New records of Batrachochytrium dendrobatidis in the Atlantic forest of Northeastern Brazil

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Abstract. The pathogenic chytrid fungus Batrachochytrium dendrobatidis threatens the amphibian diversity at global scale. Three different strains of B. dendrobatidis have been already found in Brazil, the country with the greatest diversity of anurans worldwide, and it is widely spread in the Atlantic rainforest. Here, we report the occurrence of B. dendrobatidis for the state of Alagoas, the second record from northeastern Brazil. We describe for the first time B. dendrobatidis infection in Aplastodiscus sibilatus, a Brazilian tree frog species. The impact of B. dendrobatidis on populations of Brazilian anurans is unknown and information on spatial and taxonomic distribution of this fungus in Brazil is urgently needed for the development and implementation of amphibian conservation action plans.

Key words: Chytrid fungal pathogen, Aplastodiscus sibilatus, Atlantic rainforest, state of Alagoas.

The worldwide decline in amphibian populations is one of the most striking features of the global biodiversity crisis (Houllahan et al. 2000) although untangling the multiple causes for this phenomenon has proved challenging (Collins & Storfer 2003). As with many other taxa, habitat loss has driven many amphibian populations to extinction (Stuart et al. 2004, Wake & Vredenburg 2008). These effects have been compounded by a range of other factors such as pollution, climate change, ultraviolet radiation, animal trade, invasive species and infectious diseases (Stuart et al. 2004, Young et al. 2004, Blackburn et al. 2010).

The latest contemporary threats to amphibian populations to be identified is the emergence of Chytridiomycosis, a fungal disease caused by infection of Batrachochytrium dendrobatidis (Bd) in keratinized tissues of both adult amphibians and larvae (Berger et al. 1998, Longcore et al. 1999). This disease causes hyperkeratosis, hyperplasia of the skin and disturbs the skin osmoregulatory function and may lead to the host death (Rosenblum et al. 2008, Voyles et al. 2009). Due to its enormous geographical distribution, Bd is recognized as global threat to amphibian diversity and is thought to be the main cause of enigmatic declines and extinctions of populations of amphibians in pristine and well-protected areas (Berger et al. 1998, Daszak et al. 2003, Skerratt et al. 2007, Lips et al. 2008, Wake & Vredenburg 2008). Bd thrives in moist habitats such as tropical rainforests (Lips et al. 2008, Woodhams et al. 2008), biomes that are also noted for their high diversity of amphibians (Duellman 1999).

In Brazil, three Bd strains have been already genotyped (Schloegel et al. 2012, Vieira et al. 2012) and 41 anuran species have been found infected by Bd (Carnaval et al. 2006, Toledo et al. 2006a, Toledo et al. 2006b, Sluys et al. 2007, Gründler et al. 2012, Vieira et al. 2012). So far, records of Bd in Brazil have been almost exclusively restricted to south and southeastern portions of the Atlantic rainforest - there is a single record of Bd from the northeast of the country (municipality of Jaqueira, state of Pernambuco; Carnaval et al. 2006) and an apparent distributional gap of ca. 1,700 km in the Brazilian distribution of Bd. Here, we describe the second record of B. dendrobatidis in the northeast of Brazil (and the first for Alagoas state) and report for the first time susceptibility of Aplastodiscus sibilatus to Bd infection.

On May 2012, tadpoles of Hypsiboas freicanecae (n=7) and Aplastodiscus sibilatus (n=17) were collected with a dip net at the bottom of a permanent stream (approximately 20 cm depth) at Mata da Bananeira, Estação Ecológica de Murici (ESEC Murici) (Murici, Alagoas state, 9°12'00" S
Batrachochytrium dendrobatidis in Alagoas

Figure 1. Batrachochytrium dendrobatidis diagnosis by macroscopical (lack of keratin on the jaw sheaths) and histological assessments of tadpole mouthparts of Aplastodiscus sibilatus (A, C) and Hypsiboas freicanecae (B, D) collected in Murici (Alagoas, Brazil). Arrow indicates B. dendrobatidis sporangia (C, D).

35°52’00” W, DATUM WGS84; 551 m elev.). The tadpoles were preserved in 10% neutral buffered formalin and analyzed under a stereomicroscope (Leica MZ6). All the tadpoles were found to lack keratin (in different degrees) in their mouthparts. To confirm the presence of Bd, a tadpole of each species was subjected to histological assessment following the protocol of Berger et al. (1998).

Detailed histological analysis confirmed that the collected tadpoles of H. freicanecae and A. sibilatus were infected by Bd. In addition to the lack of keratin in oral structures observed during macroscopical examinations, we also observed a lack of structural organization in the epithelium and the presence of zoosporangia of Bd during histological assessment (Fig. 1). All specimens were deposited at Coleção Herpetológica do Museu de História Natural da Universidade Federal de Alagoas, Brazil (MUFAL 10779 for Hypsiboas freicanecae, and MUFAL 10780 for Aplastodiscus sibilatus). This is the first report of infection of the tree frog Aplastodiscus sibilatus by Bd.

This report represents the first published record of Bd for the state of Alagoas and it extends Bd distribution 65 km southeast from the northernmost record of Bd in Pernambuco state (Hypsiboas freicanecae at Jaqueira municipality; Carnaval et al. 2006) and 1,650 km north from the closest southern record in Rio de Janeiro state (Bokermannohyla circumdata at Petrópolis municipality; Toledo et al. 2006a, Fig. 2).
The presence of *Bd* in ESEC Murici, a State Park of ca. 60 km², should be of particular concern because this area represents the largest rainforest fragment of Alagoas state (Assis 2000), and contains several poorly known anuran species such as *Crosodactylus dantei*, *Phylodryas gyrinaethes*, *Physalaemus caete*, *Scinax muriciensis* and *Agalychnis granulosa*. The latter species is considered threatened according to the Red List of Brazilian fauna (Haddad 2008).

*Batrachochytrium dendrobatidis* has been found in more than 200 species worldwide (Skerratt et al. 2007). In Brazil, *Bd* has been there since at least 1981 (Toledo et al. 2006a); it appears widespread in the highly fragmented Atlantic rainforest biome, it has already been found in over 40 species and in 18 of the 27 federal states (Carnaval et al. 2005, Carnaval et al. 2006, Toledo et al. 2006a, Toledo et al. 2006b, Sluys et al. 2007, Gründler et al. 2012, Vieira et al. 2012). This is very worrying, as in a recent work, Schloegel et al. (2012) identified a novel lineage of *Bd* (Bd-Brazil) in the Atlantic rainforest of Brazil that is associated with the global trade in bullfrogs, is capable of sexual reproduction, and is also detected on bullfrogs introduced into Japan. Given the small sample size of this report, much more extensive sampling will be required to provide sufficiently detailed taxonomic, spatial and genetic information about *Bd* in northeastern Brazil to allow strategic conservation planning.
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