INTRODUCTION

Globally, biodiversity is decreasing at an alarming rate even in seemingly pristine and protected environments (Barnosky et al., 2011; Novacek & Cleland, 2001). Species declines are driven by numerous anthropogenic actions, acting alone or synergistically with natural threats (Hooper et al., 2012; Rödder, Kielgast, & Lötters, 2010; Sala et al., 2000). Previous studies suggest that immediate conservation...
Craugastor taurus

Golfito robber frog (FIGURE 1) Female individual of the Critically Endangered species (hereafter Ctenosaura similis) from our study sites) and Puerto Armuelles (Panama). This species was very common in lowlands of Southern Costa Rica but catastrophically declined during the 1980s and 1990, presumably due to chytridiomycosis. Currently, it is only present in Punta Banco (one of our study sites) and Puerto Armuelles (Panama).

The strong elevational gradients in the mountain ranges of Central America (Savage, 2002) create habitat heterogeneity and high endemism of amphibians in midlands and highlands (>800 m elevation). The cool and moist environments in tropical highlands provide suitable conditions for the Bd epizootic that occurred in Central America during the 1980s and 1990s, causing the extinction of an unknown number of amphibian species, especially highland stream-breeding species (Cheng, Rovito, Wake, & Vredenburg, 2011; Lips, Diffendorfer, Mendelson, & Sears, 2008; Pounds et al., 2006; Pounds & Crump, 1994; Rovito, Parra-Olea, Vasquez-Almazan, Papenfuss, & Wake, 2009). Historical declines in montane amphibian species reflect why most studies on amphibian host-Bd dynamics in the tropics have been conducted in premontane and upper elevation localities (Lips, 1999, 1998; Puschendorf, Bolaños, & Chaves, 2006; Ryan, Lips, & Eichholz, 2008). For example, a considerable amount of Bd infection data has been opportunistically collected from montane ecosystems, increasing the focus of conservation actions on highlands while overlooking other potential environments where amphibians may also be impacted by Bd (Puschendorf, Hodgson, Alford, Skerratt, & VanDerWal, 2013). For example, the suitability of lowland ecosystems for the spread of Bd has been frequently disregarded (Puschendorf et al., 2009) even though it is known that some amphibian species (Figure 1) and clades have suffered dramatic unexplained declines in these zones (Chaves et al., 2014; La Marca et al., 2005; Puschendorf et al., 2009; Ryan et al., 2008; Whitfield et al., 2007; Zumbado-Ulate, Bolaños, Gutiérrez-Espeleta, & Puschendorf, 2014).

Despite the focus on highlands for most Bd-related studies, the few studies conducted in lowlands of Central America have found new locations where this pathogen occurs, suggesting that Bd is more widely distributed than previously thought (Flechas, Vredenburg, & Amézquita, 2015; Kilburn et al., 2010; von May, Catenazzi, Santa-Cruz, & Vredenburg, 2018; Whitfield et al., 2013; Whitfield, Kerby, Gentry, & Donnelly, 2012; Woodhams et al., 2008; Zumbado-Ulate et al., 2014). Predictive models and abiotic suitability for Bd across heterogeneous landscapes (Brannely, Martin, Llewelyn, Skerratt, & Berger, 2018; García-Rodríguez, Chaves, Benavides-Varela, & Puschendorf, 2012; Puschendorf et al., 2009; Rödder et al., 2014).
The dataset contains 19 bioclimatic variables generated by land area interpolations of climate point data from 1950 to 2000. These variables were derived from monthly precipitation and temperature data at weather stations around the world and describe annual means (e.g., annual precipitation and temperature) and average of extreme environmental values (e.g., maximum temperature of warmest month) (Hijmans et al., 2005). Thus, combining information on infection prevalence and abiotic conditions (e.g., from the WorldClim dataset) across the entire geographic distribution of a host can provide a more informative distribution of both the host and pathogen to identify potential hotspots of future disease outbreaks and potential environmental refuges from disease (Green, 2017; James et al., 2015; Rödder et al., 2010).

In this study, we sampled for Bd at four tropical lowland locations in Costa Rica and contrasted Bd prevalence and intensity of infection across study sites. We hypothesized that different host–pathogen dynamics occur across study sites because they exhibit latitudinal and altitudinal variation (Kriger & Hero, 2008; Kriger et al., 2007). We extracted all 19 bioclimatic variables of the WorldClim to describe the different ranges of temperature and precipitation across study sites, which are the main environmental variables that affect Bd growth and dispersal (Nowakowski et al., 2016; Savage, Zamudio, & Sredl, 2011). Additionally, we hypothesized that all study sites would exhibit low levels of Bd prevalence and intensity of infection suggesting stable enzootic infections of Bd (Retallick, McCallum, & Speare, 2004; Scheele, Hunter, Brannelly, Skerratt, & Driscoll, 2017; Woodhams et al., 2008). Finally, we also expected a higher prevalence of Bd in amphibian assemblages occurring in permanent streams than in ephemeral ponds and terrestrial assemblages, as has been found in previous studies (Kriger & Hero, 2007a; Lips et al., 2003).

2 | METHODS

2.1 | Lowland sampling sites

We sampled four assemblages of amphibians between November and December 2011, at four tropical lowland locations in Costa Rica. We contrasted Bd prevalence and intensity of infection across study sites. We hypothesized that different host–pathogen dynamics occur across study sites because they exhibit latitudinal and altitudinal variation (Kriger & Hero, 2008; Kriger et al., 2007).
Rica (Figure 2). We defined tropical lowlands as all tropical locations within 0–800 m elevation according to the Holdridge Life Zone System (Holdridge, 1967). Study sites consisted mostly of tropical moist forest and tropical wet forest with transitional ecosystems including semi-deciduous and evergreen forests, with temperature and precipitation ranges characteristic of these life zones. Our four sampling sites grouped into two main zones:

2.1.1 | Caribbean zone

Here, we sampled at Tirimbina Private National Wildlife Refuge at La Virgen, Sarapiqui, on the north Caribbean lowlands (10.41N, −84.11W, 0–200 m elevation), and at the Costa Rican Amphibian Research Center, at Guayacan, Siquirres (10.06N, −83.55W, 400–600 m elevation).

2.1.2 | Pacific zone

Here, we focused on the areas surrounding the small towns of Rincon de Osa (8.71N, −83.52W, 0–50 m elevation) and Punta Banco (8.36N, −83.15W, 0–50 m elevation), where we sampled across patches of coastal forest. Our sampling in this zone was limited because we were only able to access private farms upon the authorization of landowners.

2.2 | Pathogen detection

At each site, four people systematically searched for amphibians for 36–48 hr during the day and night (9–12 hr/person). Within each site, we conducted visual encounter surveys of amphibians (Heyer, Donnelly, McDiarmid, Hayek, & Foster, 1994) and classified them by the habitat where they were captured: stream-dwellers (permanent flowing water), pond-dwellers (standing ephemeral waterbodies such as swamps, pools, and ditches), and forest-dwellers (leaf-litter, tree holes, or bromeliad plants in the understory, and canopy). Caught amphibians were stored individually in clean, unused plastic bags. Each individual was inspected for visible signs of chytridiomycosis, such as hyperplasia, hyperkeratosis, abnormal shedding, depigmentation, and lethargic behavior (Berger et al., 1998; Voyles et al., 2009) and swabbed to detect Bd with a cotton swab (Medical Wire and Equipment, MW–113) using nitrile gloves. To swab, we ran a total of 20 strokes on every individual as follows: five strokes on one hand, five strokes on the ventral patch, five strokes on one foot, and five strokes along inner thigh. Swabs were stored dry in 1.5 ml Eppendorf tubes and frozen at -20°C until DNA extraction. All amphibians were immediately released after sampling. During this study, we followed field protocols (Kriger, Hines, Hyatt, Boyle, & Hero, 2006; Skerratt et al., 2008) which were approved by the National System of Conservation Areas of Costa Rica (SINAC, research permit 001-2012-SINAC) which ensures that animals are being cared for in accordance with standard protocols and treated in an ethical manner.

We extracted DNA from swabs using PrepMan Ultra (Boyle, Boyle, Olsen, Morgan, & Hyatt, 2004). All extractions were diluted 1:10 in 0.25X TE buffer and run in singlicate (Kriger, Hero, & Ashton, 2006) following diagnostic quantitative PCR (qPCR) standard protocols (Boyle et al., 2004) using an Applied BioSystems Prism 7300 Sequence Detection System to test for the presence and quantity of Bd genome equivalents. All Bd-positive samples were run again in singlicate confirmatory assay. Negative controls (DNase/RNase-free distilled water) were run in triplicate on every 96-well PCR plate. We used 100, 10, 1, and 0.1 zoospore quantification standards to produce a quantification curve. We multiplied the qPCR score by 80 to calculate the zoospore genomic equivalents in the original sample and calculated the average value from the two singlicate assays (Vredenburg, Knapp, Tunstall, & Briggs, 2010; Warne, LaBumbard, LaGrange, Vredenburg, & Catenazzi, 2016).

2.3 | Data analysis

We were interested in understanding how Bd prevalence and intensity varied among our study sites and habitats (predictor variables). For our analyses, we pooled all species together instead of using species as predictor or running independent tests for each species because the samples sizes per species were highly variable (from 1–44). This high variance in the sample size could produce significant models that may be an artifact of opportunistic sampling instead of a real pattern. Therefore, we analyzed habitat as a proxy of amphibian community composition, because the species variable was 100% correlated to habitat. To contrast Bd prevalence, we used fix-effects generalized linear models (GLMs) to find the most suitable model using binomial response variables (infected or not infected). Candidate models were ranked according to the Akaike’s information criterion (AIC) to determine the relative importance of predictor variables within each model set. The model with the lowest AIC was considered the most robust (Burnham & Anderson, 2004). To compare infection intensity among locations and habitats (predictors), we generated fix-effects general linear models (LMs) with data only from infected individuals. We built our models using the log-transformed Bd load (estimated number of genomic equivalents) as a response variable and included site and habitat as predictors. Candidate models were ranked according to the coefficient of regression ($R^2$), with the model with the highest $R^2$ considered the most robust (Zar, 2013). For the most robust GLM, we tested the significance of the predictors using an ANOVA with a chi-square approximation to find the probabilities of predictor variables within the most suitable models, and for the most robust LM we used an ANOVA. Finally, we conducted post hoc, pairwise comparisons (Tukey test) to confirm where the differences occurred between significant predictors.

To describe the local abiotic environment for the sampled lowland sites, we generated buffers (radius = 10km) around each one of our four study sites. Because we wanted to achieve a full description of the abiotic environment, we extracted values for all the cells occurring within each buffer (mean = 355 cells/site, Table 1) from all 19 bioclimatic variables of WorldClim (version 1.4; www.worldclim.org) at a spatial resolution of 30 arc-s (Hijmans et al., 2005).
We compared the abiotic environment among sites using a principal component analysis (PCA). To contrast climatic dissimilarities between lowland study sites, we also generated a pairwise matrix of Euclidean distances between the centroids of climatic envelopes. All analyses were conducted in R v.3.5.1 (R Core Team, 2014).

3 | RESULTS

We screened a total of 348 adult amphibians from 47 species for Bd (346 frogs and two salamanders, Table 2). From this list, a total of 44 species are classified as least concern and three are categorized as threatened: Oophaga granulifera is classified as vulnerable (VU), Agalychnis lemur and Craugastor taurus are classified as critically endangered (CR) according the International Union for Conservation of Nature (IUCN) (Red List of Threatened Species, version 2017–1; http://www.iucnredlist.org/). Overall, 33 species (70.2% of sampled species) tested positive for Bd and total prevalence of Bd was 54.6%. We did not detect Bd on three of the amphibian families sampled, including Plethodontidae, the only family of Salamanders in the Neotropics; however, the sample size for these families was very small.

Prevalence of infection showed high heterogeneity among sites with values ranging from 0.0% in Rincon de Osa to 68.6% in Punta Banco (Table 3). This variation in Bd prevalence was best explained by the interaction effects model (Table 4), which showed significant effects of locality ($p < 0.01$), and that the variation of Bd prevalence by site depends on the habitat ($p < 0.001$; Figure 3a). Despite being close in proximity, amphibian assemblages from Sarapiquí showed significant higher prevalence of Bd than assemblages from Siquirres ($p < 0.01$, Figure 3a, Tables 3 and 5). There was a nonsignificant trend for Bd prevalence to be lower in Siquirres than Punta Banco ($p = 0.06$). We also found high prevalence of Bd across habitats (Table 3), but no significant differences between habitats in our model ($p = 0.20$).

Similarly, the differences in the infection intensity across study sites (Figure 3b, Table 3) were best explained by the interaction model ($R^2 = 0.19$, Table 4), which also showed significant effects of location ($F_{2,166} = 15.5$, $p < 0.001$) and the interaction between habitat and location ($F_{3,166} = 3.6$, $p < 0.01$). Levels of infection intensity were significantly lower in Sarapiquí (Figure 3b, Tables 3 and 5) when compared to Punta Banco ($p < 0.001$) and Siquirres ($p < 0.01$). Overall, the infection intensity ranged from 0.1 to 63,861 genome equivalents and four individuals had more than 10,000 zoospore genomic equivalents, a theoretical number that is considered a threshold that results in mass mortality and rapid population decline (Vredenburg et al., 2010). However, none of the sampled individuals including the

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### TABLE 1

| Bioclimatic variables | Punta Banco | Rincon de Osa | Sarapiquí | Siquirres | PC1 | PC2 |
|-----------------------|-------------|---------------|-----------|-----------|-----|-----|
| BIO1 = Annual Mean Temperature | 25.5 (0.7) | 25.6 (0.6) | 25.4 (0.7) | 24.4 (1.1) | 0.1 | -0.1 |
| BIO2 = Mean Diurnal Range | 10.1 (0.7) | 11.0 (0.2) | 9.0 (0.0) | 9.0 (0.0) | 0.0 | -0.4 |
| BIO3 = Isothermality | 75.4 (0.9) | 76.6 (0.7) | 77.3 (0.7) | 79.4 (0.7) | -0.1 | 0.4 |
| BIO4 = Temperature Seasonality | 77.9 (5.5) | 78.0 (1.8) | 73.3 (5.6) | 76.1 (2.5) | 0.0 | -0.6 |
| BIO5 = Max Temperature of Warmest Month | 32.8 (0.8) | 33.2 (0.7) | 31.6 (0.7) | 30.4 (1.1) | 0.1 | -0.5 |
| BIO6 = Min Temperature of Coldest Month | 19.2 (0.9) | 18.9 (0.9) | 19.8 (0.7) | 19.0 (1.2) | 0.1 | 0.1 |
| BIO7 = Temperature Annual Range | 13.8 (1.0) | 14.2 (0.4) | 12.0 (0.2) | 11.2 (0.4) | 0.0 | -0.6 |
| BIO8 = Mean Temperature of Wettest Quarter | 25.0 (0.7) | 25.1 (0.7) | 25.3 (0.9) | 24.2 (1.2) | 0.1 | -0.1 |
| BIO9 = Mean Temperature of Driest Quarter | 25.8 (0.6) | 25.8 (0.7) | 25.9 (0.7) | 25.1 (1.2) | 0.1 | -0.1 |
| BIO10 = Mean Temperature of Warmest Quarter | 26.6 (0.8) | 26.7 (0.8) | 26.4 (0.8) | 25.5 (1.1) | 0.1 | -0.2 |
| BIO11 = Mean Temperature of Coldest Quarter | 24.7 (0.8) | 24.8 (0.8) | 24.6 (0.6) | 23.6 (1.1) | 0.1 | -0.1 |
| BIO12 = Annual Precipitation | 3,112.0 (134.0) | 3,976.4 (430.3) | 4,085.4 (185.5) | 3,784.4 (245.8) | 128.1 | 31.4 |
| BIO13 = Precipitation of Wettest Month | 586.3 (47.2) | 712.7 (51.4) | 460.4 (19.1) | 440.1 (23.5) | 13.8 | -49.9 |
| BIO14 = Precipitation of Driest Month | 54.0 (14.8) | 60.7 (19.5) | 163.6 (13.4) | 182.1 (18.7) | 3.6 | 24.8 |
| BIO15 = Precipitation Seasonality | 64.5 (6.4) | 62.8 (4.9) | 50.0 (1.5) | 27.2 (2.1) | -0.7 | -7.4 |
| BIO16 = Precipitation of Wettest Quarter | 1,351.0 (87.7) | 1,719.3 (130.8) | 1,277.3 (56.9) | 1,173.9 (65.2) | 41.7 | -88.5 |
| BIO17 = Precipitation of Driest Quarter | 176.8 (49.1) | 237.4 (65.2) | 589.9 (40.9) | 625.1 (53.4) | 14.8 | 82.8 |
| BIO18 = Precipitation of Warmest Quarter | 528.5 (27.6) | 707.5 (82.4) | 724.5 (41.9) | 772.7 (72.4) | 21.7 | 19.6 |
| BIO19 = Precipitation of Coldest Quarter | 1,071.8 (132.0) | 1,348.7 (152.2) | 1,163.9 (71.9) | 1,089.1 (60.4) | 38.4 | -36.0 |

Notes. Temperature variables are measured in Celsius (environmental variables 1–11) and precipitation variables in mm (environmental variables 12–19). *Bioclimatic variables with higher contribution.
### Table 2: List of species and number of individuals tested for *Batrachochytrium dendrobatidis* in amphibian assemblages from four lowland sites in Costa Rica

| Species                  | Habitat  | N (Bd positive) | Prevalence % (95% CI) | Genomic equivalents (±SE) |
|--------------------------|----------|-----------------|------------------------|---------------------------|
|                          |          |                 |                        | Sarapiqui | Siquirres | Punta Banco |
| Agalychnis callidryas    | Pond     | 11 (5)          | 45.5 (16.7–76.6)       | x          | 249.2 ± 214.1 x |
| Agalychnis lemur         | Pond     | 5 (2)           | 40.0 (5.3–85.3)        | x          | 12.3 ± 4.9 x |
| Agalychnis spurrelli     | Pond     | 5 (1)           | 20.0 (5.0–71.6)        | x          | 10.3 ± 0.0 x |
| Anotheca spinosa         | Forest   | 1 (1)           | 100.0 (0.2–100.0)      | x          | 112.3 ± 0.0 x |
| Boana rufitela           | Pond     | 10 (8)          | 80.0 (44.4–97.5)       | 8.4 ± 3.9 | x          | x          |
| Bolitoglossa colonnaea   | Forest   | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Centrolenella ilex      | Stream   | 1 (1)           | 100.0 (0.2–10.0)       | x          | 57.4 ± 0.0 x |
| Cochranella granulosa    | Stream   | 1 (1)           | 100.0 (0.2–100.0)      | 3.9 ± 0.0  | x          | x          |
| Craugastor transfordi    | Forest   | 24 (19)         | 79.2 (57.8–92.9)       | 31.6 ± 13.8 | 74.9 ± 112.2 x |
| Craugastor crassidigitus| Forest   | 6 (2)           | 33.3 (4.3–77.7)        | 3.0 ± 0.0  | 18.5 ± 0.0 x |
| Craugastor fitzingeri    | Forest   | 44 (26)         | 59.1 (43.2–73.7)       | 148.8 ± 321.2 | 14.1 ± 5.6  | 65.4 ± 22.5 |
| Craugastor megacephalus  | Forest   | 2 (1)           | 50.0 (12.6–98.7)       | 0.6 ± 0.0  | x          | x          |
| Craugastor stejnegerianus| Forest   | 6 (2)           | 33.3 (4.3–77.7)        | x          | x          | 2.2 ± 0.9  |
| Craugastor taurus        | Stream   | 15 (12)         | 80.0 (51.9–95.7)       | x          | x          | 11,632.5 ± 6,285.2 |
| Cruziohyla calcarifer    | Forest   | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Dendrobates auratus      | Forest   | 7 (1)           | 14.3 (0.4–57.9)        | x          | 4.9 ± 0.0   | x          |
| Dendropsophus ebraccatus | Pond     | 22 (15)         | 68.2 (45.1–86.1)       | x          | 130.3 ± 59.1 | x |
| Dendropsophus phlebodes  | Pond     | 1 (1)           | 100.0 (0.2–10.0)       | x          | 15.9 ± 0.0  | x          |
| Diasporus diastema      | Forest   | 9 (4)           | 44.4 (13.7–78.8)       | x          | 1994.3 ± 1724.7 x |
| Diasporus vocator       | Forest   | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Duellmanohyla rufioculis| Stream   | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Engystomops pustulosus  | Pond     | 10 (0)          | 0.0 (0.0–30.8)         | x          | x          | x          |
| Hylinothyrachium fleischmanni | Stream | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Hylinothyrachium valerioi| Stream   | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Hyliscirtus palmeri     | Stream   | 1 (1)           | 100.0 (0.2–100.0)      | x          | 231.2 ± 0.0 x |
| Incilius melanochlorus   | Pond     | 8 (1)           | 12.5 (0.3–52.6)        | 3.3 ± 0.0  | x          | x          |
| Leptodactylus fragilis  | Pond     | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Leptodactylus insularum | Pond     | 3 (0)           | 0.0 (0.0–70.7)         | x          | x          | x          |
| Leptodactylus poecichilus| Pond     | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Leptodactylus savagei   | Pond     | 3 (0)           | 0.0 (0.0–70.7)         | x          | x          | x          |
| Lithobates vaillanti    | Pond     | 2 (0)           | 0.0 (0.0–84.2)         | x          | x          | x          |
| Lithobates warszewitschii| Stream  | 26 (14)         | 53.8 (33.4–73.3)       | 51.8 ± 39.1 | 1,391.1 ± 704.7 x |
| Oedipina gracilis       | Forest   | 1 (0)           | 0.0 (0.0–97.5)         | x          | x          | x          |
| Oophaga granulifera     | Forest   | 1 (1)           | 100.0 (0.2–100.0)      | x          | x          | 114.0 ± 0.0 |
| Oophaga pumilio         | Forest   | 23 (18)         | 78.3 (56.3–92.5)       | 625.2 ± 479.5 | x          | x          |
| Pristimantis cerasinus  | Forest   | 7 (4)           | 57.1 (18.4–90.1)       | 3.6 ± 0.5  | x          | x          |
| Pristimantis ridens     | Forest   | 6 (3)           | 50.0 (11.8–88.2)       | 3.0 ± 0.0  | 6.4 ± 3.2  | x          |
| Rhaebo haematiticus     | Stream   | 27 (17)         | 63.0 (42.4–80.6)       | 3.1 ± 0.8  | x          | x          |
| Rhinella horribilis     | Pond     | 4 (0)           | 0.0 (0.0–60.2)         | x          | x          | x          |
| Scinax boulengeri       | Pond     | 4 (1)           | 25.0 (63.1–80.6)       | 195.2 ± 0.0 | x          | x          |

(Continues)
four that were heavily infected showed any evident signs of disease. Remarkably, three of these heavily infected individuals belong to the Critically Endangered species *Craugastor taurus*.

In our PCA analysis of the 19 bioclimatic variables, we retained the first two axes (Table 1) because they accounted for 98% of the total variance of our data. A tridimensional representation of PCA axes 1 and 2 (PCA 3 included as reference) shows four separated clusters of points, each one representing a study site (Figure 4). As expected, we found the highest similarity in climatic conditions occurred among sites in each zone (Appendix A). We found that bioclimatic variables associated with precipitation (Annual Precipitation, Precipitation of Wettest Quarter, Precipitation of Driest Quarter) make a higher contribution in the variance of our climatic data than other variables (Table 1).

### 4 | DISCUSSION

We found Bd infections at three of the four lowland sites sampled and in 70.2% of the 47 sampled species for an overall Bd prevalence of 54.6% (Tables 2 and 3). Furthermore, we did not detect signs of disease in heavily infected individuals during the study and found low levels of infection in most of our samples. Similar community composition and population dynamics observed during our study and later visits (unpublished data) suggest that host-pathogen dynamics in surveyed lowlands are exhibiting enzootic dynamics, rather than epizootic dynamics (Brem & Lips, 2008; Briggs, Knapp, & Vredenburg, 2010; Perez et al., 2014; Woodhams et al., 2008). Our findings also suggest that the distribution of Bd in Costa Rica is wider than historically considered (Puschendorf et al., 2009) and that the population declines during the 1980s and 1990s may not have been restricted to highlands. Comparable results were found in lowlands of Panama where Bd has been detected in multiple lowland sites (Kilburn et al., 2010; Perez et al., 2014; Woodhams et al., 2008). We suggest that future studies should include replicated sampling across seasons and sites that are outside the optimal environmental conditions for Bd growth, especially since most of these optimal conditions have been estimated from lab studies. Additionally, under potential scenarios of climate change, sites that are currently considered unsuitable for Bd may experience future outbreaks of chytridiomycosis if environmental conditions become closer to ideal ranges for Bd growth (AlMutairi, Grossmann, & Small, 2019; Enquist, 2002). Furthermore, conducting more studies and replicated samplings in neglected sites or locations that are assumed to be pathogen-free may help to better describe spatial dynamics of both the host and pathogen. These proposed studies could reduce the effect of opportunistically collected data from montane ecosystems and help develop more effective conservation tools and actions for amphibians in a broader range of habitats (Garner

### TABLE 2 (Continued)

| Species             | Habitat  | N (Bd positive) | Prevalence % (95% CI) | Genomic equivalents (±SE) |
|---------------------|----------|-----------------|-----------------------|---------------------------|
|                     |          |                 |                       | Sarapiqui | Siquirres | Punta Banco |
| Scinax elaeochroa   | Pond     | 6 (3)           | 50.0 (11.8–88.2)      | x          | 2.3 ± 0.4 | x           |
| Smilisca phaeta     | Pond     | 5 (2)           | 40.0 (5.3–85.3)       | x          | 9.8 ± 2.3 | x           |
| Smilisca sordida    | Stream   | 1 (1)           | 100.0 (0.2–100.0)     | 430.4 ± 0.0 | x          | x           |
| Tlalocohyla loquax  | Pond     | 15 (11)         | 73.3 (44.9–92.2)      | x          | 1,566.8 ± 1,020.7 | x |
| Teratohyla spinosa  | Stream   | 4 (2)           | 50.0 (6.8–93.2)       | 4.8 ± 1.2 | x          | x           |
| Teratohyla pulverata| Stream   | 3 (1)           | 33.3 (84.0–90.6)      | x          | 39.8 ± 0.0 | x           |
| Total               |          | 348 (190)       | 54.6 (49.2–59.9)      |            |            |             |

Notes. For every species, the table shows the habitat where the species was captured, the sample size, the overall prevalence (95% CI), and the average (SE) of genomic equivalents of *Batrachochytrium dendrobatidis* zoospores quantified per study site estimated from Bd-positive samples.

*Endangered species according the International Union for Conservation of Nature (IUCN).* 

Prevalence value previously reported in Chaves et al. (2014).

### TABLE 3

| Prevalence (95% CI) and infection intensity (SE) of *Batrachochytrium dendrobatidis* in amphibian assemblages from four lowland sites and three lowland habitats of Costa Rica |
|---------------------------------------------------------------|
| **Predictors** | **n** | **Prevalence (95% CI)** | **Infection intensity (SE)** |
|----------------|-------|-------------------------|------------------------------|
| Site           |       |                         |                              |
| Rincon de Osa  | 25    | 0.0 (0.0–13.7)          | 0 (0.0)                      |
| Punta Banco    | 35    | 68.6 (50.7–83.2)        | 2.0 (0.2)                    |
| Sarapiqui      | 144   | 67.4 (51.1–75.5)        | 0.9 (0.1)                    |
| Siquirres      | 144   | 47.9 (39.5–56.4)        | 1.5 (0.1)                    |
| Habitat        |       |                         |                              |
| Forest         | 150   | 62.7 (54.4–70.4)        | 1.2 (0.1)                    |
| Pond           | 116   | 39.7 (30.7–49.2)        | 1.3 (0.1)                    |
| Stream         | 82    | 61.0 (49.6–71.6)        | 1.2 (0.2)                    |
et al., 2016; Grenyer et al., 2006; Scheele et al., 2014; Woodhams et al., 2011).

The three endangered species sampled (Craugastor taurus, Agalychnis lemur, and Oophaga granulifera) tested positive for Bd (Table 2). The populations of C. taurus and A. lemur that we surveyed also tested positive in past surveys (Briggs et al., 2010; Whitfield et al., 2017). The continuous occurrence of these endangered species and the lack of clinical signs of chytridiomycosis in Bd-infected individuals (Berger et al., 1998; Voyles et al., 2009) suggest these populations are capable of surviving with enzootic Bd dynamics (Whitfield et al., 2017). Remarkably, infection levels in several individuals of the robber frog (C. taurus) were above 10,000 Bd genomic equivalents, a theoretical threshold that has been linked to epizootic outbreaks, population die-offs, and local extinctions (Vredenburg et al., 2010). There are several explanations for these high infection loads without signs of population decline or disease. For example, it is possible that these populations can coexist with Bd because they carry cutaneous bacteria that release anti-Bd compounds, although none have been detected in individuals of the relict populations of the Golfito robber frog (Madison et al., 2017) or in a similar critically endangered species (C. ranoides) which also catastrophically declined in the 1980s (Puschendorf et al., 2009; Zumbado-Ulate, Bolaños, Willink, & Soley-Guardia, 2011). Additionally, antimicrobial peptides and immune defenses (innate and adaptive) may play a role in this host–pathogen coexistence (Rollins-Smith, 2017; Woodhams et al., 2016). Alternatively, persistence of these populations could be associated with behavioral adaptations that rapidly clear infection or to local dry conditions that constrain Bd growth allowing susceptible frogs to coexist with low levels of Bd infection (Chaves et al., 2014; Puschendorf et al., 2011). Further studies on these endangered

### Table 4: Candidacy generalized linear models (GLMs) and linear models (LMs) used to determine the best predictors of prevalence of Batrachochytrium dendrobatidis and infection intensity in amphibian assemblages from four lowland sites and three lowland reproductive habitats in four lowland sites of Costa Rica

| Model                      | AIC (GLMs) | $R^2$ (LMs) |
|----------------------------|------------|-------------|
| Site*habitat (interaction model) | 422.03     | 0.19        |
| Site + habitat (additive model) | 431.40     | 0.14        |
| Site                      | 432.90     | 0.13        |
| Habitat                   | 467.70     | 0.00        |

Note: The most robust models were selected according the highest values for the Akaike information criteria (AIC) for the generalized linear models (GLMs) and the coefficient of regression ($R^2$) for the linear models (LMs).

### Table 5: Matrix of pairwise comparisons showing p values obtained from a post hoc analysis (Tukey test) to explain prevalence and infection intensity of Batrachochytrium dendrobatidis in amphibian assemblages from four lowland sites of Costa Rica

|                | Punta Banco | Sarapiqui | Siquirres |
|----------------|-------------|-----------|-----------|
| Bd prevalence  | Punta Banco | Sarapiqui | Siquirres |
| Punta Banco    | p < 0.001   | p < 0.01  |
| Siquirres      | p < 0.0001  | p < 0.001 |

Note: The table does not show results for Rincon de Osa because Bd prevalence at that site was 0%.

### FIGURE 3

Prevalence and intensity of infection of Batrachochytrium dendrobatidis in amphibian assemblages from four surveyed lowland sites in Costa Rica. The line plots show (a) prevalence of B. dendrobatidis among surveyed lowland sites per habitat (with 95% binomial confidence intervals) and (b) average infection intensity (SE) of B. Dendrobatidis in amphibian assemblages among surveyed lowland sites per habitat. The figure does not show results for Rincon de Osa because Bd prevalence at that site was 0%. Similarly, the plots do not display results for the category pond at Punta Banco because we did not collect any individuals from ponds at that location.
lowland populations can lead to management plans that protect and stabilize these relict populations.

The absence of Bd in fourteen surveyed species could be an artifact of the small sample sizes (1–10 individuals, Table 2) because some of these species have tested positive in other studies in Costa Rica and nearby Panama (e.g., Engystomops pustulosus, Duellmanohyla rufioculis, Anotheca spinosa, Leptodactylus poecilocichilus) (Picco & Collins, 2007; Rodríguez-Brenes, Rodríguez, Ibáñez, & Ryan, 2016; Zumbado-Ulate et al., 2014). Low sample sizes were caused by low detectability during the survey period for some of the common species (e.g., Rhinella horribilis, Smilisca sordida, Lithobates vaillanti, Leptodactylus savagei) or due to the low year-round detectability for the more cryptic and rare species (e.g., fossorial and canopy dwellers like Oedipina gracilis, Bolitoglossa colonnea, Cruziophyla calcarifer). To increase species detectability and/or sample size, future studies in lowlands and neglected sites should conduct surveys restricting or focusing the sampling on threatened species (Thorpe et al., 2018; Whitfield et al., 2017), to describe host-pathogen population dynamics, or preferably survey multiple species across seasons to obtain more accurate estimates of prevalence and infection intensity for all species within the amphibian community (Brannelly et al., 2015; Kinney et al., 2011; Vredenburg et al., 2010).

We found common lowland species with high prevalence of Bd (e.g., Lithobates warszewitschii, Craugastor fitzingeri, Rhaebo haematiticus, Oophaga pumilio, Dendropsophus ebraccatus). The species L. warszewitschii, C. fitzingeri, and D. ebraccatus also inhabit the montane ecosystems where historical enigmatic declines occurred. These species and others not sampled here (e.g., Isthmohyla pseudopoma) or with a small sample size (e.g., Smilisca sordida) seem to be highly tolerant to Bd and may function as competent reservoirs (Oostfeld & Keesing, 2000; Reeder, Pessier, & Vredenburg, 2012; Scheele et al., 2017), amplifying Bd infection in the community (Searle, Biga, Spatafora, & Blaustein, 2011). Therefore, the high infection prevalence in these species that we found at lowland sites suggests that Bd is common and persists in these locations.

Our results showed that Bd was widespread across lowlands during the time of study, but Bd prevalence and intensity might exhibit seasonal dynamics. However, to detect a seasonality effect, multi-season studies collecting samples from a variety of amphibian assemblages must be conducted (Kinney et al., 2011; Phillott et al., 2013; Savage et al., 2011). Similar studies conducted in lowlands of Costa Rica also suggest seasonal dynamics. For example, remnant populations of the lowland robber frog C. ranoides in the tropical dry forest of Costa Rica exhibited infection prevalence values that varied from <1 to 60% across a dry season (December to May) (Whitfield et al., 2017; Zumbado-Ulate et al., 2014). Similarly, prevalence of Bd varied from <5% to around 35% in an amphibian assemblage in tropical lowland forest across 1-year period (Whitfield et al., 2012). Therefore, follow-up studies across lowlands in Costa Rica are needed to identify seasonal dynamics of Bd in Costa Rica, which may help design more suitable conservation strategies for lowlands endangered populations.

We did not find Bd in our samples from Rincon de Osa, and a similar study also reported a very low prevalence of Bd in the same study sites and nearby zones across the Osa Peninsula (Goldberg, Hawley, & Waits, 2009). Although our detected prevalence in Rincon de Osa was 0%, our binomial confidence interval (0%–95%) overlaps with the prevalence value presented in this study. Therefore, our result for Rincon de Osa might be an artifact of our low sample size (n = 24) which is not large enough to achieve 95% certainty of detecting 1 positive individual, based on the minimum disease prevalence of ≥5% in infected amphibian assemblages (Skerratt et al., 2008). Climatic conditions at Rincon de Osa might constrain the dispersal and growth of Bd allowing coexistence between susceptible frogs and Bd (i.e., environmental refuge from chytridiomycosis, Puschendorf et al., 2011). However, the extirpation of the Golfito robber frog in this area, where it was abundant before the 1980s and 1990s (Chaves et al., 2014), suggests this may not be the case. We also found the highest levels of Bd prevalence in the Caribbean sites which coincide with studies conducted in the nearby locations within the same geographic zone (Whitfield et al., 2017, 2013, 2012). Thus, even within lowland zones, there is large variation in Bd prevalence across zones and sites.

Our statistical models showed no differences among habitats in relation to prevalence and infection intensity. However, there was a trend for higher prevalence of Bd in forest assemblages than in aquatic assemblages (lotic and lentic, Table 3), which differs from other similar studies (Brem & Lips, 2008; Kriger & Hero, 2007a; Lips et al., 2003). Some of the sampled species (e.g., Craugastor fitzingeri, Oophaga pumilio, Rhaebo haematiticus, Rhinella horribilis) may forage or move through different habitats that do not match their dwelling
habitat, which may have affected our results. Previous studies have shown the highest infection prevalence and intensity in permanent streams, suggesting that continuous streamflow provides more suitable conditions for the spread of Bd than other habitats (Kriger & Hero, 2007a; Lips et al., 2003). Lentic environments are more exposed to sunlight, resulting in temperatures >30°C (Adams et al., Hero, 2007a; Lips et al., 2003). Lentic environments also sustain invertebrates that feed on zoospores reducing the proportion of infected individuals (e.g., Daphnia spp., Searle, Mendelson, Green, & Duffy, 2013). However, our findings suggest that the role of terrestrial lowland ecosystems in the dispersal of Bd might have been underestimated. (but see Whitfield et al., 2012; Whitfield et al., 2013). Therefore, multiseason studies contrasting Bd dynamics across habitats are needed to elucidate the role of microhabitats in sustaining Bd.

We found significant evidence that every site of study represents an independent local abiotic environment according to the 19 environmental predictors that we used in our analysis (Figure 4). This climatic independence was consistent with the heterogeneous prevalence of Bd, which suggests that every site exhibits a different host-pathogen dynamic in response to local environmental conditions. However, irregularity in elevation gradient across our study sites (Kriger & Hero, 2008), especially in the study site of Siquirres, where elevations varied from 400 to 600 m, could have influenced the differential prevalence we found across lowlands. We recommend controlling for elevational gradients (Kilburn et al., 2010) in follow-up studies. Seasonality and particularly differences in precipitation (Table 1) may also play an important role in differential Bd prevalence between the Caribbean and South Pacific zones. The south Pacific zone, where Punta Banco and Rincon de Osa occur, presents a dry season extending from December to April, which coincided with our sampling. Conversely, the Caribbean zone does not have a well-established dry season, and the rainy season starts in December, when we conducted our surveys (Herrera, 1985). Other studies conducted at larger scale have also shown seasonal and latitudinal variation of Bd prevalence and infection (Brannelly et al., 2015; Kinney et al., 2011; Kriger et al., 2007; Phillott et al., 2013). Future studies should evaluate the effect of elevational gradients on the amphibian host-Bd dynamics.

Our results suggest that researchers should expand their sampling across the entire distribution of focal species and communities instead of only focusing on sites of historical declines. An adequate seasonal description of the suitable abiotic environment of pathogens across the host amphibian home range may help identify disease-free sites for effective repatriation or to determine instances were more technical strategies are needed to secure maintenance of declined populations (e.g., antifungal treatments to clear infection, bioaugmentation with commensal bacteria, habitat manipulation, ex-situ conservation) (Garner et al., 2016; Scheele et al., 2014). Furthermore, conducting more seasonal sampling in lowlands will increase the record of presence-absence datasets on Bd and can be used to generate more robust species distribution models (SDMs) from nonopportunistically collected data (Puschendorf et al., 2013). SDMs can help identify hotspots for future outbreaks of Bd and can be used to predict potential locations for amphibian rediscoveries (García-Rodríguez et al., 2012; Puschendorf et al., 2009). Recent validation surveys have led to the discovery of relic peripheral populations that occur in potential environmental refuges from disease (Puschendorf et al., 2011; Raffel & Fox, 2018; Scheele, Hunter, Skerratt, Brannelly, & Driscoll, 2015), validating increased surveys outside the boundaries of core geographic distributions (Abarca, Chaves, García-Rodríguez, & Vargas, 2010; Chaves et al., 2014; González-Maya et al., 2013; Jiménez & Alvarado, 2017; Nishida, 2006). A comprehensive assessment of a pathogen's distribution, prevalence, and infection intensity can lead to more effective disease-management strategies based on specific locations, habitats, and species.

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CONFLICT OF INTEREST

The authors declare no conflict of interest exists.

AUTHOR CONTRIBUTIONS

HZ-U, AG-R, CS, and VV developed the ideas and designed methodology; HZ-U and AG-R conducted data collection and data analysis; HZ-U, AG-R, CS, and VV led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

The data associated with this publication (Data files title: Bd_lowlands_Costa_Rica) are deposited at Dryad data repository. Provisional https://doi.org/10.5061/dryad.8t267j0.

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APPENDIX A

Cluster analysis of four lowland sites in Costa Rica generated from a matrix of Euclidean distances between the centroids of climatic envelopes. The cluster shows higher similarities between Sarapiqui and Siquirres (Caribbean side) and Rincon de Osa-Punta Banco (Pacific side).

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