

## THE OCCURRENCE OF *BATRACHOCHYTRIUM DENDROBATIDIS* IN BRAZIL AND THE INCLUSION OF 17 NEW CASES OF INFECTION

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**ABSTRACT:** Several studies have associated the chytrid fungus *Batrachochytrium dendrobatidis* with worldwide anuran population declines. Recently, six species have been reported to be infected by chytridiomycosis in Brazil, presenting a wide range of distribution, of about 2,400 km over the Atlantic Forest. However, in a country such as Brazil, information about this disease is still beginning to accumulate. Based on morphological and histological data, we found evidence of *B. dendrobatidis* infection in 16 Brazilian anuran species, members of the families Cycloramphidae and Hylidae. We analyzed tadpoles lacking teeth that were collected from 1964 to 2005, to seek for chytridiomycosis. Our results extend the distribution of the fungus in Brazil ca. 630 km southward (straight-line distance), reaching the southernmost limits of the Atlantic rainforest. We also speculate about its distribution in the Cerrado and Pantanal.

**KEYWORDS:** Chytrid fungus, *Batrachochytrium dendrobatidis*, anurans, Brazil, conservation

### INTRODUCTION

Anuran chytridiomycosis, an emerging infectious disease caused by the fungus *Batrachochytrium dendrobatidis*, has been linked to several amphibian population declines over the globe (Speare and Berger, 2000; Daszak *et al.*, 2003). Although recent studies have shown that the lethality of the fungus can be increased in the presence of optimal environmental conditions (Pounds *et al.*, 2006), many uncertainties about the effects and modes of action of this pathogen remain unsolved (McCallum, 2005). Recently, six anuran species have been reported to be infected by this fungus in the Brazilian Atlantic forest (Carnaval *et al.*, 2005, 2006; Toledo *et al.*, 2006). Although alarming in the first moment, the presence of infected populations does not necessarily imply anuran population decline (e.g., Toledo *et al.*, 2006; Retallick *et al.*, 2004). The insufficient data about the conservation status of Brazilian anurans limit speculations and predictions. We here add original data on the distribution of *B. dendrobatidis* in Brazil and review the present knowledge of infected species over the South American continent.

### METHODS

A total of 41 tadpoles of 28 species were selected for the analysis. Thirty-five tadpoles had been collected in the field and deposited in the Célio F. B. Haddad

anuran collection (CFBH), Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, state of São Paulo, Brazil. The remaining six tadpoles were obtained in a frog farm in Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil. Tadpole screening was not at random. First, we selected cold stream-dwelling tadpoles that had deformed oral discs; these were mainly species of the family Cycloramphidae. Then, we looked for other families and tadpoles that inhabit open areas in the interior of Brazil. Most of the specimens were collected in Atlantic forest sites, from the states of Minas Gerais and Rio de Janeiro down to the state of Rio Grande do Sul (Table 1).

Tadpole histology was performed as described in Toledo *et al.* (2006). Identification of the presence of *Batrachochytrium dendrobatidis* follows the indication provided by Berger *et al.* (2000). Furthermore, it was confirmed by comparisons with previous descriptions, particularly the presence of zoosporangia in four stages of development and the occurrence of colonial morphology in the zoosporangia (Berger *et al.*, 1998, 2000; Longcore *et al.*, 1999, Pessier *et al.*, 1999, Fellers *et al.*, 2001; Toledo *et al.*, 2006).

### RESULTS

Fungal structures consistent with *B. dendrobatidis* in the epidermis adjacent to tooth rows occurred in 20 out of 41 tadpoles, associating 16 species to the myco-

Table 1: Tadpole species surveyed for *Batrachochytrium dendrobatidis* infection in Brazil. Locality, number of screened tadpoles, number of infected tadpoles, year of collection, and voucher number. State acronyms are: MG: Minas Gerais; MS: Mato Grosso do Sul; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SC: Santa Catarina; and SP: São Paulo.

Species	Number of tadpoles analysed (number of individuals infected)	Municipality, State	Year of collection	Voucher numbers (CFBH)
<b>Bufonidae</b>				
<i>Chaunus ictericus</i>	1(0)	Jundiá, SP	1988	9145
<i>Chaunus ornatus</i>	1(0)	Jundiá, SP	1988	9143
<i>Chaunus rubescens</i>	1(0)	São Roque de Minas, MG	2005	9248
<b>Cycloramphidae</b>				
<i>Hylodes dactylocinus</i>	1(1)	Peruíbe, SP	2004	9054
<i>Hylodes meridionalis</i>	1(1)	Lauro Müller, SC	1999	12135
<i>Hylodes meridionalis</i>	1(1)	São Francisco de Paula, RS	1998	12134
<i>Hylodes perplucatus</i>	1(1)	São Bento do Sul, SC	1998	12137
<i>Hylodes phyllodes</i>	1(1)	São Luiz do Paraitinga, SP	2004	12161
<i>Hylodes</i> sp. (aff. <i>sazimai</i> )	1(1)	São Luiz do Paraitinga, SP	2005	12165
<i>Megaelosa</i> cf. <i>boticariana</i>	2(2)	Caçapava, SP	2002-2003	6292; 6293
<i>Megaelosa goeldii</i>	2(0)	Teresópolis, RJ	1964	12160
<i>Megaelosa massarti</i>	2(2)	Santo André, SP	1990	9055; 12140
<i>Megaelosa</i> sp.	1(0)	Pindamonhangaba, SP	2001	9044
<i>Proceratophrys boiei</i>	1(0)	Treviso, SC	2005	11079
<i>Thoropa taophora</i>	1(1)	Ubatuba, SP	1998	9041
<b>Hylidae</b>				
<i>Aplastodiscus callipygius</i>	2(1)	Camanducaia, MG	2002	6596
<i>Aplastodiscus</i> cf. <i>leucopygius</i>	2(2)	São Luiz do Paraitinga, SP	2004	12162; 12164
<i>Bokermannohyla hylax</i>	1(1)	São Luiz do Paraitinga, SP	2005	12163
<i>Bokermannohyla circumdata</i>	1(1)	Petrópolis, RJ	2005	12136
<i>Hypsiboas albopunctatus</i>	1(1)	Rio Claro, SP	1998	9114
<i>Hypsiboas semilineatus</i>	1(1)	Camanducaia, MG	1995	9069
<i>Phrynomedusa</i> cf. <i>marginata</i>	1(1)	São Luiz do Paraitinga, SP	2004	12158
<i>Scinax albicans</i>	1(1)	Petrópolis, RJ	2005	12138
<b>Leptodactylidae</b>				
<i>Leptodactylus labyrinthicus</i>	1(0)	Rio Claro, SP	2001	9084
<i>Leptodactylus chaquensis</i>	1(0)	Corumbá, MS	2000	9098
<i>Leptodactylus podicipinus</i>	1(0)	Corumbá, MS	2001	9105
<b>Microhylidae</b>				
<i>Chiasmocleis leucosticta</i>	1(0)	Ribeirão Branco, SP	1994	9149
<i>Elachistocleis ovalis</i>	1(0)	Rio Claro, SP	1999	9148
<b>Ranidae</b>				
<i>Lithobates catesbeianus</i>	2(0)	São Luiz do Paraitinga, SP	2004	12159
<i>Lithobates catesbeianus</i>	6(0)	Jaboticabal, SP	2005	–

sis (Table 1; Fig. 1). Different stages of *B. dendrobatidis* were identified; an early phase containing a central spherical basophilic mass, a zoospore-filled phase, empty spherical zoosporangia with internal septa, as well as a later stage where the empty zoosporangium had collapsed into an irregular shape (Fig. 2E and 2F). Zoosporangia were present at greater density in the areas of the epidermis of tooth rows and jaw sheaths that showed the most abnormal histology, consisting of hyperkeratosis (Fig. 2C and 2D) and loss of superficial epidermis (Fig. 2B and 2C). Some tadpoles presented remains of keratinized denticles (Fig. 2D), but in most cases these structures were com-

pletely destroyed. Digestive systems of tadpoles were full independently of the absence of keratin in their mouthparts.

## DISCUSSION

Stream-dwelling anurans, such as the cycloramphids and hylids surveyed in the present study, seem to be especially vulnerable to *B. dendrobatidis* infection, since all but one specimen examined were infected. Rather than implicating a phylogenetic effect, this might reflect the habitat of these species, which generally corresponds to cold streams in closed for-



Figure 1: Distribution of *Batrachochytrium dendrobatidis* in Brazil. Dark circles indicate infected populations reported by in the present study; gray circles indicate infected populations reported by Carnaval *et al.* (2006); the gray triangle is the same locality (Camanducaia, MG) reported by Toledo *et al.* (2006) and in the present study; and the white circle is the same locality (Peruíbe, SP) reported by Carnaval *et al.* (2006) and in the present study.

ests (e.g., Heyer *et al.*, 1990; Pombal Jr. and Haddad, 2005). This habitat seems to be the most probable to find *B. dendrobatidis* (e.g., Lips *et al.*, 2005; Carnaval *et al.*, 2006; Toledo *et al.*, 2006), even though this fungus can also be found in open area ponds (Herrera *et al.*, 2005; present study). Nevertheless, our results are consistent with the previous predictions made for South America, especially that the fungus

may be spread over the Cerrado and Pantanal (see Ron, 2005).

The observation of *B. dendrobatidis* in a tadpole of *Thoropa miliaris* collected in Ubatuba, state of São Paulo, provides the second record of an infected population of this species (for the record for Perúíbe, state of São Paulo, see Carnaval *et al.*, 2006). Carnaval *et al.* (2006) also surveyed tadpoles from

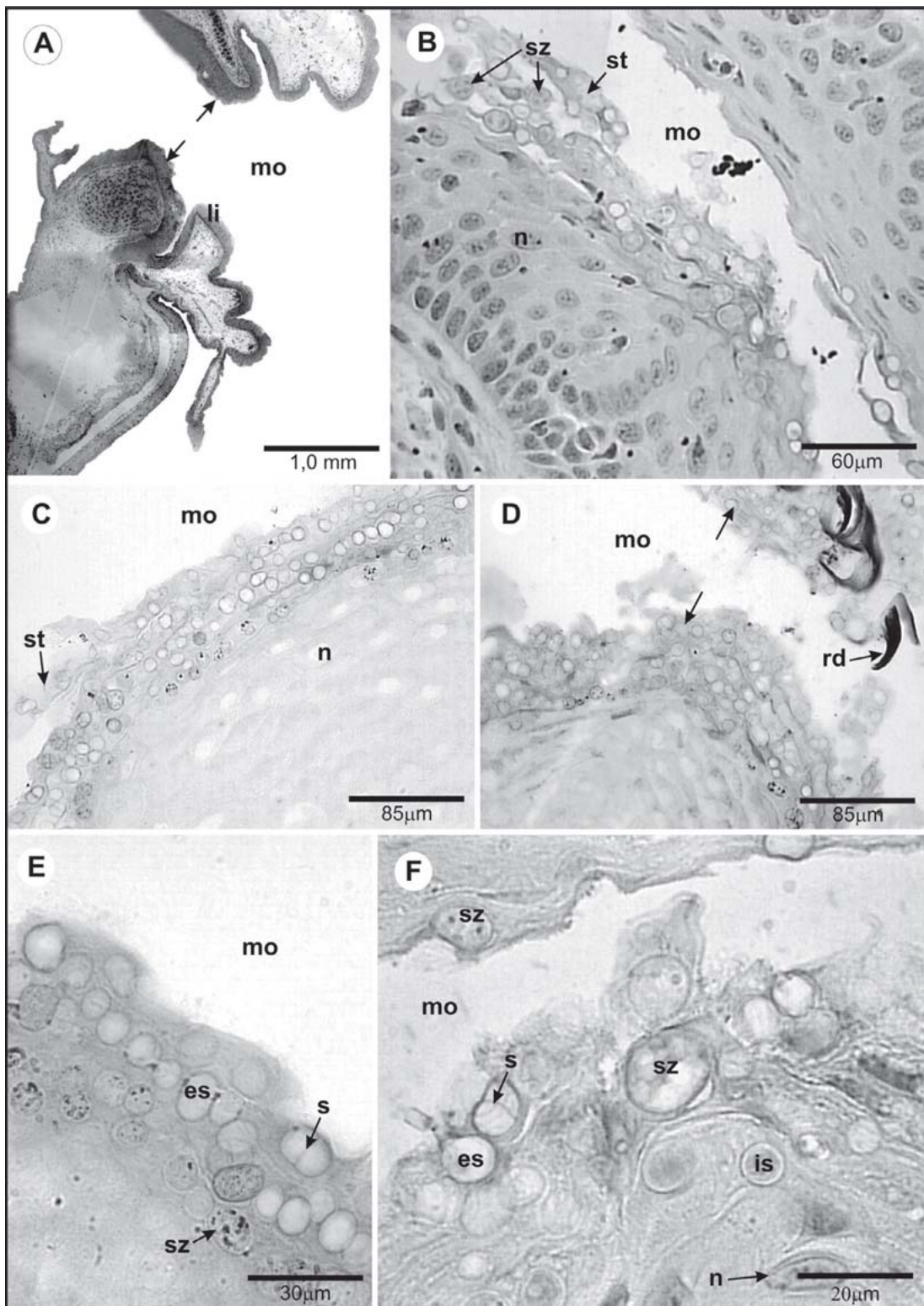


Figure 2: (A) General view of the tadpole mouth of *Hylodes meridionalis*, showing the main area of infection (arrows) by *B. dendrobatidis*; (B) Section of the mouth from a heavily infected tadpole of *Hypsiboas albopunctatus* showing sloughed tissue; (C) Section of the mouth of a heavily infected tadpole of *Hylodes perplicatus* showing sloughed tissue; (D) Mouth of *Scinax albicans* showing the remaining keratinized denticle near infected areas (arrows); (E) Mouth of *H. meridionalis* showing the different developmental phases of *B. dendrobatidis*; (F) Detail of a mouth of *H. albopunctatus* showing the different developmental phases of *B. dendrobatidis*. Samples in figures A, B and F were stained with haematoxylin and eosin; samples in figures C, D and E were stained with PAS technique. (es – empty sporangium; is – immature sporangium; li – lips; mo – mouth opening; n – nucleus of the epidermic cells; rd – remaining keratinized denticle; s – internal septum; st – sloughed tissue; sz – sporangium with zoospores).

Ubatuba; however, they did not find any infected specimen. The tadpoles used in that study were from 1968 and ours from 1998, therefore it may indicate that *B. dendrobatidis* appearance in this part of the Brazilian coast was after 1968. However, this hypothesis should be considered carefully, because of the reduced sample size in both studies (the present and in Carnaval *et al.*, 2006) and due to the possibility of false negatives caused by the limited sensitivity of histological techniques (see Puschendorf and Bolaños, 2006).

Besides extending the actual distribution of *B. dendrobatidis* southward to São Francisco de Paula in the state of Rio Grande do Sul, located about 630 km (straight-line distance) from the previous southernmost record (Carnaval *et al.*, 2006), our findings agree with Ron (2005) in providing additional evidence that the fungus may be highly widespread among Brazilian anurans. Although we have selected which species to screen for infections, the high percentage of infected specimens (nearly 60%) is a strong evidence that the real number of infected species and populations in Brazil is very large. Furthermore, the observation of *B. dendrobatidis* infections in species that typically breed in open areas and lentic waters such as *Hypsi-boas albopunctatus* (present study) and *Leptodactylus ocellatus* (Herrera *et al.*, 2005) (Bastos *et al.*, 2003 and Prado *et al.*, 2002, respectively), suggest that *B. dendrobatidis* may be able to spread over the Cerrado and Pantanal biomes as well. These biomes are inhabited both by *H. albopunctatus* and *L. ocellatus* (Frost, 2006), and were predicted to be occupied by *B. dendrobatidis* in a recent modeling study (Ron, 2005).

This large distribution does not support the hypothesis that the chytrid fungus is being introduced by alien species such as *Lithobates catesbeianus* and/or *Xenopus laevis* (e.g., Hanselmann *et al.*, 2004; Weldon *et al.*, 2004; Rachowicz *et al.*, 2005) in the biomes of Brazil. These species may be found in several localities throughout the country (e.g., Borges-Martins and Di-Bernardo, 2002; Instituto Hórus, 2006; present study), but not in all of the localities where

*B. dendrobatidis* was found (pers. obs.). Although restricted by the small sampling effort, the lack of fungus infection, both in captivity and free living populations of *L. catesbeianus* (present study), brings uncertainties about the hypothesis of alien species as pathogen carriers in Brazil.

The pathologic effects of *B. dendrobatidis* in Brazilian anuran populations are poorly known. Carnaval *et al.* (2006) found the presence of *B. dendrobatidis* in dead animals encountered in the field. We found *B. dendrobatidis* in tadpoles with unkeratinized oral discs (Toledo *et al.*, 2006; this study), which may be strongly related to the infection (see Knapp and Morgan, 2006). However, we do not know whether infected tadpoles can survive after metamorphosis, as observed in other species (e.g., Berger *et al.* 1998, 1999), or whether infected tadpoles are affected by the lack of keratin in the oral region as proposed by Toledo *et al.* (2006). As there is a lack of data on population dynamics over long periods for the present species we can not infer population declines from our data.

We still lack good samples of infected species and information about the distribution of *B. dendrobatidis* in very large Brazilian biomes such the Cerrado and Pantanal. Even in the Atlantic forest our knowledge is still limited. The same lack of information is valid for South America as a whole, where only about 20 species were reported to be infected in six countries (see Hanselmann *et al.*, 2004; Herrera *et al.*, 2005; Seimon *et al.*, 2005; and additional references in Ron, 2005). This number is certainly underestimated and further screening for chytridiomycosis must be considered urgent, especially in unexplored biomes and countries.

As future steps in the improvement of our knowledge on *B. dendrobatidis* infection in South American anurans we propose:

- 1) A historical search for the presence of chytrid fungus in South America, by examining material from scientific collections. The oldest report for South America is from 1980 reporting on the infection in a bufonid from Ecuador (Ron and Merino-Viteri, 2000) and the oldest known presence of *B. dendrobatidis* in Brazil is 1981 in an anrobatid (Carnaval *et al.*, 2006). However, the presence of the fungus in Brazil can be older than the eighties, and further surveys are needed to look for older reports;
- 2) The search for *B. dendrobatidis* in Brazilian biomes such as the Cerrado and Pantanal, which have been predicted to have cases of infection (Ron, 2005; present study);
- 3) The search for *B. dendrobatidis* in unexpected biomes, such as the Brazilian Amazonia, which has

been predicted not to have cases of infection (Ron, 2005);

- 4) The study of effects of *B. dendrobatidis* infection in natural and laboratory populations associated with long term studies, monitoring infected populations to evaluate possible population declines caused by this mycosis.

The results of these studies may help to prevent population declines, improving conservation actions in Brazil and South America.

### RESUMO

Diversos estudos têm associado o fungo quitrídio, *Batrachochytrium dendrobatidis*, ao declínio de diversas populações de anfíbios ao redor do mundo. Recentemente, a presença desse fungo foi relatada em seis espécies de anuros da mata atlântica no Brasil, apresentando uma distribuição de cerca de 2.400 km. Ainda, em um país megadiverso como o Brasil, o conhecimento sobre a epidemia encontra-se em estado incipiente. Sendo assim, buscamos sinais de infecção do fungo em girinos coletados entre 1964 e 2005 que apresentavam falta de queratina nos dentículos. Baseado em dados morfológicos e histológicos nós encontramos evidências de *B. dendrobatidis* em 16 espécies de anuros brasileiras, membros das famílias Cycloramphidae e Hylidae. Estendemos cerca de 630 km a distribuição do fungo no Brasil até o limite meridional da Mata Atlântica e especulamos sobre a distribuição do fungo no Cerrado e Pantanal.

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