INTRODUCTION

The amphibian chytrid fungus (\textit{Batrachochytrium dendrobatidis} - \textit{Bd}) is a skin parasite that can cause the fatal disease amphibian chytridiomycosis (Berger et al., 1998; Lips et al., 2006). \textit{Bd} has been implicated in rapid declines of $\geq 200$ 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 \textit{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); \textit{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 \textit{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 \textit{Bd} being detected from museum specimens of African pipid frogs (genus \textit{Xenopus}) (Weldon et al., 2004; Soto-Azat et al., 2010), anurans that have been exported widely around the world (Weldon et al., 2007). \textit{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 \textit{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 \textit{Bd} as an indigenous amphibian parasite in Africa remains uncertain: sampled \textit{Bd} isolates from Africa (although few in number and almost exclusively from South Africa) were no more heterogeneous than isolates from other continents where \textit{Bd} has caused declines, suggesting Africa may not be the endemic focus of this pathogen (James et al., 2009). In regions where \textit{Bd} has become endemic post-outbreak, this pathogen undergoes seasonal fluctuations in prevalence (Retallick et al., 2004; Kriger & Hero, 2007a).
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.

<table>
<thead>
<tr>
<th>Region</th>
<th>Locality (habitats)</th>
<th>Altitude (m)</th>
<th>Coordinates</th>
<th>Dates sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bale</td>
<td>Magano (marsh in clearing)</td>
<td>1907</td>
<td>6.63858 39.73394</td>
<td>21/6/09</td>
</tr>
<tr>
<td></td>
<td>Shawe bridge (river/streams in forest)</td>
<td>1890</td>
<td>6.645556 39.731389</td>
<td>21/6/09</td>
</tr>
<tr>
<td></td>
<td>Katcha (streams/grassland in clearing)</td>
<td>2364–2370</td>
<td>6.716389–6.71697</td>
<td>30/7/08; 21/6/09</td>
</tr>
<tr>
<td></td>
<td>WWF (degraded woodland; stream)</td>
<td>2788–2830</td>
<td>6.750033–6.757222</td>
<td>30/7/08; 19/6/09</td>
</tr>
<tr>
<td></td>
<td>Rira (stream; degraded open woodland; village)</td>
<td>2880–2936</td>
<td>6.763056–6.773611</td>
<td>21/7/08; 19/6/09</td>
</tr>
<tr>
<td></td>
<td>Fute (streams; forest, some degraded)</td>
<td>3060–3165</td>
<td>6.755–6.763056</td>
<td>21/7/08; 18/8/08</td>
</tr>
<tr>
<td></td>
<td>Tulla Negresso (degraded forest; stream)</td>
<td>3225</td>
<td>6.776111–6.7775</td>
<td>15/7/08; 21/6/09</td>
</tr>
<tr>
<td></td>
<td>Dinsho park HQ (woodland)</td>
<td>3168</td>
<td>7.095833 39.79</td>
<td>15/7/08</td>
</tr>
<tr>
<td>Kaffa</td>
<td>Bonga town (small town)</td>
<td>1789</td>
<td>7.26719 36.25898</td>
<td>7/6/09</td>
</tr>
<tr>
<td></td>
<td>Bonga stream (stream; farmland)</td>
<td>1727</td>
<td>7.27198 36.26</td>
<td>7/6/09</td>
</tr>
<tr>
<td></td>
<td>Bonga marsh (marsh)</td>
<td>1734</td>
<td>7.24932 36.2554</td>
<td>7/6/09</td>
</tr>
<tr>
<td></td>
<td>Mankira (disturbed forest; stream)</td>
<td>1620</td>
<td>7.19815 36.2854</td>
<td>8/6/09</td>
</tr>
<tr>
<td></td>
<td>Koma forest stream (forest; stream)</td>
<td>1889</td>
<td>7.31803 36.07816</td>
<td>9/6/09</td>
</tr>
<tr>
<td></td>
<td>Koma marsh (marsh)</td>
<td>1905</td>
<td>7.310556 36.079444</td>
<td>9/6/09</td>
</tr>
<tr>
<td></td>
<td>Wush Wush marsh (marsh)</td>
<td>1895</td>
<td>7.31005 36.1205</td>
<td>10/6/09</td>
</tr>
<tr>
<td></td>
<td>Saja forest (forest; river; streams)</td>
<td>2027</td>
<td>7.48705 36.09404</td>
<td>13/6/09</td>
</tr>
</tbody>
</table>
Batrachochytrium dendrobatidis in Ethiopia

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

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**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).**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>IUCN Status</th>
<th>Region</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthroleptidae</td>
<td>Leptopelis</td>
<td>gramineus</td>
<td>Least Concern</td>
<td>B</td>
<td>BPa</td>
</tr>
<tr>
<td>(1)</td>
<td>Leptopelis</td>
<td>razazzii</td>
<td>Vulnerable</td>
<td>B</td>
<td>BPa</td>
</tr>
<tr>
<td></td>
<td>Leptopelis</td>
<td>vannuterlii</td>
<td>Vulnerable</td>
<td>K</td>
<td>BPa</td>
</tr>
<tr>
<td>Brevicipitidae</td>
<td>Balebreviceps</td>
<td>hillmani</td>
<td>Endangered</td>
<td>B</td>
<td>DD***</td>
</tr>
<tr>
<td>Bufonidae</td>
<td>Altiphyynoides</td>
<td>malcolmii</td>
<td>Endangered</td>
<td>B</td>
<td>BPt</td>
</tr>
<tr>
<td>Hyperoliidae</td>
<td>Africalus</td>
<td>enseticola</td>
<td>Vulnerable</td>
<td>K</td>
<td>BPa</td>
</tr>
<tr>
<td></td>
<td>Africalus</td>
<td>clarkei</td>
<td>Vulnerable</td>
<td>K</td>
<td>BPa</td>
</tr>
<tr>
<td></td>
<td>Africalus</td>
<td>sp.</td>
<td>-</td>
<td>B</td>
<td>BPa</td>
</tr>
<tr>
<td></td>
<td>Hyperolius</td>
<td>cf. kivuensis</td>
<td>-</td>
<td>K</td>
<td>BPa</td>
</tr>
<tr>
<td>Phrynobatrachida</td>
<td>Phrynobatrachus</td>
<td>minutus</td>
<td>Least Concern</td>
<td>K</td>
<td>BPa</td>
</tr>
<tr>
<td></td>
<td>Phrynobatrachus</td>
<td>natalensis</td>
<td>Least Concern</td>
<td>K</td>
<td>BPa</td>
</tr>
<tr>
<td>Pipidae</td>
<td>Xenopus</td>
<td>clivii</td>
<td>Least Concern</td>
<td>B, K</td>
<td>BPa</td>
</tr>
<tr>
<td>Ptychadenida</td>
<td>Ptychadena</td>
<td>erlangeri</td>
<td>Near Threatened</td>
<td>B</td>
<td>BPa</td>
</tr>
<tr>
<td></td>
<td>Ptychadena</td>
<td>neumanni</td>
<td>Least Concern</td>
<td>B, K</td>
<td>BPa</td>
</tr>
</tbody>
</table>
Table 3. Frogs sampled for *Batrachochytrium dendrobatidis* (*Bd*) in Ethiopia in 2008 and 2009. CI=confidence interval.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Sample size</th>
<th>Bd Positive</th>
<th>Prevalence of <em>Bd</em> (95% CI)</th>
<th>Max/Mean Genome Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afrixalus</td>
<td>enseticola</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Afrixalus</td>
<td>clarkei</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0.40 (0.0–0.83)</td>
</tr>
<tr>
<td>Afrixalus</td>
<td>sp.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.92/0.59</td>
</tr>
<tr>
<td>Altiphrynoides</td>
<td>malcolmi</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Balebrviceps</td>
<td>hillmani</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td>0.67 (0.13–1.20)</td>
</tr>
<tr>
<td>Ericabatrachus</td>
<td>balensis</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Hyperolius</td>
<td>cf. kivuensis</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Hyperolius</td>
<td>viridiflavus</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Leptopelis</td>
<td>gramineus</td>
<td>9</td>
<td>4</td>
<td>13</td>
<td>0.56 (0.23–0.88)</td>
</tr>
<tr>
<td>Leptopelis</td>
<td>ragazzii</td>
<td>10</td>
<td>9</td>
<td>19</td>
<td>0.56 (0.23–0.88)</td>
</tr>
<tr>
<td>Leptopelis</td>
<td>vannutellii</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td>0.5 (0.26–0.75)</td>
</tr>
<tr>
<td>Paracassina</td>
<td>obscura</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Phrynobatrachus</td>
<td>minutus</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Phrynobatrachus</td>
<td>natalensis</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ptychadena</td>
<td>erlangeri</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0.40 (0.0–0.83)</td>
</tr>
<tr>
<td>Ptychadena</td>
<td>neumanni</td>
<td>2</td>
<td>13</td>
<td>15</td>
<td>0.50 (0.0–1.19)</td>
</tr>
<tr>
<td>Xenopus</td>
<td>clivii</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

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.
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

### 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.

<table>
<thead>
<tr>
<th>Region</th>
<th>Locality</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Bd +ve</td>
</tr>
<tr>
<td>Bale</td>
<td>Magano</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Shawe bridge</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Katcha</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Rira</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Fute</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Tulla Negresso</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dinsho park HQ</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bonga town</td>
<td></td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Bonga stream</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Bonga marsh</td>
<td></td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Mankira</td>
<td></td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Koma forest stream</td>
<td></td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Koma marsh</td>
<td></td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Wush Wush marsh</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Saja forest</td>
<td></td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Regional Total</td>
<td></td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 r² 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).

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.

<table>
<thead>
<tr>
<th>Reproductive Mode</th>
<th>Sampled</th>
<th>Bd +ve</th>
<th>Prevalence (95% CI)</th>
<th>Mean GE</th>
<th>Median GE</th>
<th>st dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>With aquatic larvae</td>
<td>97</td>
<td>47</td>
<td>0.48 (0.39–0.58)</td>
<td>69.68</td>
<td>1.36</td>
<td>439.14</td>
</tr>
<tr>
<td>Terrestrial</td>
<td>23</td>
<td>4</td>
<td>0.17 (0.02–0.33)</td>
<td>22.47</td>
<td>15.80</td>
<td>26.91</td>
</tr>
<tr>
<td>With aquatic larvae</td>
<td>95</td>
<td>46</td>
<td>0.48 (0.38–0.58)</td>
<td>71.22</td>
<td>1.59</td>
<td>443.98</td>
</tr>
<tr>
<td>Terrestrial</td>
<td>25</td>
<td>5</td>
<td>0.20 (0.04–0.36)</td>
<td>22.47</td>
<td>15.80</td>
<td>26.91</td>
</tr>
</tbody>
</table>
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 (*Spinophrynoideas 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 *Eriacbatrachus baleensis*. Other species sampled here that are deemed to have a high probability of susceptibility to *Bd*-related decline are *Afriraxalus clarkei* (0.97), *Ptychadena erlangeri* (0.95) and *Afriraxalus 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.
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