09	10/6/09
	Koma marsh (marsh)	1905	7.310556	36.079444	9/6/09	9/6/09
	Wush Wush marsh (marsh)	1895	7.31005	36.1205	10/6/09	13/6/09
	Saja forest (forest; river; streams)	2027	7.48705	36.09404	13/6/09	13/6/09

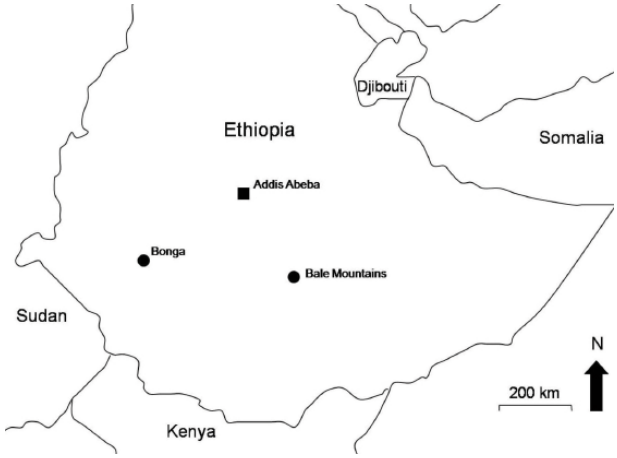

Fig. 1. Map showing Kaffa (in vicinity of town of Bonga) and Bale Mountains regions where frogs were surveyed for *Batrachochytrium dendrobatidis*.

species (62 anurans and one gymnophionan), of which 25 are endemics restricted to high elevation regions, and 23 species either endangered, vulnerable or near threatened with extinction (Largen, 2001; Largen & Spawls, 2010; IUCN et al., 2010). The major threats cited include habitat loss and climate change, with the role of emerging infectious disease so far unassessed in the field. In this paper we report high prevalence of *Bd* infection in a diversity of frog genera and species in two highland regions of Ethiopia.

METHODS

Fieldwork was conducted in multiple localities in two main regions in Ethiopia, either side of the Rift Valley, (1) the Bale Mountains from 15/07/2008 to 18/08/2008, and 19/06/2009 to 22/06/2009; (2) the Kaffa region in, and no more than 30 km from, the town of Bonga, from 07/06/2009 to 13/06/2009 (Fig 1; localities listed with GPS co-ordinates in Table 1). All but one of the localities sampled in the Bale Mountains are within a radius of 8 km of each other, within the Harenna Forest region of the southern escarpment; the other locality (only one specimen) was about 50 km north of the southernmost Harenna locality. Habitats sampled included moderately to severely disturbed forest; agricultural land; streams, ponds and marshes; towns and villages (Table 1). No undisturbed habitats were found. A total of 40 frogs (6 species in 4 genera) were sampled opportunistically in 2008; 80 in 2009 (32 from Bale: 11 species, 7 genera; 48 from Kaffa: 11 species, 7 genera). An overview of species sampled is provided in Table 2. Locality elevations range from: 1,890 to 3,225 m.a.s.l. in Bale; 1,620 to 2,027 m.a.s.l. in Kaffa. Both field seasons took place during the wet season.

The primary aim of the fieldwork was to collect amphibian samples and data for systematic studies, but also abundance data for some endemic taxa (Gower et al., in press), and so the chytrid study was consequently superficial and opportunistic. Frogs were collected by hand without gloves during visual encounter surveys, and

Table 2. Frog species sampled for *Bd* in Ethiopia 2008–2009. Regions sampled: B - Bale; K - Kaffa. Development Mode: BPa - biphasic with aquatic larvae; BPt - biphasic with terrestrial larvae; DD - direct-developing. Family classification follows Frost et al. (2006). *The family assignment of *Ericabatrachus baleensis* is debatable (Gower et al., in press). **The reproductive mode of *E. baleensis* is unknown; Largen (1991) suggested it was possibly direct-developing, but potential close relatives (Petropedetidae, Phrynobatrachidae, Pyxicephalidae) are mostly biphasic with aquatic larvae. ***Mode estimated based on Largen & Drewes (1989) and condition in other brevicipitids (Müller et al., 2007).

Family	Genus	Species	IUCN Status	Region	Development
?*	*Ericabatrachus*	*baleensis*	Endangered	B	?**
Arthroleptidae	*Leptopelis*	*gramineus*	Least Concern	B	BPa
	Leptopelis	*ragazzii*	Vulnerable	B	BPa
	Leptopelis	*vannutellii*	Vulnerable	K	BPa
Brevicipitidae	*Balebreviceps*	*hillmani*	Endangered	B	DD***
Bufonidae	*Altiphrynoides*	*malcolmi*	Endangered	B	BPt
Hyperoliidae	*Afrixalus*	*enseticola*	Vulnerable	K	BPa
	Afrixalus	*clarkei*	Vulnerable	K	BPa
	Afrixalus	sp.	-	B	BPa
	Hyperolius	cf. *kivuensis*	-	K	BPa
	Hyperolius	*viridiflavus*	Least Concern	K	BPa
	Paracassina	*obscura*	Least Concern	K	BPa
Phrynobatrachidae	*Phrynobatrachus*	*minutus*	Least Concern	K	BPa
	Phrynobatrachus	*natalensis*	Least Concern	K	BPa
Pipidae	*Xenopus*	*clivii*	Least Concern	B, K	BPa
Ptychadenidae	*Ptychadena*	*erlangeri*	Near Threatened	B	BPa
	Ptychadena	*neumanni*	Least Concern	B, K	BPa

Table 3. Frogs sampled for *Batrachochytrium dendrobatidis* (*Bd*) in Ethiopia in 2008 and 2009. CI=confidence interval.

		Sample size			*Bd* Positive			Prevalence of *Bd* (95% CI)			Max/Mean Genome Equivalent		
Genus	Species	2008	2009	Total	2008	2009	Total	2008	2009	2008 + 2009	2008	2009	2008+09
Afrixalus	*enseticola*	0	1	1	-	0	0	-	0	0	-	-	-
Afrixalus	*clarkei*	0	5	5		2	2	-	0.40 (0–0.83)	0.40 (0.0–0.83)	-	0.92/0.59	0.92/0.59
Afrixalus	sp.	0	1	1	-	0	0	-	0	0	-	-	-
Altiphrynoides	*malcolmi*	5	6	11	0	2	2	0	0.33 (0–0.71)	0.18 (0–0.41)	-	29.73/15.26	29.73/15.26
Balebreviceps	*hillmani*	9	3	12	0	2	2	0	0.67 (0.13–1.20)	0.17 (0–0.38)	-	57.49/29.69	57.49/29.69
Ericabatrachus	*baleensis*	0	2	2	-	1	1	-	0.50 (0–1.19)	0.50 (0–1.19)	-	0.42/0.42	0.42/0.42
Hyperolius	cf. *kivuensis*	0	2	2	-	1	1	-	0.50 (0–1.19)	0.50 (0–1.19)	-	1.62/1.62	1.62/1.62
Hyperolius	*viridiflavus*	0	1	1	-	1	1	-	1	1	-	4.19/4.19	4.19/4.19
Leptopelis	*gramineus*	9	4	13	5	3	8	0.56 (0.23-0.88)	0.75 (0.33–1.17)	0.62 (0.35–0.88)	4.82/2.48	68.2/29.42	68.2/12.58
Leptopelis	*ragazzii*	10	9	19	2	5	7	0.20 (0.0-0.45)	0.56 (0.23–0.88)	0.37 (0.15–0.59)	1.87/1.07	1.08/0.65	1.08/0.77
Leptopelis	*vannutellii*	0	16	16	-	8	8	-	0.5 (0.26–0.75)	0.5 (0.26–0.75)	-	15.59/4.28	15.59/4.28
Paracassina	*obscura*	0	3	3	-	1	1	-	0.33 (0–0.87)	0.33 (0–0.87)	-	2.57/2.57	2.57/2.57
Phrynobatrachus	*minutus*	0	8	8	-	5	5	-	0.63 (0.29–0.96)	0.63 (0.29–0.96)	-	6.46/1.42	6.46/1.42
Phrynobatrachus	*natalensis*	0	1	1	-	0	0	-	0	0	-	-	-
Ptychadena	*erlangeri*	5	2	7	2	2	4	0.40 (0.0-0.83)	1	0.57 (0.20-0.94)	1.13/0.72	2982.4/1494.29	2982.4/747.51
Ptychadena	*neumanni*	2	13	15	1	7	8	0.50 (0.0-1.19)	0.54 (0.27-0.81)	0.53 (0.28-0.79)	28.61/28.61	22.07/4.27	7.31
Xenopus	*clivii*	0	3	3	-	1	1	-	0.33 (0-0.87)	0.33 (0-0.87)	-	3.78/3.78	3.78
Total		40	80	120	10	41	51	0.25 (0.12-0.38)	0.51 (0.4-0.62)	0.43 (0.34-0.51)	28.61/4.46	2982.4/81.27	2982.4/65.90

Table 4. Regional, local, and temporal variation in the prevalence (Prev) and genomic zoospore equivalents (GE) of *Batrachochytrium dendrobatidis* (*Bd*) in Ethiopia. 95% confidence intervals given in parentheses. *n*=number of individuals sampled.

		2008					2009				
Region	Locality	*n*	*Bd* +ve	Prev	GE mean	GE median	*n*	*Bd* +ve	Prev	GE mean	GE median
Bale	Magano						2	0	0		
	Shawe bridge						3	3	1.00	994.54	1.08
	Katcha	2	1	0.50 (0–1.19)	0.32	0.32	9	6	0.67 (0.36–0.97)	15.89	6.26
	WWF	9	3	0.33 (0.03–0.64)	2.05	1.59	2	0	0		
	Rira	21	6	0.29 (0.09–0.48)	6.4	2.05	5	2	0.40 (0–0.83)	0.69	0.69
	Fute	6	0	0			10	4	0.40 (0.1–0.7)	8.20	1.33
	Tulla Negresso	1	0	0			1	1	1.00	57.49	57.49
	Dinsho park HQ	1	0	0							
	Regional Total	40	10	0.25 (0.12–0.38)	4.46	1.73	32	16	0.50 (0.33–0.67)	198.17	1.48
Kaffa	Bonga town						4	2	0.50 (0.01–0.99)	0.15	0.15
	Bonga stream						2	1	0.50 (0–1.19)	5.08	5.08
	Bonga marsh						16	8	0.50 (0.26–0.75)	0.98	0.67
	Mankira						6	4	0.67 (0.29–1.04)	10.38	9.59
	Koma forest stream						7	3	0.43 (0.06–0.8)	2.61	1.03
	Koma marsh						6	3	0.50 (0.1–0.9)	2.24	0.15
	Wush Wush marsh						2	2	1.00	3.98	3.98
	Saja forest						5	2	0.40 (0–0.83)	2.66	2.663
	Regional Total						48	25	0.52 (0.38–0.66)	3.33	1.32

placed into clean plastic bags, mostly individually but occasionally in groups of up to four individuals, almost always of a single species. A subset of collected specimens (selected randomly within each species) was surveyed for *Bd*, with only post-metamorphic individuals included in the screening. Frogs were sampled for *Bd* using sterile clinical swabs (MW100-100; Medical Wire & Equipment Co, Crosham, UK), firmly applied approximately three to four times each to the ventral surfaces of the pelvic region and thighs, and digits of a single fore and single hind limb. Swabbing sessions were generally brief and for fewer than 10 frogs per session. Swabs were stored individually, dry in separate tubes and mostly away from light and at temperatures between 10 and 20 ºC prior to

processing. DNA extraction and diagnostic PCR assays took place in May 2010.

In the laboratory, DNA was extracted from swabs following the protocol given by Boyle et al. (2004). Samples were subjected to quantitative real time polymerase chain reaction (qPCR) diagnostic assay, using *Bd* primers specific to the ITS-1/5.8S region of ribosomal gene (Boyle et al., 2004) and an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Positive controls of known concentration of *Bd* DNA (100, 10, 1 & 0.1 *Bd* zoospore genomic equivalents - GE, supplied by Department of Infectious Disease Epidemiology, Imperial College, London) were run as standards along with the samples, as were negative controls. Standard curve slopes for each PCR had r^2 values exceeding 0.95, with mean critical threshold values of: 25.9±0.47 for 100 zoospores; 29.4±0.49 for 10 zoospores; 32.9±0.56 for 1 zoospore; and 35.7±1.08 for 0.1 zoospores. Samples were run in duplicate on PCR plates and, if necessary, were repeated until both wells for each sample gave the same result (positive or negative). *Bd*-positive samples display a sigmoid amplification in the real time PCR, negative samples show no such amplification (e.g., Soto-Azat et al., 2010). Positive amplifications of GE<0.1 were considered to have fallen out of range of the standards, attributed either to random amplification of non-*Bd* DNA or to primers binding to each other and not to *Bd* DNA. Mean and median GE values are reported for positive amplifications only. Taxon and locality details and qPCR results have been uploaded to the *Bd*-Maps online database (www.bd-maps.net).

Prevalence (proportion of individuals infected) and intensity of parasite load (GE) was compared among species, field visits (for the Bale Mountains only), regions, and reproductive modes. The latter involved a comparison between species that are terrestrially reproducing (direct-developing or biphasic with a terrestrial tadpole) and those that are biphasic with aquatic tadpoles, based on known information, or extrapolated from known reproductive modes in known/presumed close relatives (see Table 2). For statistical analyses these variables typically had a non-normal distribution based on a Kolmogorov-Smirnoff test, and therefore median values were compared using a non-parametric Mann-Whitney *U*-test (using Minitab ® v. 14). Confidence intervals (CIs) for prevalence were calculated following Thrushfield (2007).

RESULTS

Of the 120 frogs sampled, 51 were positive for *Bd*: 10 sampled in 2008 (prevalence 25 %, 95% CI 12–38%); 41 in 2009 (51 %, 95% CI 40–62%) (Table 3). At least one specimen of all sampled genera and all but three putative species were positive for *Bd* (Table 3), and only one individual each was sampled for the three species that were *Bd* negative. *Bd* results were positive for at least one specimen from all localities except Dinsho and Magano in the Bale Mountains, from where sample sizes were only 1 and 2, respectively. Neither prevalence (p=0.56) nor GE (p=0.52) are significantly different between the Bale Mountains and Kaffa samples in 2009 (Table 4). Neither prevalence (p=0.2) nor GE (p=0.68) differed significantly between years sampled (2008, 2009) for the Bale Mountains. Among species for which more than one specimen was sampled, the highest *Bd* prevalence was in *Phrynobatrachus minutus* at 67% (95% CI 26–96%) and *Leptopelis gramineus* at 62% (95% CI 35–88%); lowest for *Balebreviceps hillmani* at 17% (95% CI 0–38%) and *Altiphrynoides malcomi* at 18% (95% CI 0–41%) (Table 3). The highest prevalence per genus was for *Hyperolius* at 67% (95% CI 13–120%), followed by *Phrynobatrachus* at 56% (95% CI 23–88%); the lowest prevalence per genus was for the monotypic *Altiphrynoides* and *Balebreviceps* (see above) and *Afrixalus* (29%, 95% CI 0–62%). Mean parasite load was highest in *Ptychadena erlangeri* (GE 747) and *B. hillmani* (29.69), and lowest for *Ericabatrachus baleensis* (0.42). Given the small sample sizes and large ranges, median GE values might be more informative, being highest for *B. hillmani* (26.69) and *A. malcomi* (15.26) and lowest for *Phrynobatrachus minutus* (0.42) and *E. baleensis* (0.18). As in several other studies (e.g., Kielgast et al., 2010) the range of GE values was sometimes large such that rare high values substantially raised means above medians, and maximum values above means (Tables 3 and 4).

The terestrially reproducing species (*A. malcolmi* and *B. hillmani*) had a notably lower *Bd* prevalence and mean (but higher median) GE than biphasic species (Table 5), but these differences are not significant, whether (p=0.2 and 0.15 respectively) or not (p=0.41 and 0.33) *E. baleensis* is considered biphasic. No dead, dying, or obviously sick frogs were encountered during fieldwork, although a few specimens of *A. malcolmi* had small (ca.

Table 5. Reproductive modes of frogs that tested positive for *Batrachochytrium dendrobatidis* (*Bd*) in Ethiopia in 2008 and 2009. The upper two rows are where the two sampled *Ericabatrachus baleensis* (one *Bd* +ve) are classified as biphasic with aquatic larvae, the lower two rows with *E. baleensis* classified as terrestrially reproducing. CI=confidence interval; GE=genomic equivalents; st dev=standard deviation.

Reproductive Mode	Sampled	*Bd* +ve	Prevalence (95% CI)	Mean GE	Median GE	st dev
With aquatic larvae	97	47	0.48 (0.39–0.58)	69.68	1.36	439.14
Terrestrial	23	4	0.17 (0.02–0.33)	22.47	15.80	26.91
With aquatic larvae	95	46	0.48 (0.38–0.58)	71.22	1.59	443.98
Terrestrial	25	5	0.20 (0.04–0.36)	22.47	15.80	26.91

1–2 mm in diameter) raised, reddish blister-like lesions similar to those caused by mesomycetozoan parasites.

DISCUSSION

The opportunistic nature of the sampling, relatively small sample sizes and relaxed sterile technique dictate that the raw data are not open to in-depth, robust interpretation. The detection of *Bd* in Kaffa and Bale confirms predictions from bioclimatic modelling (Rödder et al., 2009) that this parasite infects frogs in (especially the highlands of) Ethiopia, extending its known distribution in East Africa beyond Kenya (Kielgast et al., 2009), Uganda (Goldberg et al., 2007), eastern Democratic Republic of Congo (Greenbaum et al., 2008), the Udzungwa Mountains of Tanzania (Weldon & du Preez, 2004) and Malawi (Soto-Azat et al., 2010). The overall prevalence of *Bd* in our Ethiopian samples (43 %) is higher than that recorded from western Uganda (22%, Goldberg et al., 2007) and Kenya (31.5%, Kielgast et al., 2009), these differences might be explained by climatic and seasonal factors (Kriger & Hero, 2007a) and/or sampling artefacts. It is possible that prevalence was artificially elevated by contamination in the field, but it is also possible that prevalence has been underestimated because none of the diagnostic PCR assays was run with bovine serum albumin (BSA), which reduces amplification inhibition and potentially reveals more positive results (Garland et al., 2010).

Differences in *Bd* prevalence between taxa and surveys for our Ethiopian samples are large in some instances but our sampling was too sparse to make robust interpretations. Other regions of Ethiopia remain unsurveyed for *Bd*, but the occurrence of *Bd* in northern Kenya adjacent to the Ethiopian border (www.spatialepidemiology.net/bd/), as well as its occurrence (this report) in two areas c. 400 km apart and either side of the Rift Valley, suggests that this pathogen is widespread throughout the country, at least in highland areas. A substantial part of Ethiopia is highland (forming nearly 80% of African land >3,000 m South of the Tropic of Cancer; Yalden, 1983) and the climatic conditions of the majority of the country are predicted to be highly suitable for the persistence of *Bd* (Rödder et al., 2009). The higher prevalence of *Bd* that we recorded in species with aquatic tadpoles than those that are terrestrially reproducing (though not statistically significant) is consistent with data from most studies conducted elsewhere, with lower occurrence of infection and *Bd*-caused decline in more terrestrial species in Panama (Lips et al., 2003; 2006), Australia (Kriger & Hero, 2007b) and the USA (Longcore et al., 2007).

At least some of the Bale Mountains frogs have declined significantly in at least some localities, and one previously commonly encountered species (*Spinophrynoides osgoodi*) has been seen only once this century despite several attempts at 'rediscovery' (Gower et al., in press). Identifying cause(s) of declines here (substantial for some species, Gower et al., in press) is non-trivial given the lack of longitudinal studies, lack of observation of dead/dying frogs, lack of data on ecology of many species and on possible climate change in specific localities and the extensive habitat destruction that has occurred recently through deforestation and a surge in the human population (we have found no pristine habitats in Bale in surveys carried out since 2006). More research is urgently required to establish accurate and precise conservation assessments for Ethiopian amphibians, and in particular to determine the impacts of *Bd* infection. Although *Bd* has been clearly implicated as a cause of amphibian declines globally, it is important that such associations are tested thoroughly. For example, the presence of *Bd* is not necessarily the proximate cause of declines, but other factors, such as environmental change, might be more likely to be implicated as the cause (e.g., Daszak et al., 2005; Whitfield et al., 2007). Similarly, although *Bd* was detected in the declining population of the Kihansi Spray Toad, *Nectophrynoides asperginis* in Tanzania, this decline occurred following a population bottleneck caused by substantial habitat deterioration (Weldon & du Preez, 2004).

Based on an analysis of 12 ecological and environmental variables for the world's anurans, several Ethiopian species sampled here share traits with species known to have declined in association with *Bd* elsewhere (Bielby et al., 2008). Ethiopian species inferred to have a probability of 1.0 to decline following an outbreak of *Bd* (Bielby et al., 2008) include the Bale Mountains endemic, declining (Gower et al., in press) and endangered *Altiphrynoides malcolmi*, *Balebreviceps hillmani* and *Ericabatrachus baleensis*. Other species sampled here that are deemed to have a high probability of susceptibility to *Bd*-related decline are *Afrixalus clarkei* (0.97), *Ptychadena erlangeri* (0.95) and *Afrixalus enseticola* (0.88) (Bielby et al., 2008). Other Ethiopian species we did not sample but which are considered to have a high estimated probability (P=0.8–1.0) of *Bd*-related decline include *Spinophrynoides osgoodi*, *Leptopelis susanae*, *L. yaldeni*, *Ptychadena cooperi*, *P. filwoha*, *P. harenna*, *P. nana*, *P. wadei* and *Xenopus largeni*. These species are predominately narrowly-distributed, often highland, threatened endemics whose populations and *Bd* infection status should be assessed to determine whether this pathogen is having an impact on their populations, whether or not this pathogen proves to be indigenous. Further studies on surviving populations in the wild are required, but another potentially fruitful avenue for research is clinical infection trials using Ethiopian isolates on Ethiopian species.

Without confirmation that the strain(s) of *Bd* present in Ethiopia is a benign parasite of Ethiopia's amphibians there, the threat from this pathogen here should not be underestimated. Testing the endemic versus novel pathogen hypotheses for Ethiopia will require an investigation of archived amphibians collected in previous surveys in addition to isolation and characterization of the Ethiopian *Bd* strain(s). Few archived anuran specimens from Ethiopia have been examined thus far for the presence of *Bd*, with only three *Xenopus largeni* (from the 1970s) and 15 *X. clivii* (from the early 1900s) sampled and no *Bd* detected (Soto-Azat et al., 2010). The discovery of the amphibian chytrid fungus in populations of endangered Ethiopian amphibians now requires further investigation of the impact of this pathogen on these imperilled species.

ACKNOWLEDGEMENTS

We thank staff of the Ethiopia Wildlife Conservation Authority for practical assistance and issuing of research and export permits, in particular Daniel Pawlos, Yeneneh Teka, Kifle Agraw and Birutesfa Yimer. Biologists of the University of Addis Ababa provided important support towards this project and are thanked, especially Dr Abebe Getahun, Professor Afework Bekele, Dr Araya Asfaw, and Dr Satish Kumar. The Swiss Tropical Institute provided important logistical and financial support, especially Dr Juerg Utzinger. Funding for survey work was supported by National Geographic [CRE Grant #8532-08: Amphibians of the Fractured Dome] and the Conservation Leadership Programme. Further funding from the following institutes was important in conducting the surveys and the taxonomic work: Institute of Biogeography, University of Basel; Stipendienkommission für Nachwuchskräfte aus Entwicklungsländern, Basel; the Natural History Museum, London. Our work in the field would not have been possible without the help of many individuals, in particular Anoushka Kinahan and Thadaigh Baggallay (both Frankfurt Zoological Society), Michael Geiser, Fikirte Gebresenbet, Yoseph Assefa, Red Jackal Tour Operators and our Harenna guides Ahmed, Hussein, Hussein, Issa and Mohammed. Matthew Perkins and Frances Clare provided invaluable assistance to T.D-B in the laboratory.

REFERENCES

Bell, B.D., Carver, S., Mitchell, N.J. & Pledger, S. (2004). The recent decline of a New Zealand endemic: how and why did populations of Archey's frog *Leiopelma archeyi* crash over 1996–2001? *Biological Conservation* 120, 189–199.

Bell, R.C., Gata Garcia, A.V., Stuart, B.L. & Zamudio, K.R. (2011). High prevalence of the amphibian chytrid pathogen in Gabon. *EcoHealth* 8, 116–120.

Berger, L., Speare, R., Daszak, P., Green, E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G. & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences USA* 95, 9031–9036.

Bielby, J., Cooper, N., Cunningham, A.A., Garner, T.J. & Purvis, A. (2008). Predicting susceptibility to future declines in the world's frog. *Conservation Letters* 1, 82–90.

Boyle, D.G., Boyle, D.P., 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.

Daszak, P., Scott, D.E., Kilpatrick, A.M., Faggioni, C., Gibbons, J.W. & Porter, D. (2005). Amphibian population declines at Savannah River site are linked to climate, not chytridiomycosis. *Ecology* 86, 3232–3237.

Fisher, M.C., Garner, T.W.J. & Walker, S.F. (2009). Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time and host. *Annual Review of Microbiology*, 63, 291–310.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sà. R.O., Channing, A., Wilkinson, M., Donnellan, S.C. Raxworthy, C.J., Campbell, J.A., Blotto, B., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J., Green, D.M. & Wheeler, W.C. (2006). The amphiban tree of life. *Bulletin of the American Museum of Natural History* 297, 1–370.

Garland, S., Baker, A., Phillott, A.D. & Skerratt, L.F. (2010). BSA reduces inhibition in a TaqMan assay for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, 92, 113–116.

Goldberg, T.L., Readel, A.M. & Lee, M.H. (2007). Chytrid fungus in frogs from an equatorial African montane forest in Western Uganda. *Journal of Wildlife Diseases* 43, 521–524.

Gower, D.J., Aberra, R.K., Schwaller, S., Largen, M.J., Collen, B., Spawls, S., Menegon, M., Zimkus, B.M., de Sá, R., Mengistu, A., Gebresenbet, F., Moore, R.D., Saber, S.A. & Loader, S.P. (in press) Long-term data for endemic frog genera reveal potential conservation crisis in the Bale Mountains, Ethiopia. *Oryx*

Greenbaum, E., Kusamba, C., Aristote, M.M. & Reed, K. (2008). Amphibian chytrid fungus infections in *Hyperolius* (Anura: Hyperoliidae) from Eastern Democratic Republic of Congo. *Herpetological Review* 39, 70–73.

Hopkins, S. & Channing, A. (2003). Chytrid fungus in Northern and Western Cape frog populations, South Africa. *Herpetological Review* 34, 334–336.

Kielgast, J., Rödder, D., Veith, M. & Lötters, S. (2010). Widespread occurrence of the amphibian chytrid fungus in Kenya. *Animal Conservation* 13, 1–8.

Kriger, K.M. & Hero, J.M. (2007a). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology* 271, 352–359.

Kriger, K.M. & Hero, J.M. (2007b). The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Diversity and Distributions* 13, 781–788.

James, T.Y., Litvintseva, A.P., Vilgalys, R., Morgan, J.A.T., Taylor, J.W., Fisher, M.C., Berger, L., Weldon, C., du Preez, L. & Longcore, J.E. (2009). Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *PLoS Pathogens* 5, e1000458.

IUCN, Conservation International & NatureServe (2010). Global Amphibian Assessment. www.globalamphibians.org. Downloaded on 15 August 2010.

Largen, M.J. (2001). Catalogue of the amphibians of Ethiopia, including a key for their identification. *Tropical Zoology* 14, 307–402.

Largen, M.J. & Spawls, S. (2010). *The amphibians of Ethiopia and Eritrea*. Frankfurt am Main: Edition Chimaira. 693pp.

Lawson, D.P. & Klemens, W. (2001). Herpetofauna of the African Rain Forest: overview and recommendations for conservation. In: *African Rainforest Ecology and Conservation*, Weber, W., White, L.J.T. & Naughton-Treves, L., Eds. Yale University Press, pp. 291–307.

Lips, K.R., Reeve, J.D. & Witters L.R. (2003). Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17, 1078–1088.

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.

Lips, K.R., Diffendorfer, J., Mendelson, J. & Sears, M. (2008). Riding the wave: Climate change, emerging infectious disease and amphibian declines. *PLoS Biology* 6(3), e72.

Longcore, J.R., Longcore, J.E., Pessier, A.P. & Halteman, W.A. (2007). Chytridiomycosis widespread in anurans of northeastern United States. *Journal of Wildlife Management* 71, 435–444.

Lötters, S., Kielgast, J., Bielby, J., Schmidtlein, S., Bosch, J., Veith, M., Walker, S.F., Fisher, M.C. & Rödder, D. (2010). The link between rapid enigmatic amphibian decline and the globally emerging chytrid fungus. *Ecohealth* 6, 358–372.

Müller, H., Loader, S.P., Ngalason, W., Howell, K.M. & Gower, D.J. (2007). Reproduction in brevicpitid frogs (Amphibia: Anura: Brevicipitidae) – evidence from *Probreviceps m. macrodactylus*. *Copeia* 2007, 726–733.

Rachowicz, L.J., Hero, J.M. Alford, R.A., Taylor, J.W., Morgan, J.A.T., Vredenburg, V.T., Collins, J.P. & Briggs, C.J. (2005). The novel and endemic pathogen hypotheses: competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology* 19, 1441–1448.

Reeder, N.M.M., Cheng, T.L., Vredenburg, V.T. & Blackburn, D.C. (2011). Survey of the chytrid fungus *Batrachochytrium dendrobatidis* from montane and lowland frogs in eastern Nigeria. *Herpetology Notes* 4, 83–86.

Retallick R.W.R., McCallum, H. & Speare, R. (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biology* 2(11), e351.

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

Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D., Hines, H.B. & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* [DOI: 10.1007/s10393-007-0093-5].

Soto-Azat, C., Clarke, B.T., Poynton, J.C. & Cunningham, A.A. (2010). Widespread historical presence of *Batrachochytrium dendrobatidis* in African pipid frogs. *Diversity and Distributions* 16, 126–131.

Thrushfield, M. (2007). *Veterinary Epidemiology 3rd Edition*. Blackwell Science, Ltd., pp. 242–244.

Weldon, C., du Preez, L.H., Hyatt, A.D., Muller, R. & Speare, R. (2004). Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* 10, 2100–2105.

Weldon, C. & du Preez, L.H. (2004). Decline of Kihansi spray toad, *Nectophrynoides asperginis*, from the Udzungwa mountains, Tanzania. *Froglog* 62, 2–3.

Weldon, C. de Villiers, L. & du Preez, L.H. (2007). Quantification of the trade in *Xenopus laevis* from South Africa, with implications for biodiversity conservation. *African Journal of Herpetology* 56, 77–83.

Weldon, C., du Preez, L. & Vences, M. (2008). Lack of detection of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in Madagascar. *Monografie del Museo Regionale di Scienze Naturali di Torino, XLV* 2008, 95–106.

Whitfield, S.M., Bell, K.E., Philippi, T., Sasa, M., Bolaños, F., Chaves, G., Savage, J.M. & Donnelly, M.A. (2007). Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Sciences, U.S.A.* 104, 8352–8356.

Yalden, D.W. (1983). The extent of high ground in Ethiopia compared to the rest of Africa. *Sinet: Ethiopian Journal of Science* 6, 35–39.

Accepted: 13 June 2012