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Worrying News for Brazilian Caatinga: Prevalence of *Batrachochytrium dendrobatidis* in Amphibians

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Abstract

The chytrid fungus *Batrachochytrium dendrobatidis* (Bd) has been linked to the declines of more than 500 amphibian species globally. In Brazil, Bd has been identified in several regions but predominantly in the Atlantic Forest. Data on the occurrence of this amphibian pathogen in northeastern Brazil are scarce, specifically from the Caatinga ecoregion, where there is only one study that reports Bd in the region. This study is the first to show a high prevalence of Bd in Caatinga's species and includes two new records of species infected by Bd: *Rhinella granulosa* and *R. jimi*. In addition, we discuss the possibility of amphibian with terrestrial habits serving as potential Bd reservoirs in semiarid climate regions.

Keywords

amphibian, chytridiomycosis, Caatinga, *Rhinella*, reservoir

Amphibians are the most threatened vertebrate group in the world (Monastersky, 2014). One of the major threats to amphibian populations is chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd). This pathogen has been linked to the declines of more than 500 amphibian species globally and represents the greatest documented loss of biodiversity attributable to a pathogen (Scheele et al., 2019). In Brazil, most studies concerning Bd have been concentrated in the Atlantic Forest (e.g., Greenspan et al., 2018; James et al., 2015; Jenkinson et al., 2016; Rodriguez, Becker, Pupin, Haddad, & Zamudio, 2014), where there seems to be a higher proportion of Bd-infected amphibians (see Carvalho, Becker, & Toledo, 2017). On the other hand, recent studies (e.g., James et al., 2015) have suggested that, instead of focusing on pathogen *hot spots*, we need to identify pathogen *cold spots* so that we can better understand what limits the pathogen's distribution.

The Caatinga is a well-recognized ecological region that lies in the semiarid hinterland of northeastern Brazil. The term *Caatinga* refers mostly to a seasonally dry tropical forest that presents different physiognomies (Silva, Barbosa, Leal, & Tabarelli, 2017). This complex

physiognomic form (i.e., vegetation types) is distributed in mosaics with high temperatures, strong potential evapotranspiration, and a well-defined seasonality, presenting a long drought period and a low and irregular rainfall period (Coutinho, 2006). Despite being the major Brazilian natural region, it is the third most affected landscape by anthropic actions. This region is highly threatened by habitat loss and desertification, and it is the most neglected in terms of conservation efforts and

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the most vulnerable ecoregion in Brazil regarding the number of fully protected areas (Guedes, Sawaya, & Nogueira, 2014; Leal, Silva, Tabarelli, & Lacher, 2005; Tabarelli & Silva, 2003; Zanella & Martins, 2005). Only recently, greater attention has been given to the Caatinga region in studies that focus on landscape and biodiversity conservation (e.g., Camardelli & Napoli, 2012; de Albuquerque et al., 2012; Garda et al., 2017; Guedes et al., 2014; Leal et al., 2005; Silva et al., 2017; Silva, Tabarelli, Fonseca, & Lins, 2004). The anuran fauna of this ecoregion is still poorly known, needing further studies involving systematic, natural history, biogeography, community and population ecology, physiology, and phylogenetic analyses (Benício, da Silva, & Fonseca, 2015; Garda et al., 2017; Heyer, 1988).

Data on Bd occurrence in amphibians in northeastern Brazil are scarce, specifically for the Caatinga ecoregion. Only one study reports its existence in *Odontophrynus carvalhoi* (Savage & Cei, 1965), *Bokermannohyla oxente* (Lugli & Haddad, 2006), and *Pseudopaludicola mystacalis* (Cope, 1887) in the region (Carvalho et al., 2017). From October to November 2017, we sampled anurans in an area of Caatinga, specifically, at the Federal University of Piauí (7.082114° S, 41.435973° W, WGS84, 210 m asl), Picos municipality, Piauí state, northeastern Brazil. The region's climate is semiarid with an average annual rainfall lower than 900 mm, irregularly distributed from January to April, and with an annual average temperature of 27.3°C (Lima, Abreu, & Lima, 2000). We collected 20 individuals of *Rhinella granulosa* (Spix, 1824) and 20 individuals of *Rhinella jimi* (Stevaux, 2002). To detect and quantify the incidence of Bd in the toads, we used sterile gloves for each animal and swabbed the individuals five times in each of the following regions: right and left inguinal areas and interdigital membranes of right and left upper and lower limbs (Hyatt et al., 2007; Lambertini, Rodrigues, Brito, Leite, & Toledo, 2013). After swabbing, we released the

individuals at the same collection site. We placed swab samples in cryotubes with saline solution at -20°C and sent them to the Laboratório de Biologia Molecular at Federal University of Piauí for further molecular analysis. We then extracted DNA from each swab using PrepMan Ultra[®] (Life Technologies) and proceeded with TaqMan[®] qPCR assay for Bd detection (Boyle, Boyle, Olsen, Morgan, & Hyatt, 2004). We ran samples in triplicate. We determined Bd presence, by considering Bd positive when all of the three triplicate analyses revealed Bd zoospore genome equivalents ≥ 0.1 , and the amplification curves have a sigmoidal shape (Kriger, Ashton, Hines, & Hero, 2007).

Overall Bd prevalence was 25% ($n = 10$), being the Bd prevalence in *Rhinella granulosa* 20% (95% CI [19.83, 20.17]) and in *Rhinella jimi* 30% (95% CI [29.79, 30.20]; Table 1). This is the first record of Bd in *R. granulosa* and *R. jimi* from Caatinga, increasing to five the number of amphibian species with confirmed occurrence of Bd at this ecoregion (Figure 1 and Table 1). These are alarming results for the Caatinga, as the detection of Bd is within a priority area for conservation—municipality of Picos—and close to two areas of extreme biological importance—Parque Nacional da Serra da Capivara and Parque Nacional da Serra das Confusões (Silva et al., 2004). All of these areas harbor a singular assemblage of species. The municipality of Picos (which is the type locality of *Dendropsophus soaresi* and *Elachistocleis piauiensis*; Caramaschi & Jim, 1983) presents high species richness (~ 24 species; Benício et al., 2015). For the Parque Nacional da Serra da Capivara and Parque Nacional da Serra das Confusões, the presence of water-dependent and forest-dependent species suggest that these areas harbor faunas associated with relictual rainforest fragments (Cavalcanti et al., 2014). These areas also exhibit high levels of diversity and uniqueness with low faunal similarities between each other (Dal Vechio, Teixeira, Recoder, Rodrigues, & Zaher, 2016).

Table 1. Species of Amphibians With Confirmed Occurrence of *Batrachochytrium dendrobatidis* in the Caatinga Ecoregion, Northeastern Brazil.

Species	Positive/Sampled	Prevalence (%)	Prevalence 95% CI	Locality, state	Life stage	Year
Bufonidae						
<i>Rhinella granulosa</i>	4/20	20	[19.83, 20.17]	Picos, Piauí ^a	Adult	2017
<i>Rhinella jimi</i>	6/20	30	[29.79, 30.20]	Picos, Piauí ^a	Adult	2017
Hylidae						
<i>Bokermannohyla oxente</i>	–	–	–	Lençóis, Bahia ^b	Tadpole	1999
Odontophrynidae						
<i>Odontophrynus carvalhoi</i>	–	–	–	Maracás, Bahia ^b	Tadpole	1978
Leptodactylidae						
<i>Pseudopaludicola mystacalis</i>	–	–	–	Brejo do Piauí, Piauí ^b	Tadpole	2006

Note. CI = confidence interval.

^aThis study.

^bCarvalho et al. (2017).

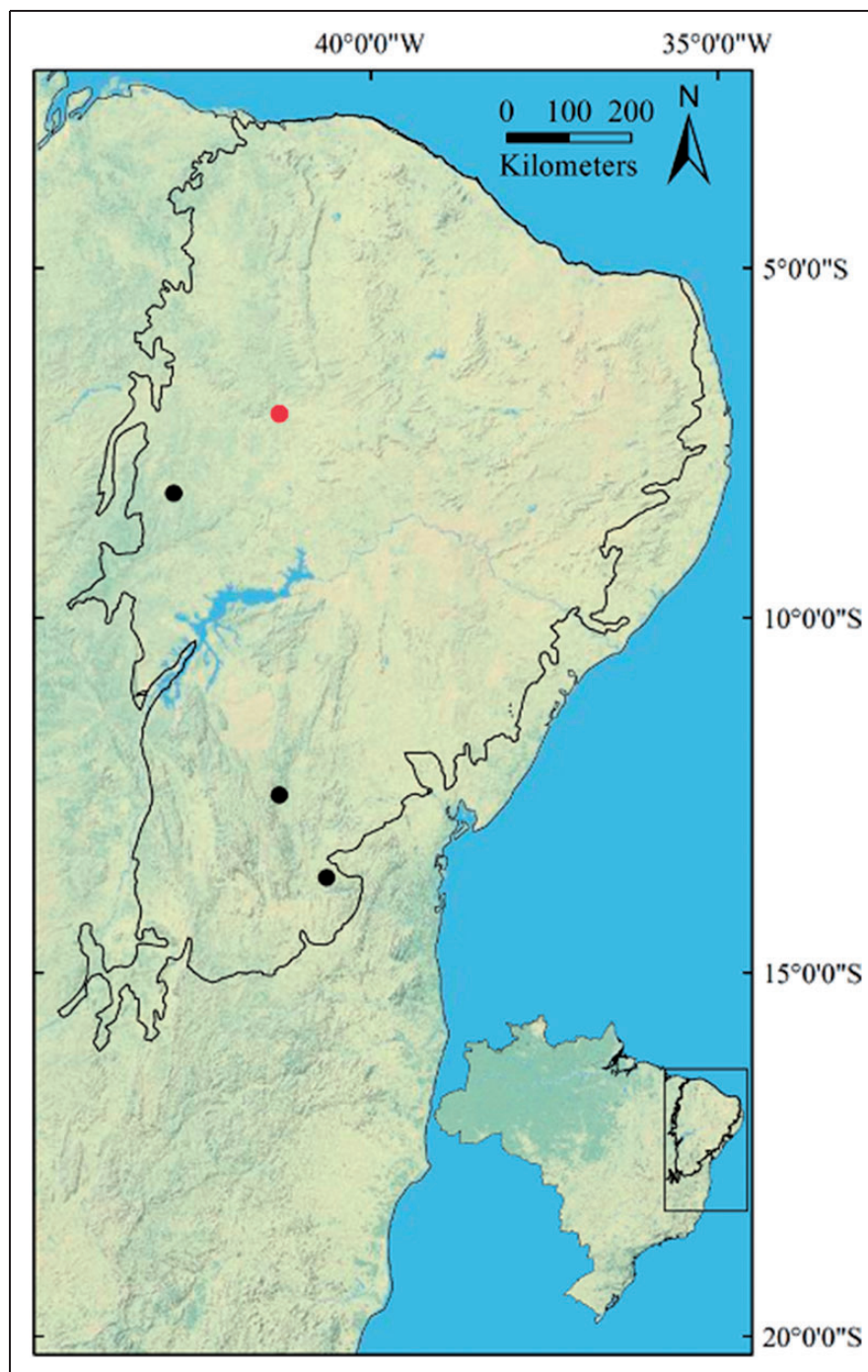


Figure 1. Distribution of *Batrachochytrium dendrobatidis* (Bd) in Caatinga ecoregion, northeastern Brazil. Black dots represent the localities with confirmed occurrence of Bd in the Caatinga. Red dot represents this new record.

Other records with confirmed Bd occurrence in the Caatinga are at the municipality of Maracás, Bahia, which is the type locality of at least 11 species and nowadays is seriously degraded (Camardelli & Napoli, 2012), and at the Parque Nacional Chapada

Diamantina, which is also a priority area for conservation with high anuran species richness (31 species) and endemism (Magalhães et al., 2015). Thus, Bd presence in these areas with endangered species and a high number of endemisms with limited geographical

distributions may have serious impacts on Caatinga amphibian fauna.

Distribution models of the Bd fungus predict a low probability of its occurrence in the Caatinga ecoregion (Becker, Rodriguez, Lambertini, Toledo, & Haddad, 2016; James et al., 2015; Rödder et al., 2009; Ron, 2005). Recent studies reported lower Bd prevalence (approximately 2%) when compared with other Brazilian ecoregions (see Carvalho et al., 2017). This fact is due to the unfavorable environmental conditions of the Caatinga ecoregion for fungi development, such as high temperatures, low rainfall, and low humidity (James et al., 2015). Bd fungus grows and reproduces between 4°C and 25°C, presenting optimum growth between 17°C and 25°C and 50% mortality rate and decreased growth in cultures exposed to 30°C (Piotrowski, Annis, & Longcore, 2004). In addition, to the chytrid fungus complete its life cycle, at least one body of water is required for the swimming zoospore (aquatic phase) to find a new host, a process hampered by the marked seasonal dryness of the Caatinga (see James et al., 2015). Annual mean temperature above the optimum range for Bd, in addition to a maximum daily temperature often above 30°C and a marked seasonal drought, could make the Caatinga an amphibian refugee against Bd in Brazil.

The chytrid fungus infects the keratinized epidermal layers of adult amphibians and synchronizes the release of zoospores with amphibian skin desquamation (see Berger, Hyatt, Speare, & Longcore, 2005). Higher temperatures (i.e., >25°C) increase the rate of epidermal renewal (Piotrowski et al., 2004), which may result in the loss or reduction of Bd infection. This way, the fungus does not have enough time to complete its life cycle before being shed with the epidermal layer (Berger et al., 2004; McDonald, Mendez, Müller, Freeman, & Speare, 2005). Therefore, high temperatures can modify the physiology of amphibians, negatively affecting the reproductive success of Bd (Piotrowski et al., 2004).

Despite the potential unfavorable conditions for Bd growth and transmission, here we report Bd prevalence in the Caatinga at least 10 times higher than expected (Carvalho et al., 2017). In addition, Ohmer et al. (2019) showed that the rate of epidermal renewal of amphibians varies across phylogenetic groups and that the Bufonidae family (mostly *Rhinella* species) presents the longest interval (between 7 and 20 days) among all families examined. By testing the effect of temperature on the rate of epidermal renewal, Meyer, Cramp, Bernal, and Franklin (2012) reported that individuals of *Rhinella marina* showed a desquamation interval of 15 days when exposed to a temperature range of 10°C to 20°C and that this interval was reduced to 8 days when exposed to 20°C and 30°C the range. Thus, even if accelerated by high temperatures, the long interval between desquamations expected for the genus *Rhinella*

could provide sufficient time for Bd to complete its cycle, which varies between 4 and 5 days (Berger et al., 2005).

Despite the high prevalence of Bd reported here, there are no records of *R. granulosa* and *R. jimi* population declines associated with chytridiomycosis (Carvalho et al., 2017; Scheele et al., 2019). Possible presence of anti-Bd bacteria, as found in other species of the Bufonidae family, may contribute to the control of the infection burden, allowing these species to coexist with the pathogen (Park, Collingwood, St-Hilaire, & Sheridan, 2014). Herein, *Rhinella granulosa* and *R. jimi* may be acting as Bd reservoir species during the dry seasons of the Caatinga. Future studies should address this question by sampling additional species and assessing the seasonal variations in Bd prevalence and infection intensity.

Our results indicate a high prevalence of Bd in the northeastern semiarid region and the possible role of amphibians with terrestrial habits acting as Bd reservoirs. Interestingly, the Bd prevalence observed in our study on these Caatinga species is similar to the highest prevalence reported in the Brazilian Atlantic forest (Carvalho et al., 2017). However, our results are still preliminary and limited, which reduces our ability to estimate the impact of Bd in this tropical dry area (i.e., Caatinga). Also, though most of the Bd-induced amphibian declines in the world have been more severe in species from wet regions (e.g., Neotropics) and strongly related to perennial aquatic habitats (Scheele et al., 2019), here we present a relatively high Bd persistence of an aquatic fungus in a dry area. The possibility of Bd spreading in semiarid areas and reaching susceptible species during rainy seasons should be taken into account for the development of conservation plans. Future efforts should be redirected to the Caatinga ecoregion, increasing the number of study species in different seasons to understand the temporal/space dynamics of Bd on this ecoregion and avoid possible outbreaks of chytridiomycosis.

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