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Amphibian Chytrid Fungus Broadly Distributed in the Brazilian Atlantic Rain Forest

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Abstract: To investigate the occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* in Brazil, we conducted histological screenings of 96 preserved specimens of anurans collected at 10 sites in the Atlantic rain forest. Data show this fungus to be widely distributed. Infected specimens included *Colostethus olfersioides* (Dendrobatidae), *Bokermannohyla gouveai* and *Hypsiboas freicanecae* (Hylidae), as well as *Thoropa miliaris* and *Crossodactylus caramaschii* (Leptodactylidae), extending the area of *B. dendrobatidis* occurrence in Brazil approximately 1,600 km N, 200 km S, and 270 km E. The altitudinal range of the chytrid is broad, spanning from less than 100 m (Estação Ecológica Juréia-Itatins, Reserva Biológica do Tinguá) to about 2,400 m (Parque Nacional do Itatiaia). An infection record dating to 1981 roughly coincides with the time of the first observations of amphibian declines in the country. Widespread occurrence of *B. dendrobatidis* in the Atlantic Forest adds to the challenge of conserving an already endangered biome given the potential risk of further local biodiversity loss. Further research is needed to understand how environmental and genetic factors relate to chytridiomycosis in leading to or preventing local die-offs. Protected sites at mid and high elevations may be particularly threatened, while lowland populations may be functioning as reservoirs. Conservation efforts should also involve monitoring studies and habitat protection.

Key words: *Batrachochytrium dendrobatidis*, Brazil, Atlantic Forest, amphibian decline, museum specimen

INTRODUCTION

Amphibian declines have been tied to climate change, chemical contamination, habitat loss, infectious diseases, overexploitation, species introduction, and ultraviolet radiation; and it is becoming increasingly clear that interactions among some of these factors play a key role in population fate (Beebee and Griffiths, 2005). In pristine areas, declines have been frequently associated with outbreaks of the chytrid fungus *Batrachochytrium dendrobatidis*, which has been diagnosed in Africa, Australia, Europe, North America, Central America, and South America (Berger et al., 1998; Lips, 1999; Longcore et al., 1999; Ron and Merino-Viteri, 2000; Bosch et al., 2001; Green and Sherman, 2001; Hopkins and Channing, 2003; Lane et al., 2003; La Marca et al., 2005).

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B. dendrobatidis is known to kill frogs in laboratory settings, developing in keratinized portions of their skin and in the oral region of tadpoles (Berger et al., 1998). In culture, it can be killed when exposed to temperatures above 30°C (Longcore et al., 1999). Resistance to this fungus varies among amphibian taxa, and species which had formerly declined may currently coexist with the disease (Retallick et al., 2004). There are also known cases of infected species declining at high elevations but not below 400 m of altitude (McDonald and Alford, 1999). Although experimental data are lacking, these observations suggest that the impact of *B. dendrobatidis* is mediated by environmental factors. Climate anomalies have been proposed as triggers of die-offs by providing opportunities for fatal chytrid outbreaks (Burrowes et al., 2004; Pounds and Puschendorf, 2004; McCallum, 2005; Pounds et al., 2006).

Although *B. dendrobatidis* has been implicated in amphibian declines throughout the neotropics (Berger et al., 1998; Lips, 1999; Puschendorf, 2003; La Marca et al., 2005; Mendelson et al., 2005), little is known about its current and historical distribution. In South America, this fungus has been diagnosed from a few localities in Ecuador (Ron and Merino-Viteri, 2000; Merino-Viteri, 2001; Ron, 2005) and single sites in Uruguay (Mazzoni et al., 2003), Venezuela (Bonaccorso et al., 2003), Peru (Seimon et al., 2005), Argentina (Herrera et al., 2005), and Brazil (Carnaval et al., 2005; Toledo et al., 2006). Ecological niche modeling nonetheless predicts *B. dendrobatidis* to be widely distributed, occurring, among other sites, along the entire range of Brazil's Atlantic rain forest (Ron, 2005).

Population declines or extinctions have been suggested to affect more than 30 taxa endemic to Brazil's Atlantic rain forest, and several of these reports were based on the lack of sightings in protected areas regularly visited since the 1970s (Heyer et al., 1988; Weygoldt, 1989; Bertoluci and Heyer, 1995; Guix et al., 1998; Pombal and Haddad, 1999; Izecksohn and Carvalho-e-Silva, 2001; Eterovick et al., 2005; Verdade et al., 2006). Habitat and climate change, acid rain, intraspecific interactions, and inadequate sampling have been suggested as causes of these observations (Heyer et al., 1988; Weygoldt, 1989; Bertoluci and Heyer, 1995; Papp and Papp, 2000; Bertoluci et al., 2005; Verdade et al., 2006). Although chytridiomycosis has never been proposed as a cause of local declines, the single record of *B. dendrobatidis* in Brazil shows that the fungus has been locally present at least since 2004 (Carnaval et al., 2005; Toledo et al., 2006). To provide baseline data on the occurrence of *B. dendrobatidis* in Brazil, particularly in the Atlantic rain forest, we performed histological screenings of preserved amphibian specimens deposited in three Brazilian herpetological collections. This is the first multispecies, multisite study of preserved specimens in the country and one of the few in South America.

Methods

Amphibian specimens were gathered from the following collections: Eugenio Izecksohn (Departamento de Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Brazil; EI), Museu Nacional do Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP); four specimens had been originally deposited at Universidade Estadual de Campinas, Departamento de Zoologia (ZUEC) and later transferred to EI (Appendix 1). To maximize the probability of finding infections, we selected species or areas with reported declines in Brazil (Eterovick et al., 2005), individuals appearing sick or dying upon collection, and tadpoles with mouthpart abnormalities (since these can be caused by chytridiomycosis; Fellers et al., 2001). Throughout the text we use acronyms to refer to the Brazilian states of Espírito Santo (ES), Minas Gerais (MG), Rio de Janeiro (RJ), São Paulo (SP), and Pernambuco (PE).

Standard histological protocols were applied to skin samples harvested from the pelvic region of all specimens (Berger et al., 1999): sections (4 μm thick) were stained with hematoxylin and eosin (H&E), and doubtful infections were confirmed with Grocott's silver-methenamine or periodic acid-Schiff stains. Histological procedures as such have been used routinely to diagnose *B. dendrobatidis* (Berger et al., 1998; Longcore et al., 1999; Burrowes et al., 2004).

Results

Ninety-six specimens belonging to 25 Atlantic Forest amphibian species were screened. Of those, six specimens representing five species tested positive for *B. dendrobatidis* (Fig. 1, Appendix 1). They are *Colostethus olfersioides* (one adult; Reserva Biológica do Tinguá, Nova Iguaçu, RJ), *Thoropa miliaris* (one adult, found dead; Estação Ecológica Juréia-Itatins, Peruíbe, SP), *Hypsiboas freicanecae* (two tadpoles with mouthpart abnormalities; Reserva Particular do Patrimônio Natural Frei Caneca, Jaqueira, PE), *Crossodactylus caramaschii* (one adult, moribund; Apiaí, SP), and *Bokermannohyla gouveai* (one young specimen, found dead; Brejo da Lapa, Itamonte, MG). The oldest record dates to 1981 (*C. olfersioides*) and the most recent is from 2005 (*B. gouveai*). *B. dendrobatidis* has a broad alti-

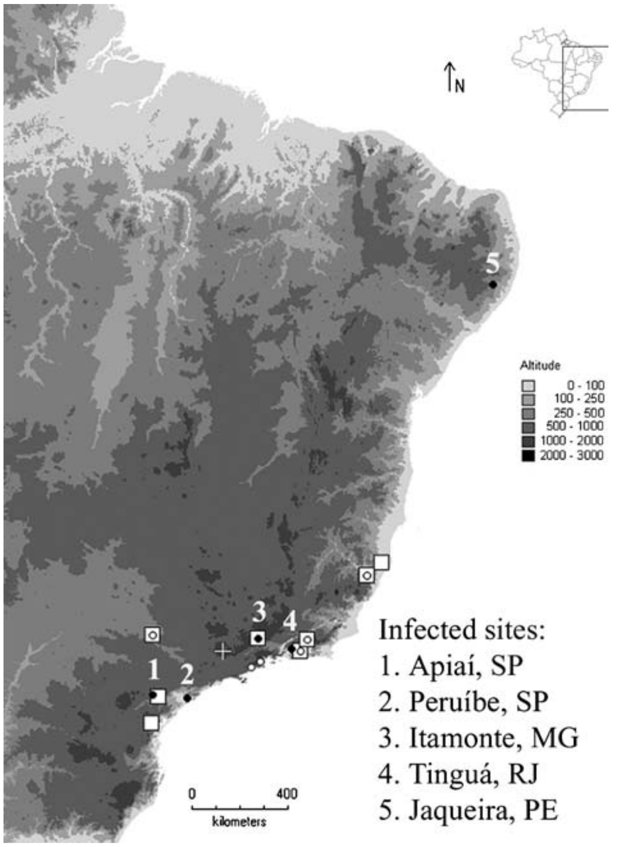

Figure 1. Chytrid screening in Brazil. Squares denote areas with reported declines in the literature. Circles represent sites sampled by this study; sites with infected individuals are depicted in black. Cross denotes single area of known chytrid occurrence prior to this study (Carnaval et al., 2005; Toledo et al., 2006).

tudinal range, occurring from less than 100 m above sea level (Nova Iguaçu, Peruíbe) and mid-altitudes around 600–900 m (Apiaí, Jaqueira) up to approximately 2,400 m (Itamonte). The maximum straight-line distance between positive localities is approximately 2,200 km.

Discussion

Our data show *B. dendrobatidis* to be widespread in the Atlantic Forest, agreeing with Ron's (2005) model inferences. The results extend the distribution of the chytrid approximately 1,600 km N, 200 km S, and 270 km E (Carnaval et al., 2005; Toledo et al., 2006). Although the infected amphibian species differ in their reproductive strategies and time of activity, all of them undergo at least part of their development in streams or flowing water, as discussed below.

C. olfersioides is a diurnal leaf-litter frog whose eggs are deposited in small water depressions; when hatched, larvae are carried to nearby streams (Izecksohn and Carvalho-e-Silva, 2001). No observations exist about the status of the species in Tinguá, which is a lowland site (40–50 m altitude). However, *C. olfersioides* has been reported as missing from Maciço da Tijuca, RJ (approximately 40 km SE, up to 1,200 m; Izecksohn and Carvalho-e-Silva, 2001). In 1988, Weygoldt (1989) observed a decrease in numbers of *C.* cf. *olfersioides* in Santa Teresa, ES (approximately 430 km NE, 600–900 m).

T. miliaris breeds in quarry walls and water flowing over rocky areas (Heyer et al., 1988). There are no records of decline of *T. miliaris* at Juréia-Itatins, located nearly at sea level. However, the infected frog was found dead over a rock in a local stream (Miguel Rodrigues, personal observation). This species was reported to decline at Estação Biológica de Boracéia, SP (approximately 300 km NW, 800–900 m): adults and larvae have not been found since the early 1980s (Heyer et al., 1988; Bertoluci and Heyer, 1995; Bertoluci et al., 2005; Bertoluci and Rodrigues, 2002). *T. miliaris* is listed as extinct in Boracéia but is abundant in other areas of southeastern Brazil (Eterovick et al., 2005).

H. freicanecae is a stream-breeding tree frog known from a single montane site at 600–700 m altitude (Carnaval and Peixoto, 2004). Adults seem to be rare at the type locality and are active at dusk and night (Ana Carnaval and Oswaldo Peixoto, personal observation). Given the high proportion (91%) of tadpoles with abnormal mouthparts collected with the type material, the original description mentioned possible local chytrid occurrence (Carnaval and Peixoto, 2004).

There are no published records of declines in *Cr. caramaschii*. However, many individuals of this diurnal, stream-breeding species seemed to be dying when collected in Apiaí (approximately 900 m above sea level) or died when handled (Guerra-Fuentes and Dixo, unpublished data). Decline records exist for other *Crossodactylus* species in Boracéia (Heyer et al., 1988; Bertoluci and Heyer, 1995); Santa Teresa (Weygoldt, 1989); Paranapiacaba, SP (Verdade et al., 2006); and Campos do Jordão, SP (Vanessa Verdade and Miguel Rodrigues, 2006).

Although no decline records exist for *Bo. gouveai*, the examined specimen was found dead in a water-filled depression next to a stream in the highlands of the Parque Nacional do Itatiaia (Ana Carnaval and Oswaldo Peixoto, personal observation). Other species have been reported to decline and disappear at similar altitudes within this park (Heyer et al., 1988; Guix et al., 1998; Pombal and Haddad, 1999).

The small sample sizes employed in this study preclude detailed analyses of the prevalence of *B. dendrobatidis* in Brazil. The total number of infected specimens (6/96) is considerably smaller than that found in areas of chytrid-driven declines (e.g., Spain, 108/345; Guarner et al., 2005). However, the latter was based on polymerase chain reaction screenings, as opposed to histological diagnoses, which are known to generate false-negative results (Berger et al., 1999; Puschendorf and Bolaños, 2006). Our data are comparable to those of Lips et al. (2003), who performed a nonopportunistic histological screening of Costa Rican amphibian species for which there were records of dead or dying individuals in the field, including sites with reports of population loss. Their reports of 2/8 infected *Atelopus chiriquensis* and of 2/4 *Eleutherodactylus melanostictus* at Las Tablas parallel our findings (*Bo. gouveai* 1/8, *C. olfersioides* 1/1, *Cr. caramaschii* 1/4, *H. freicanecae* 2/4, *T. miliaris* 1/5). Similar to this study, Lips at al. (2003) failed to detect *B. dendrobatidis* in several potentially susceptible species while employing sample sizes of one to eight specimens.

Regarding amphibian crashes in Brazil, the record of 1981 is interesting given that the first observations of unexplained declines in stream-associated populations in the country fall between 1979 and 1987 (Heyer et al., 1988; Weygoldt, 1989; Pombal and Haddad, 1999). Heyer et al. (1988) associated crashes in Boracéia and Teresópolis with the severe and unusual frost of 1979. Weygoldt (1989) discussed the possibility that an extended dry period had led to declines in Santa Teresa, observing that tadpoles collected in 1987 reached metamorphosis only after receiving antibiotic treatment. Our data show *B. dendrobatidis* to be present in Brazil in the early 1980s. When tied to Heyer et al.'s (1988) and Weygoldt's (1989) observations on local climate anomalies, our results are consistent with a hypothesis of a climate-linked epidemic event leading to amphibian declines (Pounds et al., 2006). Although we encountered no infected specimens from Boracéia, Teresópolis, or Santa Teresa, this might be a result of the relatively small number of samples screened per site.

The earliest record of *B. dendrobatidis* in South America dates to December 1980 (from Ecuador; Ron and Merino-Viteri, 2000). This study shows that the pathogen has been present in southeastern Brazil at least since 1981. Given the lack of screenings of specimens collected prior to that time, these years must be interpreted as upper bounds of an arrival date of *B. dentrobatidis* in South America rather than dates of first appearances, in contrast to procedures employed elsewhere (e.g., Weldon et al., 2004). Therefore, they cannot be used in estimates of rates of spread among sites. To date, Canada has the earliest record of *B. dendrobatidis* in the Americas (1961; Ouellet et al., 2005), while the earliest global record comes from South African *Xenopus* collected in the late 1930s. While existing data suggest that *B. dendrobatidis* may be an emergent pathogen spreading globally through a suite of carriers, further studies are needed to confirm if it is indeed novel or if it has existed in the wild for longer times but only recently increased its pathogenicity or host diversity (Rachowicz et al., 2005).

Widespread occurrence of *B. dendrobatidis* in the Brazilian Atlantic rain forest adds to the challenge of conserving an already endangered biome (Câmara, 1992; Myers et al., 2000; IUCN et al., 2004). Due to the temperature tolerance of *B. dendrobatidis*, protected sites at mid and high elevations may be particularly threatened, while lowland populations may be functioning as reservoirs. Because our data show that several Brazilian anuran communities are or have already been exposed to the pathogen, it is crucial to understand how environmental and genetic factors relate to chytridiomycosis in leading to or preventing local die-offs. Conservation efforts should also involve long-term monitoring studies, providing insight on chytrid dynamics, pathogen-host interactions, and effects on population persistence. Moreover, habitat protection is imperative to minimize stress over populations and allow for rebounds.

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Appendix 1. Complete List of Screened Specimens Including Species Name, Locality Data, Year of Collection, Information on Life Stage and Condition, and Voucher Number

Species	Township (locality), state	Year collected	Life stage, observations	Voucher number
Centrolenidae				
Hyalinobatrachium eurygnathum	Itamonte (Rio das Flores), MG	1997	Adult	MNRJ 19384
Hyalinobatrachium eurygnathum	Teresópolis, RJ	1978	Adult	MNRJ 32872
Hyalinobatrachium uranoscopum	Teresópolis, RJ	1978	Adult	MNRJ 32873
Dendrobatidae				
Colostethus olfersioides[a]	Nova Iguaçu (Reserva Biológica do Tinguá), RJ	1981	Adult	MNRJ 38464
Colostethus cf. *capixaba*	Santa Teresa (Sítio dos Boza), ES	1981	Adult	EI 10961
Colostethus cf. *capixaba*	Santa Teresa (Sítio dos Boza), ES	1984	Adult	EI 10964-10966
Hylidae				
Bokermannohyla astartea	Itatiaia (Parque Nacional de Itatiaia), RJ	1982	Adult	EI 10936-10938
Bokermannohyla caramaschii	Santa Teresa (Sítio dos Boza), ES	1983	Adult	EI 9037, 9038, 9041, 9042, 9050, 9066, 9073
Bokermannohyla gouveai	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	1982	Adult	EI ex ZUEC 5251, ex ZUEC 5253
Bokermannohyla gouveai	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	1988	Adult	EI ex ZUEC 6897, ex ZUEC 6901, ex ZUEC 6905
Bokermannohyla gouveai	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	2004	Adult	EI 10974-10975
Bokermannohyla gouveai[a]	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	2005	Young, dead along stream	EI 10980
Hypsiboas freicanecae[a]	Jaqueira (Reserva Particular do Patrimônio Natural Frei Caneca), PE	2001	Tadpole, abnormal mouth	EI 9490/3, 9490/4
Hypsiboas freicanecae	Jaqueira (Reserva Particular do Patrimônio Natural Frei Caneca), PE	2001	Tadpole	EI 9490/1, 9490/2
Hypsiboas latistrigatus	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	2004	Adult	EI 10976
Phasmahyla exilis	Santa Teresa (Sítio dos Boza), ES	1981	Adult	EI 10956-10958, 10963

(Continued)

Appendix 1. Continued

Species	Township (locality), state	Year collected	Life stage, observations	Voucher number
Leptodactylidae				
Crossodactylus caramaschii	Apiaí, SP	2003	Adult, found dead or died with handling	MZUSP 133906-133908
Crossodactylus caramaschii[a]	Apiaí, SP	2003	Adult, found dead or died with handling	MZUSP 133909
Crossodactylus dispar	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	1973	Adult	MNRJ 38455, 38456, 38459, 38460, 38462
Crossodactylus gaudichaudii	Eugênio Lefreve, SP	1952	Adult	MZUSP 11361, 11374, 11384, 11399
Crossodactylus gaudichaudii	Itamonte (Parque Nacional de Itatiaia, Brejo da Lapa), MG	1986	Adult	MNRJ 3868
Crossodactylus gaudichaudii	Rio de Janeiro (Parque Nacional da Tijuca), RJ	1986	Adult	MNRJ 13689, 13693, 31864, 31917
Crossodactylus sp.	Santa Teresa (Sítio dos Boza), ES	1980	Adult	EI 10946-10952
Cycloramphus boraceiensis	Salesópolis (Estação Biológica de Boracéia), SP	1962-63	Adult	MZUSP 23786, 23787
Cycloramphus fuliginosus	Rio de Janeiro (Parque Nacional da Tijuca), RJ	1985	Adult	MNRJ 37070
Cycloramphus fuliginosus	Rio de Janeiro (Parque Nacional da Tijuca), RJ	1986	Adult	MNRJ 32502
Cycloramphus sp.	Paraty, RJ	1982	Adult	EI 10943-10945
Cycloramphus sp.	Santa Teresa (Sítio dos Boza), ES	1980	Adult	EI 10954-10955
Cycloramphus sp.	Santa Teresa (Sítio dos Boza), ES	1981	Adult	EI 10962
Eleutherodactylus sp.	Itamonte (Parque Nacional de Itatiaia, near Brejo da Lapa), MG	2004	Adult	EI 10979
Eleutherodactylus sp.	Itamonte (Parque Nacional de Itatiaia, near Brejo da Lapa), MG	2005	Adult	EI 10977-10978
Holoaden bradei	Itatiaia, RJ	1964	Adult	MZUSP 94455, 94499, 94545, 94559
Hylodes sp.	Paraty, RJ	1982	Adult	EI 10939-10942
Hylodes sp.	Santa Teresa (Sítio dos Boza), ES	1980	Adult	EI 10953
Hylodes sp.	Santa Teresa (Sítio dos Boza), ES	1981	Adult	EI 10959, 10960
Proceratophrys melanopogon	Itatiaia (Parque Nacional de Itatiaia), RJ	1984	Adult	EI 8961-8965
Thoropa miliaris[a]	Peruíbe (Estação Ecológica Juréia-Itatins), SP	1998	Adult, found dead along stream	MZUSP 133905
Thoropa miliaris	Ubatuba, SP	1968	Adult	MZUSP 27158, 27180, 27184, 27195
Thoropa petropolitana	Teresópolis, RJ	1969	Adult	MNRJ 23527, 25946

[a]Infected specimens.

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