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Chytrid fungus (*Batrachochytrium dendrobatidis*) among amphibians in an Amazonian floodplain forest

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Master of Science in Ecology

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Abstract

The increasing frequency of emerging infectious disease outbreaks is causing population declines and sparking concerns for species conservation. Chytridiomycosis is one of these diseases. It is caused by the widespread pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) found on amphibians and is one of the leading causes of amphibian decline. The fungus has gained major attention for its role in decimating amphibian populations, particularly in upland forests in the neotropical realm, where the abiotic conditions are often ideal for the fungus. However, little is known about *Bd* prevalence and dynamics in the hotter lowland forests of the neotropics, particularly in the vast and remote areas of the Amazon lowlands. This study aims to improve our understanding of *Bd* prevalence in the Amazon lowlands, focusing specifically on a relatively unexplored area along the middle Juruá River in the western Brazilian Amazon. I conducted a survey where I assessed *Bd* prevalence in 23 anuran species (n = 95) at a single site in a floodplain forest along the middle Juruá river. The study area was approximately 44 km downstream from the town of Carauari in the state of Amazonas. Data on morphology (weight and snout-vent length), taxonomical identification and habitat preference were also collected to serve as potential predictors of *Bd* prevalence. *Bd* was detected in 7 anuran individuals, each belonging to a different species and representing six anuran families. The overall prevalence of *Bd* was 7.42 %. The low prevalence detected is consistent with most other findings from the few studies conducted in the Amazon lowlands. A logistic regression analysis showed no association between *Bd* infection and morphometrics or habitat preference ($p > 0.05$). This study is the first contemporary *Bd* survey along the Juruá river. It also adds a new species to the growing list of *Bd*-infected species worldwide, with the detection of an infected *Pipa snethlageae* individual. Large parts of the Amazon lowlands remain unexplored regarding *Bd* infection prevalence in amphibians and increased sampling will help us better understand *Bd* dynamics and its effects on amphibian populations.

Keywords: Amazon; Amphibians; *Batrachochytrium dendrobatidis;* Chytridiomycosis; Brazil*;* Juruá River

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1 Introduction

Amphibians are a highly diverse group of animals found on all continents except Antarctica. They are a unique subset of vertebrates that influence both aquatic and terrestrial habitats, playing a crucial role in ecosystems as vital components of food webs throughout their life cycle (Hocking & Babbitt, 2014; West, 2018). Moreover, amphibians can act as pest and human disease controllers (Khatiwada et al., 2016; Springborn et al., 2022), contribute to medicinal research (Chen et al., 2023) and serve as reliable bioindicators due to their high sensitivity to environmental toxins and changes (Hocking & Babbitt, 2014; Lefcort et al., 1998). It is therefore particularly concerning that amphibian populations worldwide are declining at extraordinary rates and many species are currently facing extinction (Alford & Richards, 1999; Stuart et al., 2004; Wake, 1991).

Amphibian declines have been linked to several drivers, which was highlighted by Blaustein et al. (2011) as a complex issue often involving multiple stressors. The severity of decline can vary significantly across different species (Grant et al., 2020). Some of the main causes of decline include habitat loss and fragmentation (Cushman, 2006), diseases (Densmore & Green, 2007), roadkill (Hels & Buchwald, 2001), invasive species (Kats & Ferrer, 2003), climate change and pollution (Collins & Storfer, 2003). Over 40% of all amphibian species are currently threatened – more than for any other vertebrate taxon (Luedtke et al., 2023). It is therefore imperative to fully understand the mechanisms, regions, and scale of decline for effective conservation efforts.

Global emergence of fungal pathogens is a general concern for human health and ecosystems (Fisher et al., 2020). Several pathogenic fungi have been discovered and are negatively impacting various species (Seyedmousavi et al., 2018). One of the main contributing factors to amphibian decline is the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*) and the more recently discovered *Batrachochytrium salamandrivorans* (hereafter *Bsal*). Both can cause the emerging infectious disease chytridiomycosis (Berger et al., 1998; Martel et al., 2013). *Bsal* exclusively infects salamanders and newts (Urodela), whereas *Bd* has been documented in numerous species of amphibians, including Caecilians (Gymnophiona) (Gower et al., 2013; Martel et al., 2014; Scheele et al., 2019). Chytridiomycosis affects the cutaneous region of the amphibian (Berger et al., 1998, 2004). Infection occurs in keratinized epidermal cells (Berger et al., 1998), which disrupts critical aspects of skin functioning, such as electrolyte transport across the epidermis and osmoregulation. This may result in reduced fitness or lead to death of the host, often in the form of cardiac failure (Voyles et al., 2009; Bovo et al., 2016).

Several lineages of the aquatic pathogenic fungus *Bd* have been identified (Farrer et al., 2011). In some areas, the fungus is considered an enzootic pathogen, where it shows low infection intensity (Zumbado-Ulate et al., 2019). In other regions, *Bd* behaves as an epizootic, decimating amphibian populations that lack an evolutionary history with the fungus (Catenazzi, 2015; Whitfield et al., 2016). The origin of *Bd* has been traced to South-east Asia (O'Hanlon et al., 2018), where a hypervirulent lineage has spread across the world, likely due to lack of biosecurity, increased globalization and the global amphibian trade (Byrne et al., 2019; Martel

et al., 2014; Olson et al., 2013). Chytridiomycosis has shown to cause significant species declines and is described as potentially the greatest disease-induced loss of biodiversity recorded in history (Scheele et al., 2019; Skerratt et al., 2007). Even implications for human health have been uncovered because of *Bd*-induced amphibian decline, e.g., an increase in malaria cases (Springborn et al., 2022).

Since the discovery of chytridiomycosis in 1998 (Berger et al., 1998), population declines associated with the disease have been discovered and reported across many parts of the world: Africa (Weldon et al., 2019), Australia (Skerratt et al., 2007), Central America (Cheng et al., 2011; Lips, 1999), the Caribbean (Hypolite et al., 2007), Europe (Bosch et al., 2001; Martel et al., 2014) and South America (Carvalho et al., 2017a). A global review by Scheele et al. (2019) confirmed that the fungus has low host specificity, with over 500 amphibian species experiencing population declines linked to chytridiomycosis, 90 presumed species extinctions and more than 90% declines in abundance for 124 other species.

The impact of *Bd* has been greater for certain taxa and in distinct regions, with most declines and extinctions linked to frog and toad (anuran) populations in upland sites in the tropics (Scheele et al., 2019). One suggestion for his trend is that the climatic conditions that promote high amphibian diversity match those of the fungus (Whitfield et al., 2016). On the other hand, Keesing et al. (2006) suggested that species diversity, specifically high host diversity, can directly reduce disease risk. This has also been demonstrated at smaller scales with *Bd* and tropical amphibians (Becker et al., 2014). However, *Bd* associated decline is generally greater in regions with higher species richness (Olson et al., 2013; Scheele et al., 2019). It is therefore clear that the effects of *Bd* are complex; while some species go extinct, others are able to tolerate the fungus and exhibit strong resistance, showing promise for future populations (Jaynes et al., 2022). Nonetheless, this is also problematic as these species can function as natural reservoirs of the fungus, which can potentially increase infection among more susceptible hosts.

Environmental factors play an important role in *Bd* infection (Bancroft et al., 2011). Temperature is vital in the spread and prevalence of *Bd* infections. *Bd* has a temperature optimum between 17°C-25°C (Piotrowski et al., 2004) and dies, or is strongly diminished, at higher temperatures (Johnson et al., 2003; Stevenson et al., 2013). Additionally, changes in temperature and sun exposure following natural disturbances (e.g. cyclones) can reduce infection risk for some amphibians (Roznik et al., 2015). Wet and humid conditions are ideal for the fungus, and research has shown that *Bd* can potentially transmit through rain (Kolby et al., 2015). More recently, Prado et al. (2023) showed that fog may also serve as a vector for the disease. These climatic conditions particularly coincide with amphibian-diverse higher elevation sites in the tropics, which coincidently have seen heavy population declines and many extinctions (e.g., Crawford et al., 2010 or La Marca et al., 2005). In contrast, lowland forests of the neotropics have not experienced such levels of decline (Deichmann et al., 2010). Lowland forests have been considered too warm for *Bd* infection, as these areas can reach temperatures that can kill the fungus (Ron, 2005; Stevenson et al., 2013). However, there have been reports of *Bd* infection in several neotropical lowland forests (Lambertini et al., 2022;

McCracken et al., 2009; Russell et al., 2019; Whitfield et al., 2012; Zumbado-Ulate et al., 2019). This suggests that temperature may limit, but not exclude, *Bd* in neotropical lowland forests (Whitfield et al., 2012) and that reservoir hosts could play a crucial role in the persistence of the disease in low-elevation areas (Sapsford et al., 2013).

The Amazon hosts the largest rainforest and the most extensive river basin on Earth. It features biological diversity unmatched by any other region on the planet (Zapata-Ríos et al., 2021). Published records of *Bd* presence in the Amazon lowlands consist of a few retrospective studies of museum specimens in Brazil (Becker et al., 2016; Carvalho et al., 2017a; Lambertini et al., 2017) and some more recent surveys (Lambertini et al., 2022; McCracken et al., 2009; Russell et al., 2019; Smart et al., 2024; von May et al., 2018). The majority of these studies show low infection prevalence, suggesting that *Bd* might be enzootic to certain parts of the Amazon (Lambertini et al., 2022). However, much remains to be uncovered regarding *Bd* prevalence in the Amazon. Even though models have predicted that the environmental conditions of lowland Amazonia are unsuitable for the fungus (Rödder et al., 2010; Ron, 2005), higher prevalence and intense infections may still occur since understory and leaf litter microclimate may be suitable for *Bd* growth (von May et al., 2018). Overall, research on *Bd* prevalence and infection intensity in the Amazon lowlands needs more attention, particularly given the immense diversity with over 400 amphibians found in the region (Hoogmoed, M., 2024).

One of the main rivers in the western lowlands of the Brazilian Amazon is the Juruá river, a major southern tributary of the Amazon river. The Juruá is a meandering white-water river carrying large amounts of sediments eroded from the Peruvian Andes (Sioli, 1984). Seasonal flooding occurs following the onset of the rainy season and the Juruá river spills over onto the adjoining floodplain. During flooding, sediments from the river are deposited in floodplain forests called várzeas (Junk et al., 2011). Várzea forests are unique, as they can flood up to 7.5 meters in depth for extended periods (Junk et al., 2011). Climatic conditions and elevation suggest that this area has a minimal risk of *Bd* infections (Becker et al., 2016). However, larger outbreaks of the disease have been reported in the lowlands of Peruvian Amazon, raising concerns that *Bd* strains associated with amphibian declines could be spreading to nearby Amazonian regions, such as the Juruá (Becker et al., 2016; Catenazzi et al., 2011).

Little is known regarding *Bd* prevalence along the middle Juruá river. Despite the regions rich diversity of amphibians (Moraes et al., 2022), no contemporary surveys attempting to detect *Bd* along the Juruá river have been conducted. Only retrospective studies using museum samples, which include a limited number of samples from this area (Becker et al., 2016; Carvalho et al., 2017b). *Bd* has been detected in these retrospective studies, with 3 cases along the lower parts of the Juruá in the state of Acre (Becker et al., 2016). But there is only a single record of *Bd* in the middle Juruá - a tadpole specimen from the Dendrobatidae family from 1991 (Carvalho et al., 2017b).

The objectives of the current study were to 1) determine the presence of *Bd* in the várzea anuran assemblage along the middle Juruá river and 2) examine any effect of anuran habitat preference and morphometrics on the presence of *Bd*. The presence of *Bd* was examined using a highly

detection-sensitive droplet digital polymerase chain reaction (ddPCR) approach to detect *Bd* on amphibian skin and through environmental DNA (eDNA) from water samples.

2 Materials and methods

2.1 Study area

The current study took place in a várzea forest along the middle Juruá river in the western Brazilian Amazon, from 26th of September to 21st of October 2023. This coincided with the end of the dry season in the middle Juruá, which typically lasts from May to October, with the rainy season lasting from November to April (Hawes & Peres, 2016). The study area was located on the west bank of the Juruá river, approximately 44 km downstream from the town of Carauari, state of Amazonas (Fig. 1A and B). The elevation of the study area ranged from 55 to 75 meters and included a section of an oxbow lake (Fig. 1C) created by a meander cut-off from the river.

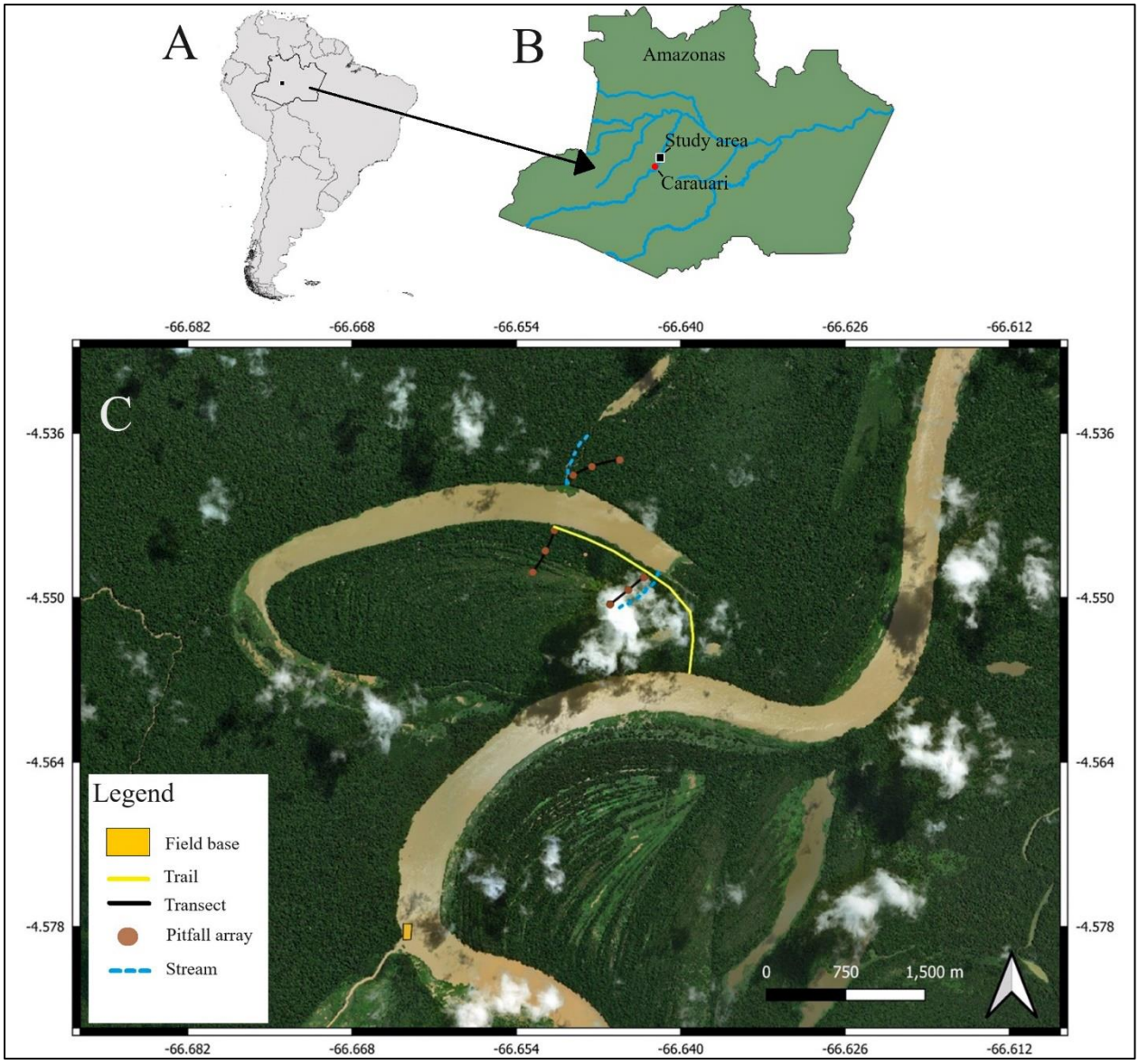

Figure 1: A) Study area in relation to the continent of South America and state of Amazonas (polygon highlighted in bold black line), B) the location of study area and town of Carauari on the middle Juruá river in the western Brazilian Amazon. C) Satellite overview of the study area. Map created with QGIS. Basemap: Bing Satellite © 2024 Microsoft (QuickMapServices QGIS plugin), Map data © 2024 TomTom, © OpenStreetMap, © 2024 Maxa.

The climatic conditions during the study were characterized by high temperatures and humidity. Temperature and humidity were recorded with data loggers (EasyLog EL-USB-1). Three data loggers placed 2 meters above the ground in the middle part of each transect (see Data collection). Recording ensued in 1-hour intervals. Recording started on September 30, 2024 for the transects on the south side of the lake and on October 3, 2024 for the transect on the north side. Unfortunately, data could only be retrieved from two of the loggers because one of the data loggers on the south side malfunctioned. Mean temperatures exceeded 27.7 degrees Celsius, with daily maximums often in the thirties and occasionally exceeding 40 degrees Celsius (Fig. 2A). Humidity levels averaged 88.5%, regularly reaching full saturation (100%; Fig. 2B).

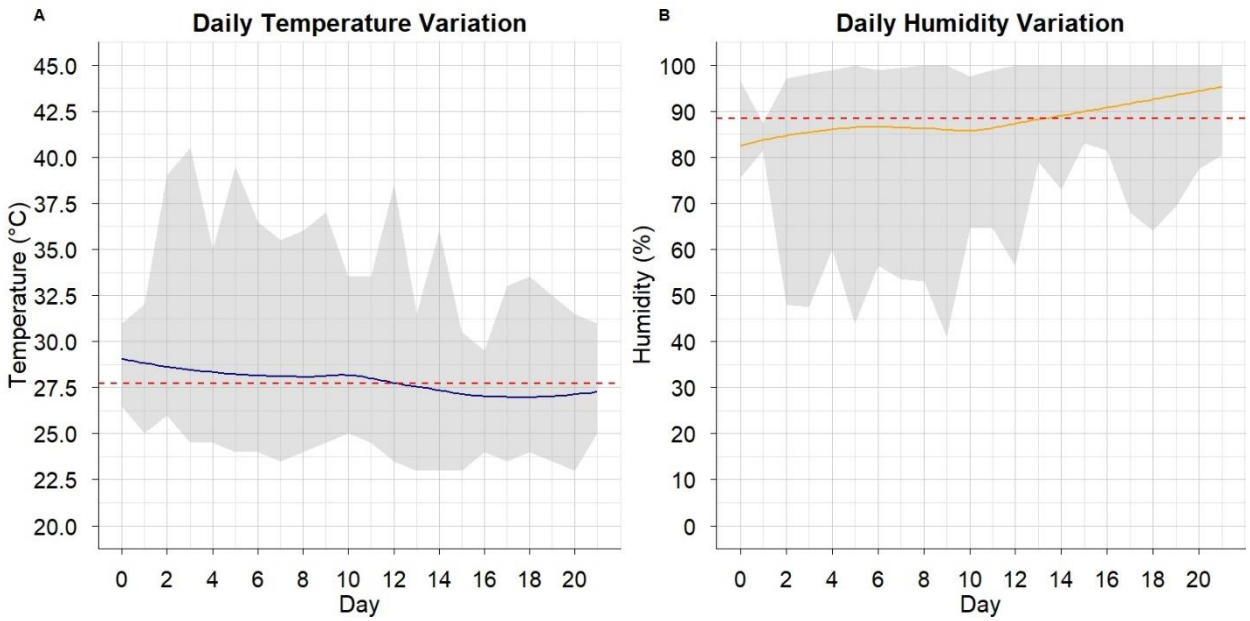

Figure 2: Mean daily (A) temperature and (B) humidity (dotted red line) with daily minimum and maximum values (shaded grey) and mean trend over time (blue and yellow line).

2.2 Data collection

To capture amphibians, three 500m transects were established in the study area; two on the south side and one the north side of the oxbow lake (Fig. 1C). A 2 km access trail was cleared on the south side of the lake, and a boat was used to cross the lake (Fig. 3A) and reach the transect on the north side. The study area contained two streams, one on either side of the lake. By the end of the fieldwork, both streams had nearly dried up due to very hot and dry conditions.

Although all species from the amphibian orders Anura, Caudata, and Gymnophiona were viable for the study, only anurans were utilized because they were the only ones found. Anurans were located and captured using pitfall traps, active searches, accidental encounters, and stream walks. Sampling for this study was authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through permit number 90415-1.

2.2.1 Pitfall traps

Pitfall traps consisted of 60-liter buckets set into the ground and arranged in a Y-shaped array (Fig. 3B) following Fisher et al. (2008). The buckets were placed approximately 3-4 m apart. Additionally, a drift fence approximately 40 cm in height, constructed from sticks and plastic

sheeting, was established to channel anurans towards the buckets. Each Y-shape consisted of 4 buckets and were located at intervals of 250 meters along each transect. Each of the three transects contained three Y-shape arrays (0m, 250m and 500m), totaling 36 buckets. Foliage was placed in each bucket along with bamboo sticks to provide protection and prevent animals from drowning in case the buckets became flooded. Small holes were drilled into the bottom of each bucket to prevent them from dislodging from the pressure caused by increasing ground water levels following rainfall. Lids were held up above the buckets with the help of sticks to avoid excess rain entering the bucket and to create shade for the hottest parts of the day. Each pitfall array was open for 12 days in the period September 29, 2023, to October 12, 2023. Every morning throughout this period each bucket was inspected to ensure individuals, including both bycatch and target species, did not remain in the bucket for too long. Anurans found in each bucket were swabbed (see below), and the specific bucket, pitfall array, and transect were recorded each time an anuran was found.

2.2.2 Active searches

Active searches in the form of time-constrained surveys (TCS) were conducted along each transect and sections of the access trail (Fig. 1C), as it is an effective method of standardizing active searches of amphibians (Guilfoyle, 2010). TCS was chosen as a survey method to allow collaboration with a fellow student examining anuran species composition, making it an effective method for both our objectives. A total of 6 surveys were conducted, one along each transect, two sections of the access trail and along the stream on the south side of the lake. Each TCS lasted 1 hour, conducted at night between 8 PM and 1 AM. The survey team consisted of 5-7 people, each person scanning for amphibians in the leaf-litter, bushes and vegetation up to 2 meters. Stream surveys involved similar survey techniques, where the team attempted to locate anurans in the stream, on understory plants near and above the stream. Anurans from the stream on the northern side of the lake were also sampled, though opportunistically and not as part of the time constrained search protocol.

2.2.3 Accidental encounters

Accidental encounters with amphibians were included and these encompassed any amphibians found outside of pitfall traps and not during designated surveys. Additionally, anurans encountered at the field base (Fig. 1C) were also included.

2.3 Skin swabbing

To assess the prevalence of *Bd* among amphibians in a várzea forest along the middle Juruá river, I swabbed the ventral skin of each captured anuran. Each captured individual was handled with a new pair of non-powder vinyl gloves to prevent transmission of *Bd* between individuals (Mendez et al., 2008). Each anuran was temporarily placed in a sterile plastic bag containing foliage from the capture site to maintain humid conditions. They were then swabbed (Fig. 3C; Thermo Scientific 4N6FLOQSwabs, regular tip, peelpouch) based on existing standardized swabbing protocols (AmphibiaWeb, 2016; Hyatt et al., 2007). This process involved five strokes on the ventral side of each hind foot, thigh, and each side of the abdomen, totaling 30 strokes. Swabs were stored at room temperature in 2.0 mL Eppendorf tubes (EP0030108078, Eppendorf® DNA LoBind tubes) with lysis buffer (ATL, 939011,

QIAGEN Buffer ATL), until the fieldwork concluded. The swabs were placed in a shaded room in the coolest part of the field base to reduce the chance of *Bd* DNA degrading due to high temperatures (Van Sluys et al., 2008). A negative field control was included, which consisted of a swab held in the air for 30 seconds. After field work, swab samples were stored in a refrigerator at 4°C prior to DNA extraction.

Each captured individual was weighed using Pesola scales. Due to the large variation in frog weight, which ranged from nearly 1g to 500g, different scales with precision of 5g, 1g, 0.5g, 0.2g, and 0.1g were utilized. The snout-vent length (SVL) of each anuran was also measured using a digital caliper (Digital vernier calipers, stainless steel, Art. 16-109, Biltema). Once the skin swabs and measurements were completed, each individual was released in proximity to where it was captured. The GPS location of each captured anuran was recorded using Garmin etrex 32x GPS. Furthermore, a brief description of the habitat of where each anuran was found was noted. This was split into categories leaf litter (LL), riverbank (RB), above ground (AG), stream (S) and field station (A). "Above ground" included any frog that was found on vegetation and "stream" included any frog that was found in or on vegetation near a stream.

2.4 Species identification

Each anuran was photographed at the capture site, in a camera cube or during handling, to allow for morphological identification. Anurans were identified in the field using field guides (Lima et al., 2005; Villacampa et al., 2017) and species inventories (Moraes et al., 2022). Weight and snout-vent length were also used for identification purposes. Photos of unknown or difficult to identify species were subsequently forwarded to experts for species confirmation. Further details regarding species identification for this study can be found in Vågen (2024).

2.5 eDNA sampling

To assess the prevalence of *Bd* in the environment, I collected water samples from two streams. Water was sampled approximately 0-20 cm below surface level using a pair of new gloves and sterile Whirl-Pak bags. The water was pressed through a filter (Whatman nitrocellulose membrane filters, diam. 25 mm, pore size 0.1 μm, plain white) using a syringe (BD Plastipak Plastic Concentric Luer-Lock Syringe). Once filter saturation was reached, the membrane filter was immediately placed in a 2.0ml tube with the use of a sterile tweezer. Tweezers were disinfected between uses with 70% ethanol and a lighter. The filters were stored at room temperature until the fieldwork phase was over, and in a refrigerator at 4°C until DNA extraction. Twelve samples, nine from the northern stream and three from the southern stream, yielded values between 20-50 mL of filtered water, and were utilized. These samples were collected on October 13th, 2023. Water samples from the northern stream were collected approximately 15 meters upstream from the lake (-4.5396532, -66.6496679). The sampling of the southern stream occurred at the location seen in Figure 3D (-4.5482044, -66.6420686).

Figure 3: A) Location of crossing on the oxbow lake featured in the study area. B) Pitfall traps arranged in a Y-shape configuration with drift fences and makeshift lids. C) Swabbing of the ventral side of a *Ameerega hahneli* individual for *Batrachochytrium dendrobatidis* detection. D) Stream on the southern side of the oxbow lake where night surveys and eDNA water sampling was carried out.

2.6 Laboratory methods

2.6.1 DNA extraction

To detect *Bd* on the anuran skin samples, the DNA from the swabs underwent extraction using standardized methods under sterile laboratory conditions. DNA extraction was performed using a Qiagen DNeasy Blood and Tissue kit following the protocol from the manufacturer (Qiagen, 2016), with some slight modifications as the swab samples were stored in ATL buffer. Adding ATL buffer in step 1 of the kit protocol was therefore skipped. The amount of proteinase K was increased to 40 µL to accommodate for the high amount of ATL buffer that the swab sample tubes were already containing. Once the incubation at 56°C in step 1 of the kit protocol was

complete, the solution was transferred to a new tube to accommodate for AL buffer and ethanol, required in step 2 of the kit protocol. For the final step, the DNA was eluted with two rounds of 100 µL AE buffer to optimize recovery. For the eDNA water samples, DNA extraction was performed using the Qiagen DNeasy PowerSoil Pro Kit following the standard kit protocol (Qiagen, 2023). The presence of DNA post extraction was assessed using agarose electrophoresis.

2.6.2 ddPCR

To identify samples with *Bd* DNA after extraction, the DNA extracts were amplified using standardized ddPCR methods, making it possible to detect even small amounts of *Bd* DNA. The DNA extracts underwent preparation for ddPCR as per the manufacturer's guidelines (Bio-Rad Laboratories, Inc, n.d.). Droplet generation was done in a total volume of 20 µL, consisting of 10 µL ddPCR Supermix for Probes (No dUTP), 1 µL of Primers/Probe (900 nm primers and 250 nM probe), 1 µL of sample DNA and 8 µL of purified water (Milli-Q) (Bio-Rad Laboratories, Inc, 2019a). For negative template controls (NTC), 1 µL purified water was added instead of DNA. The primer sets design by Boyle et al. (2004) were utilized: 5.8S (5'-AGC CAA GAG ATC CGT TGT CAA A-3'), ITS-1 3 (5'-CCT TGA TAT AAT ACA GTG TGC CAT ATG TC-3'), and the TaqMan probe MGB2. Droplet generation was conducted on the QX200 Droplet Digital PCR system where 20 µL was loaded into the sample wells and 70 µL Droplet Generation Oil (Bio-Rad Laboratories, Hercules, CA, USA) was loaded into the oil wells. Specific pipetting technique was followed as specified in the instruction manual (Bio-Rad Laboratories, Inc., 2019b). Forty µL of the droplet solution was pipetted into a sterile PCR plate which was then sealed with a PX1 PCR Plate sealer at 180C for 5 seconds. The reaction mix was immediately transferred to PCR for amplification.

The PCR amplification was performed using a Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories). Thermal cycling conditions were based on recommended instructions for ddPCR Supermix for Probes (Bio-Rad Laboratories, Inc, 2019a). The PCR thermal cycling protocol consisted of an initial enzyme activation at 95 °C for 10 minutes. This was followed by denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds and extension at 72 °C for 30 seconds. This was repeated for 40 cycles and concluded with a final enzyme deactivation step at 98 °C, with an infinite hold at 4 °C.

To determine the annealing temperature for the PCR cycling, a synthetic positive control (Sigma-Aldrich WD1153202) was utilized at different dilutions for a gradient analysis of different annealing temperatures. The annealing temperatures 42 °C, 44.4 °C, 46.7 °C, 49.6 °C, and 52 °C were used to establish the optimal annealing temperature for sample runs. Following the gradient analysis, 48 °C was deemed a suitable annealing temperature for sample runs.

Following several runs with suboptimal results of fluctuating amplitude levels, DNA samples were diluted 1/10 with purified water (Milli-Q) to reduce inhibition in the samples (McKee et al., 2015). Duplicate runs were performed for each sample to avoid false positives. Each run contained at least 2 positive controls and 2 negative controls. Additionally, several extraction controls and one field control were included. Following amplification, the PCR plate was transferred into a QX200 Droplet Reader to read the results.

2.6.3 Limit of Detection and Limit of Quantification

To be able to reliably indicate which samples were negative and which were positive, a serial dilution of the synthetic positive control was conducted to determine the Limit of Detection (LOD) and the Limit of Quantification (LOQ) (M. Hunter et al., 2016). An initial 1:1,000,000 dilution was made to avoid saturating the ddPCR. This was followed by a 1:10 dilution to create the starting point for a dilution series. The series consisted of five-fold dilutions of the initial 1:10,000,000 solution, resulting in a total of ten different diluted solutions (Brys et al., 2021). Each dilution included the same amounts of reagents as described above, with a total reaction of 20 µL of which 1 µL was DNA. The series consisted of 10 replicates of each dilution and 10 negative controls. The dilution series was run with the same ddPCR and PCR protocols as described earlier. To calculate the LOD, DNA concentration was measured from the ddPCR reactions and the expected copy number of each dilution point following the stock solution was calculated. The copy number of the stock solution was 4.04407×10^{12} copies/µL. Finally, I implemented these calculations with the designated LOD script developed by Hunter et al. (2016) in R Statistical Software (v4.2.2; R Core Team 2024) to determine the LOD. The LOD was set at 0.0768 cp/µL.

The LOQ indicates which samples can be reliably quantified and was determined as the lowest concentration where replicates show a coefficient of variation (CV) ≤ 35% (Forootan et al., 2017). The CV for each dilution was calculated by dividing the standard deviation of the concentrations by the mean concentration of the same dilution. The LOQ was conservatively set as the concentration of the dilution at 1/31,250,000,000. Therefore, the LOQ was 7.456 cp/µL.

QuantaSoft software 2.1 (Bio-Rad Laboratories) was utilized to differentiate between positive and negative droplets. A sample was considered positive only if both replicates yielded DNA concentrations above the detection threshold (LOD). Additionally, due to also detecting a low number of positive droplets in NTCs and negative extraction controls, any sample with a concentration equal to or lower than these controls were considered negative to avoid false positives. Three samples that were positive in duplicate and were above the LOD were not counted as positive, due to having DNA concentrations below the negative controls.

2.7 Statistical analysis

The estimated prevalence of *Bd* was calculated by dividing the number of *Bd*-positive individuals by the total number of individuals sampled, and for each anuran family separately. I used the Wilson score interval at 95% confidence intervals (CI) to indicate the potential true proportion of *Bd* infected individuals. This was calculated using the binom.wilson command from the "binom" package (v1.1-1.1; Dorai-Raj, 2022), in R Statistical Software (v4.2.2; R Core Team 2024).

To examine if there was an association between weight, snout-vent length, or species habitat preference on the presence of *Bd*, I used a generalized linear model (GLM) with a binomial family argument. The response variable, *Bd* presence, was encoded as a binary variable (1 for presence, 0 for absence) and modelled against the explanatory variables (weight, snout-vent

length, habitat preference). Instead of using each species as a predictor individually, or conducting separate tests for each species, I combined all species together because the sample sizes for each species varied widely (from 1 to 25). Habitat preference refers to categories based on IUCN habitat descriptions for the species in this study and included terrestrial, leaf-litter, arboreal, and aquatic (Table S1). The aquatic category was eventually excluded from the analysis as there was only one count. The model assumptions were validated by plotting residuals against predicted values (Zuur & Ieno, 2016), and I checked the collinearity, significant outliers, uniform distribution of outliers and overdispersion. Model problems were not detected. Analysis was performed using the glm function in R Statistical Software (v4.2.2; R Core Team 2024). Residuals versus fitted was plotted with the "DHARMa" package (v0.4.6; Hartig 2022).

3 Results

A total of 95 anurans, belonging to 7 families, 14 genera, and 23 species, were swabbed and analyzed for the presence of *Bd*. Most anurans swabbed were members of the *Hylidae* family (n = 64), followed by Leptodactylidae (n = 17), Bufonidae (n = 8), Dendrobatidae (n = 2), Aromobatidae (n = 2) and for the families of Centrolenidae and Pipidae only a single individual was swabbed. The swabbed anurans consisted of arboreal species (n = 65), terrestrial (n = 25), leaf-litter (n = 4), and one aquatic species.

The 4 tree-frog species (Hylidae) were the most common species swabbed*: Scinax gr. ruber* (n = 25), *Boana geographica* (n = 11), *Osteocephalus cf. leprieurii* (n = 9), and *Boana cf. steinbachi* (n = 7; Fig. 4). Five *Adenomera andreae* individuals were swabbed and 4 each of *Leptodactylus petersii, Leptodactylus leptodactyloides, Rhinella castaneotica, Rhinella marina*. The remaining 14 species were represented by ≤ 2 swabs. A full list of the swabbed anurans for this study can be found in Table S2.

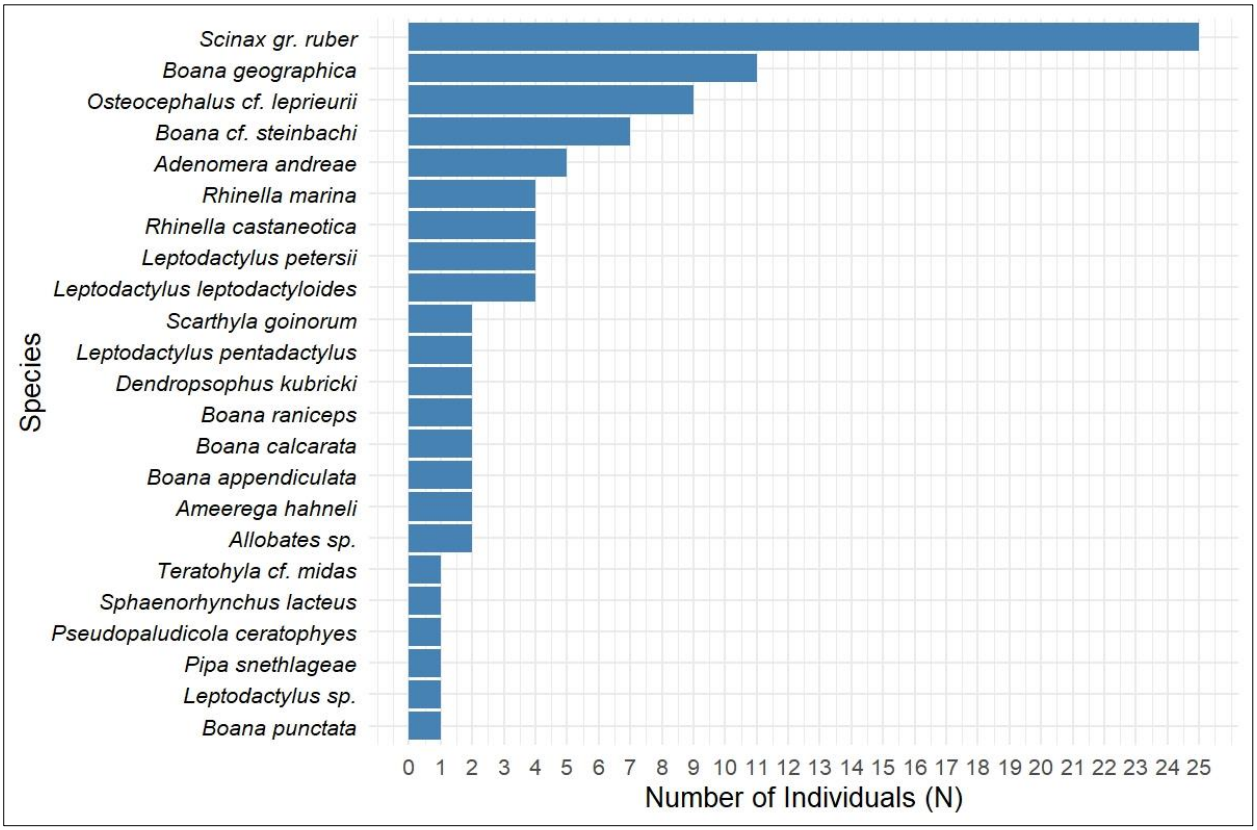

Figure 4: The total number of swabbed anuran individuals for each species in descending order.

Table 1: Overview of swabbed anurans showing family, species, number of *Bd*-positive cases per species (*Bd*/total), habitat preference, mean weight (g) and mean snout-vent length (SVL).

Family	Species	*Bd*/ total	Habitat	Mean weight (g)	Mean SVL (mm)
Aromobatidae	*Allobates sp.*	1/2	Leaf-litter	0.2 (SE ± 0.00)	14 (SE ± 0.64)
Bufonidae	*Rhinella castaneotica*	0/4	Terrestrial	1.32 (SE ± 0.17)	26.4 (SE ± 1.45)
	Rhinella marina	1/4	Terrestrial	10.28 (SE ± 4.90)	44.8 (SE ± 5.48)
Centrolenidae	*Teratohyla cf. midas*	0/1	Arboreal	0.40 (SE ± NA)	18 (SE ± NA)
Dendrobatidae	*Ameerega hahneli*	1/2	Terrestrial	0.75 (SE ± 0.25)	21.6 (SE ± 0.63)
Hylidae	*Boana appendiculata*	0/2	Arboreal	8.50 (SE ± 5.50)	53.2 (SE ± 13)
	Boana calcarata	0/2	Arboreal	0.85 (SE ± 0.15)	27.7 (SE ± 2.89)
	Boana cf. steinbachi	0/7	Arboreal	2.26 (SE ± 0.44)	34.9 (SE ± 2.19)
	Boana geographica	0/11	Arboreal	2.72 (SE ± 0.30)	38 (SE ± 1.00)
	Boana punctata	0/1	Arboreal	0.80 (SE ± NA)	23.7 (SE ± NA)
	Boana raniceps	0/2	Arboreal	10.65 (SE ± 7.15)	51.6 (SE ± 8.94)
	Dendropsophus kubricki	0/2	Arboreal	0.50 (SE ± 0.30)	21.4 (SE ± 3.2)
	Osteocephalus cf. leprieurii	0/9	Arboreal	4.17 (SE ± 0.53)	40.8 (SE ± 1.91)
	Scarthyla goinorum	0/2	Arboreal	0.55 (SE ± 0.05)	17.1 (SE ± 1.11)
	Scinax gr. ruber	1/25	Arboreal	2.93 (SE ± 0.26)	34.5 (SE ± 0.76)
	Sphaenorhynchus lacteus	0/1	Arboreal	3.20 (SE ± NA)	32.6 (SE ± NA)
Leptodactylidae	*Adenomera andreae*	0/5	Terrestrial	0.93 (SE ± 0.10)	22.2 (SE ± 0.89)
	Leptodactylus leptodactyloides	1/4	Terrestrial	4.33 (SE ± 0.46)	36.3 (SE ± 1.32)
	Leptodactylus pentadactylus	0/2	Terrestrial	262.50 (SE ± 72.50)	133 (SE ± 19.26)
	Leptodactylus petersii	1/4	Terrestrial	2.06 (SE ± 0.92)	25.5 (SE ± 4.39)
	Leptodactylus sp.	0/1	Leaf-litter	0.50 (SE ± NA)	17.6 (SE ± NA)
	Pseudopaludicola ceratophyes	0/1	Leaf-litter	0.10 (SE ± NA)	10 (SE ± NA)
Pipidae	*Pipa snethlageae*	1/1	Aquatic	50.00 (SE ± NA)	86 (SE ± NA)

3.1 *Bd* prevalence

Seven anurans tested positive for *Bd*, indicating an estimated prevalence of 7.37% (95% CI: 3.61% - 14.44%). *Bd* was detected in all but one of the anuran families swabbed (Table 1). *Bd* was detected in two individuals from Leptodactylidae, with a prevalence of 11.8%. One species

from each of Aromobatidae, Bufonidae, Dendrobatidae, Hylidae, and Pipidae was infected with *Bd*, with varying sample sizes and prevalence rates (Fig. 5). No *Bd* was detected in the one individual swabbed from Centrolenidae. Anurans from all four habitat categories were present among *Bd*-positive individuals.

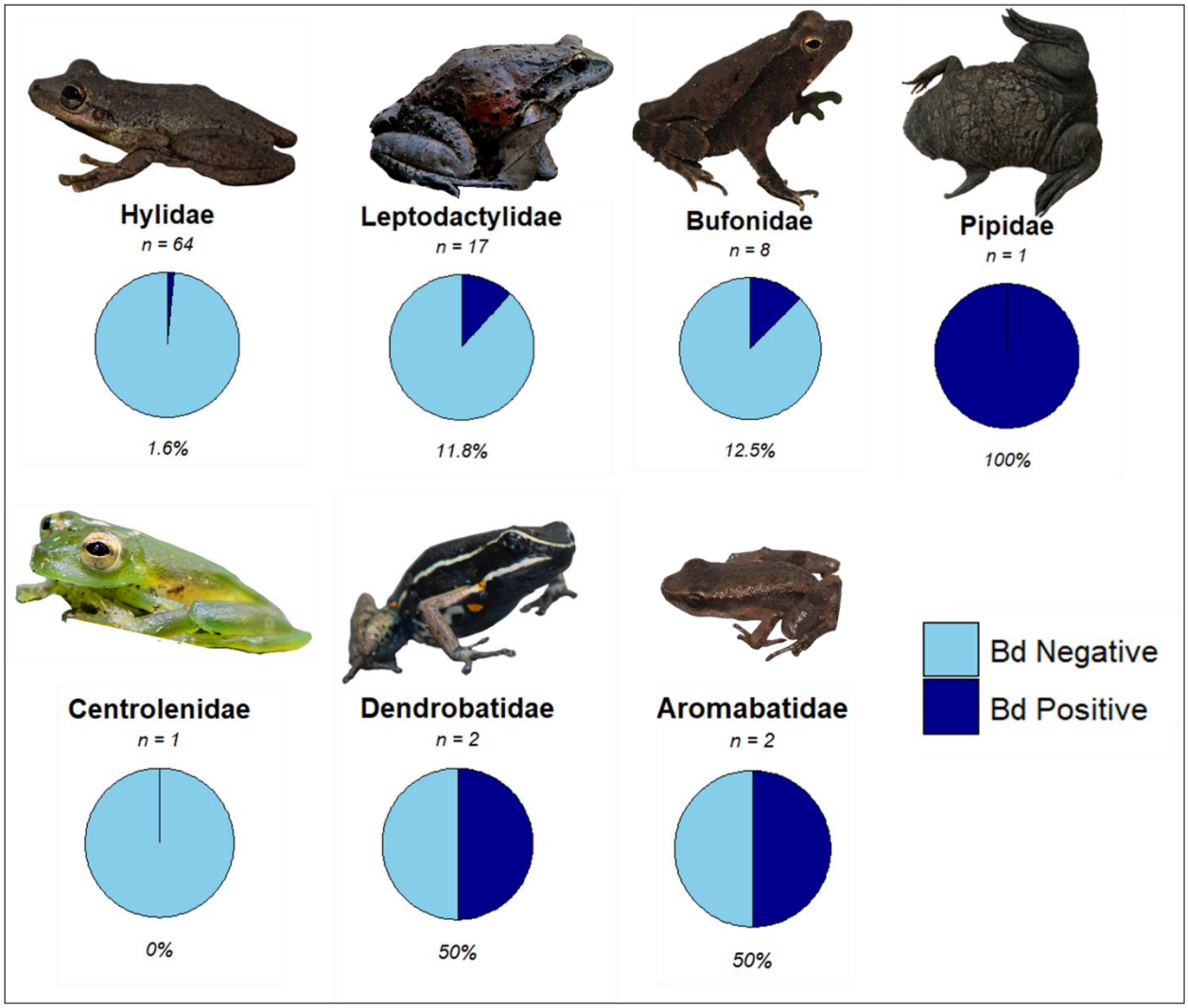

Figure 5: Prevalence of *Bd* among the anuran families that were swabbed in this study. CI can be found in Table S3. Species from left to right: *Scinax gr. ruber, Leptodactylus pentadactylus, Rhinella casteneotica, Pipa snethlageae, Teratohyla midas, Ameerega hahneli, Allobates sp.* Note that images are not to scale.

The 7 cases of *Bd* were all from different species: *Allobates sp.*, *Ameerega hahneli*, *Leptodactylus petersii*, *Leptodactylus leptodactyloides*, *Pipa snethlageae*, *Rhinella marina*, and *Scinax gr. ruber* (Fig. 6A-G). Only one individual showed clinical signs of chytridiomycosis. The ventral skin of *Pipa snethlageae* exhibited red markings as well as red colouration of fingertips (Fig. 7A and B). Additionally, this individual was observed to be missing a limb (Fig. 7A).

Figure 6: The 7 individuals with positive *Bd* detections: A) *Allobates sp.*, B) *Ameerega hahneli*, C) *Leptodactylus petersii*, D) *Pipa snethlageae*, E) *Leptodactylus leptodactyloides*, F) *Scinax gr. ruber*, and G) *Rhinella marina*.

Figure 7: Observed potential clinical signs of chytridiomycosis in *Pipa snethlageae* include: A) Red markings on fingers and a missing limb, and B) Red spots, potentially erythema, on ventral side.

3.2 Body size and habitat preference

The generalized linear model (Table S4) fitted to predict the presence of *Bd* showed no significant effect of weight (coefficient (β) = -0.0232, P = 0.895) or snout-vent length (β = -0.01601, P = 0.852) of swabbed anurans. In fact, *Bd* cases were present in all size categories of weight except for the smallest and largest extremes (Fig. 8) and a similar trend is seen for snout-vent length (Fig. 9). There was also no significant effect of habitat preference; arboreal (β = -22.638, P = 0.992), leaf-litter (β= -19.974, P = 0.993), and terrestrial (β = -20.127, P = 0.993). The performance of the model was assessed and showed that 28.2% of the variability in the response variable (*Bd*) is explained by the predictors weight, snout-vent length, and habitat preference.

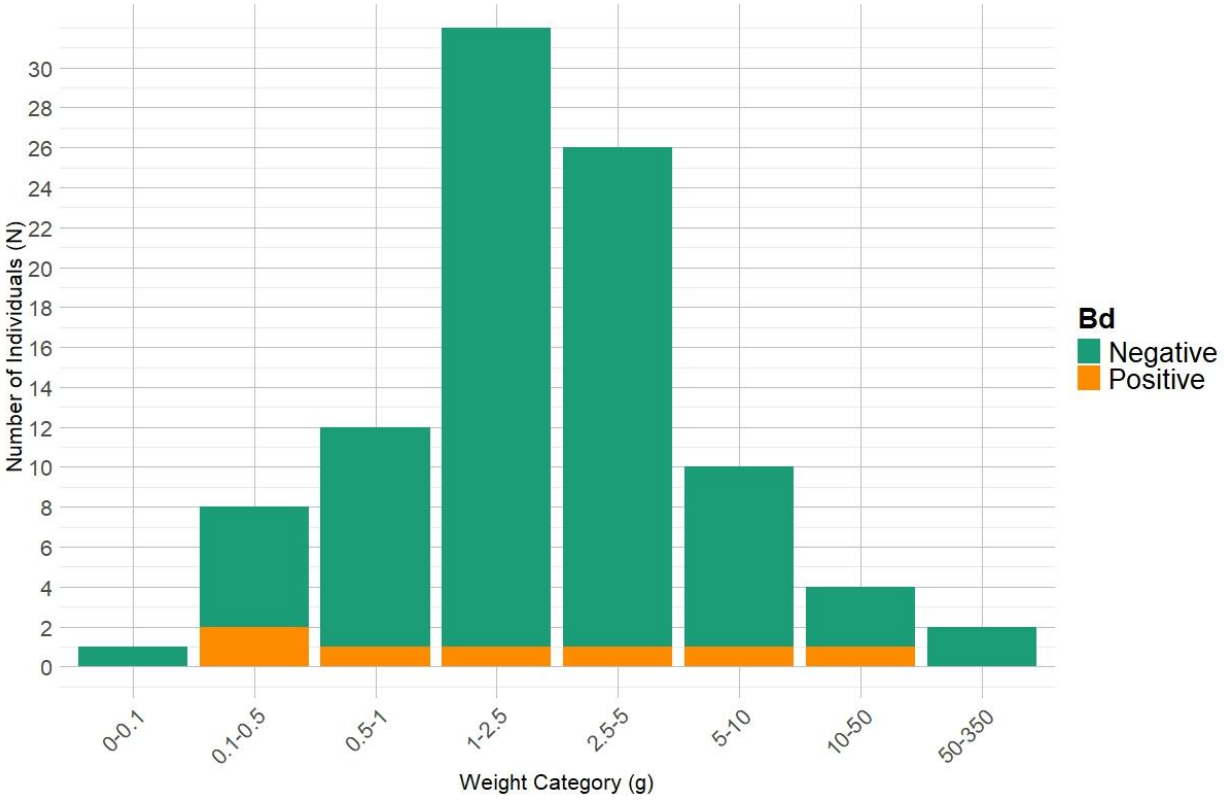

Figure 8: Bar chart visualizing the distribution of anuran weight (g), with orange color indicating positive *Batrachochytrium dendrobatidis* (*Bd*) infection status and green indicating negative.

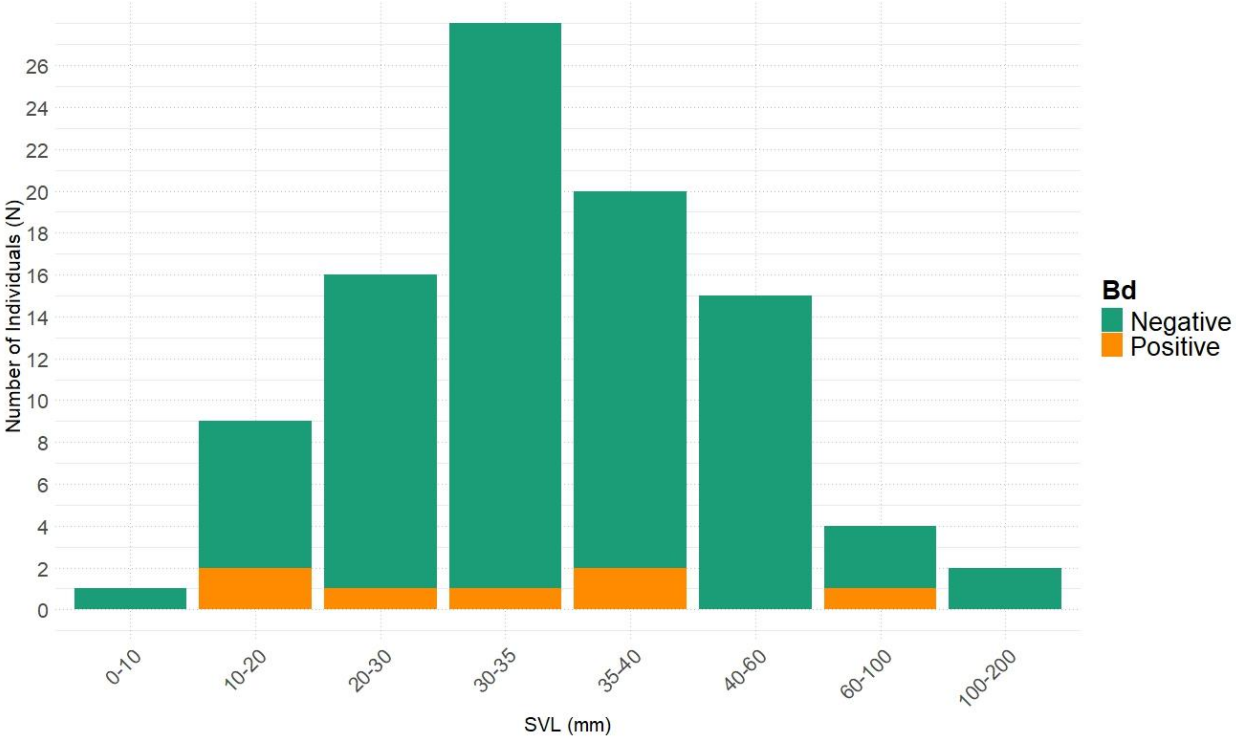

Figure 9: Bar chart visualizing the distribution of anuran snout-vent length (mm), with orange color indicating positive *Batrachochytrium dendrobatidis* (*Bd*) infection status and green indicating negative.

3.3 eDNA water samples

Of the 12 eDNA water samples collected to examine the presence of *Bd* in the environment, none of the samples yielded positive detection.

4 Discussion

4.1 *Bd* prevalence and lowland dynamics

The Amazon lowlands remain largely underexplored regarding *Bd* in amphibians. This study provides new insights into *Bd* prevalence in a highly anuran-diverse region of Brazilian western Amazon lowlands. Specifically, this is the first study of *Bd* in amphibians along the Juruá River and the first record of *Bd* infections in post-metamorphic anurans in the middle Juruá.

This study detected a very low prevalence of *Bd* infection among anurans (7.37%). The low prevalence detected is expected, as low elevation sites with high temperatures have predicted low risk of *Bd* occurrence according to models (Becker et al., 2016; Rödder et al., 2010; Ron, 2005). The prevalence is also similar to other findings in neotropical lowlands: 9.6% (Kilburn et al., 2010), 15% (Lambertini et al., 2022), 15-34% (Rebollar et al., 2014), 0.7-7.3% (von May et al., 2018) and >7.5% (Woodhams et al., 2008). Given that large parts of the neotropical lowlands are cold spots for the fungus (James et al., 2015), it is mostly higher elevation sites of the neotropics seeing severe *Bd* infections directly leading to anuran declines (Cheng et al., 2011; Lips, 1999; Scheele et al., 2019). However, some studies have found higher *Bd* prevalence in neotropical lowland forests with evidence of enzootic disease dynamics - despite the high prevalence in these studies, there have been no signs of population decline (Russell et al., 2019; Smart et al., 2024; Zumbado-Ulate et al., 2019). This has led to suggestions that lowland forests of the neotropics may hold a crucial role in the spread of *Bd*, by facilitating infections between higher elevation sites (Russell et al., 2019). Therefore, *Bd* might not be causing amphibian decline in lowland neotropical forests, but instead contributing to population declines elsewhere.

The low prevalence detected in this study may indicate that *Bd* is acting as an enzootic pathogen, which involves low intensity infections of anurans (Brem & Lips, 2008; Briggs et al., 2010). This can be the case when the fungus is of a low virulent lineage, if anurans have spent enough time with the fungus to develop resistance, or if unfavorable climatic conditions limit the fungus, among other possible factors (Briggs et al., 2010). Additionally, enzootic disease dynamics might be supported by the fact that *Bd* infection in the middle Juruá is unlikely to be recent. It has been documented along the river since the 1990s, though only a few times. The earliest detection was in 1991 from a Dendrobatidae tadpole in the middle Juruá, close to my study area (Carvalho et al., 2017b). The fungus has since been detected in lower parts of the river in three post-metamorphic anuran individuals in the municipality of Cruzeiro do Sul in the state of Acre, from specimens collected in 1993 and 2009 (Becker et al., 2016). However, studies using museum samples may underestimate *Bd* prevalence because the formalin fixation process can affect detection (Richards-Hrdlicka, 2012). As a result, some *Bd* infected anurans may have gone undetected. Comparisons of prevalence between studies should also be made cautiously, as inconsistencies can arise from the use of different DNA extraction kits. This study, like most research discussed above with respect to prevalence, used the recommended DNeasy Blood and Tissue Kit, which is generally recognized as more effective for extracting *Bd* DNA (Bletz et al., 2015).

Recent efforts to understand dynamics of *Bd* in the Brazilian Amazon have been conducted by Lambertini et al. (2022). However, their study did not include sampling in the western Brazilian Amazon. There have also been more *Bd* detections in Amazonian parts of Peru, which is where the Juruá river originates (Russell et al., 2019; Seimon et al., 2017). Catenazzi et al. (2011) suggested that *Bd* could be spreading to more central parts of Amazonia from outbreaks in Peru, which could be the source of *Bd* detected along the Juruá. However, more sampling over time is necessary to better understand the host-pathogen dynamics in the middle Juruá and determine whether *Bd* is a common occurrence among anurans. Furthermore, identifying which *Bd* lineages are present and studying genotype diversity is crucial to better understand the enzootic and epizootic *Bd* dynamics in this region (Briggs et al., 2010). I was unable to measure the intensity of infection and to establish the fungus lineage in this study. It is therefore difficult to draw definitive conclusions regarding the epizootic and enzootic *Bd* dynamics along the middle Juruá river based on my findings, albeit they provide some important insights.

4.2 Taxonomy and *Bd* infection

Field sampling encompassed 23 species from 7 anuran families (Table 1). The 7 *Bd* positive cases were from 6 different anuran families (Fig. 5) and all from different species (Fig. 6). Among the *Bd*-positive individuals, the family Leptodactylidae was the most represented, with both species belonging to the genus *Leptodactylus* (Table 1). This is unsurprising since members of this genus are frequently found in highly aquatic habitats (Sá et al., 2014), which may increase probability of infection from the fungus as the fungus zoospores infect through water (Johnson & Speare, 2003). Furthermore, this family has been utilized in *Bd* research to investigate historical *Bd* dynamics in Brazil, due to the widespread occurrence of this genus in the region and documented infection with the fungus (Becker et al., 2016). The two *Bd*-infected species from this genus were *L. petersii* (Fig. 5C) and *L. leptodactyliodes* (Fig. 5E). *Bd* has been detected in both of these species previously (e.g. Becker et al., 2016; von May et al., 2018). Published records of *Bd* infection also exist for several other *Bd*-positive species in this study: *Amereega hahneli* (von May et al., 2018), *Rhinella marina* (Acevedo et al., 2016) and *Scinax ruber* (Flechas et al., 2017), which are all commonly found species in the Amazon (Villacampa et al., 2017).

For two of the species detected with the fungus, it is difficult to find definitive published accounts of previous *Bd* infection. An individual belonging to *Pipa snethlageae* was found to be infected with *Bd.* After thoroughly reviewing the literature of *Bd* surveys conducted within the geographical range of *Pipa snethlageae* (AmphibiaWeb, n.d.), I could not find any records of this species being tested for *Bd* in the wild (Azat et al., 2022; Carvalho et al., 2017b; Lambertini et al., 2022; Soto-Azat et al., 2010). It has been swabbed for *Bd* in captivity at a French zoo but the fungus was not detected (Marquis et al., 2019). This may therefore be the first record of a *Bd* screening and *Bd* infection in *Pipa snethlageae* in its native range in the Amazon. *Pipa snethlageae* belongs to the aquatic anuran family Pipidae. These frogs, notable for their unusual appearance (Fig. 6D and Fig 7), are found in aquatic environments in South America and Africa (Cannatella & Trueb, 1988). Although I was unable to find records of *Bd* infection in *Pipa snethlageae*, other Pipidae species have been found infected with the fungus.

For example *Xeanopus laevis* (Vredenburg et al., 2013) and *Pipa pipa* (e.g. Russell et al., 2019), indicating that it is not uncommon among members of this family.

The final *Bd* infected individual belongs to the *Allobates* genus, which holds many cryptic species (Vacher et al., 2020). Several new species of this genus have been described recently (e.g. Fouquet et al., 2023; Melo-Sampaio et al., 2020). It is therefore difficult to determine whether the *Bd*-infected *Allobates sp*. from this study has been previously found to be infected with *Bd*. However, many members of the genus have been recorded with *Bd* previously (Acevedo et al., 2016; Lambertini et al., 2022).

I detected no presence of *Bd* in the swabs taken from the 16 other species. This may be due to the absence of *Bd* in these species at this location. Even though several of these species have tested positive for *Bd* previously in other areas of the Amazon (AmphibiaWeb, 2020), infections may be acting differently in the Amazon lowlands compared to other previous detections in the same species from higher elevation sites. Further investigation should be conducted to see if similar taxonomic associations with *Bd* infections are found at various elevations. Another reason for absence of *Bd* infection could be breeding strategies, because anurans breeding at permanent waterbodies are often linked with infections (Kriger & Hero, 2007). Lastly, a larger sample size of each species may elucidate whether more species are carriers of *Bd* in the middle Juruá region.

4.3 Clinical signs of disease

The one anuran showing potential clinical signs of chytridiomycosis was also the *Pipa snethlageae* individual (Fig. 7). Clinical signs of chytridiomycosis are difficult to reliably detect as infected individuals can vary from asymptomatic death to severe skin disorders (Van Rooij et al., 2015). However, this individual showed several potential signs of infection, such as erythema (Fig. 5b; Van Rooij et al., 2015) and red coloration of finger tips, which is a common location of infection (Puschendorf & Bolaños, 2006; Van Rooij et al., 2015). However, these signs can also be from other sources such as injury caused by fish, crabs, or infections with bacteria (A. Fouquet, pers, comm.) and amphibian ranaviruses, which can also cause erythema (Gray et al., 2009). This individual was captured using a pitfall trap, with there being no waterbodies in proximity to the trap, indicating it may have ventured on land, which is uncommon for Pipidae species (Cannatella & Trueb, 1988). This may have left it more exposed to predators and could be another explanation for the origin of its injuries. It is therefore difficult to say for certain that this individual was experiencing symptoms from chytridiomycosis. Measuring the zoospore load of *Bd* on the swab would have given an indication of the intensity of the infection and could help understand whether this individual was showing clinical signs of chytridiomycosis. Ultimately, potentially observing clinical signs of chytridiomycosis in an anuran infected by *Bd* at a low elevation site in the Amazon is concerning and may indicate that chytridiomycosis could pose a significant threat to some species in areas that are typically not considered to be at risk. This was also noted in McCracken et al. (2009), who observed clinical signs of chytridiomycosis in a single specimen at a lowland site in Ecuador.

4.4 A dry and hot Amazon

Field sampling took place in late 2023, a period of extreme droughts and heat anomalies across the Amazon (Espinoza et al., 2024). The temperature anomalies were at their peak during the time of the study. Despite temperatures significantly exceeding 28°C, which is the hypothesized critical maximum for *Bd* growth in the Amazon lowlands (von May et al., 2018), *Bd* was still detected in anurans. This underlines the fungus is resilient to extreme drought and heat conditions. von May et al. (2018) suggested that temperatures in the leaf litter and understory vegetation of Amazonian lowland forests can offer suitable conditions for *Bd* growth. This may therefore explain how *Bd* could still be present in the climatic conditions experienced during this study. The drought was also evident during data collection, with the two streams in the study area nearly dried up, and I observed anuran eggs and tadpoles drying out. The heat and drought could cause increased stress among anurans (Rollins-Smith & Le Sage, 2023; Walls et al., 2013), which can increase chance of infection with *Bd*. Furthermore, heat stress has been shown to adversely affect the skin microbiome of amphibians and reduce the host defense against pathogens such as *Bd* (Rollins-Smith & Le Sage, 2023). However, it is also possible that the hotter and drier conditions limited the fungus when taking into consideration the specific temperature requirements of *Bd* (Piotrowski et al., 2004). Additionally, droughts can decrease the intensity of the fungus infection (Terrell et al., 2014). Since my sampling was conducted during unusual temperature and precipitation patterns (Espinoza et al., 2024), *Bd* infection and prevalence might differ under normal weather conditions. Thus, sampling should be repeated during such periods to compare or attempt to understand the effect of the drought and heat anomalies on *Bd*.

4.5 eDNA

No *Bd* was detected in the eDNA water samples taken from the streams in the study area. This indicates that *Bd* may be rare in the floodplain environment studied, which supports the very low prevalence detected in anurans. Perhaps the temperature in the water was too high to support *Bd*, which can be the case if water temperatures reached the critical maximum for *Bd* (Stevenson et al., 2013; von May et al., 2018). Indeed, the water samples were collected from streams with minimal flow and high temperatures – although water temperature was not measured. This would be a useful addition to future studies. In addition, water filtration presented challenges due to the high sediment and algae load in the water. Filters were easily clogged and limited the amount of water that I could filter. The volume of water filtered may affect detectability of the target species (Hunter et al., 2019). The application of pre-filtration or a multi-filter methods improve should be considered in future studies (Hunter et al., 2019; Takasaki et al., 2021).

4.6 Morphometrics and habitat preference

Morphometrics (weight and snout-vent length) has previously been associated with infection of *Bd*. Some studies have found that body size may influence infection. According to Burrow et al. (2017) and Smart et al. (2024), smaller individuals have shown increased susceptibility to the disease and infection. Other studies found that older frogs with greater body size are more susceptible (Bradley et al., 2019) and have a greater risk of infection and subsequent

decline (Lips et al., 2003). I did not find similar associations. None of the variables (weight or snout-vent length) significantly explained the presence of *Bd*. Anurans infected with *Bd* were present in most weight (Fig. 8) and snout-vent length (Fig. 9) categories. This indicates that morphometrics is not a predictor for *Bd* infection at this site along the middle Juruá river.

I also found no association between *Bd* and habitat preference. *Bd* may therefore be randomly distributed among anurans inhabiting different parts of the forest, not showing any preference to specific environments. This is consistent with findings from Peruvian lowlands (Russell et al., 2019). However, in both Russell et al. (2019) and this study, aquatic species showed a 100% infection prevalence for *Bd* (although not included in either of our analysis due to 100% prevalence and n = 2 and n = 1 respectively). Interestingly, for both studies the species belonged to the genus *Pipa*. This may suggest that fully aquatic anurans, particularly those in the *Pipa* genus, are more susceptible to *Bd* infection. Their constant presence in water might increase their risk of infection. The increased vulnerability of species with high dependence on permanent waterbodies was also suggested by Kriger & Hero (2007), who found that *Bd* is non-randomly distributed in the environment, with anurans found breeding in permanent water bodies having a greater chance of infection. Further research should explore whether habitat is associated with *Bd* prevalence in lowlands, as such associations have been observed elsewhere.

5 Conclusion

The amphibian pathogenic fungus *Batrachochytrium dendrobatidis* was detected in a várzea forest along the middle Juruá river. This is the first detection of *Bd* in post-metamorphic anurans in this area of the Juruá river and one of few detections of *Bd* in this part of the western Amazon lowlands. This study involved swabbing a diverse range of species from several different anuran families. Due to the geographical scope of this study, its findings cannot be generalized to the entire region and sampling across the Amazon is needed to obtain a clearer understanding of *Bd* prevalence in the region. Overlaying prevalence data with climate and habitat data would improve predictions of trends at larger scales. The potential observation of clinical signs of chytridiomycosis in one *Bd* positive anuran does raise the possibility that chytridiomycosis could be negatively affecting amphibians in lowland Amazon forests. Furthermore, the swabbing and detection of *Bd* in the rarely captured species *Pipa snethlageae* adds to the long list of species infected with the fungus across the globe.

Detecting *Bd*, despite its low prevalence in these remote and anuran-rich areas of the Amazon, is cause for concern. It suggests that lowland Amazonia could play a role in the persistence and spread of the deadly amphibian fungus. With much still unknown about *Bd* dynamics in the lowlands, the added pressure of climate change could bring about new dynamics and highlights the need for a better understanding of the pathogens impact in this area. Understanding the role of *Bd* and its effects on amphibian decline in the Amazon lowlands is therefore critical. Future research should focus on uncovering such *Bd* dynamics so that effective disease mitigation strategies can be implemented and prevent further population declines and species extinctions.

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Supplementary materials

Table S1: Categories used for each species habitat preference with reference.

Species	Family	Habitat	Reference
Adenomera andreae	Leptodactylidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Adenomera andreae. The IUCN Red List of Threatened Species 2023: e.T56304A3037679. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T56304A3037679.en. Accessed on 10 July 2024.
Allobates sp.	Aromobatidae	leaflitter	Gonçalves Dias-Terceiro, Randolpho & Kaefer, Igor & Fraga, Rafael & Araujo, Maria & Simões, Pedro & Lima, Albertina. (2015). A Matter of Scale: Historical and Environmental Factors Structure Anuran Assemblages from the Upper Madeira River, Amazonia. Biotropica. https://doi. org/10.11 11/btp.12197
Ameerega hahneli	Dendrobatidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Ameerega hahneli. The IUCN Red List of Threatened Species 2023: e.T77187961A61396539. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T77187961A61396539.en. Accessed on 10 July 2024.
Boana appendiculata	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Boana appendiculata. The IUCN Red List of Threatened Species 2023: e.T184675833A186752119. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T184675833A186752119.en. Accessed on 10 July 2024.
Boana calcarata	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Boana calcarata. The IUCN Red List of Threatened Species 2023: e.T55426A61398018. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T55426A61398018.en. Accessed on 10 July 2024.
Boana cf. steinbachi	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Boana steinbachi. The IUCN Red List of Threatened Species 2023: e.T82498489A154175020. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T82498489A154175020.en. Accessed on 10 July 2024.
Boana geographica	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Boana geographica. The IUCN Red List of Threatened Species 2023: e.T91950241A61398586. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T91950241A61398586.en. Accessed on 10 July 2024.
Boana punctata	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Boana punctata. The IUCN Red List of Threatened Species 2023: e.T55620A61401241. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T55620A61401241.en. Accessed on 10 July 2024.
Boana raniceps	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Boana raniceps. The IUCN Red List of Threatened Species 2023: e.T88331830A61401422. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T88331830A61401422.en. Accessed on 10 July 2024.
Dendropsophus kubricki	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Dendropsophus kubricki. The IUCN Red List of Threatened Species 2023: e.T154037601A154037700. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T154037601A154037700.en. Accessed on 10 July 2024.
Leptodactylus leptodactyloides	Leptodactylidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Leptodactylus leptodactyloides. The IUCN Red List of Threatened Species 2023: e.T57140A85886282. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T57140A85886282.en. Accessed on 10 July 2024.
Leptodactylus pentadactylus	Leptodactylidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Leptodactylus pentadactylus. The IUCN Red List of Threatened Species 2023: e.T57154A85888061. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T57154A85888061.en. Accessed on 10 July 2024.
Leptodactylus petersii	Leptodactylidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Leptodactylus petersii. The IUCN Red List of Threatened Species 2023: e.T211139302A85888201.

			https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T211139302A85888201.en. Accessed on 10 July 2024.
Leptodactylus sp.	Leptodactylidae	leaflitter	Angulo, A., & Icochea, J. (2010). *Cryptic species complexes, widespread species and conservation: lessons from Amazonian frogs of theLeptodactylus marmoratusgroup (Anura: Leptodactylidae). Systematics and Biodiversity, 8(3), 357–370.* doi:10.1080/14772000.2010.507264
Osteocephalus cf. leprieurii	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Osteocephalus leprieurii. The IUCN Red List of Threatened Species 2023: e.T55798A61403731. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T55798A61403731.en. Accessed on 10 July 2024.
Pipa snethlageae	Pipidae	aquatic	IUCN SSC Amphibian Specialist Group. 2023. Pipa snethlageae. The IUCN Red List of Threatened Species 2023: e.T58164A61414969. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T58164A61414969.en. Accessed on 10 July 2024.
Pseudopaludicola ceratophyes	Leptodactylidae	leaflitter	IUCN SSC Amphibian Specialist Group. 2023. Pseudopaludicola ceratophyes. The IUCN Red List of Threatened Species 2023: e.T57312A85894407. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T57312A85894407.en. Accessed on 10 July 2024.
Rhinella castaneotica	Bufonidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Rhinella castaneotica. The IUCN Red List of Threatened Species 2023: e.T54603A184640885. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T54603A184640885.en. Accessed on 10 July 2024.
Rhinella marina	Bufonidae	terrestrial	IUCN SSC Amphibian Specialist Group. 2023. Rhinella marina. The IUCN Red List of Threatened Species 2023: e.T100099625A2951416. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T100099625A2951416.en. Accessed on 10 July 2024.
Scarthyla goinorum	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Scarthyla goinorum. The IUCN Red List of Threatened Species 2023: e.T55920A85905975. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T55920A85905975.en. Accessed on 10 July 2024.
Scinax gr. ruber	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Scinax ruber. The IUCN Red List of Threatened Species 2023: e.T55994A54348716. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T55994A54348716.en. Accessed on 10 July 2024.
Sphaenorhynchus lacteus	Hylidae	arboreal	IUCN SSC Amphibian Specialist Group. 2023. Sphaenorhynchus lacteus. The IUCN Red List of Threatened Species 2023: e.T56015A85907270. https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T56015A85907270.en. Accessed on 10 July 2024.
Teratohyla cf. midas	Centrolenidae	arboreal	IUCN SSC Amphibian Specialist Group. 2022. Teratohyla midas. The IUCN Red List of Threatened Species 2022: e.T54971A54997093. https://dx.doi.org/10.2305/IUCN.UK.2022-2.RLTS.T54971A54997093.en. Accessed on 10 July 2024.

Table S2**:** Summary of the swabbed anurans used for statistical analysis including, family, species, *Bd* detection (positive highlighted in bold) and morphometric data.

Family	Species	*Bd* status	Weight (g)	SVL (mm)
Aromobatidae	*Allobates sp.*	negative	0.2	14.68
Aromobatidae	*Allobates sp.*	**positive**	0.2	13.41
Bufonidae	*Rhinella castaneotica*	negative	1.25	24.02
Bufonidae	*Rhinella castaneotica*	negative	1	25.45
Bufonidae	*Rhinella marina*	negative	4	36.49
Bufonidae	*Rhinella castaneotica*	negative	1.25	25.46
Bufonidae	*Rhinella marina*	negative	8.15	43.42
Bufonidae	*Rhinella marina*	negative	24.7	60.59
Bufonidae	*Rhinella castaneotica*	negative	1.8	30.6
Bufonidae	*Rhinella marina*	**positive**	4.25	38.52

Centrolenidae	*Teratohyla cf. midas*	negative	0.4	17.99
Dendrobatidae	*Ameerega hahneli*	negative	0.5	21
Dendrobatidae	*Ameerega hahneli*	**positive**	1	22.26
Hylidae	*Boana cf. steinbachi*	negative	4.5	45.45
Hylidae	*Scinax gr. ruber*	negative	4.5	40.67
Hylidae	*Scinax gr. ruber*	negative	2	33.09
Hylidae	*Scinax gr. ruber*	negative	2.75	35.8
Hylidae	*Boana cf. steinbachi*	negative	1.75	31.56
Hylidae	*Scinax gr. ruber*	negative	2	34.27
Hylidae	*Osteocephalus cf. leprieurii*	negative	6.1	47.77
Hylidae	*Osteocephalus cf. leprieurii*	negative	3.75	38.55
Hylidae	*Boana geographica*	negative	5.25	46.68
Hylidae	*Boana geographica*	negative	3.7	39.91
Hylidae	*Boana geographica*	negative	2.1	34.35
Hylidae	*Boana geographica*	negative	2.1	35.54
Hylidae	*Boana geographica*	negative	1.85	35.56
Hylidae	*Dendropsophus kubricki*	negative	0.2	18.19
Hylidae	*Boana geographica*	negative	2.9	36.76
Hylidae	*Boana geographica*	negative	2	39
Hylidae	*Osteocephalus cf. leprieurii*	negative	1.9	30.91
Hylidae	*Osteocephalus cf. leprieurii*	negative	2.3	37.04
Hylidae	*Osteocephalus cf. leprieurii*	negative	3.1	37.71
Hylidae	*Boana appendiculata*	negative	3	40.24
Hylidae	*Dendropsophus kubricki*	negative	0.8	24.58
Hylidae	*Scinax gr. ruber*	negative	2	32.22
Hylidae	*Scinax gr. ruber*	negative	2.2	33.39
Hylidae	*Scinax gr. ruber*	negative	4	36.92
Hylidae	*Scinax gr. ruber*	negative	3.8	35.75
Hylidae	*Scinax gr. ruber*	negative	5.5	41.61
Hylidae	*Scinax gr. ruber*	negative	2.6	32.24
Hylidae	*Scinax gr. ruber*	negative	2.2	33.86
Hylidae	*Scinax gr. ruber*	negative	5.25	40.24
Hylidae	*Scinax gr. ruber*	negative	2	33.11
Hylidae	*Scinax gr. ruber*	negative	2.1	31.97
Hylidae	*Scinax gr. ruber*	negative	4.5	40.87
Hylidae	*Scinax gr. ruber*	negative	2.1	28.38
Hylidae	*Scinax gr. ruber*	negative	2.3	31.73
Hylidae	*Scinax gr. ruber*	negative	2.6	33.45
Hylidae	*Scinax gr. ruber*	negative	6.25	41.28
Hylidae	*Scinax gr. ruber*	negative	2.25	34.59
Hylidae	*Scinax gr. ruber*	negative	2.25	34.27
Hylidae	*Scinax gr. ruber*	negative	2.25	32.54

Hylidae	*Scinax gr. ruber*	negative	2	31.58
Hylidae	*Boana raniceps*	negative	17.8	60.55
Hylidae	*Boana cf. steinbachi*	negative	1.5	33.27
Hylidae	*Boana geographica*	negative	2.3	38.16
Hylidae	*Boana geographica*	negative	2.6	38.05
Hylidae	*Boana geographica*	negative	2.4	36.99
Hylidae	*Boana geographica*	negative	2.7	36.58
Hylidae	*Boana cf. steinbachi*	negative	1.6	32.69
Hylidae	*Boana raniceps*	negative	3.5	42.68
Hylidae	*Scinax gr. ruber*	negative	1.75	28.89
Hylidae	*Boana cf. steinbachi*	negative	1.5	28.91
Hylidae	*Osteocephalus cf. leprieurii*	negative	5.4	45.75
Hylidae	*Scarthyla goinorum*	negative	0.6	18.21
Hylidae	*Boana appendiculata*	negative	14	66.24
Hylidae	*Boana calcarata*	negative	0.7	24.85
Hylidae	*Scarthyla goinorum*	negative	0.5	15.99
Hylidae	*Boana calcarata*	negative	1	30.62
Hylidae	*Osteocephalus cf. leprieurii*	negative	6	47.76
Hylidae	*Boana cf. steinbachi*	negative	3.2	40.32
Hylidae	*Osteocephalus cf. leprieurii*	negative	5.3	43.33
Hylidae	*Osteocephalus cf. leprieurii*	negative	3.7	38.08
Hylidae	*Boana cf. steinbachi*	negative	1.8	32.27
Hylidae	*Boana punctata*	negative	0.8	23.74
Hylidae	*Sphaenorhynchus lacteus*	negative	3.2	32.57
Hylidae	*Scinax gr. ruber*	**positive**	2.2	30.66
Leptodactylidae	*Adenomera andreae*	negative	1	21.98
Leptodactylidae	*Leptodactylus petersii*	negative	3.7	31.69
Leptodactylidae	*Adenomera andreae*	negative	1.25	24.36
Leptodactylidae	*Adenomera andreae*	negative	1	22.96
Leptodactylidae	*Adenomera andreae*	negative	0.7	22.92
Leptodactylidae	*Leptodactylus leptodactyloides*	negative	4.5	37.71
Leptodactylidae	*Leptodactylus leptodactyloides*	negative	3.3	33.84
Leptodactylidae	*Adenomera andreae*	negative	0.7	19.01
Leptodactylidae	*Leptodactylus leptodactyloides*	negative	4	34.36
Leptodactylidae	*Leptodactylus pentadactylus*	negative	190	113.68
Leptodactylidae	*Leptodactylus petersii*	negative	0.7	20.31
Leptodactylidae	*Leptodactylus sp.*	negative	0.5	17.58
Leptodactylidae	*Leptodactylus petersii*	negative	3.6	34.14
Leptodactylidae	*Leptodactylus pentadactylus*	negative	335	152.19
Leptodactylidae	*Pseudopaludicola ceratophyes*	negative	0.1	10
Leptodactylidae	*Leptodactylus petersii*	**positive**	0.25	15.93
Leptodactylidae	*Leptodactylus leptodactyloides*	**positive**	5.5	39.31

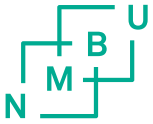
Pipidae	*Pipa snethlageae*	**positive**	50	86

Table S3: Prevelence of *Bd* infection in different anuran families. CI calculations using Wilson scoring intervals.

Anuran family	*Bd* positive	No. sampled	Prevalence	Lower	Upper
Aromobatidae	1	2	50%	0.095	0.906
Bufonidae	1	8	12.5%	0.022	0.471
Centrolenidae	0	1	0%	0	0.794
Dendrobatidae	1	2	50%	0.095	0.906
Hylidae	1	64	1.6%	0.003	0.083
Leptodactylidae	2	17	11.8%	0.033	0.343
Pipidae	1	1	100%	0.207	1

Table S4: Results of the generalized linear model (GLM) examining the relationship between host traits (Term) and *Bd* infections in anurans from the middle Juruá with the coefficient estimates for each predictor (Weight, SVL, habitat preference), standard errors, z-value and the p-value column showing the non-significant p-values for each ($p > 0.05$).

Term	Estimate	Std. error	*z value*	p-value
(Intercept)	19.104	2399.55	0.008	0.994
Weight	-0.023	0.176	-0.132	0.895
Snout-vent length	-0.016	0.086	-0.187	0.852
Habitat (Arboreal)	-22.638	2399.55	-0.009	0.992
Habitat (Leaf litter)	-19.974	2399.55	-0.008	0.993
Habitat (Terrestrial)	-20.127	2399.55	-0.008	0.993



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