INTRODUCTION

Many anuran populations worldwide have experienced severe declines (Williams & Hero 1998, Bustamante et al. 2005), and anurans are justly considered among the most threatened vertebrates (Baillie et al. 2004, 2010). Infectious diseases are among the main causes of these population declines (Baillie et al. 2004, Fisher et al. 2012). Particularly, the aquatic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has led to the decline of several amphibian populations and species worldwide (LaMarca et al. 2005, Lips et al. 2006, Skerratt et al. 2007), and it has mostly affected species with a high dependence on water (Bielby et al. 2008, Catenazzi et al. 2011). *Bd* is a generalist pathogen (Morgan et al. 2007, Walker et al. 2012).
suggest that amphibian declines associated with
infectious diseases because of increased interactions
among the pathogen and potential hosts (Keesing et
al. 2006). Data from a global amphibian assessment
in diverse locations throughout Africa, Asia, Europe,
and the Americas (Fisher et al. 2009, Olson et
al. 2013).

Species diversity may affect the prevalence of infectious diseases because of increased interactions
among the pathogen and potential hosts (Keesing et
al. 2006). Data from a global amphibian assessment
suggest that amphibian declines associated with Bd
infection are greater in regions with higher species
richness (Olson et al. 2013). However, at local scales,
higher diversity tends to attenuate infection indices
(Becker et al. 2014). Moreover, the effects of Bd vary
among species and populations worldwide (Berger et
al. 1999, Briggs et al. 2010, Kilpatrick et al. 2010,
Searle et al. 2011), probably because of a combination
of environmental factors and host traits that
impact the spread and susceptibility of species to Bd
(Walker et al. 2010, Bancroft et al. 2011). Life history
traits have also been associated with higher Bd infec-
tion rates (Bancroft et al. 2011, Guayasamin et al.
2014) among families with high diversity, broad dis-
tributions, and with both aquatic and terrestrial
stages (Corey & Waite 2008, Olson et al. 2013).

The Neotropical region of South and Central America contains the highest diversity of amphibians
(Baillie et al. 2010). This area includes the Brazilian
Atlantic forest and Cerrado (an open grassland phys-
ionomy), 2 of the most endangered, species-rich
(Myers et al. 2000) biomes and centers of endemism
in the world (Haddad et al. 2013, Valdujo et al. 2013).
Three distinct genotype lineages (including the most
ancestral) of the Bd pathogen have been found in the
Atlantic forest, and this biome may represent the
center of origin for Bd (Schloegel et al. 2012, Rosen-
blum et al. 2013). To date, 2 anuran species in Cer-
rado and 110 species in several habitats of the
Atlantic forest have been recorded as Bd-positive
(Table S1 in the Supplement at www.int-res.com/
articles/suppl/d114p061_supp.pdf), with records dat-
ing back to more than 100 yr ago (Rodriguez et al.
2014). However, the vast majority of these reports are
from the south and southeast Atlantic forest (Lisboa
et al. 2013, Rodriguez et al. 2014). In the northern
portion of the biome, the distribution and prevalence
of Bd are poorly documented (Carnaval et al. 2006,

Here we provide current information on Bd distri-
bution in north and northeastern Brazil. We have
included the first record of Bd in the state of Bahia, as
well as the first record in an anuran caught in the
wild in Brazilian Amazonia. We also used data from
previous chytrid studies in the Atlantic forest (south,
setheast, and northern regions) to test the hypothe-
sis that Bd is a generalist pathogen in this biome. If
Bd is a generalist, we would expect that the number
of species per family is positively correlated with the
number of infected species in the family.

MATERIALS AND METHODS

We conducted fieldwork in 5 Brazilian localities: 4
in the Atlantic forest in the states of Pernambuco,
Alagoas (June to December 2013 and February to
May 2014), Bahia (July 2010), and Minas Gerais
(August 2010) and 1 in the Amazon forest in the state
of Pará (February 2013). At each location, we
swabbed individuals from terrestrial (forest and open
areas) and aquatic habitats (ponds and streams) dur-
ing diurnal and nocturnal visual encounter surveys,
as per the methods of Crump & Scott (2001).

To avoid cross-contamination, each collected anu-
ran was immediately and individually maintained in
a plastic bag. Individuals were swabbed for Bd de-
tection by using sterile cotton swabs and wiping the
forelimbs, hindlimbs, and pelvic region 5 times for
each individual, as per the method described by Hy-
att et al. (2007). DNA extraction was performed using
50 µl of PrepMan® ULTRA (Life Technologies,
Thermo Fisher Scientific) for each swab, followed by
Taqman® qPCR assays for detection and quantifica-
tion of Bd loads, according to standard protocols
(Boyle et al. 2004, Lambertini et al. 2013). Bd preva-
ce was estimated as the number of Bd-positive
frogs divided by the total number of sampled frogs.
Infection intensity was estimated as the number of
zoospore equivalents (ZE) obtained using qPCR (see
Lambertini et al. 2013). The strain used for the stan-
dard curve was CLFT 023 (from Monte Verde, Ca-
manducaia, Minas Gerais), allowing for comparisons
with other localities worldwide (Longo et al. 2013).

To test the hypothesis that Bd is a generalist patho-
gen (showing low species-specificity), we combined
our data with records of previous Atlantic forest stud-
ies (Table S1 in the Supplement) to calculate the
number of infected species. We then used a linear
regression to test the correlation between the num-
ber of infected species and the total number of spe-
cies registered in the biome (from Haddad et al.
2013). Differences between Bd prevalence and load
among families were tested with analyses of variance
(ANOVA). Analyses were performed using the R sta-
tistical computing environment (R Development Core Team 2013) after normalizing data through a log transformation.

RESULTS

Out of the 27 sampled species from 8 anuran families, 22 (81.5%) tested positive for Bd (Table 1). Out of the 90 sampled individuals from 5 localities (Fig. 1), 39 (43.3%) tested positive for Bd infection. All samples collected in Bahia (n = 2), Pernambuco (n = 3), Pará (n = 1), and Minas Gerais (n = 1) tested positive for Bd (Table 1).

Table 1. Anuran species sampled for Batrachochytrium dendrobatidis presence in the Amazon and Atlantic forests (see Fig. 1). Zoospore equivalents (mean and SD) were calculated only for positive individuals. For most species, all sampled individuals were adults; the exception was Proceratophrys renalis, for which an adult and a juvenile were sampled.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality, state</th>
<th>Zoospore equivalents</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Min.–Max.</td>
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<tr>
<td>Bufonidae</td>
<td></td>
<td></td>
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<tr>
<td>Frostius pernambucensis</td>
<td>Murici, Alagoas</td>
<td>62.17</td>
<td>–</td>
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<tr>
<td>Rhinella crucifer</td>
<td>Murici, Alagoas</td>
<td>4.79</td>
<td>–</td>
</tr>
<tr>
<td>Rhinella hoogmoedi</td>
<td>Murici, Alagoas</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Craugastoridae</td>
<td></td>
<td></td>
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<tr>
<td>Pristimantis vinhai</td>
<td>Itacaré, Bahia</td>
<td>2.37</td>
<td>–</td>
</tr>
<tr>
<td>Pristimantis ramagii</td>
<td>Murici, Alagoas</td>
<td>25.51</td>
<td>3.53–135.72</td>
</tr>
<tr>
<td>Dendrobatidae</td>
<td></td>
<td></td>
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<tr>
<td>Adelphobates galactonotus</td>
<td>Santa Cruz Dos Martírios, Pará</td>
<td>1.56</td>
<td>–</td>
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<tr>
<td>Hemiphractidae</td>
<td>Gastrotheca sp.</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hylidae</td>
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<tr>
<td>Aplastodiscus sibilatus</td>
<td>Murici, Alagoas</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dendropsophus minutus</td>
<td>Murici, Alagoas</td>
<td>12.4</td>
<td>–</td>
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<tr>
<td>Hypsiboa albo-marginatus</td>
<td>Murici, Alagoas</td>
<td>1.05</td>
<td>–</td>
</tr>
<tr>
<td>Hypsiboa crepitans</td>
<td>Poção, Pernambuco</td>
<td>34.62</td>
<td>2.2–67.05</td>
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<tr>
<td>Hypsiboa exastis</td>
<td>Murici, Alagoas</td>
<td>7.88</td>
<td>–</td>
</tr>
<tr>
<td>Hypsiboa semilineatus</td>
<td>Murici, Alagoas</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Phyllodytes cf. acuminatus</td>
<td>Murici, Alagoas</td>
<td>16.33</td>
<td>–</td>
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<tr>
<td>Phyllodytes cf. edelmoi</td>
<td>Murici, Alagoas</td>
<td>25.56</td>
<td>–</td>
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<tr>
<td>Phyllodytes edelmoi</td>
<td>Murici, Alagoas</td>
<td>60.22</td>
<td>2.95–227.27</td>
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<tr>
<td>Phyllodytes grynaeathes</td>
<td>Murici, Alagoas</td>
<td>359.16</td>
<td>1.72–1276.49</td>
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<td>Phyllodytes tuberculatus</td>
<td>Itacaré, Bahia</td>
<td>2.93</td>
<td>–</td>
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<tr>
<td>Phyllomedusa nordestina</td>
<td>Murici, Alagoas</td>
<td>72.63</td>
<td>3.30–141.95</td>
</tr>
<tr>
<td>Scinax cf. eurydice</td>
<td>Carangola, Minas Gerais</td>
<td>1.64</td>
<td>–</td>
</tr>
<tr>
<td>Scinax nebulosus</td>
<td>Murici, Alagoas</td>
<td>4.32</td>
<td>–</td>
</tr>
<tr>
<td>Scinax pachycrus</td>
<td>Poção, Pernambuco</td>
<td>1.88</td>
<td>–</td>
</tr>
<tr>
<td>Hylodidae</td>
<td>Crossodactylus dantei</td>
<td>2793.2</td>
<td>264.98–5321.50</td>
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<tr>
<td>Leptodactylidae</td>
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<tr>
<td>Physalaemus cf. cuvieri</td>
<td>Murici, Alagoas</td>
<td>7.91</td>
<td>4.27–11.55</td>
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<tr>
<td>Leptodactylus natalensis</td>
<td>Murici, Alagoas</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Leptodactylus cf. mystaceus</td>
<td>Murici, Alagoas</td>
<td>5.1</td>
<td>–</td>
</tr>
<tr>
<td>Odontophrynidae</td>
<td>Proceratophrys renalis</td>
<td>6.01</td>
<td>–</td>
</tr>
</tbody>
</table>

Among families with the greatest number of samples, Bd prevalence was higher in Craugastoridae (56%; 2 species; 16 individuals) and Hylidae (42%; 15 species; 52 individuals) and lower in Leptodactylidae (38%; 3 species; 8 individuals) and Bufonidae (25%; 3 species; 8 individuals), although Bd prevalence among families did not differ statistically ($F = 0.80$, df = 3, $p = 0.50$). Infection load was the highest in Craugastoridae (log $ZE = 1.39 \pm 1.51$, $n = 9$ individuals) and Hylidae (log $ZE = 1.05 \pm 0.87$, $n = 21$ individuals), although loads did not differ between families ($F = 0.92$, df = 5, $p = 0.47$). The highest intensity of infection (log $ZE = 1.79–5.20$) was detected in Crossodactylus.
dantei and Phyllodytes gyrinaethes, followed by Phyllomedusa nordestina, Frostius pernambucensis, Phyllodytes edelmoi, Hypsiboas cimicola, Scinax eurydice, Adelphobates galactonotus, and Pristimantis ramagi. The following species had the lowest loads: Rhinella crucifer, Scinax nebulosus, Phyllodytes tuberculosus, Pristimantis vinhais, Scinax pachycrus, Scinax cf. eurydice, Adelphobates galactonotus, and Hypsiboas almarmoratus (log ZE = 0–1.40). Bd was not detected in Rhinella hoogmoedii, Gastrotheca sp., Aplastodiscus sibiliatus, Hypsiboas semilineatus, and Leptodactylus natalensis (only 1 individual was tested for each of these species). The sampled individual of A. galactonotus collected from Pará, Amazonia, was infected.

Anuran richness per family was positively correlated with the number of infected species in the Atlantic forest ($r^2 = 0.753, p < 0.001, df = 14$, Fig. 2). The richest families in the Atlantic forest (Hylidae, Hylodidae, and Leptodactylidae) had greater numbers of infected species (Table S2 in the Supplement).
Brazil) have been present at least since 1897 (Rodriguez et al. 2014) in terrestrial and aquatic habitats without lethal effects on anuran populations (Toledo et al. 2006b, Gründler et al. 2012, Rodriguez et al. 2014). In the northern region, information on Bd has been limited to reports of anuran infection from the states of Alagoas, Bahia, and Pernambuco (Carnaval et al. 2006, Lisboa et al. 2013, present study). Our findings of Bd in Bahia fill a gap of 1600 km between Minas Gerais and Alagoas, indicating that Bd is widespread across a broad host range throughout the Atlantic forest. Bd prevalence and load (Table 1) among anurans in the northern region may be regarded as moderate. However, there is a clear need for more surveys in this region, since small sample sizes and differences in sampling effort may lead to a bias in estimating prevalence and pathogen load (Ohst et al. 2013). Climatic and forest characteristics can affect Bd infection (Rödder et al. 2008, Fisher et al. 2009, Gründler et al. 2012), and the differing climatic dynamics in northern and southern parts of the Atlantic forest may therefore affect Bd dynamics in these regions (Câmara 2003, Carnaval et al. 2014).

Our Bd data for the northern region of the Atlantic forest are consistent with an enzootic pattern where there is no detectable effect of Bd on anuran survival (Briggs et al. 2010). The same pattern was found in the south Atlantic forest (Rodriguez et al. 2014) and other areas (Canada: Forzán et al. 2010, Cameroon: Doherty-Bone et al. 2013, Germany: Ohst et al. 2013). These findings contradict epizootic events reported in Australia (Berger et al. 1998), Costa Rica (Lips et al. 2003), Mexico (Lips et al. 2004), Panama (Lips 1999), Spain (Bosch et al. 2001), the USA (Vredenburg et al. 2010), and Venezuela (La Marca 2005), where high prevalence (Lips et al. 2006) and high Bd infection loads (Vredenburg et al. 2010) have been associated with local mass extinctions and fatal declines.

The Atlantic forest is among the most threatened forests in the world, where more than 80% of the remaining fragments are smaller than 0.5 km² (Ribeiro et al. 2009). Nevertheless, this biome has high anuran species richness (522 species) in Brazil, presumably because of its diverse geographical and climatic characteristics (Ribeiro et al. 2009, Toledo & Batista 2012, Haddad et al. 2013, Toledo et al. 2014). This high diversity of species and habitats may favor Bd infections by providing diverse hosts and microhabitats (Becker & Zamudio 2011). As in Costa Rica and Australia (Becker & Zamudio 2011), the number of species per family was positively correlated with the number of infected species in the Atlantic forest (Fig. 2). Even though several anuran species in the Atlantic forest still need to be screened for Bd infection, our data support the hypothesis that this fungus is a generalist pathogen (Fisher et al. 2009) that spreads rapidly (Morgan et al. 2007) and infects many different host species types (Crawford et al. 2010, Walker et al. 2010). In contrast, Bd infections in Europe and the USA are associated with amphibian families that display similar life-history traits (Corey & Waite 2008, Bancroft et al. 2011, Baláž et al. 2014). For example, species of the family Ranidae are more susceptible to Bd infection because of their similarities in body shape and habitat use for breeding (Bancroft et al. 2011).

We found that Hylidae and Leptodactylidae have the greatest number of Bd-positive species in the Atlantic forest (Table S2 in the Supplement). These families exhibit the greatest diversity of species and reproductive modes in this biome and include species that spawn aquatic and terrestrial eggs and use different types of lotic, lentic, and terrestrial habitats during the breeding season (Haddad & Prado 2005). In southern and southeastern Brazil, species that breed in lentic and terrestrial habitats are characterized by a higher Bd prevalence when compared with species that breed in lotic habitats (Gründler et al. 2012, Rodriguez et al. 2014). Likewise, North American ranids (another species-rich family) that are dependent on water bodies (i.e. lentic habitats) for laying eggs and tadpole development seem to be more prone to Bd infection than strictly terrestrial or direct-development species (see also Becker et al. 2014).

The Atlantic forest extends from 3° to 31° S and 35° to 60° W in Brazil (Ribeiro et al. 2009); to date, only 38% of the anuran species present in 10 states have been screened for Bd infection, of which 25% tested positive. Several taxa (mainly in the north and northeastern regions) have not been sampled, and long-term monitoring of anurans in the Brazilian biomes is clearly needed to fill geographical, taxonomic, and ecological gaps. These data reveal infection trends in the wild, providing sine qua non information that can be directly integrated into amphibian conservation plans.

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