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Source: South American Journal of Herpetology, 33(1) : 24-33

Published By: Brazilian Society of Herpetology

URL: <https://doi.org/10.2994/SAJH-D-22-00032.1>

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South American Journal of HERPETOLOGY

VOLUME 33 | DECEMBER 2024



SOCIEDADE BRASILEIRA DE
HERPETOLOGIA

Detection of the fungus *Batrachochytrium dendrobatidis* in anurans from the semiarid region of Brazil: new infection records for endemic species

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Abstract. Chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), has affected amphibians globally since the 1970s, being considered the main cause of their decline worldwide. The present study is the first to assess the presence of *Bd* in anuran populations in dry and wet tropical forests in Ceará state, northeastern Brazil. Samples were collected in different environmental landscapes throughout the dominant dry forests of the Caatinga and the relict moist tropical forest (*brijos de altitude*) in the localities of the Monte Alegre Private Natural Heritage Reserve, Ibiapaba Plateau, Serra de Maranguape, Araripe Plateau, Farias Brito, and Campos Sales. The dry forests of Caatinga harbor amphibian species typical of open areas, while relict moist tropical forests possess endemic and potentially threatened species. The Caatinga is a peculiar Brazilian biome that has been neglected in scientific research and conservation efforts, highlighting the importance of our study. Anuran skin samples were collected from adults using swabs, and DNA was extracted and amplified by PCR using *Bd*-specific primers. In total, 125 samples were analyzed, comprising 28 species of eight anuran families, with 20 (71%) of the sampled species testing positive for *Bd*. This is the first record on *Bd* infection for *Adenomera juikitam*, *Boana raniceps*, *Dendropsophus nanus*, *D. soaresi*, *Leptodactylus troglodytes*, *L. vastus*, *Physalaemus cicada*, *Pristimantis relictus*, *Proceratophrys ararype*, *Pseudopaludicolamystacalis* and *Scinax x-signatus*. Particularly the *Bd*-positive species *Proceratophrys ararype* and *Pristimantis relictus* can be considered as greatest concern, as they were recently described and their known distribution is restricted to the high elevation relictual moist forests.

Keywords. Amphibians; Caatinga; Ceará; Chytridiomycosis; Pathogen; *Bd*.

INTRODUCTION

Brazil is home to a significant portion of the world's biodiversity and boasts the greatest amphibian richness on Earth (Segalla et al., 2021; Frost, 2023). Global biodiversity is, however, constantly threatened by anthropic actions, and decreased biodiversity leads to severe consequences including alterations in ecosystem goods and services and increased risks of pathogen dispersal (Costanza et al., 1997; Berger et al., 1998; Cragg and Newman, 2013).

Among vertebrates, amphibians are the most threatened group, as declines have been documented for 501 species worldwide, comprising 6.5% of all described amphibian species (Fisher and Garner, 2020). These declines include 90 extinctions (Scheele et al., 2019), the highest extinction rate among vertebrates (Stuart et al., 2004; Carvalho et al., 2017). Habitat loss, climate change, the introduction of exotic species and pollution are among the amphibian decline causes (Collins and Storfer, 2003).

However, the main cause for high rates of amphibian decline are the pathogenic fungi of the genus *Batrachochytrium* (Burrowes and De la Riva, 2017; Scheele et al., 2019). They comprise two different species

Batrachochytrium dendrobatidis Longcore et al., 1999 (*Bd*), and *B. salamandrivorans* Martel et al., 2013 (*Bsal*). *Bsal* is currently restricted to Europe, but *Bd* has spread across several continents and resulted in the death of huge numbers of amphibians worldwide (Fisher and Garner, 2020). These fungi cause chytridiomycosis, a severe skin disease in which the fungi attack the epidermis, causing hyperkeratosis and resulting in the thickening of the outer skin layer (Fisher and Garner, 2020). Amphibian skin acts not only as a physical barrier, but also performs important roles in respiration and osmoregulation (Duellman and Trueb, 1994). Hyperkeratosis spreads quickly and overwhelmingly (Fisher et al., 2009), causing osmotic imbalances, electrolyte disturbances, respiratory difficulties, cardiac arrest, and death (Berger et al., 1998; Voyles et al., 2009).

Studies of *Bd* genome sequences have identified at least six distinct strains of this fungus: the panzootic strain *Bd-GPL*, the Swiss lineage *Bd-CH*, the Cape strain *Bd-Cape*, the Asian strains *Bd ASIA-1* (which is ancestral to other strains), and *Bd ASIA-3*, and the Brazilian strain *Bd-Brazil* (Fisher and Garner, 2020). Phylogenetic analyses indicate that the *Bd-Brazil* lineage was among the earliest

How to cite this article: Mendes M.S., Oliveira F.A.S., Castro L.G.Z., Ávila R.W., Monteiro F.A.C., Melo V.M.M., Cascon P., Hissa D.C. 2024. Detection of the fungus *Batrachochytrium dendrobatidis* in anurans from the semiarid region of Brazil: new infection records for endemic species. *South American Journal of Herpetology* 33: 24–32. <http://doi.org/10.2994/SAJH-D-22-00032.1>

to diverge from other lineages (Rosenblum et al., 2013). Fisher and Garner (2020) also described additional *Bd* variant strains, such as *Bd* ASIA-2/BRASIL, which has been identified in both Korea and Brazil. Furthermore, a hybrid genotype of the *Bd*-GPL and *Bd*-Brazil lineages has been detected in the Atlantic Forest biome, providing evidence of sexual reproduction in *Bd* populations (Schloegel et al., 2012; Fisher and Garner, 2020).

In Brazil, few studies have investigated the number of amphibian species infected with *Bd* and their geographic distribution (Rodriguez et al., 2014; Carvalho et al., 2017). *Bd* has been reported throughout the country, with the highest number of infected populations recorded in the Atlantic Rainforest biome (Carvalho et al., 2017), likely due to sampling bias, as most studies have been conducted in this region. However, the number of *Bd* occurrences and affected locations has recently increased, with new records from other biomes raising concerns about the spread of the disease across Brazil (Amorim et al., 2019; Benício et al., 2019).

Beyond its impact on anuran diversity, *Bd* infection has broader ecological implications, as amphibians play a vital role in the trophic network. Their decline can disrupt pest control and lead to population reductions in amphibian predators, such as snakes (Shuman-Goodier et al.,

2019; Zipkin et al., 2020), ultimately threatening the integrity of Brazil's biodiversity. Therefore, it is crucial to assess the presence of *Bd* in underexplored regions of Brazil and to monitor areas where the fungus has already been detected. Such efforts are essential to subsidize strategies for global amphibian conservation. This study aimed to investigate the presence of *Bd* in anuran populations inhabiting drylands and wet tropical forests in the semiarid region in the state of Ceará, northeastern Brazil.

MATERIALS AND METHODS

Data sampling

Ceará is predominantly composed of arid environments with dry forests (Caatinga sensu stricto) that support amphibian species typical of open areas. However, endemic and potentially threatened amphibian species also occur in isolated, high-elevation regions covered by relict moist forests (*brejos de altitude*; Roberto and Loebmann, 2016). The biodiversity of the Caatinga biome has historically been underestimated, despite hosting a significant number of animal species, including endemics. The amphibian fauna includes at least 98 species, 20 of which

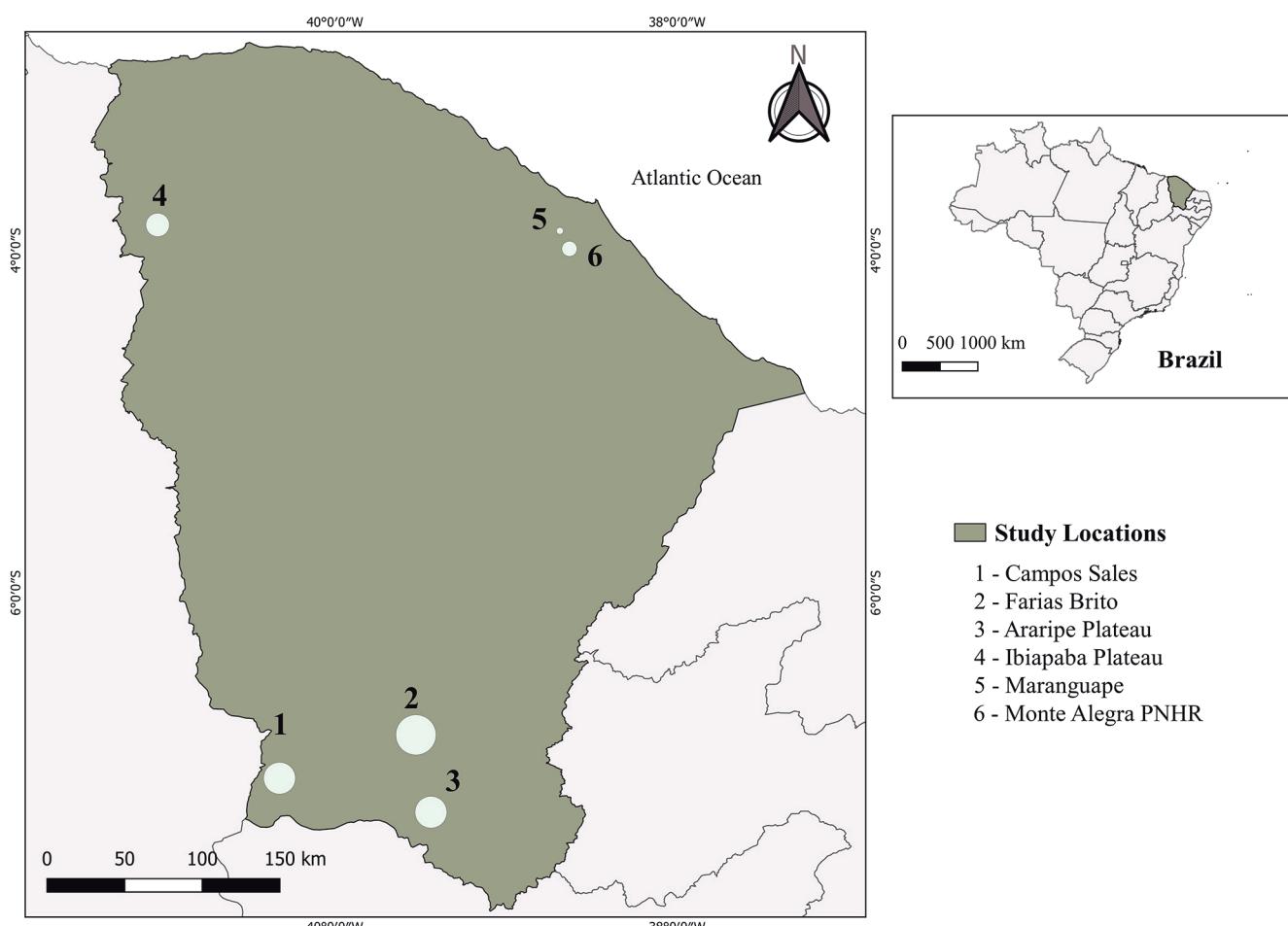


Figure 1. Map of the state of Ceará, Brazil, indicating the sampling locations investigated herein. The circle diameters are proportional to the percentage of *Bd*-contaminated species detected at each location. Number of species sampled/number and percentage of species with positive results: (1) Campos Sales 11/7 (63.64%). (2) Farias Brito 8/7 (87.5%). (3) Araripe Plateau 9/6 (66.6%). (4) Ibiapaba Plateau 14/6 (42.96%). (5) Maranguape 14/1 (7.14%). (6) Monte Alegre 15/5 (38.46%).

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are endemic (Garda et al., 2017). Despite increased efforts to assess the Caatinga herpetofauna in recent years, the Caatinga remains one of Brazil's most neglected regions in terms of conservation initiatives (Roberto and Loebmann, 2016; Silva Neta et al., 2018).

Sampling was conducted in the dry season (July–August 2019) and the rainy season (January–March 2020) in the state of Ceará, northeastern Brazil (Fig. 1). This region is characterized by low annual rainfall, high evapotranspiration, and a prolonged drought lasting 7–10 months each year. The predominant vegetation is the Caatinga biome, a phytogeographic domain unique to Brazil (Silva et al., 2017). Specimens were collected from multiple localities across Ceará, covering diverse phytophysiognomies, including typical Caatinga vegetation (Farias Brito and Campos Sales) and relict wet tropical forests (Serra de Maranguape, Monte Alegre Private Natural Heritage Reserve [PNHR], Chapada do Araripe, and Planalto da Ibiapaba). Specifically, amphibians were sampled from six localities, including both Caatinga sensu stricto areas and relict moist forests, as follows: (1) Monte Alegre PNHR in Pacatuba ($03^{\circ}59'22.1''S$, $38^{\circ}37'44.5''W$; relict moist forest); (2) Araripe Plateau in Crato ($07^{\circ}16'47.5''S$, $39^{\circ}26'18''W$; relict moist forest); (3) Farias Brito ($06^{\circ}49'41.8''S$, $39^{\circ}31'33.7''W$; Caatinga); (4) Serra de Maranguape ($03^{\circ}50'12.2''S$, $40^{\circ}53'55.2''W$; relict moist forest); (5) Ibiapaba Plateau ($03^{\circ}50'16.2''S$, $40^{\circ}53'55.2''W$; relict moist forest); (6) Campos Sales ($07^{\circ}04'53.6''S$, $40^{\circ}19'23.7''W$; Caatinga). We visited each site once during rainy season, except for Monte Alegre PNHR, where three field expeditions were carried out during the rainy season and two during the dry season.

Anurans were captured by hand during active searches. Upon capture, each specimen was rinsed with ultrapure water, photographed, and identified to species. Skin samples were collected using ready-to-use sterile swabs, which were passed 5–7 times across the ventral and inguinal regions, thighs, and feet to ensure reliable sampling (Hyatt et al., 2007). Individual gloves were used for each specimen to prevent cross-contamination. Swabs were placed in 1.5 mL dry cryogenic tubes, stored in ice-cooled coolers, and transported to the laboratory, where they were stored at $-20^{\circ}C$ until DNA extraction.

Captured individuals were examined for clinical signs of chytridiomycosis, such as abnormal cutaneous pigmentation, epidermal desquamation, irregular epithelial tissue, erosions (hyperkeratosis), or hyperplasia (Alvarado-Rybäk et al., 2021). After inspection and sampling, all animals were released.

Genetic material extraction

DNA extraction from swabs was performed following the protocol by Boyle et al. (2004), with modifications by Lambertini et al. (2013). Briefly, 50 μ L of PrepMan ULTRA® reagent (Applied Biosystems) was added to each Eppendorf tube containing the swabs. The tubes were vortexed for 45 s, centrifuged for 30 s at 12,000 rpm, vortexed again for another 45 s, and centrifuged for 30 s at the

same speed. The samples were then heated in a boiling water bath for 10 minutes, cooled at room temperature for 2 minutes and centrifuged for 1 minute at 12,000 rpm. Swabs were inverted in the cryotube using flame sterilized forceps for each inversion, centrifuged for 5 minutes at 12,000 rpm, and then discarded. Subsequently, a quick centrifugation was performed (for a few seconds), and 45 μ L of the solution transferred to new tubes. The solution was centrifuged one final time for 10 minutes at 12,000 rpm to ensure purification.

DNA amplification and sequencing

Bd-specific primers (*Bd* 1a: 5'-CAGTGTGCCATATGT-CACG-3', *Bd* 2a: 5'-CATGGTTCATATCTGTCCAG-3') designed to Annis et al. (2004) were employed to amplify a 300 base pairs product. PCR was performed following the protocol by Kosch and Summers (2013), with modifications. The reaction mixture consisted of 5 μ L of 5 \times GoTaq Buffer (Promega), 3 μ L of MgCl₂ (25 mM), 0.5 μ L of dNTPs (1,000 μ M), 5 μ L of each primer (5 μ M), 0.2 μ L of Taq Polymerase (5 U), and 1.3 μ L of water, resulting in a 20 μ L mix. This mixture was combined with bovine serum albumin (BSA) to a final concentration of 400 ng/ μ L per tube containing the PCR mixture. Additionally, 5 μ L of sample DNA (diluted to 100 ng) was added, bringing the total reaction volume to 35 μ L, including BSA. A negative control was included, replacing the DNA with distilled water. Thermocycler conditions were set as follows: an initial denaturation at $95^{\circ}C$ for 5 minutes, followed by 44 cycles of 45 seconds at $93^{\circ}C$, 45 seconds at $60^{\circ}C$, and 1 minute at $72^{\circ}C$. The protocol concluded with a final extension step at $72^{\circ}C$ for 10 minutes. Following PCR, 8 μ L of each amplified sample was run on a 2% agarose gel, alongside a DNA ladder for size comparison. The gel was stained with SYBR Safe (Invitrogen) and visualized using a UV photodocumentation system.

One of the most common challenges in PCR analysis is sample inhibition, often caused by swab contamination with soil, debris, and components naturally present on the amphibian epidermis (Hyatt et al., 2007). Hyatt et al. (2007) reported that approximately 25% of their PCR experiments showed complete inhibition in detecting *Bd*, with the potential for false negatives. Phenolic compounds, tannic acids, and humic acids are among the most significant PCR inhibitors. These substances are derived from the decomposition of plant material and are commonly found in soil, sediments, and natural environments containing water and debris (Garland et al., 2010). To mitigate this problem, PCR additives such as bovine serum albumin (BSA) can be used to counteract inhibition (Wilson, 1997). In this study, BSA was employed and demonstrated to be effective in overcoming PCR inhibition.

To validate the PCR protocol, selected amplified products were purified using potassium acetate and ethanol. Impurity removal was confirmed based on final DNA concentrations exceeding 50 ng/ μ L, as well as 260/230 nm and 260/280 nm absorbance ratios, measured using a

Nanodrop® ND-1000 spectrophotometer (Nanodrop, Wilmington, DE, USA). The purification process involved mixing 72 µL of each PCR product with 7.2 µL of a 3 M potassium acetate solution (pH 5.5) and twice the total volume of 100% ethanol. The mixtures were homogenized by inversion and then incubated at –80°C for 30 minutes. Samples were then centrifuged at 14,000 rpm for 15 minutes at 4°C, after which the supernatants were discarded. The resulting pellets were resuspended in 150 µL of cold 70% ethanol, centrifuged at 14,000 rpm for 5 minutes at 4°C, and the supernatants were discarded. The pellets were dried on a heating block at 36°C for approximately 20 minutes to evaporate residual ethanol. Finally, the purified DNA was resuspended in 30 µL of DNase- and RNase-free ultrapure water.

DNA sequencing was performed using the Sanger sequencing method by Macrogen Inc., Seoul, Korea. The sequencing reactions were performed using *Bd*-specific primers (*Bd* 1a: 5'-CAGTGTGCCATATGTCAG-3', *Bd* 2a: 5'-CATGGTTCATATCTGTCCAG-3') designed by Annis et al. (2004). The samples chosen for sequencing were CS11

and CS13 from Campos Sales; FB02 from Farias Brito; PA27 from Monte Alegre PNHR; PI01 from Ibiapaba Plateau; and CA18 from Araripe Plateau. The partial sequences provided by Macrogen Inc. were of high quality (Phred > 20) and used to generate consensus sequences using Codon Code Aligner version 9.0.2 program (Codon Corporation, 2021). Consensus sequences were subsequently queried against the GenBank (Sayers et al., 2019) nucleotide collection database for identification employing the BLAST local alignment tool (Altschul et al., 1990).

RESULTS

Samples were collected from 210 individuals of 28 species, 57 sampled at the Monte Alegre PNHR, 17 at Campos Sales, 18 at Araripe Plateau, 22 at Farias Brito, 42 at the Serra de Maranguape, and 54 at the Ibiapaba Plateau (Tables 1, 2). The samples of each species were analyzed by locality until *Bd* was detected, resulting in a total of 125 samples subjected to DNA extraction and PCR anal-

Table 1. Sampled anuran species, their respective families, and localities. NC indicates species not collected at a given locality; “+” represents a species that tested positive for *Batrachochytrium dendrobatidis* (*Bd*); “–” represents a species that tested negative for *Bd*. Numbers in parentheses indicate the ratio of infected individuals to the total individuals tested. *Indicates the first *Bd* record for the species.

Family	Species	Campos Sales	Araripe Plateau	Farias Brito	Ibiapaba Plateau	Monte Alegre PNHR	Maranguape
Bufonidae Gray, 1825	<i>Rhinella diptycha</i> Cope, 1862	– (0 / 3)	+(3 / 5)	– (0 / 1)	+(1 / 2)	– (0 / 3)	– (0 / 2)
	<i>Rhinella granulosa</i> Spix, 1824	– (0 / 2)	– (0 / 1)	NC	– (0 / 1)	– (0 / 3)	NC
Craugastoridae Hedges et al., 2008	* <i>Pristimantis relictus</i> Roberto et al., 2022	NC	NC	NC	+(2 / 2)	– (0 / 1)	– (0 / 6)
Eleutherodactylidae Lutz, 1954	<i>Adelophryne maranguapensis</i> Hoogmoed et al., 1994	NC	NC	NC	NC	NC	– (0 / 2)
Hylidae Rafinesque, 1815	* <i>Boana raniceps</i> Cope, 1862	NC	NC	+(1 / 1)	+(1 / 1)	NC	– (0 / 3)
	<i>Corythomantis greeningi</i> Boulenger, 1896	NC	NC	NC	+(1 / 1)	NC	NC
	<i>Dendropsophus minusculus</i> Rivero, 1971	NC	NC	NC	NC	– (0 / 1)	– (0 / 1)
	<i>Dendropsophus minutus</i> Peters, 1872	NC	+(1 / 1)	NC	– (0 / 1)	+(1 / 1)	– (0 / 2)
	* <i>Dendropsophus nanus</i> Boulenger, 1889	NC	NC	+(1 / 2)	+(1 / 1)	NC	NC
	* <i>Dendropsophus soaresi</i> Caramaschi and Jim, 1983	+(1 / 1)	NC	NC	– (0 / 2)	NC	NC
	* <i>Scinax x-signatus</i> Spix, 1824	– (0 / 1)	+(1 / 2)	+(1 / 1)	– (0 / 5)	+(1 / 3)	NC
	<i>Trachycephalus typhonius</i> Linnaeus, 1758	NC	NC	NC	NC	NC	– (0 / 1)
Leptodactylidae Werner, 1896 (1838)	* <i>Adenomeria juikitam</i> Carvalho and Giaretta, 2013	NC	+(1 / 1)	NC	NC	– (0 / 1)	– (0 / 1)
	<i>Leptodactylus fuscus</i> Schneider, 1799	+(1 / 1)	NC	+(1 / 1)	NC	– (0 / 1)	NC
	<i>Leptodactylus macrosternum</i> Miranda-Ribeiro, 1926	+(1 / 2)	NC	+(1 / 2)	– (0 / 1)	NC	– (0 / 1)
	<i>Leptodactylus mystaceus</i> Spix, 1824	NC	NC	NC	NC	+(1 / 1)	– (0 / 2)
	* <i>Leptodactylus troglodytes</i> Lutz, 1926	+(1 / 1)	– (0 / 1)	NC	+(1 / 1)	NC	NC
	* <i>Leptodactylus vastus</i> Lutz, 1930	NC	– (0 / 1)	NC	NC	+(1 / 4)	– (0 / 1)
	<i>Physalaemus albifrons</i> Spix, 1824	+(2 / 2)	NC	– (0 / 2)	NC	NC	NC
	* <i>Physalaemus cicada</i> Bokermann, 1966	+(2 / 2)	NC	NC	NC	NC	NC
	<i>Physalaemus cuvieri</i> Fitzinger, 1826	NC	+(2 / 3)	+(1 / 1)	– (0 / 2)	+(1 / 2)	+(1 / 4)
	* <i>Pseudopaludicola mystacalis</i> Cope, 1887	NC	NC	+(1 / 1)	NC	NC	NC
Microhylidae Günther, 1858 (1843)	<i>Pseudopaludicola pocoto</i> Magalhães et al., 2014	– (0 / 1)	NC	NC	NC	NC	NC
	<i>Elachistocleis cf. piauiensis</i> Caramaschi and Jim, 1983	NC	NC	NC	NC	– (0 / 1)	NC
Odontophrynidae Lynch, 1969	* <i>Proceratophrys ararype</i> Mângia et al., 2018	NC	+(3 / 3)	NC	NC	NC	NC
	<i>Proceratophrys cristiceps</i> Müller, 1883	NC	NC	NC	– (0 / 3)	– (0 / 2)	– (0 / 1)
	<i>Proceratophrys renalis</i> Miranda-Ribeiro, 1920	NC	NC	NC	NC	NC	– (0 / 2)
Phyllomedusidae Günther, 1858	<i>Pithecopus gonzagai</i> Andrade et al., 2020	+ (1 / 1)	NC	NC	– (0 / 2)	NC	NC
Total species sampled		11	9	9	14	13	14

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Table 2. Results of GenBank queries for consensus 5.8S rRNA sequences from samples CS11, CS13, FB02, PA27, PI01 and CA18. Abbreviations: bp, base pairs; CS, Campos Sales; FB, Farias Brito; PA, Monte Alegre PNHR; PI, Ibiapaba Plateau, CA = Araripe Plateau.

Samples	bp	Most similar species (accession number)	Identity	Coverage	E-value
CS11	292	<i>Batrachochytrium dendrobatidis</i> (HQ176489.1)	99%	100%	3.00E-147
CS13	293	<i>Batrachochytrium dendrobatidis</i> (JQ582937.1)	99%	100%	6.00E-130
FB02	296	<i>Batrachochytrium dendrobatidis</i> (JQ582937.1)	100%	100%	1.00E-151
PA27	291	<i>Batrachochytrium dendrobatidis</i> (JN870749.1)	99%	92%	2.00E-144
PI01	285	<i>Batrachochytrium dendrobatidis</i> (JQ582940.1)	99.85%	99%	1.00E-141
CA18	295	<i>Batrachochytrium dendrobatidis</i> (JQ582937.1)	100%	97%	3.00E-147

ysis, among which 40 tested positive. Table 2 presents the molecular identification of six samples randomly chosen for DNA sequencing, all of which were identified as *Bd*.

In total, 20 (71%) of the 28 anuran species tested positive for *Bd*. The results indicate a broad distribution among the sampled localities; however, only one individual, which was positive for *Bd*, displayed clinical signs consistent with chytridiomycosis (Fig. 2).

There is no clear pattern in the prevalence of *Bd* among the different sampled localities. However, the southernmost localities in the state had a higher percentage of infected individuals (> 50%). The 20 *Bd*-positive species are distributed among six of the eight families collected: Bufonidae, Craugastoridae, Hylidae, Leptodactylidae, Odontophrynidae and Phyllomedusidae. The 20

anuran species with *Bd*-contaminated individuals in the present study exhibit varied distribution patterns. From broad range species—like *Physalaemus cuvieri*, found in almost all localities—to the relict moist forest endemic *Proceratophrys ararype*, found at only one locality (Table 3).

DISCUSSION

This study is the first to investigate the presence of the fungus *Bd* in anuran populations in the state of Ceará, Brazil and provides novel records of *Bd* in Brazil that expand our knowledge of the presence of this pathogen in anuran populations from the semiarid region. Specifically, the cur-

Table 3. Anuran species that presented *Batrachochytrium dendrobatidis*-positive individuals in the present study, with their distribution areas, Biome/Vegetation of occurrence and conservation status according to the IUCN (2021). Source: Frost, 2023.

Species	Distribution	Biome/Vegetation	IUCN
<i>Rhinella diptycha</i>	Pará, Northeast Brazil south to Argentina, Uruguay, Bolivia, Paraguay	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Pristimantis relictus</i>	Northeast Brazil	Endemic to relict moist tropical forests (<i>brijos de altitude</i>)	Not listed
<i>Boana raniceps</i>	Amazonian Colombia, Venezuela, French Guiana, Northeast Brazil (Ceará), Paraguay, northern Argentina, eastern Bolivia	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Corythomantis greeningi</i>	Northeast Brazil, Tocantins, Goiás	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Dendropsophus minutus</i>	Cis-Andean South American from Venezuela to Argentina	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Dendropsophus nanus</i>	North and Northeast Brazil to Argentina, Paraguay, Bolivia	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Dendropsophus soaresi</i>	Northeast Brazil, Minas Gerais	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Scinax x-signatus</i>	Colombia, Venezuela, Guyana, Suriname and Northeast, Southeast, and South Brazil	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Adenomera juikitam</i>	Amazonia and Northeast Brazil relict moist tropical forests (<i>brijos de altitude</i>)	Wet Forests	Least Concern
<i>Leptodactylus fuscus</i>	Panama and cis-Andean South America to northern Argentina	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Leptodactylus macrosternum</i>	South America, Amazonia to northern Argentina	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Leptodactylus mystaceus</i>	Amazonia. Relict moist tropical forests (<i>brijos de altitude</i>) in Northeast Brazil. Minas Gerais, São Paulo and Paraná in Atlantic Forest	Wet Forests/Cerrado	Least Concern
<i>Leptodactylus troglodytes</i>	Northeast Brazil, Minas Gerais, Goiás	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Leptodactylus vastus</i>	Northeast Brazil	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Physalaemus albifrons</i>	Northeast Brazil, Minas Gerais	Caatinga and Cerrado	Least Concern
<i>Physalaemus cicada</i>	Northeast Brazil, Minas Gerais	Caatinga and Caatinga/Cerrado transition zones	Least Concern
<i>Physalaemus cuvieri</i>	Northeast, Center-West and South Brazil, Argentina, Bolivia, Paraguay, Venezuela	Wet Forests/Cerrado/Caatinga	Least Concern
<i>Pseudopaludicola mystacalis</i>	Northeast, Southeast and South Brazil, Argentina, Paraguay	Relict moist tropical forests (<i>brijos de altitude</i>)/Wet Forests	Least Concern
<i>Proceratophrys ararype</i>	Northeast Brazil in Chapada do Araripe, Ceará	Relict moist tropical forests (<i>brijos de altitude</i>)	Not listed
<i>Pithecopus gonzagai</i>	Northeast Brazil north of São Francisco River	Wet Forests/Cerrado and Caatinga	Least Concern

Detection of the fungus *Batrachochytrium dendrobatidis* in anurans from the semiarid region of Brazil: new infection records for endemic species
Mirian dos Santos Mendes, Francíscia Andréa da Silva Oliveira, Luzia Gabrielle Zeferino de Castro, Robson Waldemar Ávila, Felipe Augusto Correia Monteiro, Vânia Maria Maciel Melo, Paulo Cascon, Denise Cavalcante Hissa

rent study used PCR to study 125 samples of anurans representing 28 species of eight families, yielding positive results for 40 samples (32%) across 20 species. The samples were collected in five regions of Ceará, encompassing both milder and drier climates. Additionally, we report the finding of an animal in the field exhibiting white patches on the skin, a sign of chytridiomycosis (Amorim et al., 2019). To our knowledge, this is the first record of amphibians with visible signs of the disease in the Caatinga biome (Fig. 2). Among the 20 *Bd* positive species, 11 represent the first records of *Bd* infection in Brazil, including *Adenomera juikitam*, *Boana raniceps*, *Dendropsophus nanus*, *D. soaresi*, *Leptodactylus troglodytes*, *L. vastus*, *Physalaemus cicada*, *Proceratophrys ararype*, *Pristimantis relictus*, *Pseudopaludicola mystacalis* and *Scinax x-signatus*.

Since the discovery of chytridiomycosis, multiple detection techniques have been described and refined, and technical advances have significantly increased their reliability. Detection by PCR identifies *Bd* quickly and with degree sensitivity and specificity using a non-invasive sample collection method (Boyle et al., 2004). Annis et al. (2004) developed specific *Bd* primers, greatly contributing to the speed and accuracy of this test. In the present study, our PCR assay was validated by sequencing the PCR products of six samples, confirming that our assay was actually detecting the presence of *Bd* (Table 2).

The occurrence of *Bd* in anurans has also been investigated in other states within the semiarid region of northeastern Brazil. In Piauí, Benício et al. (2019) analyzed 20 samples each of *Rhinella granulosa* and *R. diptycha*, detecting *Bd* in 20% and 30% of the samples, respectively. Amorim et al. (2019) tested 190 individuals, representing 85 species collected in the state of Bahia and preserved in collections, and detected *Bd* in 16 samples of 14 species.

In other regions of Brazil, several studies have investigated the presence of *Bd*.

Valencia-Aguilar et al. (2015) collected 90 individuals representing 27 species, 22 of which (39 specimens)

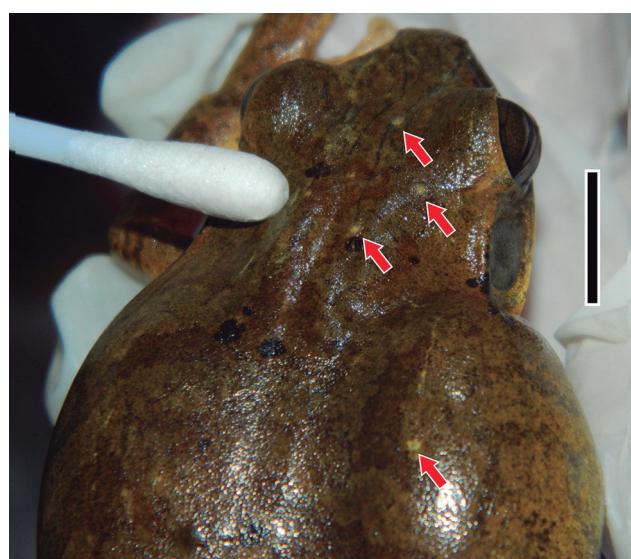


Figure 2. An individual of *Boana raniceps*, the only captured animal showing symptoms of chytridiomycosis, displaying white spots on its skin (arrows). Scale bar: 10 mm. Photo: Thabata Cavalcante.

were *Bd*-positive. This study evaluated the incidence of the fungus at four localities in poorly explored regions of the Brazilian Atlantic Forest of Bahia, Pernambuco, Minas Gerais, and Alagoas, as well as a locality in the Amazon Forest in Pará. Importantly, this study provided the first positive record of *Bd* in a wild-caught Amazonian anuran (*Adelphobates galactonotus* Steindachner, 1864).

Some studies have examined a larger number of Brazilian localities and species, including both adult frogs and tadpoles. For example, Rodriguez et al. (2014) tested 2,799 post-metamorphic frogs collected from 1894 to 2010, focusing on the Atlantic Forest, and recorded the earliest known *Bd* occurrence in Brazil in 1894. Similarly, Becker et al. (2016) investigated the presence of *Bd* in 1,391 preserved post-metamorphic frogs from Amazonia and other ecoregions, with samples ranging from 1895 to 2014, and Carvalho et al. (2017) extended the scope to tadpoles, evaluating 32,551 museum specimens collected between 1930 and 2015.

When comparing studies using material obtained from preserved specimens in museum collections to those using fresh samples tested shortly after collection, it is important to note the differences in their outcomes. Studies based on collection materials have the advantage of providing historical data on the presence of *Bd* across different years, thereby offering insights into the history of *Bd* infection at specific localities, as demonstrated by Carvalho et al. (2017). However, the effectiveness of *Bd* assays on collection specimens depends on the preservation quality and history of the samples. Conversely, studies involving field-collected samples provide real-time data on current *Bd* infection in a given area (Lambertini et al., 2021).

It is known that environmental temperature, pH, salinity, and rainfall can significantly influence the onset and severity of chytridiomycosis (Haver et al., 2022), and that in some regions, forest loss is linked to the occurrence and spread of *Bd* in hosts (Carvalho et al., 2024). Amphibians inhabiting fragmented forests are immunogenetically weakened, showing higher prevalence than those inhabiting preserved forests (Belasen et al., 2022), which raises great concern as symptomatic amphibians have been observed in fragmented and deforested areas.

Among the 20 species that tested positive for *Bd* in the present study, approximately half ($n = 11$) are broadly distributed in multiple biomes across much of South America and, while the reamining species (viz., *Corythomantis greeningi*, *Dendropsophus soaresi*, *Leptodactylus troglodytes*, *L. vastus*, *Physalaemus albifrons*, *Ph. cicada*, *Pithecopus gonzagai*, *Pristimantis relictus*, and *Proceratophrys ararype*) are restricted to the Caatinga biome of northeastern Brazil, with some extending into neighboring northeastern states. Among species restricted to the Northeast Region and neighboring states, *C. greeningi*, *D. soaresi*, *L. troglodytes*, *L. vastus* and *P. gonzagai* occur both in wet forests and open vegetation formations (Cerrado and Caatinga), *Ph. albifrons* and *Ph. cicada* occur only in Cerrado and Caatinga areas, *Pri. relictus* and *Pro. ararype* are endemic to wet forests

located in relict moist forests, with *P. ararype* being endemic to the Araripe Plateau.

The Maranguape mountain locality is particularly relevant, as it presented the highest number of sampled species ($n = 14$), only one of which (*Physalaemus cuvieri*) was positive for *Bd*. It is important to note that *Adelophryne maranguapensis*, an endemic species to this relict moist forest and considered threatened with extinction (Silvano, 2004), tested negative for *Bd*.

Among the species analyzed here, *Pristimantis relictus* inhabits humid forests, occurring in the Serras da Meruoca, Uruburetama, Baturité, Maranguape and Aratanha and Planalto da Ibiapaba (Roberto and Loebmann, 2016; Roberto et al., 2022). The captured individuals were located in trees or over the ground. Individuals positive for *Bd* were detected in the Planalto de Ibiapaba, while samples obtained in Maranguape and Monte Alegre PNHR were negative. Species of the genus *Pristimantis* lay terrestrial eggs presenting direct development. Terrestrial species that reproduce out of water are less exposed to contamination by an aquatic fungus such as *Bd*, although infection has been reported for some anurans that have these characteristics (Moura-Campos et al., 2021). On the other hand, studies have shown that lower exposure can lead to a lack of adaptive responses to the fungus and, consequently, greater susceptibility compared to species that develop from aquatic larvae (Mesquita et al., 2017).

This makes the record of this pathogen in *Pristimantis relictus* particularly worrisome, as this species is restricted to relict moist forests that already suffer significant anthropic impacts. *Bd* records for other *Pristimantis* species were registered by Valencia-Aguilar et al. (2015) for *P. ramagii* (Boulenger, 1888) and *P. vinhai* (Bokermann, 1975) and by Amorim et al. (2019) for *P. paulodutrai* (Bokermann, 1975).

Proceratophrys ararype (Odontophrynidae) was recently described for the Chapada do Araripe plateau, in the northeastern Caatinga ecoregion. As this species is endemic to this high-altitude wet relictual forest, which suffers strong pressure from anthropic activities, it is probably already threatened with extinction (Mângia et al., 2018) and infection by the *Bd*, as discovered herein, further aggravates the conservation status of this species.

Adelophryne maranguapensis (Eleutherodactylidae), an anuran endemic to the wet forests of the Serra de Maranguape (Roberto and Loebmann 2016) was a noteworthy *Bd* negative species. Besides its restricted distribution area, this species presents a specialized mode of reproduction, performing oviposition in bromeliads and presenting direct development (Cassiano-Lima et al., 2011), which makes it even more vulnerable, due to high bromeliad extraction for commercial purposes, in addition to deforestation (Roberto and Loebmann 2016). No *Bd* infection records for *Adelophryne* species in Brazil are available.

Studies have shown that climatic variations, extreme temperatures, and significant climate events like El Niño negatively affect amphibians' immune response to chytrid infection (Raffel et al., 2015). Adams et al. (2017) reinforce that rising temperatures and prolonged droughts can ben-

efit the *Bd* fungus, making such climates suitable for the pathogen's growth over large areas and consequently affecting more amphibian hosts. In this study, we observed that locations experiencing greater temperature changes and lower altitudes compared to others exhibited a higher number of positive results.

In summary, populations of 20 anuran species in the state of Ceará are infected by *Bd* in Caatinga biome areas and relict moist forests (*brejos de altitude*). Our finding that more than 70% of the tested species were positive for *Bd* raises concerns about the conservation of the biodiversity of northeastern Brazil. This study also highlights the need for further research on the region's amphibian diversity, including the monitoring of tadpoles, which was not addressed here. Our findings are highly relevant for both the scientific community and decision-making processes related to biodiversity conservation. As chytridiomycosis is recognized as one of the leading causes of amphibian population declines and extinctions worldwide—including in Brazil—these results emphasize the urgency of conservation efforts in this region.

ACKNOWLEDGMENTS

This study was supported by the Cearense Foundation for Support to Scientific and Technological Development (FUNCAP) through a scholarship granted to MSM, by the National Council for Scientific and Technological Development through CNPq projects 407456/2018-0 and 407783/2023-7 assigned to DCH., as well as by the Coordination for Improvement Higher Education Personnel (CAPES). RWA was supported by CNPq productivity research grants (PQ 305988/2018-2; 307722/2021-0). The authors thank researchers Cícero Ricardo de Oliveira, Ana Carolina Brasileiro Melo, and Roberta Braga for field support and anuran identification and Lucia Castro Cunha for permission to work at Monte Alegre PNHR. Collection permits were issued by the Brazilian Ministry of Environment (Sistema de Autorização e Informação em Biodiversidade permit numbers 68784 and 29613).

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