1   # Prevalence of the fungal pathogen *Batrachochytrium dendrobatidis* in

2   # amphibians of Costa Rica predated first-known epizootic

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Abstract

Emerging infectious diseases are a growing threat to biodiversity worldwide. Outbreaks of the infectious disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), have caused the decline and extinction of numerous amphibian species. In Costa Rica, a major decline event occurred in 1987, more than two decades before this pathogen was discovered. The loss of many species in Costa Rica is assumed to be due to *Bd*-epizootics, but there are few studies that provide data from amphibians in the time leading up to the proposed epizootics. In this study, we provide new data on *Bd* infection rates of amphibians collected throughout Costa Rica, in the decades prior to the epizootics. We used a quantitative PCR assay to test for *Bd* infection in 1016 specimens collected throughout Costa Rica. We found *Bd*-infected hosts collected as early as 1964, and a infection prevalence average per decade of just 4%. The infection prevalence remained relatively low and geographically constrained until the 1980s when epizootics are hypothesized to have occurred. After that time, infection prevalence increased three-fold and *Bd*-infected hosts we collected throughout the entire country. Our results, suggest that *Bd* may either have invaded Costa Rica earlier than previously known, and spread more slowly than previously reported, or that an endemic lineage of the pathogen may exists. To help visualize areas where future studies should take place, we provide a *Bd* habitat suitability model trained with local data. Studies that provide information on genetic lineages of *Bd* are needed to determine whether an endemic lineage of *Bd* or the Global Panzootic Lineage (identified from mass die off sites globally) was present in Costa Rica and responsible for the epizootics that caused amphibian communities to collapse.

Introduction

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47   Amphibians are experiencing a global extinction event [1,2]. Though many factors contribute to

48   population declines, the emergence of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*)

49   is one of the most important [3]. The disease chytridiomycosis, caused by the fungal pathogen

50   *Batrachochytrium dendrobatidis* (hereafter *Bd*), was first described in 1999 and has since been

51   found all over the world [3–5]. Interestingly, *Bd* is composed of many genetic lineages that vary

52   in virulence and affect host species differently. The panzootic disease is attributed to *Bd*-GPL, a

53   Global Panzootic Lineage of *Bd* associated with *Bd* epizootics and host population collapse [6].

54   Other lineages of *Bd* have been shown to be less virulent and have been identified in areas

55   lacking epizootics [7]. *Bd* infects the skin of the amphibian and causes hyperkeratosis, the

56   thickening of skin which disrupts the amphibian's osmotic balance; leading to death by cardiac

57   arrest in highly infected individuals [8,9].

58   The dynamics of *Bd* and its hosts, including pathogen invasion and the host-pathogen

59   interactions that follow, are still not fully understood. For example, in some areas (e.g. South

60   Korea, Brazil, and South Africa), *Bd* appears to be in an enzootic state with amphibian hosts

61   [10–12], while in others (eg. western North America [13], Central America and South America)

62   there are repeated examples of epizootics and die offs of hosts. In these areas, *Bd*-GPL is

63   associated with epizootics [14]. South Korea was recently proposed as a region of high *Bd*

64   genetic diversity, with one of the *Bd* lineages identified as exhibiting genetic hallmarks that may

65   be the source of the panzootic *Bd* (*Bd*-GPL) that emerged in the 20th century [15]. Many of the

66   reported declines attributed to *Bd*-GPL in the new world occurred decades before *Bd* was

67   described, thus, retrospective studies can help create a timeline for *Bd* emergence and spread.

68   Causes of amphibian declines in Costa Rica, where some of the earliest reported declines

69   of amphibians occurred, have been debated in the literature [16,17]. Some studies proposed *Bd*

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70 epizootics occurred when environmental factors weakened host immune systems making them

71 more susceptible to endemic *Bd* [18]. Other studies refute this and show that an invasive *Bd*

72 pathogen caused the epizootics [19,20]. Costa Rica had one of the earliest amphibian declines

73 (1980s and 1990s) that was later associated with *Bd* epizootics [21–23]. These declines mostly

74 affected stream-dwelling species at elevations between 1000 and 2500 meters and include sites

75 such as Monteverde, where the amphibian community collapsed a decade before *Bd* was

76 described [24]. At this site, around the year 1987, half of all amphibian species, along with the

77 Costa Rican golden toad (*Incilius periglenes*), disappeared [22].

78   Like many other areas experiencing *Bd* epizootics, anuran (frogs and toads) species in

79 Costa Rica experienced differential susceptibility to *Bd*. Whether this is due to different immune

80 responses by hosts or possibly exposure to different lineages of *Bd* is not known [25]. For

81 example, all nine frog species within the *Craugastor punctariolus* clade (robber frogs) [26,27]

82 declined across all their elevational range, from 0 to 2300 meters a.s.l. [28], and yet decades later

83 they appear to be slowly recovering from past *Bd* epizootics [25,29,30]. Similar cases of

84 catastrophic decline followed by apparent recovery have been observed in some highland

85 populations of harlequin frogs, tree frogs and ranid frogs [31,32]. Population fluctuations such as

86 these elicit questions regarding the role of *Bd* transmission, virulence, and lineage in this disease

87 system. Recent studies have shown that *Bd*-GPL is unlikely to be endemic to Costa Rica though

88 it is possible that other endemic *Bd* lineages occur in Costa Rica and throughout the Americas

89 [33].

90   Retrospective studies analyzing the presence of *Bd* in specimens preserved in natural

91 history collections have been useful to describe *Bd* invasions that may have led to amphibian

92 declines [20,34] as well as situations where *Bd* has been present for a century [10,35,36].

93   Museum collection data has also contributed to tracking and identifying declined species [37–

94   39]. The utilization and analysis of accurate collections and databases is crucial to understanding

95   the historical context of population declines and can result in more applicable conservation plans.

96   We conducted a retrospective survey using a *Bd* qPCR assay effective on museum specimens

97   [20] to describe the spatial and temporal patterns of *Bd* of anurans in Costa Rica from 1961-

98   2011. We used logistic regression analysis to examine possible environmental factors correlated

99   with *Bd* infection occurrences. Based on our data, we also constructed a habitat suitability model

100   for *Bd* in Costa Rica using a MaxEnt model in order to visualize *Bd* habitat suitability for the

101   region.

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Materials and Methods

Data collection

105   We sampled 1016 formalin-fixed, ethanol-preserved museum specimens including thirty-four

106   species of frogs from five taxonomic families. All specimens were collected in Costa Rica

107   between 1961 and 2011 and are housed in the Museum of Zoology (UC Berkeley) and at the

108   Universidad de Costa Rica (UCR). We focused our sampling efforts on anuran species that were

109   reported to have declined during the 1980s and 1990s [40]. Most of the *Craugastor* species have

110   not recovered from population declines and are still classified as critically endangered or extinct

111   according the IUCN [41]. However, we also chose species whose populations initially declined

112   and were subsequently observed to be recovering by around 2010 or later (*Agalychnis annae,*

113   *Agalychnis lemur, Lithobates vibicarius* and *Lithobates warszewitschii*). The data from our skin

114   swabs, including qPCR results from our survey can be freely accessed on the amphibian disease

115   portal (AmphibiaWeb.org) [42].

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Quantitative PCR Assay

118   We collected skin swabs from formalin-fixed frogs following a standardized protocol that

119   reduces chances of cross-contamination between specimens [20]. Each museum specimen was

120   removed from the holding jar using flame-sterilized forceps and thoroughly rinsed with 70%

121   ethanol to remove any contaminants from other animals stored in the same jar. Flame-sterilized

122   forceps were used to hold the specimen while swabbing 5 times each of the following locations

123   for a total of 25 strokes, using sterile synthetic cotton swabs; l) the ventral surface from mid

124   abdomen to cloaca, 2) each inner thigh, and 3) the bottom side of the webbing between each toe.

125   Swabs were kept at -4˚ Celsius until processing in the laboratory. Latex or nitrile gloves were

126   used at all times when handling tubes, jars, and specimens. Gloves were changed between

127   handeling every specimen. *Bd* was extracted from swabs using the Prepman Ultra and Real-Time

128   PCR protocol in the Vredenburg Lab at San Francisco State University [20,43,44]. Positive and

129   negative controls were run in triplicate on every 96-well PCR plate and standard curves were

130   constructed by using 100, 10, 1, and 0.1 *B. dendrobatidis* zoospore quantification standards.

131   Samples were run on an Applied Biosystems 7300 Real-Time PCR thermocycler. We calculated

132   the number of zoospores in terms of Zswab (i.e., estimated *Bd* zoospore genomic equivalents on

133   each swab) by multiplying qPCR results by 80 to account for sample dilution (40 μL Prepman ×

134   10 dilution/ 5 μL for reaction = 80). A *Bd*-positive sample was described as having a Zswab

135   score greater than zero.

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Statistical Analyses

138   We performed all statistical analyses using the software R (version 3.4.2). To characterize the

139   temporal and spatial occurrence of *Bd* in Costa Rica, we calculated 95% confidence intervals for

140   *Bd* prevalence for each decade sampled based on a binomial probability distribution. We also

141   performed a linear regression for *Bd* infection status as a response variable, assuming a binomial

142   distribution as individuals are either infected or non-infected. Lastly, we used MaxEnt to

143   estimate *Bd* habitat suitability using the significant variables from the linear regression model

144   [45].

145   For the linear regression, we used the elevation and 19 bioclim variables available on

146   WorldClim (http://www.worldclim.org) and reduced the number of variables by performing a

147   Pearson-correlation test to eliminate highly correlated factors (>0.9 or <-0.9). The following

148   variables were then used; annual mean temperature, mean diurnal temperature range, day-to-

149   night temperature oscillations relative to the annual oscillations (isothermality), temperature

150   seasonality, annual precipitation, precipitation of the wettest month, precipitation of the warmest

151   quarter, precipitation of the driest quarter, and precipitation of the coldest quarter. We then

152   performed a stepwise regression to choose the best-fit model based on the AIC [46,47].

Results

154   Our qPCR analysis of the 1016 museum specimens analyzed revealed sixty-eight *Bd*-positive

155   anuran samples and 948 *Bd*-negative anurans for an overall infection prevalence of 6.7%

156   (supplementary table 1). The earliest records were detected in four *Lithobates vibicarius*

157   specimens collected in 1964 from the central volcanic mountain range, on the hillsides of Poas

158   Volcano (fig 1a, supplementary table 2). UCR museum records of the species included in this

159   study begin in the 1960s, thus our retrospective survey begins with the oldest specimens

160   collected in 1961 (n= 2). When grouped by decades we find relatively low *Bd* prevalence in the

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161   1960s and 1970s (4.31%, n= 348 and 4.57%, n= 372, respectively), followed by an increase in

162   the 1980s to 11.70%, n= 171. By the late '80s and '90s, *Bd* was detected in museum specimens

163   collected throughout the entire country, whereas earlier positive samples were obtained only

164   from frogs collected in the central regions of the country. Thus, *Bd* became more common

165   throughout the country in the late 1980s and 90s (figure 1a, figure 2). Overall, the majority of the

166   *Bd* positive samples were found in mountains throughout Costa Rica at elevations ranging from

167   32–2550 meters a.s.l. We found that *Bd* prevalence between species ranged from 0.0% to 46.7%.

168   The species with the highest percentage of *Bd* positive samples came from species that are highly

169   dependent on water for reproduction or live in close proximity to water. For example, we found

170   46.7% *Bd* infection prevalence (n= 15) in the stream-breeding frog *Hyloscirtus palmeri*, and

171   45.9% prevalence (n= 37) in *Lithobates vibicarius,* a highland pond-breeding frog. The lowest

172   *Bd* prevalence occurred in the Dendrobatidae and Bufonidae families, in species that spend much

173   of their time on land rather than in water. However, we sampled only a small number of

174   Dendrobatidae specimens (n= 7), and no samples were *Bd*-positive, whereas in Bufonidae we

175   sampled 171 specimens and found that 0.6% were *Bd* positive (supplementary table 1). Overall,

176   the Ranidae family showed the highest percentage of positives (22.4%), followed by

177   Craugastoridae (7.4%). Most Craugastoridae samples were taken from direct developing

178   streamside-breeding species of the *Craugastor punctariolus* clade, which are critically

179   endangered across their entire distribution. *Craugastor andi* however, a species not categorized

180   within the *punctariolus* clade, is also found near streams [28]. In the Craugastoridae family*,* only

181   four *C. escoces* individuals tested positive out of sixty-three specimens. All *C. escoces* samples

182   were collected before the 1987 Costa Rican amphibian population decline epidemic [24]. The

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183   earliest *Bd*-positive *C. escoces* specimens were collected in 1975, and the last was collected in

184   1986.

185   Our power analysis showed that we had enough samples within each time period to have

186   a robust statistical test (p < 0.01 across all time periods sampled; table 1). In the model with the

187   best AIC (AIC = -2853.44), we found that infection status has a positive relationship with

188   elevation and annual mean temperature (p<0.001 and p<0.001; respectively). We also found that

189   *Bd* infection status has a negative relationship with mean diurnal temperature range (p<0.001).

190   Infection status was not shown to have a significant relationship with the following factors:

191   isothermality, precipitation of the warmest quarter, precipitation of the coldest quarter,

192   precipitation of the wettest month, annual precipitation, temperature seasonality, and

193   precipitation of the driest quarter (table 2). Areas predicted by the *Bd* habitat suitability model to

194   be suitable for *Bd* occurrence, includes mid-elevation ranges across central Costa Rica (fig 3).

195   The areas predicted to be unsuitable include the lowland regions and the coasts.

Discussion

197   Chytridiomycosis has severely impacted anuran biodiversity worldwide, with hundreds of

198   species affected [3,48]. Though our knowledge of the pathogen is incomplete, our understanding

199   continues to grow through studies of *Bd* pathogen and host dynamics. Our retrospective study

200   revealed that *Bd* was present in Costa Rica twenty-three years before declines were discovered at

201   Monteverde [19]. We found an increasing prevalence of *Bd*-infected hosts collected throughout

202   Costa Rica beginning in the 1970s (fig 1a), with prevalence escalating during and after the

203   known epizootic time period (1980s), which supports the pattern expected with an invasive

204   pathogen.

205   Previous studies describe the epizootic as a wave that progressed from north-west to

206   south-east Costa Rica and into Panama [20,49,50]. Here, we show a potentially contrasting

207   spatial pattern where *Bd* appears to be present at low prevalence across some of the region (fig

208   1a) before the purported wave passed through the country. This could be indicative of an

209   invasive pathogen that invades unsuccessfully for decades before epizootics develop, or it could

210   be that the earlier *Bd* infections were the result of a non-virulent lineage of *Bd*, such as has been

211   identified in other parts of the world [35,51,52]. Consistent with an invading pathogen, our

212   limited data show a pattern of spread across the entire time of the study. We found *Bd*-positive

213   specimens only in and around central Costa Rica in the earlier time period (1960s), but by the

214   1970s, we found *Bd*-positive individuals across a larger area, from north western areas to

215   southern areas of Costa Rica. Our results from the 1980s show the largest expansion of *Bd*, with

216   *Bd*-positive individuals found on the eastern coast of Costa Rica and near the Panama-Costa Rica

217   border to the south-east and all the way north close to Nicaragua. This pattern might reflect the

218   *Bd*-epizootics that are proposed from that time period. In the more recent decades (e.g. 1990s),

219   there were fewer specimens in museum collections that we could test. Thus, constructing a

220   robust statement regarding the spatial distribution of *Bd* in the more recent time period is not

221   possible given the available specimens. Our samples are not free from sampling biases, since

222   museum specimens were collected for reasons unrelated to our study.

223   In some areas (e.g. South Korea, Brazil, and South Africa), where both the Global

224   Panzootic Lineage (GPL) and an endemic strain of *Bd* occur sympatrically, direct competition

225   between pathogen strains and potential cross immunity of hosts may explain the lack of

226   epizootics [11,53]. Our study provides evidence that *Bd* was present in Costa Rica before the

227   1987 epizootic in Monteverde, but we acknowledge that there may have been previous

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228   undetected epizootics especially since the pathogen was yet described. The few specimens

229   collected in the 1970s showed relatively high levels of infection and, by the 1980s, both

230   prevalence and zoospore equivalents increased (fig 1a, 2). Our data do not refute the studies that

231   show that epizootics in Central America are associated with *Bd* invasion, but they do help

232   provide further data for interpretation. For example, identifying the *Bd* lineage of our earlier

233   positive *Bd* samples would be extremely helpful, since having multiple pathogens that are closely

234   related to each other in a population of hosts may help us understand why *Bd* has had such

235   variable effects on hosts, even in known-epizootic areas. Sub-lethal effects from fighting off the

236   infection of one pathogen can suppress host immunity against other stressors, causing a larger

237   effect [54–56]. However, populations can also benefit by being exposed to a lower virulence

238   pathogen before being exposed to a similar yet more virulent pathogen. Direct competition

239   between pathogens and/or cross immunity has been shown to assist the hosts in acquiring partial

240   or total immunity to one pathogen from a previous infection by another closely related pathogen

241   [57]. Additionally, climate change and the stress of an inconsistent environment may negatively

242   affect amphibians and result in suppressed immune systems, which could make amphibians more

243   vulnerable to chytridiomycosis [69–71]. Future studies involving *Bd* genotyping are required to

244   determine whether *Bd* found in Costa Rica are of a single lineage and whether or not the *Bd*

245   found in epizootics and enzootics are of the same lineage.

246   Consistent with other studies, our linear regression results found that *Bd* infection status

247   has a positive relationship with elevation and mean temperature [58,59], but contrary to other

248   studies, our best linear regression model (table 2), did not show a relationship between

249   precipitation and *Bd* occurrence [60–63]. This may be due to unintended sampling bias. For

250   example, frogs in the genus *Craugastor* made up a large proportion of available specimens (434

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251   of 1016) and yet most were negative. This genus of frogs are direct developers that do not have a

252   free-swimming aquatic tadpole phase of development, but instead hatch from terrestrial eggs as

253   fully metamorphosed froglets. This more terrestrial lifestyle may decrease exposure to the

254   aquatic pathogen *Bd*, although terrestrial life history alone is not associated with susceptibility to

255   infection [64,65].

256   Our data show higher *Bd* prevalence in mid to high elevation species, which is possibly

257   due to a more suitable climate for *Bd* [66–68]. The *Bd* habitat suitability model we produced

258   from our data alone, predicts suitable habitat similarly to previous studies [72,73] (fig 3), where

259   the model indicates high habitat suitability for *Bd* where epizootics occurred. For example, in

260   figure 3, we identify the locations with documented *Bd* epizootics occurred in Costa Rica in

261   1987, 1993, and 1994 (fig 3, blue circles 1-4). Our model also identifies high elevations along

262   the central mountain range as having the highest *Bd* suitability and should be prioritized for

263   further research and monitoring.

264   The zoospore equivalents (i.e. the Zswab, infection intensity or host infection load) and

265   prevalence of *Bd* observed in this study are typically consistent with epizootic or enzootic

266   dynamics [74]. Nonetheless, our results challenge the hypothesis that *Bd* invaded Costa Rica

267   immediately before epizootics began and suggests that more research is needed to understand

268   and document the role of past potentially failed invasions and/or potential endemic lineages in

269   describing current dynamics of *Bd* and amphibian hosts. Our results show that although *Bd* was

270   present in the 1960s, the significant increase in *Bd*-positive individuals did not begin in the

271   samples available until the 1980s (when the epizootics began). The data we provide in this study

272   are not well-suited to test the novel vs endemic pathogen hypotheses for *Bd* (fig 3) [17];

273   however, these samples could be used to test for *Bd* lineage in future studies that may shed light

274 on this question [51]. The steady increase in prevalence of *Bd* throughout all elevations in Costa

275 Rica after 1990 suggests that *Bd* has become more broadly established throughout the country

276 [25,75] than it was previously. The recent rediscovery of some remnant populations of frogs

277 once thought extinct provides new opportunities to assess the current impact of *Bd* in highly

278 susceptible species [25,29,30,32]. Consistent with previous studies, we propose that *Bd*

279 epizootics in amphibians began in the central range of Costa Rica, affecting stream-breeding and

280 pond-breeding species that inhabited this region (supplementary tables 1 and 2) such as

281 *Lithobates vibicarius, Isthmohyla angustilineata, I. tica, I. xanthosticta, I. rivularis,*

282 *Duellmanohyla uranochroa Craugastor fleischmanni, C. ranoides, C. escoces, C. sp. (C.*

283 *punctariolus* clade), *C. melanostictus, C. andi, Atelopus varius, A. senex* (Harlequin frogs), and

284 *Incilus holdrigei.*

285 In this study we discovered *Bd*-infected frogs in Costa Rica twenty-three years before

286 enigmatic amphibian declines occurred. These infected animals could represent failed pathogen

287 invasions (e.g. "pathogen fade out" theory [76,77]), slower than expected invasion dymaics

288 resulting in epizootics, or endemic lineages of *Bd* that may exhibit enzootic pathogen/host

289 dynamics. New studies that sequence and identify *Bd* lineages from our data could help create a

290 more complete understanding of the lineage and spread patterns of *Batrachochytrium*

291 *dendrobatidis* in Central America.

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509

510   **Figure 1a)** Amphibian museum specimens (1964-2011) that tested positive for *Bd* infection in

511   Costa Rica. Monteverde (red circle) experienced major population declines 1986-89. *Bd*-positive

512   amphibians were collected (n= 32) in the two previous decades in the northern and central

513   mountain range South of Monteverde, but no population studies are available in those areas.

514

515   **Figure 1b.** Amphibian museum specimens (1961-2011) that tested negative for *Bd* infection in

516   Costa Rica (n= 948).

517

518   **Fig 2**. **Emergence of *Bd* in Costa Rica from 1961-2011.** Infection prevalence (solid blue line)

519   and infection intensity (broken red line) patterns over time along with the maximum zoospore

520   equivalents value per time period (red diamonds). Gray bars represent number of samples

521   analyzed per time period. Dashed line at Log10 Zoospore equivalents = 4 marks the Vredenburg

522   10,000 value [13].

523

524   **Table 1**. ***Batrachochytrium* dendrobatidis (*Bd*) prevalence in museum specimens collected in**

525   **Costa Rica**. Pr (no *Bd*) is the probability of finding no *Bd*-positive samples in each time period if

526   *Bd* was present with an enzootic prevalence of 11.0% [35].

527

528   **Table 2**. **Linear regression output. Environmental factors and their relationship to *Bd***

529   **infection status**. (+) next to the variable name indicates a positive relationship between factor

530   and *Bd* presence. (-) indicates a negative relationship between factor and *Bd* presence. The model

531   with the lowest AIC value (1st model; AIC = -2853.44) was considered the best model.

532

533   **Figure 3. *Bd* habitat suitability model** Areas in Costa Rica predicted to have *Bd* suitability.

534   Increased intensity from white to green indicates increased suitability while blue dots are *Bd*-

535   epizootic localities. (1) Monteverde, where the 1987 decline occurred and (2-4) indicates
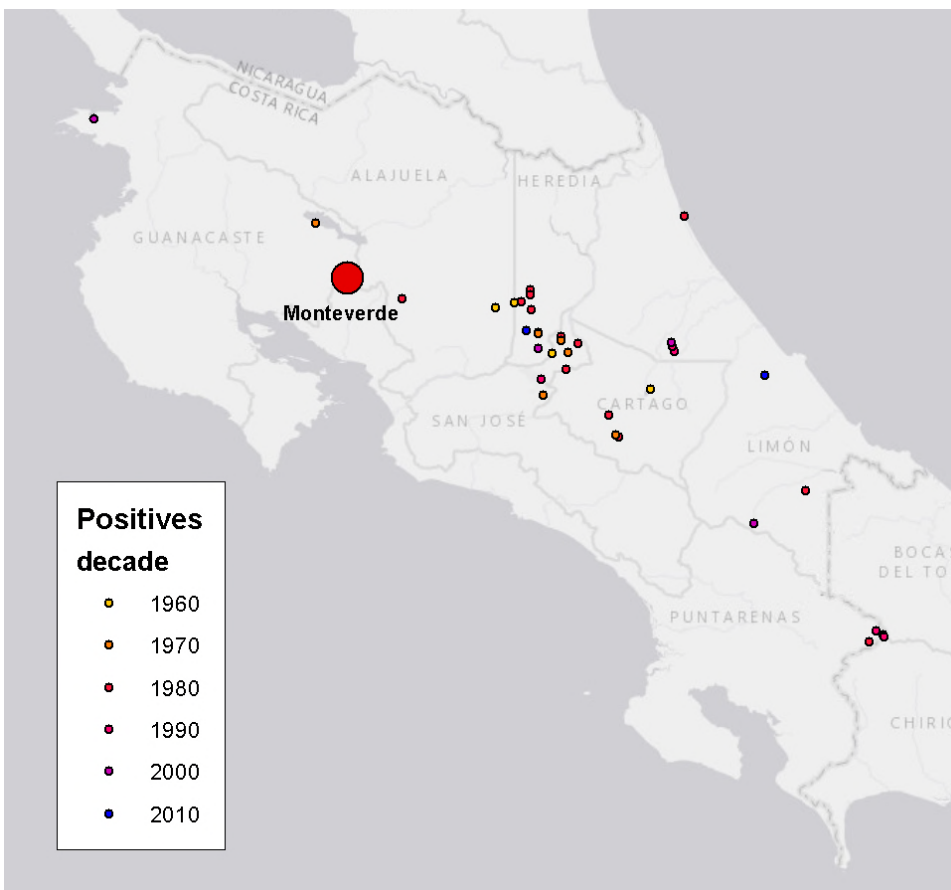
536   declines between 1993-1994.
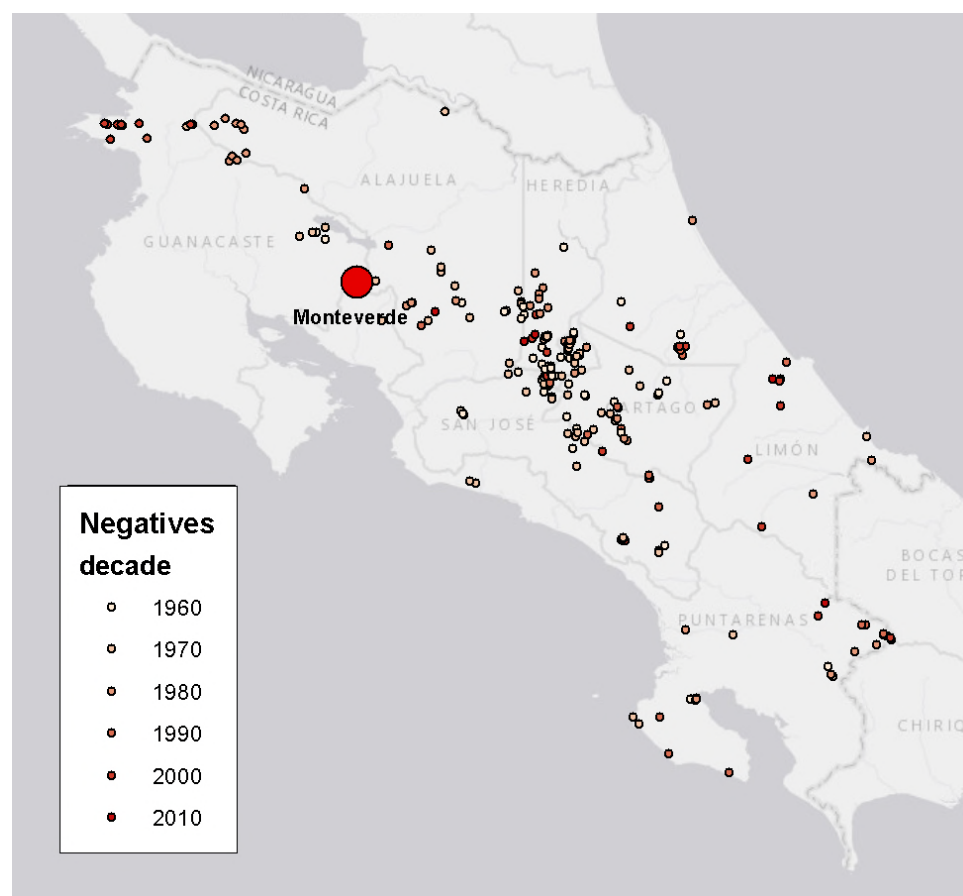
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539   Figure 1a.

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542   Figure 1b.

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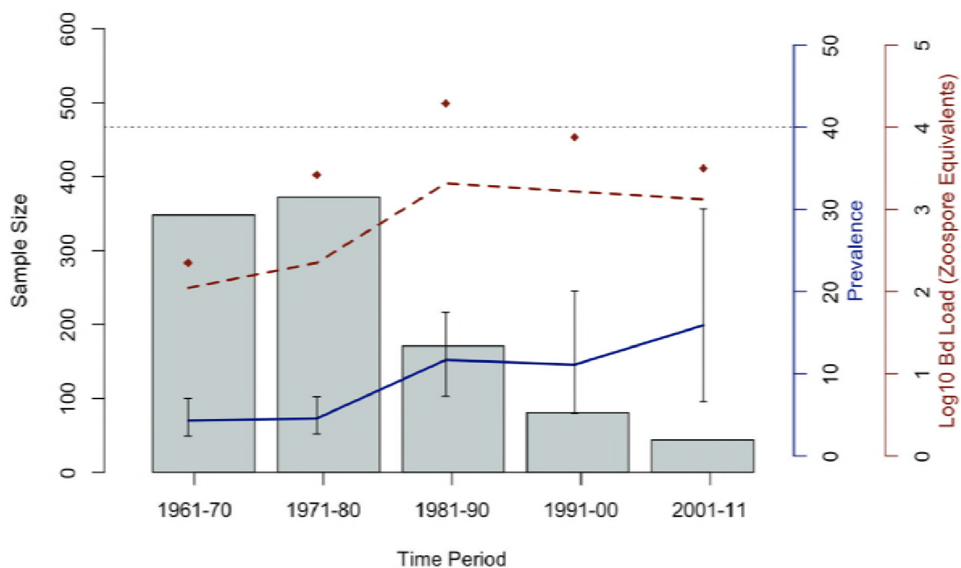544

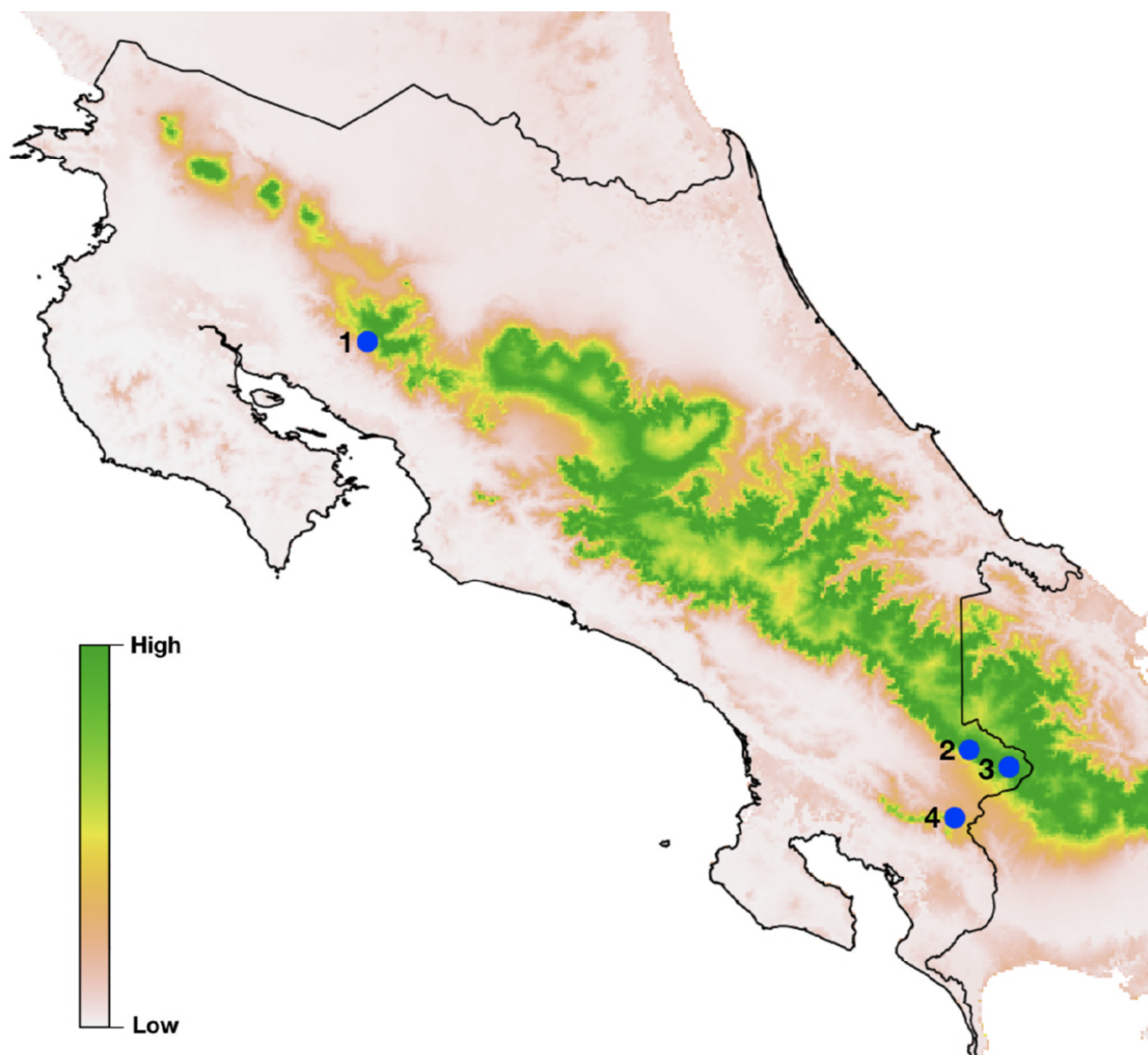545   Figure 2.

546

547   Figure 3

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549

550

551

552 **Supplementary Table 1.** *Bd* observed in in museum specimens from Costa Rica. The

553 table shows surveyed species, conservation status and proportion of samples with *Bd*

554 including 95% binomial confidence intervals.

Family	Genus species	N Red List	Total # positive)	% Positive (95% CI)
Bufonidae			**171 (1)**	**0.6 (0.0-3.2)**
	Atelopus chiriquiensis	CR	39 (0)	--
	Atelopus senex	CR	30 (0)	--
	Atelopus varius	CR	30 (0)	--
	Incilius fastidiosus	CR	19 (1)	5.3 (0.1-26.1)
	Incilius holdridgei	CR	41 (0)	--
	Incilius periglenes	EX	12 (0)	--
raugastoridae			**453 (33)**	**7.3 (5.1-10.1)**
	Craugastor andi	CR	20 (1)	5 (0.1-24.9)
	Craugastor angelicus	CR	57 (1)	1.8 (0.0-9.4)
	Craugastor catalinae	CR	1 (1)	100 (2.5-100)
	Craugastor escoces	EX**	63 (4)	5.4 (1.8-15.5)
	Craugastor fleischmanni	CR	92 (9)	9.8 (4.6-17.8)
	Craugastor melanostictus	LC	25 (5)	20 (6.8-40.7)
	Craugastor obesus	EN	5 (1)	20 (0.1-71.6)
	Craugastor ranoides	CR	98 (5)	5.1 (1.7-11.5)
	raugastor rhyacobatrachus	EN	6 (1)	16.7 (0.4-64.1)
	Craugastor taurus	CR	31 (0)	--
	Craugastor sp.	CR*	36 (4)	11.1 (3.1-26.1)
	ristimantis caryophyllaceus	NT	20 (1)	5 (0.1-24.9)
Dendrobatidae			**7 (0)**	**--**
	Silverstoneia nubicola	NT	7 (0)	--
Hylidae			**300 (15)**	**5.0 (2.8-8.1)**
	Agalychnis annae	EN	47 (2)	4.3 (0.5-14.5)
	Agalychnis lemur	CR	54 (0)	--
	Duellmanohyla rufioculis	LC	32 (0)	--
	Duellmanohyla uranochroa	EN	19 (0)	--
	Hyloscirtus colymba	CR	5 (0)	--
	Hyloscirtus palmeri	LC	15 (7)	6.7 (21.3-73-4)
	Isthmohyla angustilineata	CR	12 (1)	8.3 (2.1-38.5)
	Isthmohyla calypsa	CR	1 (1)	100 (2.5-100)
	Isthmohyla pictipes	EN	37 (2)	4 (0.66-18.19)
	Isthmohyla rivularis	CR	40 (2)	(0.61-16.92)
	Isthmohyla tica	CR	26 (0)	--
	Isthmohyla xanthosticta	DD	1 (0)	--
	Ptychohyla legleri	EN	11 (0)	--
Ranidae			**85 (19)**	**2.4 (14.1-32.7)**

	Lithobates vibicarius	CR	37 (17)	3.7 (32.4-65.2)
	Lithobates warszewitschii	LC	48 (2)	4.2 (0.5-14.3)
Total	**34**		**016 (68)**	**6.7 (5.2-8.4)**

555   EX= extinct, CR= critically endangered, EN= endangered, NT= near threatened, LC= least

556   concern, DD= data deficient, *= this population, **= rediscovered

557

558   **Supplementary Table 2.** Geographic and time data for all *Bd*-positive species surveyed in UCR

559   museum specimens from Costa Rica.

Species	# Positive	Year collected	Elevation (m)	Localities+
Incilius fastidiosus	1	1990	2400	Las Tablas
Craugastor andi	1	1986	1300	La Virgen
Craugastor angelicus	1	1967	1370	Cinchona
Craugastor catalinae	1	1990	1800	Las Tablas
Craugastor escoces	4	1975(3), 1986	1500, 2000 (3)	Chompipe (3), PNBC
Craugastor fleischmanni	9	1974(2), 1975(2), 1977, 1982, 1984, 1986, 2010	900, 1260, 1300, 1700, 1800, 2000, 2011, 2110 (2)	Chompipe (3), Desamparados, Chompipe, Hondura, San Ramón (2), Porrosati
Craugastor melanostictus	5	1968 (2), 1970 (2), 1986	1500, 2000 (4)	Chompipe (4), Varablanca
Craugastor obesus	1	1984	700	Nimaso
Craugastor ranoides	5	1968, 1985 (2), 1986, 2009	1 (2), 32, 600, 900	Tortuguero (2), Reserva, Murciélago, Turrialba
Craugastor rhyacobatrachus	1	1983	1260	Mellizas
Craugastor sp.	4	1971, 1984 (3)	1240, 1600, 1640	Tapantí
Pristimantis caryophyllaceus	1	2007	1500	Telire
Agalychnis annae	2	1992, 2004	1150, 1500	San Francisco, Concepción

Hyloscirtus palmeri	7	1984, 1997, 1999 (3), 2002, 2011	360, 400, 600, 775 (3), 1400	Guayacán (5), Veragua, Carrillo
Isthmohyla angustilineata	1	1984	1600	Tapantí
Isthmohyla calypsa	1	2002	2300	Las Tablas
Isthmohyla pictipes	2	1969, 1971	2000, 2110	Chompipe (2)
Isthmohyla rivularis	2	1984, 1990	1240, 1800	Tapantí, Las Tablas
Lithobates vibicarius	17	1964 (4), 1966, 1968 (5), 1971 (2), 1976, 1979, 1984, 1986, 1990	1400, 1500, 1700, 2000 (5), 2050, 2110 (3), 2400, 2550 (4)	Poás (4), Chompipe (8), San Jerónimo, Cascajal (2), Varablanca, Las Tablas
Lithobates warszewitschii	2	1970, 1984	650, 1400	Tilarán, La Mula

560