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<th>Journal:</th>
<th>Animal Conservation</th>
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<td>Manuscript ID</td>
<td>ACV-05-19-R-103.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Review</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>20-Aug-2019</td>
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<td>Complete List of Authors:</td>
<td>Doherty-Bone, Thomas; Royal Zoological Society of Scotland, Conservation Programs; Natural History Museum London, Life Sciences Cunningham, Andrew; Zoological Society of London, Institute of Zoology Fisher, Matthew; Imperial College London, South Kensington Campus; Garner, Trent; Institute of Zoology, Zoological Society of London; North-West University, Unit for Environmental Sciences and Management Ghosh, Pria; Imperial College London, School of Public Health; North-West University, Unit for Environmental Sciences and Management Gower, David; Natural History Museum (London), Life Sciences department Verster, Ruhan; North-West University, Unit for Environmental Sciences and Management Weldon, Ché; North-West University, Faculty of Natural Sciences</td>
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<tr>
<td>Keywords:</td>
<td>emerging infectious disease, conservation, chytridiomycosis, Batrachochytrium, Anura, caecilian</td>
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Amphibian chytrid fungus in Africa - Realigning hypotheses and the research paradigm

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Abstract

The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), responsible for numerous amphibian declines and extinctions, was previously thought to originate from the African continent. This was based on infected museum specimens from early 20th Century South Africa, Cameroon and Uganda. Further research on archived specimens from other continents eventually revealed early 20th Century records also in Brazil and Japan. Recent robust analysis of genomic diversity and phylogeny of *Bd* has shown origin from Asia to be more plausible. This raises the issue that the threat of *Bd* for African amphibians as a novel pathogen has been underestimated. There are now cases where dramatic amphibian declines in disparate mountains on the continent could be attributed to *Bd*, and this necessitates an urgent realigning of hypotheses and the research agenda for amphibian conservation on the continent. Notably, hotspots of amphibian host naivety include West Africa where this pathogen has so far not been detected. We discuss research gaps that amphibian conservationists might focus on, notably more genomic sequencing of the *BdCAPE* lineage to determine its date of emergence, and assessing the susceptibility of different amphibian species to infection, disease and decline to better prioritise conservation actions.

Main text
Based on its phylogeny and genomic diversity, the amphibian chytrid fungal pathogen (*Batrachochytrium dendrobatidis*; hereafter *Bd*), a causative agent of the lethal disease, amphibian chytridiomycosis, appears to have recently originated from Asia (O’Hanlon et al. 2018). This discovery challenges some of the working hypotheses about *Bd* and its likely conservation impacts (Weldon et al. 2004, Rachowicz et al. 2005, Lips et al., 2008). One new perspective, for example, is that this widespread and destructive pathogenic chytrid fungus has been recently introduced to the African continent. Prior to the latest study by O’Hanlon et al. (2018), *Bd* was hypothesised to originate from Africa, based on: records of infected frogs in the early 20th Century in disjunct countries (Weldon et al. 2004, Soto-Azat et al. 2010, Vredenburg et al. 2013, Seimon et al. 2015); widespread occurrence in contemporary amphibian communities on the continent either side of the Sahara in the absence of invasive alien amphibian species (Kielgast et al. 2010, El Mouden et al. 2011, Tarrant et al. 2013); scant support for obvious amphibian declines in the presence of *Bd*; and the presence of multiple lineages of *Bd* in South Africa suggesting broad genetic diversity of a source pathogen population (Farrer et al. 2011). Additionally, substantial international export of clawed frogs (*Xenopus* spp.) from South Africa was initiated in the early 20th Century (Weldon et al. 2007), which is contemporaneous with the posited time of emergence and initial global spread of *Bd* (Farrer et al. 2011; O’Hanlon et al. 2018). In addition to O’Hanlon et al.’s (2018) study, the “chytrid out of Africa” hypothesis had been challenged by an absence of *Bd* in surveys in Africa west of the Dahomey Gap (the dry savanna separating moist forests of Ghana and western Nigeria, forming a separate West African biogeographic hotspot) (Penner et al. 2013). Furthermore, *Bd* has been detected in archived amphibian specimens collected in the early 20th Century from South America (Carvalho et al. 2017), North America (Talley et al. 2015) and Asia (Japan) (Goka et al. 2009). Results from the examination of museum specimens, however, should be treated with caution because, within such collections, there often are numerous contemporaneous and historical (many of which are unknowable) opportunities for cross-contamination between specimens (of even different
species) from various points in time and space, and these are rarely mitigated (i.e. by rinsing specimens with ethanol before sampling) by studies of historical Bd “infection”.

If, as now appears to be the case, Bd is a novel pathogen to the African continent, it could be a substantially underestimated threat to African amphibian biodiversity. Research on amphibian population trends in Africa is rare, and there have been few reports of so-called “enigmatic declines” where previously abundant amphibians vanish in relatively unchanged habitat. A notable exception is the Kihansi Spray Toad (*Nectophrynoides asperginis*) that was driven to extinction in the wild in Tanzania during a rapid Bd epidemic wave (Weldon et al. preprint-2019). The susceptibility of this species to lethal Bd infection has repeatedly been demonstrated in captive populations (McAloose et al. 2008; Makange et al. 2014). Although mass mortalities of the Lake Clawed Oku frog (*Xenopus longipes*) in Cameroon have been observed, Bd was ruled out as a causative factor (Doherty-Bone et al. 2013). More recently, however, the presence of Bd has been found to correlate with amphibian declines elsewhere in Cameroon, with puddle frogs (*Phrynobatrachus* spp.), long-fingered frogs (*Cardioglossa* spp.) and small-tongue toads (*Werneria* spp.) being particularly affected (Hirschfeld et al. 2016, Scheele et al. 2019, Tchassem et al. in press). These previously-common species have declined dramatically in both forest and agricultural habitats in the space of four years, with no evidence of recovery (Doherty-Bone & Gvoždík 2017). The Bd infection loads in these Cameroon species were lower than in other frog populations succumbing to chytridiomycosis (*sensu* Briggs et al. 2010), but this might be explained by low lethality thresholds of infection loads, as might occur in *Rhinoderma darwinii* in Chile (Valenzuela-Sánchez et al. 2017). In South Africa, where Bd is known to have been present over a long period of time and where amphibians are relatively well studied, sporadic Bd-associated mortality events appear to be exacerbated by environmental conditions in Poynton’s River Frog (*Amietia poyntoni*) from Namaqualand and the Eastern Cape Province (Hopkins & Channing, 2003, Lane et al. 2003), and in a high altitude species in the Drakensberg Mountain range, the Phofung River Frog (*Amietia hymenopus*) (Griffiths et al. 2018). Conversely, the cause(s) of enigmatic declines of the Western Cape endemic Rose’s Mountain Toad (*Capensibufo rosei*), which
has disappeared from all but two historical sites, remain(s) unknown (Cressey et al. 2014), but Bd is
known to cause amphibian population declines and extinctions in the absence of notable mortality
events (Valenzuela-Sánchez et al. 2017).

Given the recent discovery that Bd is an introduced pathogen to Africa (O’Hanlon et al. 2018), the
population monitoring of a wide range of African amphibian species in concert with
contemporaneous Bd surveillance is urgently required. Although species already of conservation
concern should be monitored (restricted range species, mountain specialists), the monitoring of less
obviously threatened species should also be considered due to the uncertainty of which species
might be susceptible to Bd-driven declines. The species putatively impacted by Bd in Cameroon
include frogs (Phrynobatrachus sp.) that were previously abundant, assessed to be Vulnerable by the
IUCN, and broadly distributed across mountains (Hirschfeld et al., 2016, Doherty-Bone & Gvodik
2017). Collection of data on environmental parameters that can influence host-pathogen dynamics,
such as temperature, rainfall and hydroperiod, also should be considered in these further studies
(Doddington et al. 2013; Bosch et al. 2018; Hudson et al. 2019). Knowledge of environmental
parameters is useful for differentiating causes of dramatic declines that have occurred both in the
presence of Bd and extensive habitat loss, such as in the highlands of Ethiopia (Gower et al. 2012,
2013a). This knowledge can be used to mitigate declines should severity of Bd infection interact with
habitat change, such as habitat management that influences microhabitat quality or density of
amphibian hosts. Understanding environmental parameters is also vital to explain population
decreases that occur in what might be considered an apparently stable host-pathogen system, such as
those influenced by season and latency of infection severity (Longo & Burrowes 2010, Longo et al.

Genotyping Bd has become a valuable tool in understanding virulence and the potential outcome of
infection. Several lineages of Bd have been found in Africa (Fig. 1). The hypervirulent global
panzootic lineage (BdGPL) is known to occur in South Africa, Uganda and Ethiopia, while the
hypovirulent BdCAPE lineage was found initially in South Africa (Farrer et al, 2011), and more-
recently in frogs and caecilians within, and originating from, Cameroon (O’Hanlon et al. 2018, Fisher et al. 2018). In South Africa, there is evidence of hybridization between these two lineages. Although the virulence of this hybrid is unknown, the only other recorded hybrid lineage (from Brazil), can be more virulent than either of the parent strains (Greenspan et al. 2018). Assessing the distribution of these (and possible additional) lineages across the rest of Africa should be a priority for better understanding the evolutionary history of BdCAPE and other strains, and to determine possible routes of spread.

Within parts of Africa (e.g. the Albertine Rift, Cameroon, Gabon and the Gulf of Guinea islands), the presence of BdGPL has been identified via sequencing of the ITS region (Seimon et al. 2015, Hydeman et al. 2017, Miller et al. 2018). Use of the ITS region to identify Bd lineages, however, has been found to be inaccurate compared to genomic sampling (O’Hanlon et al. 2018), demonstrating the need for more genomic sampling and/or an alternative assay to identify Bd lineages (Bletz et al. 2015). O’Hanlon et al. (2018) were unable to estimate the date of emergence of BdCAPE due to a paucity of isolates, however the emergence of BdGPL was confidently dated to the late 19th/early 20th Century, thus this lineage has only recently arrived in Africa as elsewhere. Studies of archived amphibians have identified the presence of Bd in Cameroon since at least 1933, Kenya 1934, Uganda 1935, in South Africa since 1938, the eastern Democratic Republic of Congo 1950 and Bioko island 1966 (Weldon et al. 2004, Soto-Azat et al. 2010, Vredenburg et al. 2013, Seimon et al. 2015, Hydeman et al. 2017), but in each case the PCR techniques used could not identify the lineage of Bd present. Assuming these results are a true reflection of historical Bd presence in Africa, and are not a result of specimen contamination, establishing a technique to establish the strain from preserved specimens would facilitate our understanding of the history and likely future impact of Bd on African amphibians.

In the meantime, amphibian and wildlife disease biologists in Africa should shift their research paradigm from assuming BdGPL is indigenous to the continent, to the latest phylogenetically driven hypothesis that it is an introduced pathogen that may invade naïve host populations as it expands its
distribution into new regions. There are relatively few historical records of *Bd* on the continent, which may or may not be records of *BdGPL*, therefore one research priority is to identify the mechanisms of spread of *BdGPL* within Africa and whether incursions into Africa are continuing. Where capacity is not yet available, research should be facilitated by collaboration between established laboratories with facilities to undertake necessary molecular techniques and researchers based in African nations or institutions with more limited scientific resources.

It has been hypothesised that *BdGPL* could have been spread globally from Africa in the early 20th Century via the international trade in *Xenopus* spp. frogs for biomedical purposes (Weldon *et al.* 2004) with pet trade a more-recent pathway (Wombwell *et al.* 2016; O’Hanlon *et al.* 2018). It is notable, however, that the one known example of international spread of *Bd* (*BdCAPE*) from Africa was due to a conservation programme. Frogs endemic to South Africa (*Xenopus gilli*) were being captive-bred in the same facility in Jersey Zoo as Mallorcan midwife toads (*Alytes muletensis*). It appears that the former infected the latter, so that when captive-bred midwife toads were released on Mallorca, they co-introduced *BdCAPE* (Walker *et al.* 2008). Although the Mallorcan midwife toad conservation programme is considered to be a success, there is some evidence that *BdCAPE* is now restricting the population growth of the species in the wild (Doddington *et al.* 2013).

Understanding variation of host responses among African amphibian species to infection with different lineages of *Bd* is a clear research gap. *Ex situ* exposure of Malagasy frogs to *BdGPL* has demonstrated extremely high susceptibility and rapid proliferation of infection, although trials were terminated with the onset of humane endpoints before lethality could be determined (Bletz *et al.* 2015). Other African species have been found to succumb to lethal infection by *Bd*, including caecilians, such as *Geotrypetes seraphini* (from a *BdCAPE* strain), as well as dwarf clawed frogs (*Hymenochirus curtipes*) (unknown strain) albeit possibly confounded by stress from captivity in these cases (Gower *et al.* 2013b, Murphy *et al.* 2015). Research on other amphibian taxa that are possibly susceptible to infection is now needed, especially West African endemic species, and high elevation species of puddle frogs (*Phrynobatrachus* spp.), small-tongued toads (*Werneria* spp.) and...
long-fingered frogs \( \textit{Cardioglossa} \) spp.) that have dramatically declined in the Cameroonian mountains, as well as other mountains where frog species have disappeared, such as Ethiopia (Gower \textit{et al.}, 2013a, Penner \textit{et al.}, 2013, Hirschfeld \textit{et al.} 2016, Doherty-Bone & Gvoždík 2017, Scheele \textit{et al.} 2019, Tchassem \textit{et al.} In Press). A trait-based approach is likely to facilitate the prioritisation of species for research into resilience to \( \text{Bd} \) infection (Gervasi \textit{et al.} 2017). In addition to long term population monitoring with contemporaneous \( \text{Bd} \) surveillance, studies should take into account long-term deleterious effects, including impacts on population demographics where techniques like skeletochronology or less-invasive mark-recapture studies can reveal a reduced age structure in a \( \text{Bd} \)-impacted population (Scheele \textit{et al.} 2016a, Lampo \textit{et al.} 2017). Sub-lethal effects of \( \text{Bd} \) infection should also be investigated, including impacts on growth rate and reproductive effort (Roznik \textit{et al.} 2015, Campbell \textit{et al.}, 2019). In addition to species that are susceptible, those that are tolerant of \( \text{Bd} \) infection, and that are likely reservoirs for reinfection of vulnerable species, should be considered (Woodhams \textit{et al.}, 2011, Scheele \textit{et al.} 2016b). Susceptibility should be assessed for species with high extinction risk to enable pro-active preparations for the event of disease incursion, such as the Mount Nimba toad \( \textit{Nimbaphrynoides occidentalis} \) which is already threatened by mining (Penner \textit{et al.} 2013).

Africa’s amphibian diversity is still being described and currently comprises 886 species: 859 anurans, 23 caecilians and four salamanders (AmphibiaWeb, 2019). This diversity is subdivided by mountains, river basins, savanna and forests to form biodiversity hotspots in West African forests, the Highlands of Cameroon and Nigeria, the Congo Basin, the Albertine Rift, the Ethiopian Highlands, Eastern Arc Mountains, East African coastal forests and South Africa. Inclusion of Madagascar to this region adds a further 348 species (AmphibiaWeb, 2018). Of the total of 848 species on the African continent so far assessed by the IUCN, 212 are threatened with extinction (i.e. vulnerable, endangered, critically endangered, extinct in the wild). An additional 145 species (from 313 species assessed) are threatened on Madagascar, Comoros and Mayotte. Threats have been identified by the IUCN as habitat loss, overexploitation and climate change. Given the recent finding that \( \text{Bd} \) is an
introduced pathogen, action is urgently needed to better understand the conservation risk it poses to amphibian diversity in Africa.

Acknowledgments

We thank Stefan Lötters and an anonymous reviewer for constructive criticism of an earlier version of this manuscript.

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Figure 1. Distribution of strains of Batrachochytrium dendrobatidis on the African continent.