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Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis

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The recent increase in emerging fungal diseases is causing unprecedented threats to biodiversity. The origin of spread of the frog-killing fungus *Batrachochytrium dendrobatidis* (*Bd*) is a matter of continued debate. To date, the historical amphibian declines in Brazil could not be attributed to chytridiomycosis; the high diversity of hosts coupled with the presence of several *Bd* lineages predating the reported declines raised the hypothesis that a hypervirulent *Bd* genotype spread from Brazil to other continents causing the recent global amphibian crisis. We tested for a spatio-temporal overlap between *Bd* and areas of historical amphibian population declines and extinctions in Brazil. A spatio-temporal convergence between *Bd* and declines would support the hypothesis that Brazilian amphibians were not adapted to *Bd* prior to the reported declines, thus weakening the hypothesis that Brazil was the global origin of *Bd* emergence. Alternatively, a lack of spatio-temporal association between *Bd* and frog declines would indicate an evolution of host resistance in Brazilian frogs predating *Bd*'s global emergence, further supporting Brazil as the potential origin of the *Bd* panzootic. Here, we *Bd*-screened over 30 000 museum-preserved tadpoles collected in Brazil between 1930 and 2015 and overlaid spatio-temporal *Bd* data with areas of historical amphibian declines. We detected an increase in the proportion of *Bd*-infected tadpoles during the peak of amphibian declines (1979–1987). We also found that clusters of *Bd*-positive samples spatio-temporally overlapped with most records of amphibian declines in Brazil's Atlantic Forest. Our findings indicate that Brazil is post epizootic for chytridiomycosis and provide another piece to the puzzle to explain the origin of *Bd* globally.

1. Introduction

The anthropogenic movement of parasites outside their natural ranges (pathogen pollution) is one of the largest threats to biodiversity [1,2]. Because diversity of hosts (i.e. species, populations) is positively associated with diversity of pathogens (i.e. species, genotypes) around the globe [3], many Anthropocene panzootics have a tropical origin [2]. In humans, this hypothesis is supported by an out-of-Africa emergence for many epidemics [2]. For most wildlife, the tropics are the areas of highest host diversity [4], and therefore, areas of high likelihood of pathogen emergence [2,5]. For instance, South America is a hotspot of amphibian diversity; Brazil alone harbours 1080 of the 7546 described species globally [6,7]. As the most diverse amphibian place in the world, Brazil is also a likely hotspot for amphibian pathogens, and a candidate point of origin of the wildlife disease that has caused the largest number of population declines and extinctions in the recorded history [8].

Chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), has been linked to declines of hundreds of amphibian species globally [4]. Although many studies indicate that amphibian declines are linked

to the introduction of *Bd* to several areas of naive host populations (i.e. the novel spreading pathogen hypothesis; [9]), *Bd* is known to have been present in several continents before frog die-offs were reported (i.e. endemic pathogen hypothesis; [10–15]). Despite studies supporting both regional spread and endemicity in different regions, there is still substantial debate about the origin of the *Bd* panzootic [16–18]. To date, several regions have been suggested as the point of origin of hypervirulent *Bd* based on historical records of the pathogen from museum-preserved specimens [10], absence of population declines in the wild [18] and diversity of *Bd* lineages [19,20]. The absence of amphibian declines in Brazil attributed to chytridiomycosis, coupled with the high diversity of hosts and *Bd* lineages [14,18,21], raised the hypothesis that a hypervirulent *Bd* genotype might have spread from Brazil to other continents causing the recent global amphibian crisis [14,21].

Amphibian population declines and extinctions were reported for seemingly pristine forests of Brazil, but to date, these declines were mostly enigmatic and occurred before the discovery of chytridiomycosis [22]. *Bd* infection loads in Brazil are higher in pristine closed-canopy forests [23], where dozens of amphibian species experienced drastic reductions in population sizes, with a number of species going locally extinct after the late 1970s [22,24,25]. Amphibians declined in several regions, but the extinctions reported in montane protected areas in the States of São Paulo, Rio de Janeiro, Minas Gerais and Espírito Santo were among the more severe ones. Specifically, population declines were reported for over 13 species at Estação Biológica de Boraceia, state of São Paulo, after 1979 [24]. Declines were also observed during the same period of time at Parque Nacional da Serra dos Órgãos and Parque Nacional do Itatiaia (Brazil's first national park), in the state of Rio de Janeiro and Minas Gerais [24–26]. Peter Weygoldt not only reported amphibian declines at Reserva Ecológica Santa Lúcia, state of Espírito Santo, after 1981, but also speculated about a potential disease-causing agent [25]. The majority of these declines (i) occurred in pristine montane sites of the Atlantic Forest; (ii) disproportionately affected amphibian species with aquatic larvae (mostly stream breeders), and (iii) took place within a narrow time period (between 1979 and 1987).

In other Neotropical regions, many population declines attributed to *Bd* coincided temporally with declines in Brazil [9,27,28]. Furthermore, montane stream-breeding frogs were also at higher risk of local extinctions in Central America and Australia [9,28,29]. For instance, dozens of amphibians from the genus *Atelopus* declined or disappeared along high-elevation streams after outbreaks of chytridiomycosis in Central America and the tropical Andes [9]. Similarly, several frogs with aquatic larval development went extinct in the wild in eastern Australia after the emergence of *Bd* in natural forests [27,28,30,31]. Relict amphibian communities affected by *Bd* epizootics are expected to show the same signs of the 'Ghost of Epizootics Past' (e.g. disproportionate loss of highly aquatic species) [18]. The declines caused by *Bd* in Central America, the Tropical Andes and eastern Australia are similar to the enigmatic declines in Brazil's Atlantic Forest, supporting the hypothesis that historical declines observed in the Atlantic Forest were also caused by chytridiomycosis.

Here, we *Bd*-screened over 30 000 museum-preserved tadpoles collected in Brazil between 1930 and 2015 and quantified spatio-temporal aggregations of *Bd*-positive samples. We tested whether spatio-temporal aggregations of *Bd* overlapped with areas of historical amphibian population declines and extinctions. A spatio-temporal convergence between *Bd* and historical declines would support that amphibian declines in Brazil were caused by chytridiomycosis, indicating that Brazilian amphibians were not adapted to *Bd*, and thus weakening the hypothesis that Brazil was the global origin of *Bd* emergence. Alternatively, a lack of spatio-temporal association between *Bd* and the historical declines would further contest *Bd* as the cause of the enigmatic declines in Brazil and would give additional support for Brazil as the potential origin of a hypervirulent *Bd* genotype. We used a combination of SaTScan cluster analysis and spatio-temporal randomizations to test whether areas of declines showed higher proportions of *Bd*-infected tadpoles. We also tested whether the incidence of *Bd*-infected tadpoles was higher during the peak of declines along Brazil's Atlantic Forest. Finally, we used Akaike information criterion (AIC) model averaging to test whether *Bd* in tadpoles responds to the impact of macro-environmental variables in the same manner as *Bd* in post-metamorphic anurans. Our results reveal the potential role of chytridiomycosis as the leading cause of catastrophic amphibian declines and extinctions observed in Brazil after the late 1970s, and provide another piece to the puzzle of the origin of the *Bd* panzootic.

2. Material and methods

(a) Sampling

We analysed 32 551 tadpoles (stored among 5597 individual vials) collected between the years of 1930 and 2015. We screened tadpoles from 13 families across 923 localities spanning the six Brazilian ecoregions; see the electronic supplementary material for the list of collections and museums from which samples were obtained. We extracted precise geographical coordinates of collection locality for the majority of analysed specimens. For those specimens without precise locality information, we extracted the municipality's geographical centroid using Geonames (http://www.geonames.org/).

(b) Disease assessment

Retrospective surveys of museum-preserved specimens have been widely used to describe historical *Bd* dynamics in several regions [10,14,15,32]. Thus far, retrospective studies have focused on adult frogs, despite the fact that tadpoles could be constantly exposed to waterborne *Bd* [33]. *Bd* attacks keratinized tissue, which in tadpoles is concentrated in the mouthparts [27]. Infection in tadpoles consequently causes depigmentation in both the jaw sheath and tooth rows [34–39]. Although depigmentation may also result from exposure to environmental contaminants [40] or to very low temperatures [41], the depigmentation pattern due to *Bd* infection is unequivocal; *Bd* causes patchy depigmentation with complete loss of keratin in localized areas compared with fully keratinized surrounding areas [36]. Recent studies found an overwhelming proportion of tadpoles with highly depigmented mouthparts attributed to *Bd*; infection prevalence was estimated at 95% in the Atlantic Forest torrent frog *Hylodes japi* and in the American bullfrog *Lithobates catesbeianus* [39], 100% in the mountain yellow-legged frog *Rana muscosa* from California [37] and 96% in several amphibian species from Australia [42]. In addition, more than 100 *Bd* genotypes were isolated from tadpoles with depigmented mouthparts across

Brazil's Atlantic Forest [21]. Therefore, patterns of mouthpart depigmentation can be effectively used as a proxy for *Bd* infections in Brazil.

We screened individual tadpoles for *Bd* by visually inspecting their buccal apparatus using a dissecting microscope [43]. We included specimens ranging from Gosner stages 25 to 40 in the analyses [44]. All visualizations were performed by the same person (T.C.) for standardization purposes. We considered specimens *Bd*-undetected when they exhibited fully pigmented tooth rows and jaw sheaths. We considered specimens *Bd*-positive if they exhibited patterns of full or partial depigmentation of the buccal apparatus compatible with depigmentation due to chytridiomycosis [36,39,42]. We did not include specimens that exhibited jaw sheaths or tooth rows with slight depigmentation or with tooth rows that were easily detached by gentle manipulation.

To validate our screening method, we (i) performed both histological and qPCR screening [45] of the buccal apparatus of a subset of tadpoles with normal and depigmented mouthparts (electronic supplementary material, figure S1) and (ii) compared data of *Bd* isolation success from tadpoles with normal and depigmented mouthparts. Because histological screening of *Bd* in museum-preserved tadpoles damages specimens, we only received permission to screen 20 tadpoles from Museu de Zoologia 'Prof. Adão José Cardoso', Universidade Estadual de Campinas (ZUEC): 10 tadpoles with mouthparts partially or fully depigmented, and 10 tadpoles with normally pigmented mouthparts (electronic supplementary material, table S1). We sectioned each specimen's buccal apparatus, fractioned tissue (approx. 2 mm^2 fragments) using a scalpel, placed tissue fragments on a glass slide with a drop of distilled water, and screened for *Bd* zoosporangia using a microscope at 400× magnification for 30 min according to [46]. Using a two-way contingency analysis, we confirmed that the probability of finding *Bd* zoosporangia is greater in tadpoles showing signs of mouthpart depigmentation ($\chi^2 = 21.024$; $N = 20$; $p < 0.0001$; electronic supplementary material, table S2).

Additionally, we collected 14 live tadpoles with fully pigmented and 10 tadpoles with fully depigmented mouthparts. We sectioned each specimen's buccal apparatus, fractioned tissue (approx. 4 mm^2 fragments) using a scalpel, and extracted DNA using Prepman Ultra®. We tested samples for *Bd* in singlicate using Taqman qPCR [40], with standards ranging from 0.1 to 1000 zoospore genomic equivalents (g.e.) to determine *Bd* infection loads of each specimen; specimens with g.e. ≥ 1 were considered *Bd*-positive. We confirmed that the probability of detecting *Bd* using qPCR is greater in tadpoles showing signs of mouthpart depigmentation ($\chi^2 = 17.143$; $N = 24$; $p < 0.0001$; electronic supplementary material, table S2).

We also compared *in vitro* cultivation success of *Bd* from live tadpoles collected by LFT's laboratory in Brazil during the last 5 years. We confirmed that the probability of isolating *Bd* from amphibians is greater using tadpoles showing signs of mouthpart depigmentation ($\chi^2 = 167.299$; $N = 174$; $p < 0.0001$; electronic supplementary material, table S2).

(c) Spatio-temporal analyses

We compared the proportion of *Bd*-infected tadpoles among the six Brazilian ecoregions using a generalized linear model (GLM) with binomial distribution (logit link) and conducted detailed downstream analyses for the best-sampled ecoregion (Atlantic Forest; $n = 15\,981$ screened tadpoles). We performed a spatio-temporal analysis to test whether *Bd* is randomly distributed in a space–time setting across the Atlantic Forest using SaTScan v. 9.4 [47]. SaTScan space–time statistic is defined by a cylindrical window with a circular geographical base and with height corresponding to time. The base reflects a purely spatial screening, while the height reproduces the time period of possible clusters. The cylindrical window moves in both space and time in a way that it screens each possible geographical location and size and visits each predefined time period, evaluating all possible overlapping cylinders of different sizes and shapes throughout the study area. We built a model by applying Kulldorff's clustering algorithm [48] under a Bernoulli probability model with 0/1 event data of *Bd*-positives and negative control tadpoles, screening for geographical areas with higher- or lower-than-expected spatio-temporal trends. We set the maximum spatial cluster size at 50% of the population (software default) and lumped our temporal database into four equal intervals of 21 years because our dataset does not meet a satisfactory spatial coverage at higher temporal resolutions [15]; yet we reported on datasets with time-aggregations of 20, 18, 16, 14, 12 and 10 years. We compared clusters to the entire study area using the maximum-likelihood ratio statistic to infer statistical significance for the most likely clusters. A *p*-value was assigned to the clusters comparing the log-likelihood ratio between the most likely clusters and a randomized dataset. For more details, see [49].

We performed spatial randomizations to test whether our detected SaTScan clusters of *Bd*-positive samples overlapped spatio-temporally with sites of reported amphibian population declines and extinctions (electronic supplementary material, table S3). Our automation randomized the centroid location of each of our significant SaTScan clusters within the Atlantic Forest 100 times, and recorded the number of reported amphibian population declines and extinctions that overlapped spatio-temporally. We then statistically compared the average number of amphibian population declines obtained with our randomizations to the observed overlap with our SaTScan *Bd*-clusters at 95% CI. Our spatio-temporal database included 26 out of 64 (40.62%) amphibian species with reported population declines and extinctions.

We extracted the Studentized deviance residuals of a logistic regression of time (years) against *Bd* (positive/total observed) to use as a temporally detrended variable of *Bd* infection. We then used a one-tailed *t*-test to test whether *Bd*-infections (temporally detrended) were higher during the peak of the reported amphibian population declines and extinctions (i.e. 1979–1987) than during years of low amphibian population declines.

(d) Environmental analyses

We used model averaging based on the AIC, including *Bd* as the response variable (temporally detrended) and the following environmental factors as explanatory variables: human footprint, vegetation density, precipitation, temperature, topographic complexity and elevation; all these variables are shown to impact macroclimate associated with *Bd* persistence in the wild [23,50]. Model averaging allows us to make inferences based on a set of candidate models, not just the best-fit model. This GLM approach ranks parameter estimates from each possible model using a cut-off AIC weight quantile of 0.95, and thus allows us to detect the strength and the direction of explanatory variables that will most probably influence disease [51]. We extracted data on 11 temperature variables (bio1–bio11) and eight precipitation variables (bio12–bio19) from worldclim/bioclim [52]. These metrics were calculated based on a dense network of climatic stations throughout the world (i.e. precipitation data from 47 554 localities and temperature data from 24 542 localities). We also extracted data on human footprint [53], topographic complexity [54], elevation and vegetation density [55] for each sampling location using Arc Map v. 10.1 [56]. All rasters were generated at a scale of 1 km.

We performed two principal component analyses to consolidate climatic variables owing to their high cross-correlation. We used the scores of the first principal component

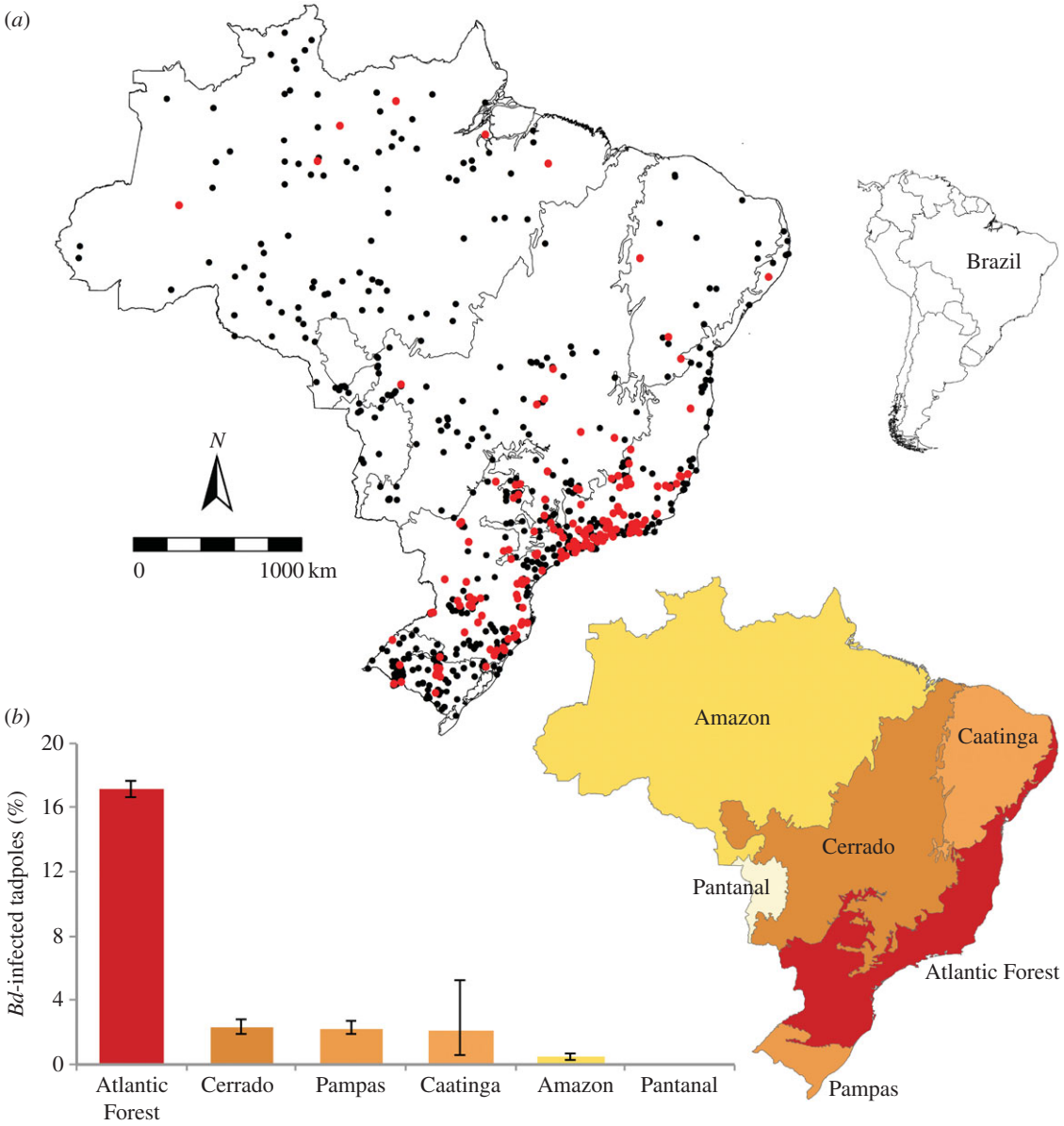

Figure 1. Geographical distribution of *Bd*-infected (red dots) and *Bd*-undetected tadpoles (black dots) collected between 1930 and 2015 (*a*). Proportion of *Bd*-infected tadpoles across Brazilian ecoregions; warmer colours indicate higher proportion of *Bd*-infected individuals (*b*); averages and 95% binomial confidence intervals are shown. (Online version in colour.)

depicting temperature and the first principal component depicting precipitation as explanatory variables in the analyses. To account for potential *Bd*-screening errors, we also conducted an independent AIC model averaging where we randomly selected 10% of the tadpoles with depigmented mouthparts (*Bd*-positive) and treated them as normal tadpoles (*Bd*-undetected). The influence of taxonomy was not considered in the analyses because the proportion of *Bd*-infected tadpoles increased with sample size similarly among sampled families (Pearson correlation $r = 0.827$, $p = 0.0005$; electronic supplementary material, figure S2).

3. Results

We found that Brazil's Atlantic Forest showed the highest proportion of *Bd*-infected tadpoles when compared with the remaining Brazilian ecoregions (Cerrado, Pampas, Caatinga, Amazon and Pantanal; $\chi^2 = 2469.65$; $N = 32\,551$; $p < 0.0001$; figure 1). Our SaTScan spatio-temporal analysis detected five clusters of *Bd*-positive samples (electronic supplementary material, table S4): one large and highly significant cluster (*Bd*-C1) in the southeastern Atlantic Forest with temporal aggregation from 1974 to 2015, and four smaller clusters in southern Brazil (figure 2). We repeated this analysis at six alternative time-aggregations of samples and results remained consistent for *Bd*-C1 (electronic supplementary material, table S5). Our null model indicated that the combined area of these five significant *Bd*-clusters had a higher-than-expected spatio-temporal overlap with most records of amphibian population declines and extinctions in Brazil's Atlantic forest (sites with reported declines: $p = 0.002$; number of populations with reported declines: $p = 0.011$; electronic supplementary material, figure S3). Furthermore, we found a higher proportion of *Bd*-positive samples (14.5%) during the peak of amphibian declines (1979–1987) than during years of low population declines (8.6%; $t = 1.992$; $N = 73$; $p = 0.035$; figure 3).

Our AIC model averaging found a positive effect of elevation (std. $\beta = 0.323$; s.e. $= 0.031$) topographic complexity (std. $\beta = 0.224$; s.e. $= 0.023$), and rainfall (std. $\beta = 0.083$; s.e. $= 0.022$), and a negative effect of temperature (std. $\beta = -0.125$; s.e. $= 0.030$) on the likelihood of *Bd* occurrence in Brazil. Our results remained unaltered after including a

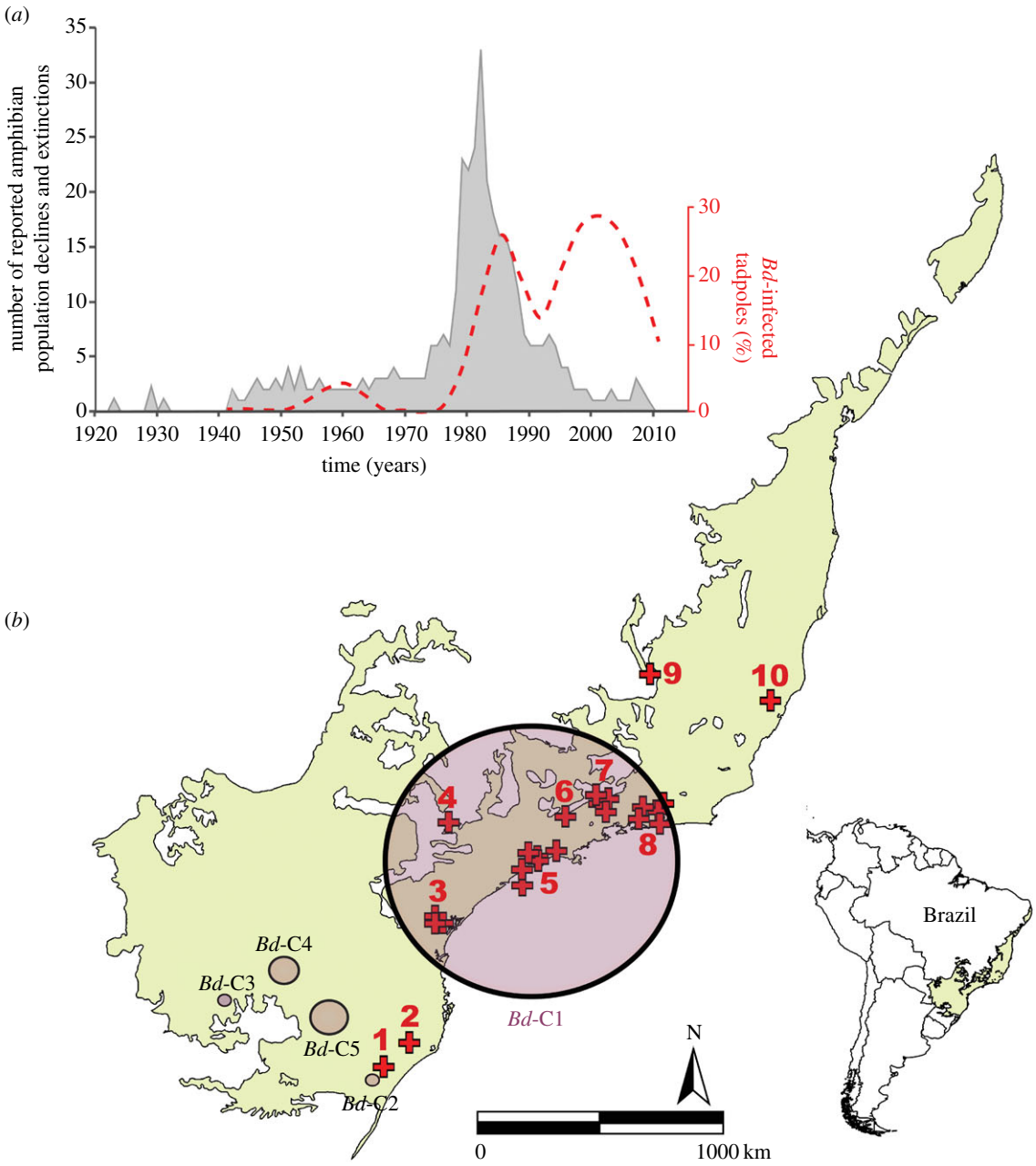

Figure 2. Amphibian population declines and extinctions reported in Brazil's Atlantic Forest during the last 95 years and historical proportion of *Bd*-infected tadpoles (*a*). SaTScan clusters of *Bd*-positive samples (C1–C5 circles) and sites of amphibian population declines and extinctions (red symbols) (*b*). Crosses represent decline sites; numbers of corresponding sites can be found in electronic supplementary material, table S3. (Online version in colour.)

conservative margin of error of 10% for potential inaccurately detecting *Bd* from depigmented mouthparts (electronic supplementary material, table S6).

4. Discussion

Our results provide strong evidence that chytridiomycosis caused most historical amphibian declines observed in Brazil. Specifically, the significant increase in *Bd* prevalence during the peak of die-offs, combined with a spatio-temporal overlap of *Bd*-infected tadpoles and areas of historical declines, support the hypothesis that *Bd* played an important role in amphibian declines across the Atlantic Forest. The most significant spatio-temporal cluster of *Bd*-infected tadpoles included the majority of sites with reported amphibian declines after the late 1970s. Because the time frame of these die-offs largely overlapped with declines observed in other regions [9,27,28], our results challenge the hypothesis of Brazil as the global origin of a hypervirulent *Bd* genotype. Our findings support The Ghost of Epizootics Past hypothesis; communities affected by chytridiomycosis in other tropical regions show the same signatures of decline as Brazil's amphibian communities. Specifically, amphibian communities in Brazil's Atlantic Forest showed a similarly high proportion of population declines among montane stream-breeding frogs (i.e. *Cycloramphus*, *Crossodactylus*, *Hylodes*, *Phrynomedusa*) when compared with communities affected by *Bd* in Central America and Australia [9,28,29].

There are at least three hypotheses to explain the link between *Bd* and amphibian population declines in Brazil after the late 1970s, including: (i) the arrival of a novel genotype of the Global Panzootic Lineage (*Bd*-GPL) [16,17] (the most virulent clade associated with declines on different continents) [9,18,27,57]; (ii) a sudden increase in virulence of a local *Bd* genotype, through genetic mutation/recombination

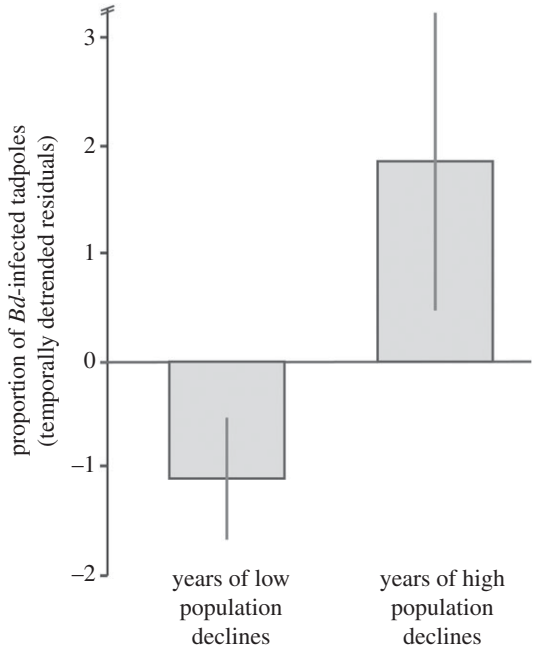

Figure 3. Proportion of *Bd*-infected tadpoles (temporally detrended residuals) during years of low (1930–1978; 1988–2015) and high amphibian population declines (1979–1987); averages and standard errors are shown.

[17,58,59] or phenotypic adaptations [60]; and (iii) a synergetic effect of multiple impacts, such as climate change, habitat alteration and pollution [61–64], which may contribute to an increase in disease incidence following the long-term presence of mainly enzootic *Bd*. These three hypotheses are not mutually exclusive and further studies are needed to investigate mechanisms responsible for the observed shift in disease dynamics in Brazil.

The arrival of a novel genotype within the *Bd*-GPL clade, or an increase in virulence of a local genotype with subsequent spread, are plausible explanations for the observed population declines of Atlantic Forest frogs. Genomic resequencing of *Bd* isolates revealed that a recently diversified and hypervirulent lineage (i.e. GPL) is associated with the observed amphibian population declines in Central America, Australia and North America [16,17]. *Bd*-GPL spread throughout Central America covering over 1500 km in 13 years [9,65]. A similar *Bd* spreading pattern in Brazil would cover enough ground to reach the major decline sites in the Atlantic Forest from 1979 to 1987 (i.e. Boraceia, Itatiaia, Serra dos Órgãos and Santa Tereza). Furthermore, the intensified bullfrog trade in southeastern Brazil after the 1970s may have also played an important role increasing the pace of the spread of *Bd* in that region [66]. The additional four narrow clusters we detected in the southern range of the Atlantic Forest also coincided with areas of bullfrog farming, which were higher in southern Brazil in the early 1990s [67]. This recent increase in bullfrog farming in the south may help explain the second peak of prevalence. Furthermore, this second peak may be also linked to local frogs evolving tolerance after the first peak and leading to enzooticity. Although *Bd*-GPL has been endemic in Brazil for over a century [14,15], our results cannot rule out the possibility of an introduction of a novel hypervirulent *Bd*-GPL genotype occurring in the late 1970s. Furthermore, the most significant *Bd* cluster overlaps with an area where *Bd*-GPL and the narrowly distributed *Bd*-Brazil overlap and hybridize [14,21]. These findings underscore the need for experimental work testing the virulence of hybrid *Bd* genotypes on the local anurofauna.

Climate change triggering independent *Bd* outbreaks in several pristine regions where *Bd* is enzootic (e.g. Atlantic Forest) would give support to the Endemic Pathogen hypothesis, which posits that *Bd* has globally coexisted with amphibians for a long time but emerged as a virulent pathogen due to environmental or other exogenous factors [61,68]. Global El Niño climatic events could cause amphibian population declines by increased temperature variability at regional scales, reducing frog defences against *Bd* [68]. Furthermore, montane frogs tend to be cold-adapted, which could make them more susceptible to the synergistic effects of disease and global climate change [69]. Although comparative genetic studies of *Bd* isolates found low genetic diversity consistent with a rapid expansion of *Bd*-GPL [70–72], climate change may well be facilitating *Bd* spread or outbreaks via reducing host defences. Deforestation is another obvious hypothesis to explain the historical amphibian declines in Brazil's heavily fragmented Atlantic Forest. Nevertheless, most declines were observed in protected parks, and *Bd* infection loads are usually higher in these pristine closed-canopy forests [23]. Furthermore, most of Atlantic Forest had been deforested before the 1960s [73], thus it is unlikely that deforestation is a confounding effect in our analyses.

Contrary to our findings linking *Bd* to the historical amphibian declines in Brazil, previous work on the historical distribution of *Bd* failed to detect an increase in pathogen prevalence during the years of decline [14,15]. Our study differs from previous research in that we focused on tadpoles while past studies focused on post-metamorphic amphibians from the Atlantic Forest [14] and the Amazon basin [15]. All anuran life stages can be infected with *Bd*, and although tadpoles rarely die [36,38], recently metamorphosed froglets are the most vulnerable life stage to *Bd*-induced mortality [38]. Thus, many tadpoles may not survive to the adult stage, and studies focused solely on adult frogs may miss important host–pathogen dynamics and may not properly detect the disease-causing agent. *Bd* infection in tadpoles could lead to decreases in population fitness, because infected tadpoles forage less, grow more slowly, and have reduced size at metamorphosis [38,74,75]. Decreases in tadpole fitness may thus cause downstream effects on population persistence, leading to slow-paced or silent population declines.

The strength of our results is supported by published environmental niche models and regression analyses. We detected higher proportions of *Bd*-infected tadpoles in areas where previous environmental niche models estimated high likelihood of *Bd* persistence, including the Atlantic Forest [15,18,50,63,76,77]. Furthermore, our interpretations are backed up by our multi-model inference. Our AIC model averaging showed that *Bd* infection likelihood in tadpoles was positively associated with elevation, rainfall and topographic complexity, and negatively associated with temperature across Brazil. These results are in agreement with previous studies that found comparable effects of the same macroenvironmental variables on *Bd* in adult anurans [15,23,50], supporting our interpretations that *Bd* is the culprit behind historical amphibians population declines in Brazil.

Our study presents novel information about the global emergence of *Bd* and provides strong evidence for another catastrophic case of chytridiomycosis impacting dozens of amphibian species. Our results indicate that Brazil's amphibian

diversity is not buffered from epizootics, and that the reported declines in the Atlantic Forest are not fundamentally different than those caused by *Bd* elsewhere. What sparked this panzootic is still an open question, and recent findings support that global climate change could trigger epizootics in regions of endemic *Bd*-GPL [69]. Therefore, further studies focused on the synergistic impacts of climate change and pathogen spread will be key to solving this problem. Studies focused on the signatures of amphibian declines in natural communities, genotype diversification, and *Bd* spatial turnover in areas of observed extinctions could also help elucidate patterns of host resistance and susceptibility to *Bd*. Understanding the history of the emergence of chytridiomycosis may not only guide efforts to prevent future amphibian die-offs, but will also contribute to our understanding of the epizootiology of other emerging fungal diseases.

Ethics. All museum-preserved specimens were used with the appraisal of the curators. No live specimens were used in the analyses.

Data accessibility. Database available from the Dryad Digital Repository at: http://dx.doi.org/10.5061/dryad.4t53n [78].

Authors' contributions. T.C., C.G.B. and L.F.T. conceived the idea of the study. T.C. collected field data. T.C., L.F.T. and C.G.B. carried the statistical analyses, data analyses interpretation and wrote the manuscript. All authors gave final approval for publication.

Competing interests. We declare no competing interests.

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