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Alba Navarro Lozano

Ecology and Biogeography of *Batrachochytrium dendrobatidis* in the Brazilian Atlantic Forest

São José do Rio Preto
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"It doesn't matter whether you're the lion or a gazelle, when the sun comes up, you'd better be running".

Cristopher McDougall

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RESUMO

O fungo quitridio *Batrachochytrium dendrobatidis* (*Bd*) é considerado uma das principais causas do declínio global dos anfíbios. *Bd* já foi encontrado em quase 700 espécies de anfíbios de todo o mundo. Embora muitos estudos tenham sido publicados desde a sua descrição em 1999, ainda há muito a ser conhecido, particularmente em relação à sua ecologia. Nossos objetivos neste estudo foram: (i) analisar os fatores bióticos e abióticos que regulam a prevalência e a intensidade de infecção de *Bd* em girinos que habitam poças e riachos no Núcleo Curucutu do Parque Estadual da Serra do Mar, sudeste da Mata Atlântica brasileira; (ii) compreender qual será o efeito das mudanças climáticas na distribuição potencial de *Bd* na Mata Atlântica brasileira; (iii) avaliar o uso de deformidades orais em girinos como indicadores confiáveis para a determinação de infecção por *Bd*; e (iv) criar uma ferramenta de divulgação para promover a transferência de conhecimento sobre *Bd* entre a universidade e a sociedade brasileira. Detectamos que a prevalência e a intensidade de *Bd* não diferiu entre poças e riachos, mas são regulados por diferentes fatores ambientais. Em riachos, a velocidade da água e a profundidade explicaram a variabilidade na prevalência de *Bd*, mas a intensidade permaneceu inexplicada. Por outro lado, a densidade do zooplâncton foi o fator chave para explicar a prevalência e a intensidade em poças. Estimamos que, hoje, cerca do 60% da área da Mata Atlântica brasileira apresenta condições climáticas adequadas para a ocorrência de *Bd*, porém diferentes cenários de aquecimento global para o ano de 2070 diminuiriam essas áreas entre um 27.5 e 42.6%. Também demonstramos que as deformidades orais em girinos não são um indicador confiável para o diagnóstico da quitridiomicose em girinos de anfíbios anuros, e que não devem ser utilizadas como ferramenta de diagnóstico isoladamente. Desenvolvimos uma página de internet (www.quitribrasil.com) para a divulgação de todos os casos de *Bd* relatados no Brasil. As informações geradas por estes trabalhos podem melhorar a compreensão da ecologia e distribuição de *Batrachochytrium dendrobatidis* em um dos biomas mais ricos e com as maiores taxas de endemismo de anfíbios no mundo.

Palavras-chave: *Batrachochytrium dendrobatidis,* Amphibia, Girino, Ecologia, Conservação, Distribuição geográfica, Mudança climática, Mata Atlântica, Morfologia

ABSTRACT

*The chytrid fungus Batrachochytrium dendrobatidis (*Bd*) is considered one of the main cause of global amphibian declines.* Bd *has been reported in nearly 700 species of amphibian worldwide. Although many studies have been published since its description in 1999, there is still much to be known, particularly regarding to its ecology. In this study, we aim to: i) analize biotic and abiotic factors that regulate prevalence and intensity of* Bd *infection in tadpoles inhabiting lentic and lotic habitats, in Nucleo Curucutu of the Parque Estadual da Serra do Mar, southeastern Brazilian Atlantic Forest; ii) understand the effect of climate change in potential future distribution of* Bd *in the Brazilian Atlantic Forest; iii) evaluate the use of oral deformities in tadpoles as reliable proxies for the determination of infection by* Bd*; and iv) create an outreach tool to communicate the knowledge of* Bd *from university to Brazilian society. We detected that the prevalence and intensity of* Bd *did not differ between ponds and streams but were driven by different environmental factors in the two habitats. In streams, water velocity and depth explained variability in* Bd *prevalence, but* Bd *intensities remained unexplained. On the other hand, zooplankton density was the key factor in explaining* Bd *prevalence and intensity in ponds. We estimated that currently, about 60% of the Atlantic Forest area shows climatic suitability for* Bd *occurrence; but in global warming scenarios for 2070 those suitable areas will decrease by between 27.5 and 42.6%. We also showed that oral deformities in tadpoles are not a trustworthy indicator for chytridiomycosis diagnosis in tadpoles of anuran amphibians, and should not be used in isolation as a diagnosis tool. We developed and launched one internet webpage (www.quitribrasil.com) to communicate all* Bd *cases reported in Brazil. Information generated by our study could improve the knowledge of ecology and distribution of Batrachochytrium dendrobatidis in one of the richest biomes with greatest values of endemism of anuran amphibians in the world.*

Keywords: *Batrachochytrium dendrobatidis, Amphibia, Tadpole, Ecology, Conservation, Geograohic distribution, Climatic change, Atlantic Forest, Morphology*

INTRODUCTION

Global loss of biodiversity is one of the most serious problem of our time (Baillie et al., 2010). Data from the International Union for Conservation of Nature (IUCN) warns that approximately 41% of the world's amphibians species are endangered, making them the most vulnerable group among vertebrates (IUCN, 2016). Whereas it is true that amphibian populations present natural abundance fluctuations (Pechmann et al., 1991; Alford & Richards, 1999), population declines occurred in the last decades have not being balanced with equivalent increases (Houlahan et al., 2000). Different studies have shown that amphibian population declines are the outcome of complex interactions of factors such as habitat loss and land-use changes (Bradford et al., 1993; Stuart et al., 2004; Cushmann, 2006), exotic species introduction (Fisher & Shaffer, 1996; Bradford 1989; Kiesecker et al., 2001), environmental contamination (Harte & Hoffman, 1989; Rohr et al., 2004; Blaustein et al., 2003), UV-radiation increase (Blaustein et al 1994, 1997; Anzalone et al 1998; Lizana & Pedraza 1998), climatic changes (Heyer et al., 1988; Laurance et al., 1996; Pounds et al, 1999, 2006; Pounds, 2001), and the emergence infectious diseases (Berger et al., 1998, 1999; Lips, 1999, 1998).

From the end of 1970’s and during the 1990’s, dramatic amphibian death events occurred in Central America and Australia (Laurence et al., 1996; Campbell et al., 1999; Pounds et al., 1997; Crump et al., 1992). What researchers studying these cases found most striking was that both events occurred in well conserved pristine forest areas. They suggested that climate change was responsible for them (Pounds et al., 1999). However, at the end of the 1990's it was detected a link between mass mortality events in Central America and Australia: the presence of fungal sporangium in upper layers of amphibians skin, a, infection called chytridiomycosis (Berger et al., 1998). Shortly after this discovery, Longcore *et al.* (1999) described *Batrachochytrium dendrobatidis* (*Bd*), a chytrid fungus responsible for those death events. Since *Bd* was first described, several dramatic declines of amphibian populations worldwide have been explained by *Bd* infections (*e.g.* Berger et al, 1998, 1999; Lips, 1999, 1998; Bosch et al., 2001; Ouellet et al., 2005; Weldon et al., 2004; Garner et al., 2005; Lips et al., 2006). Nowadays is known that *Bd* is spread worldwide, already found in 56 countries and affecting nearly 700 species of all three amphibian orders (*http://www.bd-maps.net*).

BOX 1

In 2013, Martel et al. discovered a second species of chytrid fungus, ***Batrachochytrium salamandrivorans*** (*Bsal*), which was causing lethal infections and population declines in the European *Salamandra salamandra*. Shortly after, the same research group pointed Oriental Asia as the origin of Bsal, since unlike with Bsal in Europe, the presence of the fungus do not seem to be affecting Asian urodels (Martel et al., 2014). To date, Bsal only occurs in urodeles (newts and salamanders) and not in anurans (frogs and toads) or caecilians.

Who is *Batrachochytrium dendrobatidis*? And, how does it affect amphibians?

Bd is a non-hyphal zoosporic chytrid aquatic fungus belonging to the class Chytridiomycetes and order Rhizophydiales (Longcore et al., 1999; Letcher et al., 2006). Although a saprobic phase has been suggested for *Bd*, to date only two life cycle phases has been described, a free-swimming zoosporic phase that allows *Bd* to meet a host, and a sporangial phase that results from the encystment of the zoospore into keratinizing tissue of a host, or those tissues fated to keratinize, where matures and produce new zoospores (Longcore et al., 1999; Fellers et al., 2001; Berger et al., 2005).

Bd infection is restricted to the oral region in tadpoles, the only keratinized part of this life cycle phase (Marantelli et al., 2004; Berger, 1998, 1999; Fellers et al., 2001). In adults, *Bd* colonize keratinized cells of the stratum corneum and granulosum of the epidermis, with higher concentrations in ventral and pelvic regions, hind limbs and feet (Longcore et al., 1999; Pessier et al., 1999). Superficial pathologies associated with the infection vary from swollen and dekeratinization of the oral disk (Berger et al., 1999; Fellers et al., 2001; Navarro-Lozano et al., 2018), to hyperkeratosis, hyperplasia, ulceration, depigmentation, slower rehydration and sloughing of metamorph skin (Berger et al., 1998; Voyles et al., 2009; Carver et al., 2010), as well as behavioral abnormalities such as inappetance, lethargy and loss of reflex and escape response in adults (Berger et al., 1998, 1999; Nichols et al., 2001; Pessier et al., 1999, Voyles et al., 2009). In has also been described pathophysiologies caused by the impaired performance of the amphibians skin such as significant reductions in osmolarity and concentrations of Na^+, K^+ and Cl^- in plasma, which may result in death by cardiac arrest (Voyles et al., 2009; Marcum et al., 2010). In addition, it has been demonstrated that *Bd* produces mycotoxins that block the amphibian immune response, inhibiting lymphocyte proliferation and causing cell apoptosis (Fites et al., 2013).

Not all species from affected regions have the same likelyhood to be infected (Blaustein et al., 2005; Searle et al., 2011; Lips 2006, Gahl 2012). Some species of salamanders show low susceptibility to *Bd* infection which has been linked to the presence of bacterial strains on their skin (Harris et al. 2006). Indeed, the bacteria *Janthinobacterium lividum*, found in the skin of some salamander species, produces lethal concentrations of antifungal metabolites for *Bd* (Brucker et al., 2008). Functional traits can also determine the likelyhood of species to be infected. Species inhabiting permanent waterbodies seem to be more infected by *Bd* than those inhabiting temporal waterbodies (Kriger & Hero, 2007). In turn, among species that share the same habitat, those occuping warmer and dryer microhabitats are also less likely to be infected (Rowley & Alford, 2013).

BOX 2

Despite *Bd* came into the spotlight for its detrimental effect on amphibians, this fungus has been found in other animals that share amphibian habitats as:

- **Lizards**: *Anolis humilis* and *Anolis lionotus* (Kilburn et al., 2011).
- **Snakes**: *Pliocercus euryzonus, Imantodes cenchoa* and *Nothopsis rugosus* (Kilburn et al., 2011).
- **Birds**: *Branta canadensis* and *Anser domesticus* (Garmyn et al., 2012); *Anas falvirostris, Anas georgica, Fulica ardesiaca, Fulica gigantean, Lophonetta specularioides, Plegadis ridgwayi, Rollandia Rolland* and *Spatula puna* (Burrowes & De la Riva, 2017a).
- **Arthropods**: *Procambarus* spp. and *Orconectes virilis* (McMahon et al. 2013).
- **Fishes**: *Danio rerio* (Liew et al., 2017).

Origin of *Batrachochytrium dendrobatidis*

Since chytridiomycosis was first reported, there has been a great debate to explain the origin of this disease. And although it has taken almost two decades of studies, a research with more than 200 *Bd* isolates, collected from various regions of the planet, has managed to point to East Asia as the geographical origin of *Bd* (Dr. Jaime Bosch's personal communication). This recent research by Dr. Jaime Bosch and other 52 authors, which will be published in Nature this year, highlights the presence of four divergent main lineages of Bd: Bd-Asia1, Bd-Asia2/Brazil, Bd-Cape and Bd-GPL (*Global Panzootic Lineage*). It is Bd-GPL who stands out over the rest of lineages for its hypervirulence and widely distribution, being associated with species extinction and mass mortality events of amphibians occurred in Central America, Australia and Spain (Farrer et al., 2011; Schloegel et al., 2012). From this research it is also concluded that *Bd*-GPL has a recent origin, and that its global expansion took place until the first half of the 20th century, coinciding in time with the commercialization of amphibians for scientific and feed purposes (Dr. Jaime Bosch's personal communication).

BOX 3

Until today, the international trade of amphibian species seems to be the main source of dispersion of the fungus around the world (Laurance et al., 1996; Fisher & Garner, 2007; Schloegel et al. 2012). ***Why?*** For example, *Xenopus laevis* and *Lithobates catesbeiana*, commonly commercialized for consumption, as pets or for laboratory use, can accommodate high loads of *Bd* without succumbing to the disease (Mazzoni et al., 2003; Weldon et al., 2004; Daszak et al., 2004). The lack of signs of disease and the ineffectiveness of transcontinental biosecurity have turned these species into perfect vectors for the global dissemination of *Bd*. Specimens of *L. catesbeiana* infected by the fungus been reported in markets in Asia, South and North America, and Europe (Garner et al., 2006; Bai et al., 2010).

Eighteen years after the discovery of *Bd,* many studies have been published. However, there is still much to be known, particularly regarding to its ecology. This doctoral dissertation presents a study of the distribution and ecology of *Bd* in the Brazilian Atlantic forest, which stands out as an ideal place for the study of *Bd* due to its importance as a global hotspot (Myers, 2000; Jenkins et al., 2015) and to the absence of harmful pathologies, despite of the large number of amphibian species Bd-infected. The dissertation includes four chapters: (1) ***Batrachochytrium dendrobatidis* infection of frogs is driven by different biotic and abiotic factors in ponds and streams of the megadiverse Atlantic Forest of Brazil**, in which biotic and abiotic environmental factors that influence the dynamic of *Bd* infection (prevalence and intensity) in a pristine area of the Brazilian Atlantic Areas are studied; (2) **Global warming could decrease potential geographic distributon of amphibian-killing fungus in Braziian Atlantic Forest**, in which we have analyzed the effect of climate change over *Bd* distribution in the Brazilian

Atlantic Forest; (3) ***Are oral deformities in tadpoles accurate indicators of anuran chytridiomycosis?***, in which the relationship between oral deformities and *Bd* infection in tadpoles of different species and is potential use as a proxy for the determination of *Bd* infection is studied; and (4) ***Batrachochytrium dendrobatidis in Brazil: www.quitribrasil.com***, in which we present the webpage www.quitribrasil.com, where information of all *Bd* cases reported in Brazil is provided. Finally, the conclusions section highlights the main findings that our studies have revealed.

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Conclusions

The main conclusions obtained after the work carried out during this research are listed below.

FINAL CONCLUSIONS

1) Nucleo Curucutu becomes part of the list Brazilian Atlantic Forest areas in which anuran amphibian infected by *Batrachochytrium dendrobatidis* (*Bd*) has been detected.

2) Neither signs of chytridiomycosis nor dead or dying amphibian individuals were detected in Nucleo Curucutu.

3) The prevalence and intensity of *Bd* did not differ between ponds and streams in Nucleo Curucutu.

4) In ponds, zooplankton density explained variability of *Bd* prevalence and intensity.

5) In streams, water velocity and depth were the key factors that explained *Bd* prevalence, however, *Bd* intensity remains unexplained.

6) The potential geographic distribution of *Bd* in the Brazilian Atlantic Forest reaches 665,769.2 km^2, corresponding to 58.8% of the total area of this domain.

7) Greater climatic habitat suitability for *Bd* is located in southeastern Brazil, in the states of São Paulo, Minas Gerais and Rio de Janeiro.

8) All global warming scenarios for year 2070 predict a decrease of the potential distribution of *Bd* in the Atlantic Forest.

9) All global warming scenarios predict occurrence of *Bd* in higher altitudes and latitudes of the Brazilian Atlantic Forest.

10) The dekeratinization of tooth rows themselves was not necessarily associated with presence of *Bd* in any of the anuran species studied (*Aplastodiscus albosignatus, Boana albopunctata, B. faber, Scinax hayii, Crossodactylus caramaschii*, and *Physalaemus cuvieri*).

11) Dekeratinization of jaw sheaths showed a nonrandom relationship with *Bd* infection in A*. albosignatus, B. albopunctata* and *S. hayii,* but its use as a proxy for *Bd* detection resulted in up to 30.8% false positives and up to 29.3% false negatives.

12) Dekeratinization of jaw sheaths is even worse as a proxy for *Bd* detection in species that do not show relationship between dekeratinization and *Bd* infection, generating up to 100% false positives.

13) Oral deformities in tadpoles are not an accurate indicator for chytridiomycosis in frogs.