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Detection of the *Batrachochytrium dendrobatidis* global panzootic lineage in Ecuadorian anurans of the Amazonian lowlands

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ABSTRACT: Considerable attention has been directed to studying the infection dynamics of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) affecting amphibians in the high elevations of the Neotropics. However, lowland forests of the same realm remain comparatively understudied in this context. Herein, we attempt to bridge this gap by measuring the prevalence of *Bd* via quantitative polymerase chain reaction (qPCR) in several anuran taxa inhabiting the Amazonian lowlands in the northeast of Ecuador. To this end, we sampled 207 anurans from 10 different families, 25 different genera, and 55 distinct host species originally collected in 2008. Taxonomy (at the family level), morphology (i.e. weight and snout–vent length), and life-long aquatic dependency of hosts (i.e. aquatic index) were also compiled to serve as potential predictors of *Bd* infection status. Our findings revealed a relatively high *Bd* prevalence of 58%, with 88% of sampled anuran families testing positive for the fungus at varying proportions. Model selection involving fitting and testing several different linear models, including mixed linear models, revealed a significant negative relationship between host weight and *Bd* infection status ($p < 0.01$). However, no significant associations were observed between taxonomy, aquatic dependency, snout–vent length, and *Bd* infections. In addition, we only detected the global panzootic lineage of *Bd* (*Bd*-GPL) and not the *Bd*-Asia-2/*Bd*-Brazil lineage via qPCR single nucleotide polymorphism (SNP) genotyping. Our findings contribute to the understanding of *Bd* dynamics in the Neotropical lowlands and emphasize the need for future research on the ecological factors influencing *Bd* in the Amazon and their implications for amphibian conservation.

KEY WORDS: Bd-GPL · Chytrid · Ecuador · Amazon · Bromeliads · Aquatic index · Emerging infectious disease

1. INTRODUCTION

The Neotropics, encompassing Central America, the Caribbean, and South America, boast remarkable biodiversity, surpassing the combined diversity of plants and animals in the African and Southeast Asian tropics (Harvey et al. 2020, Raven et al. 2020). Unfortunately, this rich biodiversity faces significant risks due to various factors, including global climate change, deforestation, and disease (Pounds et al. 2006, Lips et al. 2008, Newbold et al. 2015, Fisher & Garner 2020, Antonelli 2022). Amphibians, particularly anurans, constitute one of the most diverse vertebrate groups in the Neotropics (Vasconcelos et al. 2019); yet they are also one of the most threatened vertebrates in South America and also worldwide (Vitt & Caldwell 2013, Menéndez-Guerrero et al. 2020). Among the multitude of threats, emerg-

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ing infectious diseases (EIDs) have become a major challenge to amphibian conservation in the Neotropics (Menéndez-Guerrero & Graham 2013, Ortega-Andrade et al. 2021).

EIDs may spill over or jump from one taxon to another, not just threatening regional biodiversity (Daszak et al. 2000, Zipkin et al. 2020) but potentially whole ecosystems (Lips et al. 2006, Whiles et al. 2013, Rantala et al. 2015, Scheele et al. 2019). Chytridiomycosis is one such wildlife EID caused by the amphibian-killing fungus (i.e. *Batrachochytrium dendrobatidis*, hereafter *Bd*), which infects the skin of amphibians (Longcore et al. 1999, Grogan et al. 2018, Martel et al. 2018). This waterborne disease is characterized by degradation of the mouthparts of larvae or hypo- or hyper-keratinization of skin cells in adult amphibians. The resulting failure of gas exchange and electrolyte transport in the animal can eventually lead to death (Voyles et al. 2007, Van Rooij et al. 2015). *Bd* infects over 700 species (Lips et al. 2006, Scheele et al. 2019), yet susceptibility to, and prevalence of, this disease seems to be environment-, host-, population-, and strain-specific (Fisher & Garner 2020).

Amphibians in the Neotropics have been severely impacted by this fungal disease (Cheng et al. 2011, Azat et al. 2022), and Ecuador is especially relevant because it is home to one of the highest numbers of amphibian species in the Neotropics (Frost et al. 2006, Coloma et al. 2018, Ortega-Andrade et al. 2021). Furthermore, over a third of these species are considered among the most threatened in South America, as they are being extirpated at an alarming rate due to a host of risk factors which also includes *Bd* (Ortega-Andrade et al. 2021). Within Ecuador, as in other parts of Central and South America, detection of *Bd* and reports of chytridiomycosis have mostly come from the highlands (Menéndez-Guerrero & Graham 2013, Guayasamin et al. 2014, Bresciano et al. 2015). Meanwhile, studies on the ecology and epidemiology of *Bd* have less frequently focused on lowland habitats such as the Amazon region (Puschendorf et al. 2009, Zumbado-Ulate et al. 2019), which encompasses approximately 42% of Ecuador's landmass (UNDP 2022). Thus, major knowledge gaps still exist regarding *Bd* disease dynamics and their implications in these low-elevation regions of South America (Becker et al. 2016, Zumbado-Ulate et al. 2019).

Understanding the influence of host traits (e.g. behavior, size, and life history) on disease susceptibility in the Ecuadorian Amazon also needs more attention. For instance, in parts of South and Central America, the effects of *Bd* are correlated to the host's exposure to water during different life-history stages (Lips et al. 2003, Kriger & Hero 2007, Mesquita et al. 2017, Sette et al. 2020, Byrne et al. 2022). Accordingly, direct-developing (i.e. tadpole life stage absent) species that have little or no contact with aquatic environments during their ontogeny (Duellman & Trueb 1994, Altig & McDiarmid 2007) generally have lower *Bd* prevalence than their aquatic counterparts (Lips et al. 2003, Kriger & Hero 2007, Bielby et al. 2008, Brem & Lips 2008); however, there are some exceptions (Gründler et al. 2012, Ribeiro et al. 2020). Most of these data come from studies focusing on the highlands; thus, whether the same underlying factors apply to host–pathogen relationships in the Amazon Basin still requires investigation.

Globally, multiple divergent lineages of *Bd* have been identified using whole genome sequences (Farrer et al. 2011, Rosenblum et al. 2013, O'Hanlon et al. 2018). Three have so far been detected in South America, namely, the global panzootic lineage referred to as *Bd*-GPL, the *Bd*-Asia-2/*Bd*-Brazil lineage, and a hybrid lineage (Schloegel et al. 2012). *Bd*-GPL, the most prevalent and hypervirulent among all the lineages (Farrer et al. 2011, James et al. 2015, O'Hanlon et al. 2018), has been detected in at least 5 different South American countries, specifically, Colombia, Peru, Brazil, Chile, and Bolivia (O'Hanlon et al. 2018). The *Bd*-Asia-2/*Bd*-Brazil lineage has been reported in the Atlantic Forest of Brazil (Jenkinson et al. 2016) but was first detected on a frog, imported from Brazil, that was acquired at a local market in Michigan, USA (Schloegel et al. 2012). The diversity and distribution of these strains underscore the need for further genotyping studies in Ecuador to better understand the prevalence and dynamics of lineage-specific *Bd* infections, as has been previously demonstrated in other regions of the world (Byrne et al. 2017, 2019, Abarca et al. 2021, Ghosh et al. 2021, Carvalho et al. 2023).

Our study aimed to (1) investigate the association between aquatic dependency, morphology, familial taxonomy, and prevalence of *Bd* in a lowland Amazonian Forest in Ecuador; and (2) genotype *Bd* collected from various anuran species in the area. In doing so, we expand ongoing attempts at divulging crucial information on the obscure infection patterns of the amphibian-killing fungus in Neotropical lowlands. Additionally, our study highlights the need to reconsider previously identified predictors of *Bd* dynamics, such as aquatic dependency, size, and taxonomy, in the context of lowland tropical forests, with special attention to transient or permanent bromeligenous species that inhabit phytotelmata. Although

major *Bd*-driven amphibian declines have not been reported in lowland South American species, our results suggest that these sites could still contribute to the spread and persistence of the amphibian-killing fungus.

2. MATERIALS AND METHODS

2.1. Study site

Sampling was conducted at the Tiputini Biodiversity Station, Orellana Province, Ecuador (0.6379° S, 76.1498° W; 217 m elev.). Founded in 1994 by Universidad San Francisco de Quito (USFQ), the station lies along the Rio Tiputini and is adjacent to the Yasuní Biosphere Reserve, which is renowned for its biodiversity (Bass et al. 2010). The research station has 139 documented amphibian species within its 6.5 km^2 boundary, spanning the 3 orders Caudata, Gymnophia, and Anura, but there are 150 species known from the greater Yasuní Biosphere Reserve (Bass et al. 2010, USFQ 2023). The climate of the Yasuní region is typically described as aseasonal, with temperatures averaging 25°C (15°−38°C), humidity averaging 88%, and the northwestern area (study location) receiving an average of 2425−3145 mm of rainfall per year, with no less than 100 mm per month (December and January are the driest months) (Bass et al. 2010, McCracken & Forstner 2014).

2.2. Sample collection

All observations and data were collected between March and November 2008. Anurans were sampled during terrestrial surveys or tree canopy bromeliad surveys as part of a previous study to document the amphibian diversity of the Tiputini Biodiversity Station (McCracken & Forstner 2008, McCracken et al. 2009). Each individual was collected and held in a new zip-lock bag with a small amount of commercially bottled water added before being processed to prevent desiccation. Processing consisted of weighing, measuring, photographing, and taking a small tissue sample. Morphological measurements for snout−vent length (SVL) were taken with a Mitutoyo CD-S6'C digital dial caliper (0.01 mm precision), and weights were taken with a Pesola digital pocket scale (0.01 g precision) on live specimens.

Appropriate cross-contamination preventative measures were taken between each anuran processed. These included the use of new nitrile gloves, spraying calipers and surfaces (polyethylene cutting boards) with 70% ethanol and air drying afterwards, and flame-sterilizing scissors, scalpels, and forceps between individuals. For voucher specimens a small portion of thigh muscle tissue that included skin was extracted prior to fixation. A toe clip was collected from anurans being returned to their original capture location. All tissue samples were placed in 1.8 ml Nunc cryotubes (Thermo Fisher Scientific) with 95% ethanol, stored at 4°C in the field, and transferred to −80°C for long-term storage. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen), and DNA presence and quality were assessed using agarose electrophoresis.

This research was conducted in compliance with the rules overseen by the Texas State University Institutional Animal Care and Use Committee (Protocol nos. 0721-0530-7, 05-05C38ADFDB, and 06-01C694 AF). Permission and permits were issued by the Ministerio del Ambiente, Ecuador (Permit nos. 006-IC-FA-PNY-RSO, 012-IC-FA-PNY-RSO; Provincial de Orellana Fauna permit number 0018 DPO-MA; and Provincial de Napo Fauna permit number 017-IC-FAU/FLO-DPN/MA).

2.3. *Bd* detection, quantification, and genotyping via qPCR

Reactions for the quantification of *Bd* load infection intensity were run in singlicate 25 µl volumes comprising 5 µl of DNA (diluted 1:10) and 12.5 µl of TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific), 2.75 µl nuclease-free water, 0.625 µl of primer ITS1-3 Chytr (18 µM), 0.625 µl of primer 5.8S Chytr (18 µM), 0.625 µl of probe Chytr MGB2 (5 µM), and 0.50 µl bovine serum albumin (400 ng μl^{-1}) per reaction (Boyle et al. 2004, Kriger et al. 2006). A standard curve was generated using the global panzootic strain JEL423 (Fisher et al. 2012), which had a dynamic range of 0.1 to 1000 zoospore equivalents (ZE). We considered samples *Bd* positive via qPCR if the load was greater than 1 ZE.

All samples were genotyped, regardless of *Bd* presence or absence, using a single nucleotide polymorphism (SNP) assay that discriminates between *Bd*-GPL and *Bd*-Asia2/*Bd*-Brazil (SC9_200709_CT) based on 27 global *Bd* genomes (Rosenblum et al. 2013). The primers amplify a 109 base pair fragment, and dual probes target an SNP at position 200 709 on the supercont1.9 genomic scaffold of the strain JEL423 reference genome (GenBank: DS022308.1). The dual probes can detect either *Bd*-GPL (genotype TT), *Bd*-Asia2/*Bd*-Brazil (genotype CC), or a co-infection or

hybrid strain (genotype CT) (Table 1). Genotyping reactions were conducted in singlicate 15 µl volumes comprising 15 µl of TaqMan Fast Advanced Master Mix, 0.75 µl of the SNP assay (20× concentration), 4.25 µl of nuclease-free water, and 5 µl of extracted DNA (variable concentrations). The results were interpreted using the Thermo Fisher Connect cloud service and the 'Standard Curve' and 'Genotyping' applications to detect *Bd* presence/absence, *Bd* infection load, and generate the *Bd* SNP genotype calls.

2.4. Statistical analyses

All analyses were conducted in the R environment for statistical computing (version 4.3.0) (R Core Team 2021). The overall prevalence of *Bd* was determined by calculating the ratio of samples that tested positive for *Bd* to the total sample size. Additionally, the prevalence was calculated for each family of anuran sampled, and 95% Wilson binomial confidence intervals (CI) were generated for prevalence estimates using the epi.conf() function in the 'epiR' package (version 2.0.62) (Stevenson 2022). To maximize the overall sample size and account for the uncertain species-level identification of some samples, individuals were grouped by taxonomic family.

The null hypothesis regarding the absence of a significant relationship between taxonomy (at the family level), morphology (SVL and weight), aquatic index (AI) of the host, and the *Bd* infection status (presence/absence) was tested to investigate the relationship between infection status and host traits. AI assignments followed the approach of Lips et al. (2003), with modifications based on literature sources such as AmphibiaWeb (AmphibiaWeb 2022) and the IUCN Red List of Threatened Species (IUCN 2022). Anuran families were categorized into 4 AI categories: AI0 for terrestrial species with direct development (terrestrial breeders), AI1 for arboreal species that breed in water, AI2 for riparian species that breed in water, and AI3 for direct-developing bromeligenous species, introduced in this study to encompass species reliant on the moist microhabitat of phytotelmata for shelter and/or breeding, bypassing the aquatic larval stage.

The dataset was initially analyzed using summary statistics and visual inspections of variable distributions. Continuous variables that did not exhibit a normal distribution were log-transformed to enhance suitability for subsequent model fitting processes. Next, a null model was fitted using the glmer() function from the 'lme4' package (version 1.1-33) (Bates 2018), employing maximum likelihood (Laplace Approximation) with a binomial family and a logit link. This null model included only an intercept term, serving as the baseline for comparison in the analysis to assess whether adding more predictors improved the model's explanatory ability and overall fit to the data. The mixed model approach was chosen to account for the non-independence of data points due to phylogenetic correlations within families.

Subsequently, a series of general linear mixed models (GLMMs) were fitted to explore how the addition of predictor variables and their interactions influence infection status, accounting for random effects due to familial clustering. A total of 11 GLMMs were tested, including the full model (all 3 predictors) and models with individual predictors and interactions. To determine the best-fitting model, each model's performance score was computed using the compare_performance() function from the 'performance' package (version 0.12.0) (Lüdecke et al. 2021). This involved normalizing 10 different fit indices (scaled from 0 to 1) and calculating their mean value for each model (Table S1 in the Supplement; www.int-res.com/articles/suppl/d160p115_supp.pdf).

Model validation for the best-performing model was conducted using the check_model() function

Table 1. Primer and probe sequences for SC9_200709_CT (Assay ID AHGJ91E), a custom TaqMan single nucleotide polymorphism (SNP) genotyping assay (Applied Biosystems) at 40× concentration (SNP in **bold** and underlined). This assay targets the nuclear genome of *Batrachochytrium dendrobatidis* and discriminates between alleles that identify lineages *Bd*-GPL or *Bd*-ASIA2/*Bd*-Brazil

Primer/Probe	Sequence (5′–3′)	Concentration (µM)	Reporter	Quencher	Strain
Forward primer	GCG GTC ATT GTA AAG GAT ACT GAT ACT	36			
Reverse primer	CAT CAA TTG AAG TCC ATC GAC CAG AT	36			
Reporter 1	CTT TGG TTT CC**G** TCG CAT C	8	VIC	NFQ	*Bd*-ASIA2/*Bd*-Brazil
Reporter 2	CTT TGG TTT CC**A** TCGC ATC	8	FAM	NFQ	*Bd*-GPL

from the 'performance' package. This involved assessing for overdispersion, singularity, multicollinearity among random effects, collinearity among predictor variables, and heteroscedasticity. Residual diagnostics were employed to evaluate model fit and potential misspecifications, supported by simulations to visually inspect residual distributions. Six outliers were detected but disregarded due to satisfactory outcomes from other diagnostic checks, which indicated that they did not detrimentally impact the model's overall accuracy or reliability.

Lastly, an *F*-test for overall significance was conducted using the ANOVA() function in base R. This facilitated the comparison of the best-performing model against the null model. The odds ratio for the best-performing model identified through the *F*-test was generated using the or_plot() function from the 'finalfit' package (version 1.0.6) (Harrison 2023).

3. RESULTS

A total of 207 individual anurans were sampled, spanning 9 families, 25 genera, and approximately 55 known species (Table S2). The overall prevalence of *Bd* infections was 0.58 (95% CI = 0.51–0.64; n = 207). Among families with 5 or more individuals, *Leptodactylidae* had the highest *Bd* prevalence at 0.90 (95% CI = 0.80–0.95; n = 20) while prevalence for *Bufonidae, Dendrobatidae, Hylidae*, and *Microhylidae* ranged from 0.40 to 0.50 (Table 2). The single representatives of *Aromobatidae* and *Ranidae* tested positive, while the single representative of *Centrolenidae* tested negative for *Bd* (Table 2). When grouped by AI, riparian frogs that breed in water (AI2) had the highest *Bd* prevalence at 0.64 (95% CI = 0.49–0.76; n = 44), prevalence was 0.57 (95% CI = 0.46–0.70; n = 65) for terrestrial species with direct development (AI0), also 0.57 (95% CI = 0.41–0.72; n = 35) for direct-developing bromeligenous species (AI3), and 0.54 (95% CI = 0.42–0.66; n = 63) for arboreal species that breed in water (AI1) (Table 2). Prevalence was not statistically different among different AI categories (Table S1).

Neither the median weight nor SVL of the host was significantly different between infected and uninfected individuals (Fig. 1). The GLMM with weight (log-transformed to adjust for positive skewness) as the predictor variable and Family as the random effect term was identified as the best-performing model (performance score = 69%) (Table S1). The fixed effect for weight was statistically significant (estimate = −1.00, SE = 0.30, z = −3.30, p < 0.001), indicating that higher weight was associated with lower odds of infection (Table S3). The *F*-test for overall significance indicated a significant improvement in model fit when including the predictor variable weight (χ^2 = 12.99, df = 1, p < 0.001), compared to the null model containing only an intercept (Table S4). This is further supported by the odds ratio (OR) analysis for weight (OR = 0.37, 95% CI = 0.20 – 0.67, p = 0.001). This OR indicates that for each unit increase in weight, the odds of infection decrease by 63% (since 1 – 0.37 = 0.63). The confidence interval does not include 1, reinforcing that the association is statistically significant.

All DNA extractions from the toe clips showed a high molecular weight band on an agarose gel. Out of 120 *Bd*-positive samples via qPCR, 72 (60%) were genotyped as strain *Bd*-GPL based on the results of the SNP Assay. The remaining 48 positive samples did not show an amplification curve for either dye. As expected, samples that were qPCR negative also did not return a genotype. The median infection intensity for genotyped samples was 2329 ZE with a range of 458–1 048 416 ZE, and 122 ZE with a range of 1.83–2656 ZE for non-genotyped samples (Fig. S1). These median infection intensity values were significantly different (2-sample Wilcoxon test; W = 306, α = 0.05, p < 0.0001).

Table 2. Prevalence of *Batrachochytrium dendrobatidis* (*Bd*) infections, sample sizes, estimates of standard error, and lower and upper Wilson binomial confidence intervals (CIs) for 9 anuran families sampled at Tiputini Biodiversity Station, Ecuador, and for individuals grouped by their aquatic index (AI) with life-stage habitat (adult/larval)

Category	Prevalence	Sample size	SE	Wilson binomial CI
Aromobatidae	1.00	1	0.70	0.50–1.00
Bufonidae	0.50	10	0.41	0.35–0.65
Centrolenidae	0.00	1	0.70	0.00–0.50
Dendrobatidae	0.50	11	0.39	0.36–0.64
Hylidae	0.50	58	0.26	0.43–0.56
Leptodactylidae	0.90	20	0.27	0.80–0.95
Microhylidae	0.40	5	0.49	0.22–0.62
Ranidae	1.00	1	0.70	0.50–1.00
Strabomantidae	0.58	100	0.22	0.53–0.63
AI0-terrestrial/terrestrial	0.57	65	0.25	0.46–0.70
AI1-arboreal/aquatic	0.54	63	0.25	0.42–0.66
AI2-terrestrial/aquatic	0.64	44	0.27	0.49–0.76
AI3-arboreal/terrestrial	0.57	35	0.30	0.41–0.72

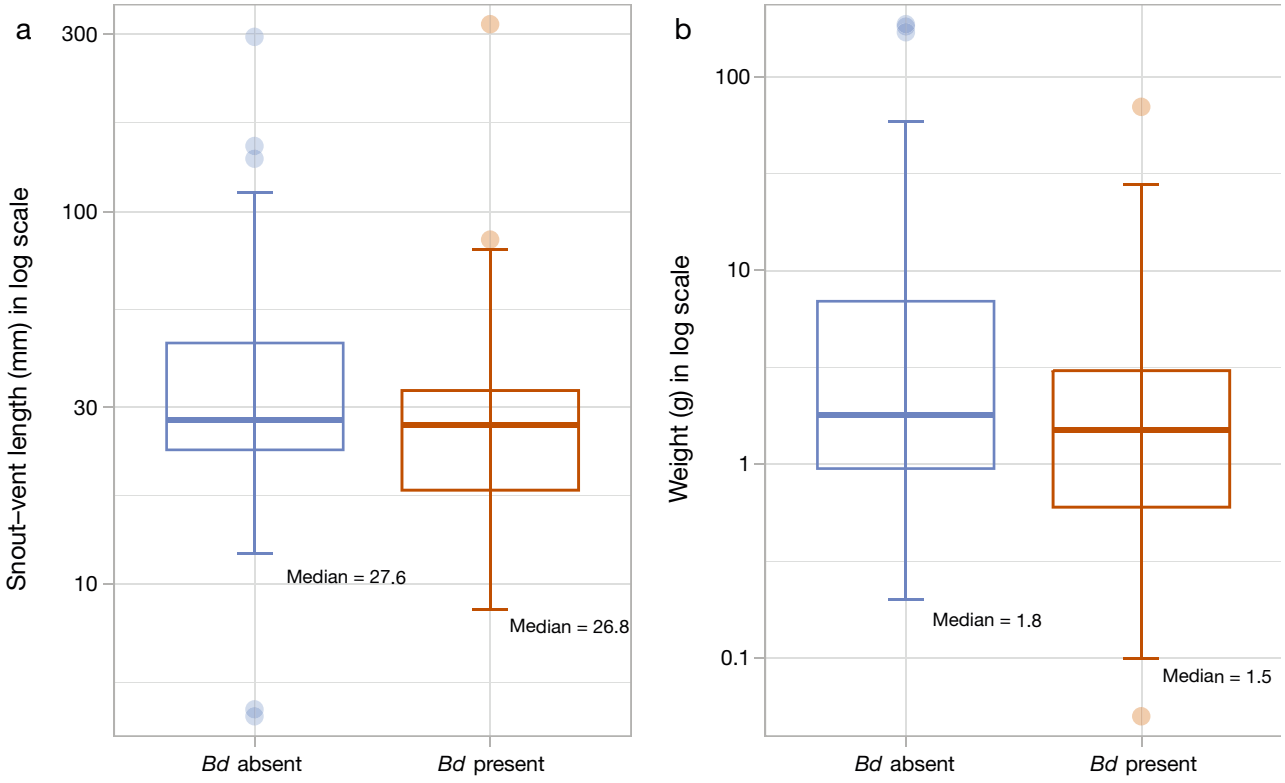

Fig. 1. Boxplots comparing (a) snout–vent length in mm and (b) body weight in g between non-infected anurans (*Bd* absent) and anurans infected with *Batrachochytrium dendrobatidis* (*Bd* present) at Tiputini Biodiversity Station, Ecuador. The *y*-axes have been log-transformed, boxes represent the interquartile range (IQR), and the horizontal lines inside the boxes indicate the median. Whiskers extend to 1.5 times the IQR from the first and third quartiles, and dots indicate outliers

4. DISCUSSION

4.1. Overview

Since *Bd* infections in amphibians first gained widespread attention in the late 1990s (Berger et al. 1998, Lips 1999, Longcore et al. 1999), a significant amount of research has been carried out on various aspects of amphibian chytridiomycosis in the Neotropics. However, the majority of these *Bd* studies, thus far, have prioritized highlands and montane ecosystems over lowland tropical forests. While these warmer lowlands might not provide abiotic conditions within the optimal physiological parameters for this pathogenic fungus (Piotrowski et al. 2004, Ron 2005, Puschendorf et al. 2009, Liu et al. 2013, Menéndez-Guerrero & Graham 2013) — however, see McCracken et al. (2009) for an exception — the role of Neotropical lowlands as putative *Bd* reservoirs or sinks has only recently begun to be investigated (Becker et al. 2016, Rodríguez-Brenes et al. 2016, Russell et al. 2019, Zumbado-Ulate et al. 2019). Our study is one amongst only a handful of contributions to a better understanding of the associations between anuran host traits and *Bd* infections in the lowlands of South America.

4.2. Overall *Bd* prevalence and genotyping

Our results indicate an overall higher *Bd* prevalence (0.58, n = 207) than those reported by earlier studies from the Amazonian lowlands; specifically, 0.38, n = 1391 (retrospective study; Becker et al. 2016), 0.34, n = 324 (Russell et al. 2019), 0.007–0.073, n = 282 (von May et al. 2018), and one study (0.20; n = 86) that was carried out at the same location (McCracken et al. 2009). Our prevalence data are comparable to other studies in the lowlands of Costa Rica in Central America (0.546, n = 348) (Zumbado-Ulate et al. 2019) and Brazil in South America (1.0, n = 40) (Ruggeri et al. 2020), although the latter only tested tadpoles of *Boana faber*. Most *Bd* studies on adult amphibians now routinely rely on DNA extracts from skin swabs of a larger body area; consequently, we may have underestimated *Bd* prevalence and infection intensity if we potentially missed low-load infections. However, some studies have shown no differences between swab and

toe extracts on *Bd* detection (at early life stages) or infection intensity estimates (Hyatt et al. 2007, Burrowes et al. 2011). Regardless, we recommend future *Bd* surveillance efforts in this region use standardized *Bd* swabbing procedures.

There is some limited evidence of *Bd*-GPL from the Andes and the east coast of Ecuador detected on swabs taken from 5 museum specimens of *Atelopus* sp. and *Telmatobius* sp. (Byrne et al. 2019). However, to the best of our knowledge, no prior information on the genotype of *Bd* was known from the Ecuadorian Amazon. Based on our results, this site only showed evidence of the global panzootic lineage with no detection of *Bd*-Brazil/Asia2. This is consistent with the genotyping results for *Bd* from the Peruvian Amazon (Russell et al. 2019) and extends the range of *Bd*-GPL to now include other parts of Ecuador along with other South American countries such as Brazil, Chile, and Colombia.

Our molecular results show a clear and strong positive association between the infection intensity (i.e. the load of *Bd*) and the odds of the *Bd* lineage being successfully genotyped (Fig. S1). This implies that samples that tested positive for *Bd* might not have returned a genotype unless the fungal load was above a given threshold (roughly between 458 and 2656 ZE). Future studies should be mindful that *Bd* infection prevalence rates in a sampled population are prone to underestimation if single SNP qPCR genotyping is used as the sole method of chytrid detection.

4.3. Body measurements and *Bd* infection status

The median weight (and SVL) across families indicated significant variation in body size among different taxonomic groups in our dataset (Table S5). To account for potential phylogenetic relationships and other family-specific factors that might affect the response variable, the taxonomic family was included as a random effect in the model. After accounting for variability between families, host weight was found to be a significant predictor of *Bd* infection status (Table S1). The median weight of uninfected individuals (1.80 g) was slightly larger than the median weight of infected individuals (1.50 g) (Fig. 1b). According to our GLMM, for each unit increase in weight, the log-odds of being infected with *Bd* decreased by 1.00, indicating that heavier individuals are less likely to be infected by *Bd*. The median SVL of uninfected anurans (27.6 mm) was also higher than that of infected anurans (26.9 mm) (Fig. 1a). Models including SVL performed poorly compared to the model with only weight (Table S3). However, because we do not have genetic data for the host, we cannot fully account for phylogenetic relatedness among host species. Including the taxonomic family as a random effect may not capture true evolutionary relationships within and between families. Although we found a significant effect of mass, any effect of mass could be an effect of species (e.g. smaller sampled species may be the ones that tend to be infected). Including family as a random effect does not fully account for multiple samples from the same species. Consequently, some observed effects might be influenced by unaccounted phylogenetic factors. Future studies with comprehensive phylogenetic data and the use of phylogenetic linear models would be better positioned to disentangle the effects of body size and phylogenetic relatedness on *Bd* infection status.

The influence of host size on the infection intensity and status of *Bd* is not clear. Some research has shown that larger (and older) hosts in several different organisms (including frogs infected with *Bd*) can have more developed immune systems and are therefore able to mount better defenses against pathogens (Møller et al. 1998, Lamirande & Nichols 2002, Wilcoxen et al. 2010). For example, Burrow et al. (2017) investigated the association between host size and *Bd* and found that smaller size in anurans increased susceptibility to diseases. Similarly, studies on Australian frogs and European anurans have uncovered an inverse relationship between the status of *Bd* infection and SVL (Kriger et al. 2006, Kriger & Hero 2007) and mass (Meurling et al. 2024), respectively. Conversely, research has also shown that larger hosts are not only more likely to be infected but also more likely to experience declines (Lips et al. 2003, Bancroft et al. 2011).

Considering the totality of available evidence, the correlation between an anuran's size/weight and its ability to combat *Bd* infections appears to be more complex than a simple linear relationship. For example, according to Lips et al. (2003), large frogs infected by *Bd* only declined at high elevations, whereas large infected lowland anurans survived. Cohen et al. (2019) examined how thermal mismatches interact with body size and other host traits and found that cold-adapted species with larger adult body sizes had higher *Bd* prevalence. These findings hint at the existence of some form of interaction between various predictors of *Bd* in their effect on the prevalence and intensity of the pathogen.

The relationship between body measurement and *Bd* infections in our data underscores the need to investigate whether size and weight drive the ability

to prevent infections, or conversely, whether infections pose constraints on how large anurans can grow. There is support for both hypotheses in the literature (Parris & Cornelius 2004, Wu et al. 2018), and clearly, more data are required before these questions can be addressed. For now, our results show a lower presence of *Bd* infections in heavier anuran species, from an important ecological site within the Ecuadorian Amazon, compared to lighter taxa.

4.4. Aquatic indices and *Bd* prevalence

Our logistic regression analyses revealed no significant association between the AI and the status of *Bd* infections in anurans from Tiputini (Table S1). Previous studies have found aquatic breeders to have a statistically higher prevalence of *Bd* especially when compared to terrestrial breeders (Lips et al. 2003, Kriger & Hero 2007, Bancroft et al. 2011, Mesquita et al. 2017). However, this does not appear to be a consistent pattern across all studies. For example, Ribeiro et al. (2020), recorded a higher *Bd* prevalence for direct developing/terrestrial frogs compared to aquatic breeders. The authors accredited this to sampling that was restricted to riparian zones, unlike some other studies, which are areas that may facilitate contact with waterborne *Bd* regardless of the type of breeding environment (Lips et al. 2006, Brem & Lips 2008, Ribeiro et al. 2020). Stream-adjacent populations of direct-developing frogs could thus be at a higher risk of infection by *Bd* than currently thought. This reinforces the need for more surveys that focus on these lowland riparian environments and their role in the host–pathogen dynamics of the amphibian chytrid fungus.

Though they did not investigate the association between exposure to water and *Bd* infections, McCracken et al. (2009) found that the prevalence of *Bd* was non-randomly distributed along the vertical axis. Specifically, the frogs that inhabited the canopy (defined as over 4 m above ground level) had the highest prevalence with as many as 33% being infected. This further corroborates our findings showing a lack of association between AI and *Bd*, considering that several canopy-inhabiting species in our dataset are either direct developers or terrestrial species that lay eggs in water (i.e. AI0 and AI2, respectively).

It has been proposed that canopy-dwelling species can also be regularly exposed to *Bd*, which may be present in standing water collected within the phytotelmata of tank bromeliads (McCracken et al. 2009). Several species of frogs exploit these water-filled plant cavities for egg or tadpole development or even spend their entire life cycle within them (Peixoto 2013, Sabagh et al. 2017, Tonini et al. 2020). To factor in the near-constant exposure to water or humidity, species occupying this niche (all belonging to the genus *Pristimantis*) sampled in our study, were assigned a separate AI category (AI3). Indeed, the prevalence of *Bd* was higher (though not significantly) in these bromeligenous frogs (A3) when compared to aquatic breeders that are arboreal and breed in water (A1) (Table 2). This is not entirely surprising because even though ambient temperatures in lowland tropical rainforests may not be optimal for the proliferation of *Bd*, McCracken et al. (2009) found the water in lowland bromeliads to be at temperatures that are conducive for the survival of *Bd*. A high prevalence of *Bd* has also been reported for frogs inhabiting phytotelma microhabitats in other Neotropical lowland forests (Ruano-Fajardo et al. 2016). While technically challenging, future amphibian chytrid research would thus benefit by investigating the role of this water-impounding foliage as reservoirs of *Bd* in lowland tropical forests.

4.5. Taxonomy and *Bd* prevalence

Exploratory data analysis revealed a significant correlation between taxonomic rank at the family level and aquatic indices in our dataset. Furthermore, a model that included only Family as a fixed effect did not perform well, indicating that Family alone does not significantly predict *Bd* infection status in this community. This suggests that *Bd* prevalence is mostly randomly distributed among taxonomic families; however, *Leptodactylidae* (n = 20) did show a very high prevenance of *Bd* (0.90: 95% CI = 0.80–0.95) among the individuals sampled. While contradictory to some previous findings (Bancroft et al. 2011, Burrowes & De la Riva 2017), this general lack of association is documented by others who have looked at phylogenetic relatedness as a predictor of *Bd* prevalence and susceptibility (Berger et al. 1998, Crawford et al. 2010). Overall, 88% (8 out of 9) of the families sampled for *Bd* were found to be infected by the fungus, compared to 43% (3 out of 7) of the amphibian families that were sampled by McCracken et al. (2009) at the same site. Similar to our findings regarding morphology, our results do not align with the existing literature on studies conducted in the highlands of the Neotropics concerning the association between taxonomy and *Bd* infections in anuran fauna and point to a lack of host specificity.

5. CONCLUSIONS

Our study contributes to the limited body of research investigating the prevalence of *Bd* infections in the lowland Amazonian rainforest of Ecuador (McCracken et al. 2009). Notably, we confirm the presence of the *Bd*-GPL strain infecting amphibians in this region and report a comparatively high prevalence of *Bd* infections among Neotropical lowland anuran fauna. Furthermore, our findings highlight the need to re-evaluate previously identified predictors of *Bd* dynamics, such as morphology, aquatic dependence, and taxonomy, which were primarily derived from studies focused on highland environments. In the context of lowland tropical forests, special attention should be given to bromeligenous amphibian species. Although major *Bd*-driven declines have not been reported in lowland South American amphibians, our results suggest that these sites could still contribute to the spread and persistence of the amphibian chytrid fungus, a pathogen responsible for one of the most devastating wildlife EIDs in modern history.

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