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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 *Bd*CAPE 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 declines 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.* 2013, Valenzuela-Sanchez et al 2017).

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 (*Bd*GPL) is known to occur in South Africa, Uganda and Ethiopia, while the hypovirulent *Bd*CAPE 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 *Bd*CAPE 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 *Bd*GPL 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 *Bd*CAPE due to a paucity of isolates, however the emergence of *Bd*GPL 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 *Bd*GPL 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 *Bd*GPL, therefore one research priority is to identify the mechanisms of spread of *Bd*GPL 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 *Bd*GPL 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* (*Bd*CAPE) 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 *Bd*CAPE (Walker *et al.* 2008). Although the Mallorcan midwife toad conservation programme is considered to be a success, there is some evidence that *Bd*CAPE 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 *Bd*GPL 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 *Bd*CAPE 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 (*Cardioglossa* spp.) that have dramatically declined in the Cameroonian mountains, as well as other mountains where frog species have disappeared, such as Ethiopia (Gower *et al.*, 2013a, Penner et al., 2013, Hirschfeld *et al.* 2016, Doherty-Bone & Gvoždík 2017, Scheele *et al.* 2019, Tchassem *et al.* In Press). A trait-based approach is likely to facilitate the prioritisation of species for research into resilience to *Bd* infection (Gervasi *et al.* 2017). In addition to long term population monitoring with contemporaneous *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 *Bd*-impacted population (Scheele *et al.* 2016a, Lampo *et al.* 2017). Sub-lethal effects of *Bd* infection should also be investigated, including impacts on growth rate and reproductive effort (Roznik *et al.* 2015, Campbell *et al.*, 2019). In addition to species that are susceptible, those that are tolerant of *Bd* infection, and that are likely reservoirs for reinfection of vulnerable species, should be considered (Woodhams *et al.*, 2011, Scheele *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 (*Nimbaphrynoides occidentalis*) which is already threatened by mining (Penner *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 *Bd* is an introduced pathogen, action is urgently needed to better understand the conservation risk it poses to amphibian diversity in Africa.

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Literature Cited

AmphibiaWeb www.amphibiaweb.org Accessed August 2019

Bletz, M.C., Rosa, G.M., Andreone, F., Courtois, E.A., Schmeller, D.S., Rabibisoa, N.H., Rabemananjara, F.C., Raharivololoniaina, L., Vences, M., Weldon, C. and Edmonds, D., Raxworthy, C.J., Harris, R.N., Fisher, M.C. & Crottini, A. (2015). Widespread presence of the pathogenic fungus *Batrachochytrium dendrobatidis* in wild amphibian communities in Madagascar. *Sci. Rep.-UK*, 5, 8633.

Bosch, J., Fernández-Beaskoetxea, S., Garner, T.W. & Carrascal, L.M. (2018). Long-term monitoring of an amphibian community after a climate change-and infectious disease-driven species extirpation. *Glob. Change Biol.*, 24, 2622-2632.

Briggs C.J., Knapp R.A. & Vredenburg V.T. (2010). Enzootic and epizootic dynamics of the chytrid fungus pathogen of amphibians. *PNAS*, 107, 9695-9700.

Campbell, L., Bower, D.S., Clulow, S., Stockwell, M., Clulow, J. & Mahony, M. (2019). Interaction between temperature and sublethal infection with the amphibian chytrid fungus impacts a susceptible frog species. *Sci. Rep.-UK*, *9*, 83.

Carvalho, T., Becker, C. G. & Toledo, L. F. (2017). Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. *Proc. R. Soc. B,* 284, 20162254.

Conradie, W., Weldon, C., Smith, K.G. & Du Preez, L.H. (2011). Seasonal pattern of chytridiomycosis in common river frog (*Amietia angolensis*) tadpoles in the South African Grassland Biome. *African Zoology,* 46, 95-102.

Cressey, E.R., Measey, G.J. & Tolley, K.A. (2014). Fading out of view: the enigmatic decline of Rose's mountain toad *Capensibufo rosei*. *Oryx*, 49, 521-528.

Doddington, B.J., Bosch, J., Oliver, J.A., Grassly, N.C., Garcia, G., Schmidt, B.R., Garner, T.W. & Fisher, M.C. (2013). Context-dependent amphibian host population response to an invading pathogen. *Ecology*, 94, 1795-1804.

Doherty-Bone, T.M., Ndifon, R.K., Nyingchia, O.N., Landrie, F.E., Yonghabi, F.T., Duffus, A.L.J., Price, S., Perkins, M., Bielby, J., Kome, N.B., LeBreton, M., Gonwouo, L.N. & Cunningham, A.A. (2013). Morbidity and mortality of the critically endangered Lake Oku clawed frog (*Xenopus longipes*). *Endanger. Species Res.*, 21, 115-128.

Doherty-Bone, T.M. & Gvoždík, V. (2017). The amphibians of Mount Oku, Cameroon: an updated species inventory and conservation review. *ZooKeys*, 643, 109-139.

El Mouden, E.H., Slimani, T., Donaire, D., Fernandez-Beaskoetxea, S. & Fisher, M.C. (2011). First record of the chytrid fungus *Batrachochytrium dendrobatidis* in North Africa. *Herp. Rev.*, 42, 71-75.

Farrer, R.A., Weinert, L.A., Bielby, J., Garner, T.W.J., Balloux, F., Clare, F., Bosch, J., Cunningham, A.A., Weldon, C., du Preez, L.H., Anderson, L., Kosakovsky Pond, S.L., Shahar-Golan, R., Henk, D.A. & Fisher, M.C. (2011). Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proc. Natl. Acad. Sci. USA,* 108, 18732−18736.

Fisher, M.C., Ghosh, P, Shelton, J., Bates, K., Brookes, L., Wierzbicki, C., Aanensen, D.M., Alvarado-Rybak, M., Bataille, A., Berger, L., Böll, S., Bosch, J., Clare, F.C., Courtois, E., Crottini, A., Cunningham, A.A., Doherty-Bone, T.M., Gower, D.J., Höglund, J., Kosch, T., Laurila, A., Lin, C.-F., Loyau, A., Martel, A., Meurling, S., Miaud, C., Minting, P., Pasmans, F., Rakotonanahary, T., Schmeller, D.S., Schmidt, B.R., Skerratt, L., Smith, F., Soto-Azat, C., Tessa, G., Valenzuela-Sánchez, A., Verster, R., Vörös, J., Waldman, B., Webb, R.J., Weldon, C., Wombwell, E., Longcore, J. & Garner, T.W.J. (2018). Development and worldwide use of a protocol for the non-lethal isolation of chytrids from amphibians. *Sci. Rep.-UK*, 8, 7772.

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

Goka, K., Yokoyama, J., Une, Y., Kuroki, T., Suzuki K., Nakahara, M., Kobayashi, A., Inaba, S., Mizutani, T. & Hyatt, A.D. (2009). Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Mol. Ecol. 18, 4757-4774.

Gower, D.J., Doherty-Bone, T.M., Aberra, R.K., Mengistu, A., Menegon, M., de Sá, R., Saber, S.A., Cunningham, A.A. & Loader, S.P. (2012). High prevalence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) across multiple taxa and localities in the highlands of Ethiopia. *Herpetol. J.*, 22, 225–233.

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.A. & Gebresenbet, F. (2013a). Long-term data for endemic frog genera reveal potential conservation crisis in the Bale Mountains, Ethiopia. *Oryx*, *47,* 59-69.

Gower, D.J., Doherty-Bone, T., Loader, S.P., Wilkinson, M., Kouete, M.T., Tapley, B., Orton, F., Daniel, O.Z., Wynne, F., Flach, E., Müller, H., Menegon, M., Stephen, I., Browne, R.K., Fisher, M.C., Cunningham, A.A. & Garner, T.W.J. (2013b). *Batrachochytrium dendrobatidis* infection and lethal chytridiomycosis in caecilian amphibians (Gymnophiona). *EcoHealth*, 10, 173-183.

Greenspan, S.E., Lambertini, C., Carvalho, T., James, T.Y., Toledo, L.F., Haddad, C.F.B. & Becker, C.G. (2018). Hybrids of amphibian chytrid show high virulence in native hosts. *Sci. Rep.-UK*, *8*, 9600.

Griffiths, S.M., Harrison, X.A., Weldon, C., Wood, M.D., Pretorius, A., Hopkins, K., Fox, G., Preziosi, R.F. & Antwis, R.E. (2018). Genetic variability and ontogeny predict microbiome structure in a disease-challenged montane amphibian. *ISME J.,* 12, 2506-2517.

Hirschfeld, M., Blackburn, D.C., Doherty-Bone, T.M., Gonwouo, L.N., & Rödel, M.O. (2016). Dramatic decline of montane frog species in a Central African biodiversity hotspot. *PLOS ONE,* 11, e0155129. doi:10.1371/journal.pone.0155129

Hopkins, S. & Channing, A. (2003). Chytrid fungus in Northern and Western Cape frog populations, South Africa. Herpetol. Rev., 34, 334-336.

Hudson, M. A., Griffiths, R. A., Martin, L., Fenton, C., Adams, S-L., Blackman, A., Sulton, M., Perkins, M., Lopez, J., Garcia, G., Tapley, B., Young, R. P. & Cunningham, A. A. (2019). Reservoir frogs: Seasonality of *Batrachochytrium dendrobatidis* infection in robber frogs. *PeerJ*, e7021.

Hydeman, M.E., Longo, A.V., Velo-Antón, G., Rodriguez, D., Zamudio, K.R. & Bell, R.C. (2017). Prevalence and genetic diversity of *Batrachochytrium dendrobatidis* in Central African island and continental amphibian communities. *Ecol. & Evol.*, 7, 7729-7738.

Kielgast, J., Rödder, D., Veith, M. & Lötters, S. (2010). Widespread occurrence of the amphibian chytrid fungus in Kenya. Anim. Conserv. 13, 36-43.

Lampo, M., Señaris, C. & García, C.Z. (2017). Population dynamics of the critically endangered toad *Atelopus cruciger* and the fungal disease chytridiomycosis. *PLOS ONE*, *12*, p.e0179007.

Lane, E.P., Weldon, C. & Bingham, J. (2003). Histological evidence of chytridiomycosis in a free-ranging amphibian, *Afrana fuscigula* (Anura: Ranidae), in South Africa. J. S. Afr. Vet. Assoc. 74, 20-21.

Lips, K.R., Diffendorfer, J., Mendelson III, J.R. & Sears, M.W. (2008). Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLOS Biol*, *6*, e72.

Longo, A.V. & Burrowes, P.A. (2010). Persistence with chytridiomycosis does not assure survival of direct developing frogs. *Ecohealth*, 7, 185-195.

Longo, A.V., Ossiboff, R.J., Zamudio, K.R. & Burrowes, P.A. (2013). Lability in host defenses:

terrestrial frogs die from chytridiomycosis under enzootic conditions. J. Wildlife Dis. 49, 197-199.

Makange, M., Kulaya, N., Biseko, E., Kalenga, P., Mutagwaba, S. & Misinzo, G. (2014). *Batrachochytrium dendrobatidis* detected in Kihansi Spray toads at a captive breeding facility (Kihansi, Tanzania). *Dis. Aquat. Organ.,* 111, 159-164.

McAloose, D., Pramuk, J., Raphael, B., Calle, P. and others (2008) Mortality summary (2000–2007) in endangered captive Kihansi spray toads *(Nectophrynoides asperginis)*. 57th Annual International Conference of the Wildlife Disease Association, Wildlife Disease Association, Edmonton, AB

Murphy, B.G., Hillman, C. & Groff, J.M., 2015. Chytridiomycosis in dwarf African frogs *Hymenochirus curtipes*. *Dis. Aquat. Organ.*, 114*,* 69-75.

O'Hanlon, S., Rieux, A., Farrer, R., Rosa, G.M., Waldman, B., Bataille, A., Kosch, T., Murray, K., Brookes, L., Brankovics, B., Martel, A., Pasmans, F., Sanchez, A., Cunningham, A., Schmidt, B., Miaud, C., Soto-Azat, C., Gower, D.J., Schmeller, D., Loyau, A., Courtois, E., Wombwell, E., Toledo, L.F., Clare, F., Spagnoletti, M., Smith, F., Höglund, J., Vörös, J., Zamudio, K.R., Berger, L., Skerratt, L., Fumagalli, M., Böll, S., Martin, M., Wales, N., Ghosh, P., Verster, R., Meurling, S., Minting, P., Doherty-Bone, T.M., Jenkinson, T., James, T.J., Gilbert, T., Shelton, J., Wierzbicki, C., Hintz, W., Tessa, G., Aanensen, D., Weldon, C., Bosch, J., Balloux, F., Garner, T.W.J. & Fisher, M.C. (2018). A 20th Century out-of-Asia origin of a panzootic threat to global amphibian biodiversity. *Science,* 360, 621-627.

Penner, J., Adum, G.B., McElroy, M.T., Doherty-Bone, T., Hirschfeld, M., Sandberger, L., Weldon, C., Cunningham, A.A., Ohst, T., Wombwell, E., Portik, D.M., Reid, D., Hillers, A., Ofori-Boateng, C., Oduro, W., Plötner, J., Ohler, A., Leaché, A.D. & Rödel, M.-O. (2013). West Africa - a safe haven for frogs? A regional assessment of the chytrid fungus (*Batrachochytrium dendrobatidis*). *PLOS One*, 8, 305 56326.

Rachowicz, L.J., Hero, J.M., Alford, R.A., Taylor, J.W., Morgan, J.A., 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. *Cons Biol*, 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. Herpet. Notes, 4, 83-86.

Roznik, E.A., Sapsford, S.J., Pike, D.A., Schwarzkopf, L. and Alford, R.A., 2015. Condition-dependent reproductive effort in frogs infected by a widespread pathogen. *Proc. R. Soc. B*, 282, 20150694.

Scheele, B.C., Hunter, D.A., Banks, S.C., Pierson, J.C., Skerratt, L.F., Webb, R. & Driscoll, D.A. (2016). High adult mortality in disease-challenged frog populations increases vulnerability to drought. *J. Anim. Ecol.*, 85, 1453-1460.

Scheele, B.C., Hunter, D.A., Brannelly, L.A., Skerratt, L.F. & Driscoll, D.A. (2017). Reservoir-host amplification of disease impact in an endangered amphibian. *Cons. Biol.*, 31, 592-600.

Scheele, B.C., Pasmans, F., Skerratt, L.F., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho, T., Catenazzi, A., de la Riva, I., Fisher, M.C., Flechas, S.V., Foster, C.N., Frías-Álvarez, P., Garner, T.W.J., Gratwicke, B., Guayasamin, J.M., Hirschfeld, M., Kolby, J.E., Kosch, T.A., La Marca, E., Lindenmayer, D.B., Lips, K.R., Longo, A.V., Maneyro, R., McDonald, C.A., Mendelson III, J., Palacios-Rodriguez, P., Parra-Olea, G., Richards-Zawacki, C.L., Rödel, M.O., Rovito, S.M., Soto-Azat, C., Toledo, L.F., Voyles, J., Weldon, C., Whitfield, S.M., Wilkinson, M., Zamudio, K.R. & Canessa, S.

(2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, 363, 1459-1463.

Seimon, T.A., Ayebare, S., Sekisambu, R., Muhindo, E., Mitamba, G., Greenbaum, E., Menegon, M., Pupin, F., McAloose, D., Ammazzalorso, A., Meirte, D., Lukwago, W., Behangana, M., Seimon, A. & Plumptre, A.J. (2015) Assessing the threat of amphibian chytrid fungus in the Albertine Rift: Past, present and future. *PLOS ONE*, 10, e0145841.

Smith, K.G., Weldon, C., Conradie, W. & du Preez, L.H. (2007). Relationships among size, development, and *Batrachochytrium dendrobatidis* infection in African tadpoles. *Dis. Aquat. Organ.*, 74**,** 159-164.

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

Talley, B.L., Muletz, C R., Vredenburg, V.T., Fleischer, R.C. & Lips, K.R. (2015). A century of *Batrachochytrium dendrobatidis* in Illinois amphibians (1888-1989). *Biol. Cons.,* 182, 254-261.

Tarrant, J., Cilliers, D., du Preez, L.H., Weldon, C. (2013). Spatial assessment of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in South Africa confirms endemic and widespread infection. *PLOS ONE*, 8, e69591

Tchassem, A.F., Doherty-Bone, T.M., Kameni, M.M., Tapondjou, W.P., Tamesse, J.L. & Gonwouo, N.L. (In Press). Enigmatic and land-use driven declines of threatened montane amphibians on Mount Bamboutos, Cameroon. *Oryx*

Valenzuela-Sánchez, A., Schmidt, B.R., Uribe-Rivera, D.E., Costas, F., Cunningham, A.A. & Soto-Azat, C. (2017). Cryptic disease-induced mortality may cause host extinction in an apparently stable host–parasite system. *Proc. R. Soc. B*, 284: 20171176.

Vredenburg, V.T., Felt, S.A., Morgan, E.C., McNally, S.V.G., Wilson, S. & Green, S.L. (2013). Prevalence of *Batrachochytrium dendrobatidis* in *Xenopus* Collected in Africa (1871–2000) and in California (2001–2010). *PLOS ONE*, 8, e63791.

Walker, S.F., Bosch, J., James, T.Y., Litvintseva, A.P., Valls, J.A.O., Piña, S., García, G., Rosa, G.A., Cunningham, A.A., Hole, S. & Griffiths, R. (2008). Invasive pathogens threaten species recovery programs. *Curr. Biol.*, 18, R853-R854.

Weldon, C., du Preez, L.H., Hyatt, A.D., Muller, R. & Speare, R. (2004). Origin of the amphibian chytrid fungus. Emerg. Infect. Dis., 10, 2100−2105.

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. *Afr. J. Herpetol.*, 56, 77-83.

Weldon, C., Channing, A., Misinzo, G. & Cunningham, A.A. (2019). Disease driven extinction in the wild of the Kihansi spray toad (*Nectophrynoides asperginis*). *bioRxiv*, p.677971. https://doi.org/10.1101/677971

Wombwell, E. L., Garner, T. W. J., Cunningham, A. A., Quest, R., Pritchard, S., Rowcliffe, J. M. & Griffiths, R. (2016). Detection of *Batrachochytrium dendrobatidis* in amphibians imported into the UK for the pet trade. EcoHealth 13, 456-466.

413 Woodhams, D.C., Bosch, J., Briggs, C.J., Cashins, S., Davis, L.R., Lauer, A., Muths, E., Puschendorf, R., Schmidt, B.R., Sheafor, B. & Voyles, J. (2011). Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Front Zool*, *8*, 8.

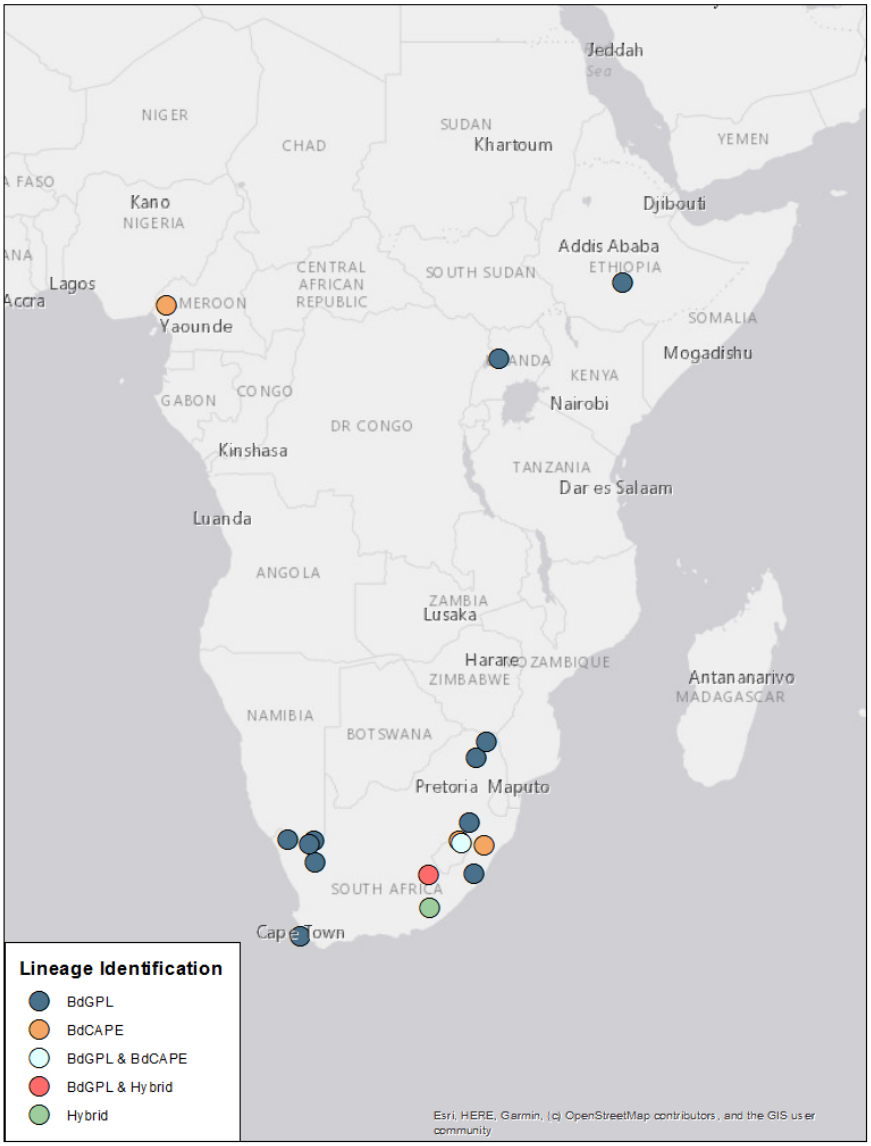

Figure 1. Distribution of strains of Batrachochytrium dendrobatidis on the African continent.