

Batrachochytrium dendrobatidis in near threatened and endangered amphibians in the southern Brazilian Atlantic Forest

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Nearly a third (32%) of the world's amphibian species are either threatened with extinction or already extinct (Stuart et al. 2004, IUCN 2014). Rapid population declines have been reported in many parts of the world (Blaustein et al. 2002, IUCN 2014, Stuart et al. 2004, Vredenburg et al. 2010) and one of the main causes is the chytridiomycosis, an infectious disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al. 1998). Currently, there are few studies that focus on the prevalence of *Bd* on threatened amphibians, and policies to minimize negative impacts on at-risk species due to *Bd* are restricted (Stuart et al. 2004). In this study, we investigated the presence of *Bd* in near threatened and endangered amphibian species in the southern portion of the Atlantic Forest biome.

We sampled anurans in a stream located within a seasonal forest fragment in the state of Santa Catarina, Brazil (26°45'03.02" S and 53°29'12.22" W, datum SAD69, 630 m above sea level; Fig. 1) from October 2012 to January 2013, months which coincide with higher temperatures and rainfall (i.e., spring and summer).

We used survey at breeding sites as a sampling technique (Crump & Scott 1994). Captured individuals were sampled by gentle sterile swab on the ventral surface of the skin, including hands and feet, thighs, abdomen (Hyatt et al. 2007) and mouth. All individuals were inspected for clinical signs of chytridiomycosis, including low body mass, lethargic behavior, and visible paleness (Berger et al. 2009).

Detection and quantification of *Bd* on sampled individuals were performed by real-time qPCR assay (Lambertini et al. 2013). Prevalence data were obtained by dividing the number of positives by the total of analyzed individuals, and the infection load by the number of genomic equivalents obtained as a result of the qPCR reactions, resulting in the number of zoospores genomic equivalents for each individual (Boyle et al. 2004). We considered threatened a species that was classified as

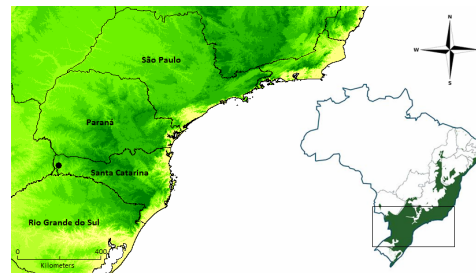


Figure 1. Sampling site in São Miguel do Oeste, state of Santa Catarina, southern Brazil.

such in at least one of the following lists: the international list of threatened species (IUCN 2014), the list of endangered species of the state of Santa Catarina (CONSEMA 2011).

Twelve adult frogs were sampled, including *Hypsiboms curupi*, *Crossodactylus schmidtii*, and *Proceratophrys bigibbosa*. Of these species, *C. schmidtii* is classified as critically endangered (CONSEMA 2011), *H. curupi* as endangered (CONSEMA 2011) and *P. bigibbosa* as nearly threatened (IUCN 2014).

None of the infected individuals exhibited clinical signs of chytridiomycosis. The infection load varied from 70 to 6,168 zoospores in infected individuals. Among this three species, *Proceratophrys bigibbosa* had the highest zoospore load (Table 1).

This is the first record of *Bd* infection in endangered or near threatened species in subtropical Atlantic Forest amphibians. The presence of *Bd* in amphibians from Santa Catarina has been previously recorded in the eastern portion (Toledo et al. 2006) and western (Rodríguez et al. 2014) portion of the state. However, our results indicate that *Bd* is more broadly distributed in the state and may occur in different vegetation types.

Table 1. Endangered or near threatened species according to recent classification lists, as well as the prevalence, and infection load – as mean zoospore genomic equivalents \pm standard deviation (number of individuals, range).

Family/Species	Prevalence (%)	Mean \pm SD	n	Range	
				Min \pm SD	Max \pm SD
Hylidae / <i>Hypsiboas curupi</i>	83.3	1007.05 \pm 1021.81	6	70.66	2520.49
Leptodactylidae / <i>Crossodactylus schmidti</i>	100	221.63	1	–	–
Odontophrynidae / <i>Proceratophrys bigibbosa</i>	40	2930.58 \pm 3008.76	5	2401.23	6168.89

The species recorded in this study are restricted to forest streams (Haddad et al. 2013). As such, their extinction risk is higher than for other generalist species (e.g., Hero et al. 2005, Williams & Hero 2001). The high degree of fragmentation in the interior of the Atlantic forest is currently the main threat to the conservation of these species, and may result in the isolation of populations and in a consequent decrease of genetic variability, as well as an increased vulnerability to disease (Ribeiro et al. 2009).

There are several reports of *Bd* causing infections in amphibians inhabiting streams (Lips et al. 2003, La Marca et al. 2005), which suggest that such populations are more frequently infected (Lips 1999, Lips et al. 2003). This is because *Bd* persists longer in humid environments, even exhibiting a resting stage (Di Rosa et al. 2007), but it is unable to survive to a prolonged desiccation (Johnson & Speare 2003, Piotrowski et al. 2004, Lips et al. 2006). The prevalence of *Bd* is historically linked to this type of environment because the canopy cover and water depth are typically greater than many other environments, buffering the exposure to temperatures higher than the *Bd* optimum temperature (Piotrowski et al. 2004, Van Sluys & Hero 2009, Raffel et al. 2010, Becker et al. 2012, Rodriguez et al. 2014).

Infection prevalence can be related to host reproductive mode and/or the environment, and environmental conditions such as landscape can exert strong effects on host-pathogen dynamics (Longo et al. 2010, Cheng et al. 2011, Whitfield et al. 2012). According to our results, the sampled anuran populations may not be in immediate danger of decline due to chytridiomycosis, as we did not detect infection loads above 10,000 zoospores (Vredenburg et al. 2010, Kinney et al. 2011).

In addition to creating new and updated records of *Bd* infection occurrence, understanding the effects of environmental variables on disease dynamics is crucial for anuran conservation. This is particularly important for endangered species and those in areas where amphibian diversity is poorly

known, such as those in the southern Brazilian Atlantic forest. Information on species in need for urgent conservation action, such as listed endangered species, may subsidize public policies, making them more effective for conservation and management of biological diversity (Langhammer et al. 2007).

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