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11/30/15

A Report on Batrachochytrium dendrobatidis (Chytrid):
Causes: Chytridiomycosis
Also Known as: *Bd*

Overview:

First described in 1998 (Berger *et al.* 1998), Chytrid is a fungal infection of amphibians, which has recently exploded onto the world stage. A 2004 report by the CDC suggests that the fungus was originally endemic to southern Africa, and that recent dissemination of the fungus — likely through the trade in pet frogs — to South America, New Zealand and Spain is heavily impacting the viability of their amphibian populations (Weldon *et al.* 2004). Chytrid infection is associated with massive amphibian mortality, population decline, and extinction. As such, the threat of Chytrid outbreak in Madagascar is particularly concerning, given that Madagascar harbors such an immense degree of amphibian biodiversity and a large number of unique species — specifically Madagascar contains around 238 different *Anuran* or frog species (Andrenone *et al.* 2008). Prevention of widespread Chytrid epidemic, and destruction of these species in Madagascar is a top conservation priority.

Introduction of Chytrid to Madagascar:

Given that Madagascar is geographically isolated, it was hoped that Chytrid would not easily spread to the island. Indeed the island was closely monitored following the identification of Chytrid in amphibian populations, and for many years it seemed as if it remained safe from the ravages of the disease. In 2008 a report by Weldon *et al.* presented data which suggested that Madagascar had not yet been reached. Their histological survey — examining the cells of the skin for evidence of the disease — of 527 frogs from multiple locations across the eastern region of the island failed to turn up any evidence of the pathogen. Nonetheless, they urged the need for continued monitoring. In 2012, a subsequent publication authored by Weldon reported that Chytrid had still yet to reach Madagascar. In this study, qPCR testing was implemented in order to attempt to detect the pathogen; this technique is far more sensitive than histological testing (Smith 2007)[1]. This study was significantly more comprehensive, sampling “300 frog specimens belonging to 53 species in 13 genera, from 12 sites throughout Madagascar spanning all of Madagascar‘s major bioclimatic regions and an array of different elevations from 20 to 2400 m above sea level (Vredenburg *et al.* 2012).” That said, it is still possible Chytrid had arrived and remain undetected at this time.

It was only a few years later in 2014, that the first evidence of Chytrid invasion on Madagascar was presented. A researcher named Jonathan Kolby from the James Cook University in Queensland, Australia published findings in PLOS ONE which included the detection of Chytrid in three Madagascar frogs — each frog was of a different species endemic to Madagascar. Kolby utilized a qPCR assay to test 565 amphibians across nine species, all of which had been exported from Madagascar to the US for sale as exotic pets. Kolby’s findings in this report were not conclusive, given that he did not present consistent results in his Chytrid assays (Kolby 2015), however his results were confirmed by a separate group in a 2015 Nature Scientific Reports article. Therein, the authors showed evidence of the Chytrid fungus across Madagascar, with initial detection occurring as early as 2010. The study was quite comprehensive, covering 4,155 individual amphibians from across the island over the course of a decade (Bletz *et al.* Feb 2015). Inconsistencies in the results of different surveillance initiatives in the period from 2010-2014 are well addressed in a clarification article written by the same group (Bletz *et al.* Oct 2015).

It appears as if the worldwide Chytrid epidemic began from a point of origin in Southern Africa, where the fungus is endemic. It was then disseminated by the trade in amphibians as exotic pets. In particular, the species *Xenopus laevis* has been blamed for carrying the disease; the species has been trafficked extensively since the 1930s as an exotic pet and is capable of carrying the disease without major symptoms. This second characteristic makes it an ideal vector for the disease, as it allows the frog to travel long distances without perishing (Weldon et al. 2004). It is unclear at this time how the infection reached Madagascar, but now that it has arrived conservationists must begin to combat the disease and mitigate the risk posed to the island’s numerous endemic amphibians.

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[1] Though it has been argued that the swabbing technique used to collect samples may result in false negatives when concentrations of the Chytrid fungus spores are low (Shin *et al.* 2014)

Destructive Potential of Chytrid:

Chytrid epidemics are quite capable of causing destruction of amphibian populations. In the late 1990s, an outbreak of Chytrid was reported to have caused mass mortality and population decline in a population toad found in a area of central Spain. This outbreak caused the frogs to disappear “from 86% of the ponds where they were known to reproduce (Bosch *et al.* 2001).” The 1998 paper in which Chytrid was first identified accurately hypothesized that the Chytrid infections they observed caused mortality by thickening the skin of amphibians, obstructing the skin’s normal physiological functions: the transport of water, gases and electrolytes into and out of the body (Berger *et al.* 1998; Voyles *et al.* 2009).

A 2006 PNAS report defined the gravity of the situation at the time in terms of species declines: Chytrid was the suspected causative factor with the recent decline of at least 93 species worldwide, many in Central America — an amphibian hotspot (Lips *et al.* 2006). Many more affected species had yet to be identified at this time, as Chytrid spread like wildfire across the globe (Weldon *et al.* 2004 - Fig 3), and only a year later, a report was published upping the number of in-decline or extinct frog species to around 200 (Skerratt *et al.* 2007; Bletz *et al.* 2015). Chytrid has been identified in deceased frogs from locales as distant as Australia and Panama (Berger *et al.* 1998). Given estimates of 2394 frogs (there exist an estimated 6010 species globally), this represents a significant loss of global biodiversity. Notably, frog species constitute the majority of amphibian species (88.3%), so their loss is quite significant for amphibians as a whole.

Potential Impact of Chytrid in Madagascar:

As previously mentioned, Madagascar is a region of immense species richness. Its geographic isolation has allowed it to evolve a host of endemic species — those species which are found nowhere else. Particularly important in the case of Chytrid is the high diversity of endemic frog species. Previous assessments have placed the number of frog species present on Madagascar at around 238 (Andrenone *et al.* 2008). Our group analyzed the amphibiaweb.org database, which listed a total of 305 frog species as endemic to Madagascar, 239 of which were assigned IUCN statuses. The majority were of least concern, though there are a substantial number of species which are already imperilled without the burden of Chytrid (Fig 1).

Effects of Chytrid Outbreak

The primary impact of Chytrid in Madagascar will be the profound loss of biodiversity. Many unique species of amphibian have the potential to perish due to Chytrid and in doing so vanish from the Earth altogether. Though the loss of such a large number of species is tragic in and of itself, there are other reasons of practical significance for conservation of amphibians like frogs.

Primarily, frogs serve an important role in the ecosystems in which they reside: impacting the population dynamics of aquatic insects, algae and aquatic predators, and the flow of matter and energy through the food web. It has also been suggested that, because tadpoles and mature frogs occupy different ecological niches, the loss of one frog species is akin to the overall loss of two species in a given ecosystem (Whiles *et al.* 2006). The loss of a large number of frog species could lead to the serious disruption of numerous aquatic ecosystems. For instance, the loss of frog species could significantly reduce the predator burden on various insect species, allowing them to grow unchecked. Some such insects may even be vectors for human diseases. It is well recognized that dramatic shifts in ecosystem dynamics can alter the rates of human infectious disease (Chivian 2002).

In addition, the unique species present on Madagascar may hold a fount of novel compounds which could be used in the development of pharmaceuticals (Flemming 2007). Frog skin has been demonstrated to secrete a host of antimicrobial compounds which may find use as antibiotics (Conlon 2012; Conlon 2004). Given the impending crisis posed by the rise of antibiotic-resistant bacteria, the identification of effective new antibiotics is essential.

Intervention to Halt Chytrid in Madagascar

It is clear that the best means of preventing the destruction of Madagascar's endemic frog species would have been the prevention of Chytrid outbreak on the island in the first place - amphibian quarantine. Given that this is no longer an option we must turn our attention to the mitigation of damage; both taxonomic and ecological. Analysis of the composition of Madagascar frog genus reveals that one particular genus *Boophis* contains a large proportion of the total species (23%) and of the family *Mantellidae* (33%), which is unique to Madagascar (Fig 2). This is particularly concerning, as preliminary infectivity studies have revealed that at least two of these species (*B. madagascariensis, B. viridis*) are susceptible to the fungus (Bletz *et al.* Feb 2015). Assessments of unique and valuable taxa must be made and vulnerability assessed in order to best target conservation attention and resources — we attempt to begin this assessment above.

Monitoring of the progress of the Chytrid epidemic will also be essential for the direction of conservation efforts. It is important to note that one has to be looking in the right places to find Chytrid: "the likelihood to detect Bd seems to be higher in the dry, cooler season and in association with mid-high elevation locations (Andreone *et al.* 2015)." Indeed, just because Chytrid is not detected, doesn't mean it is not present. In addition, monitoring agencies must track the emergence of the Chytrid Global Panzootic Lineage (*Bd*GPL) which is the highly virulent strain associated with most amphibian declines (Andreone *et al.* 2015; Farrer *et al.* 2011). As of 2015, there does not seem to be a high prevalence of this strain and minimal amphibian loss. The National Monitoring Program requires continued support in order to "gather robust data on the patterns of prevalence, intensity and distribution of Bd across the island" in addition to sustaining captive populations of valuable species (Andreone *et al.* 2015).

While treatment options are limited for an outbreak of this scale - it would be very difficult to treat the entire frog population — there is some hope that captive individuals could be treated for the fungus. Several groups have proposed the use of probiotics: harnessing several existing species of bacteria found on amphibian skin to compete with the fungus (Andreone *et al.* 2015; Harris *et al.* 2009). The treatment of captive individuals is important for the preservation of artificial populations of valuable frog species. The creation of a sort of ark for Madagascar frogs may be the best hope of preserving this bounty of evolutionary diversity, should Chytrid cause extinction in the wild.

Figures:

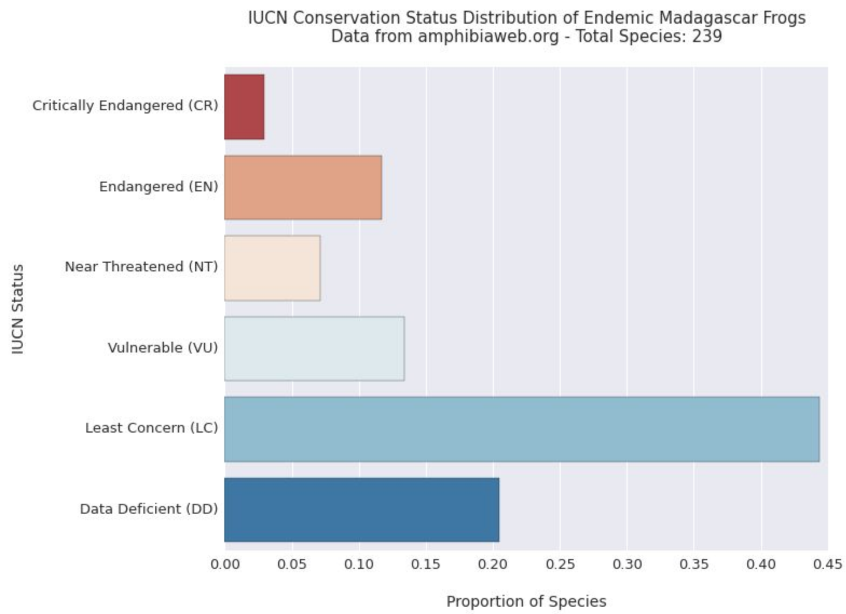

Fig 1: IUCN Status of The Frog Species Endemic to Madagascar. A total of 305 species were recovered from the amphibiaweb.org database, all of which were frog species. Of these frog species, 239 were assigned IUCN statuses, the distribution of which are presented above.

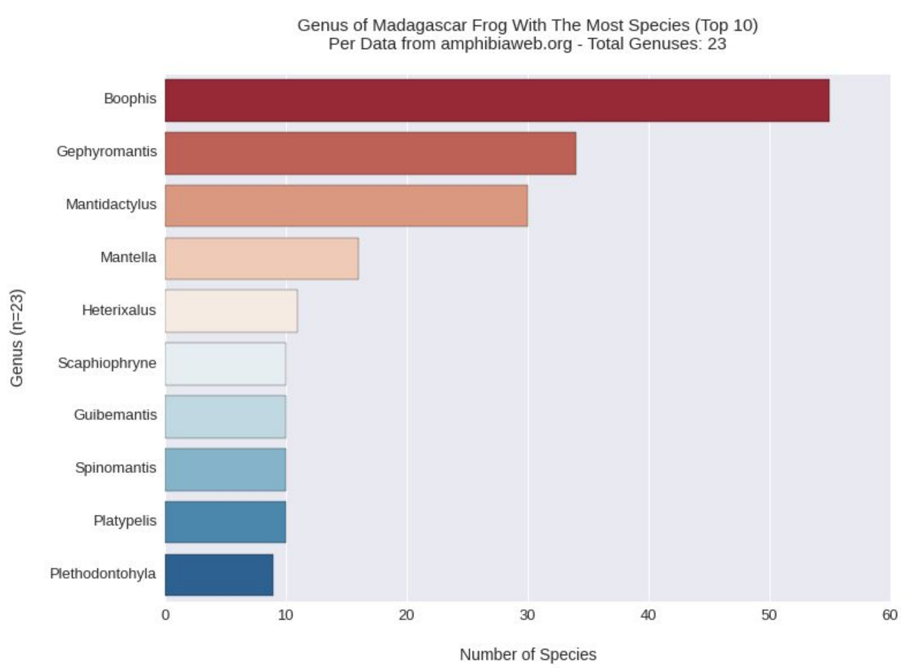

Fig 2: Of the existing genus of Madagascar frog, a small proportion hold a majority of the species. The genus Boophis contains 55 of the 239 overall IUCN recognized species, and is a member of the family *Mantellidae*, the largest of the three frog families found on Madagascar.

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