

## LETTER

# Tourism may threaten wildlife disease refugia

## 1 | INTRODUCTION

Few Neotropical amphibian communities inhabiting high elevation sites have been spared from chytridiomycosis (*Batrachochytrium dendrobatidis*, *Bd*), a fungal pathogen responsible for ongoing declines of amphibian populations worldwide (Scheele et al., 2019). Their persistence has been attributed to the supposed absence of virulent chytrid fungi (Scheele et al., 2019). Pervasive in the Andes (e.g., Lips et al., 2008), notably in the Venezuelan range (Sánchez et al., 2008), and present in the eastern Guiana Shield (Courtois et al., 2015), *Bd* has not yet been reported from the highlands of the western Guiana Shield, that is, the Pantepui biogeographical region (Mayr & Phelps, 1967). Pantepui spans three countries (Venezuela, Guyana, and Brazil) and encompasses one of the most fascinating and inaccessible landscapes on Earth (Figure 1). The region harbors a uniquely ancient and peculiar biology, and is often dubbed “the Lost World”, a direct reference to the eponymous novel by Sir Arthur Conan Doyle (McDiarmid & Donnelly, 2005). This stunning region is most celebrated for its iconic “tepui”, Precambrian, nutrient-poor, sandstone tabletop mountains renowned for their high floral and faunal endemism (Berry et al., 1995). Dozens of these “islands in the sky”, reaching up to 3000 m elevation and physiographically and ecologically strongly isolated from the more fertile surrounding environments, form one of the now rare “OCBILs” (Old, Climatically Buffered, Infertile Landscape) that persist on Earth (Hopper, 2009). Terrestrial vertebrate life on the highest tepui tops (>2500 m elevation) is usually depauperate and often consists of a few endemic early branching lineages (Kok et al., 2017). Because of their inaccessibility, many of these sky islands have been less visited than the moon (Kok et al., 2012). Although revered and believed to be the houses of the Gods, and therefore often taboo in the Amerindian culture (McPherson, 2008), the few tepui summits accessible on foot are nowadays subjected to the impacts of poorly regulated tourism development (e.g., Safont et al., 2014). Hitherto this pressure seems to have been confined to the very limited available infrastructure (i.e., trails and a sparse

number of basic camp sites), but incursions outside these areas are increasingly frequent, and illegal mining has been detected (Safont et al., 2014), even in areas reported as being mostly devoid of human presence (Kok, in litt. to Environmental Protection Agency Guyana, 5/09/2018).

The remoteness and isolation of tepui summits may protect against infections that underpin global amphibian declines. Indeed, tepui summits are so remote that they are usually little affected by human-induced impacts. A recent increase in recreational pressure on tepui summits and their slopes allows testing of anthropogenic pathogen incursion in supposedly immunologically naïve communities. We, therefore, initiated the first survey of the presence of *Bd* in this mostly pristine ecosystem and tested if tourist incursions predict the prevalence of *Bd* load in amphibians in the region.

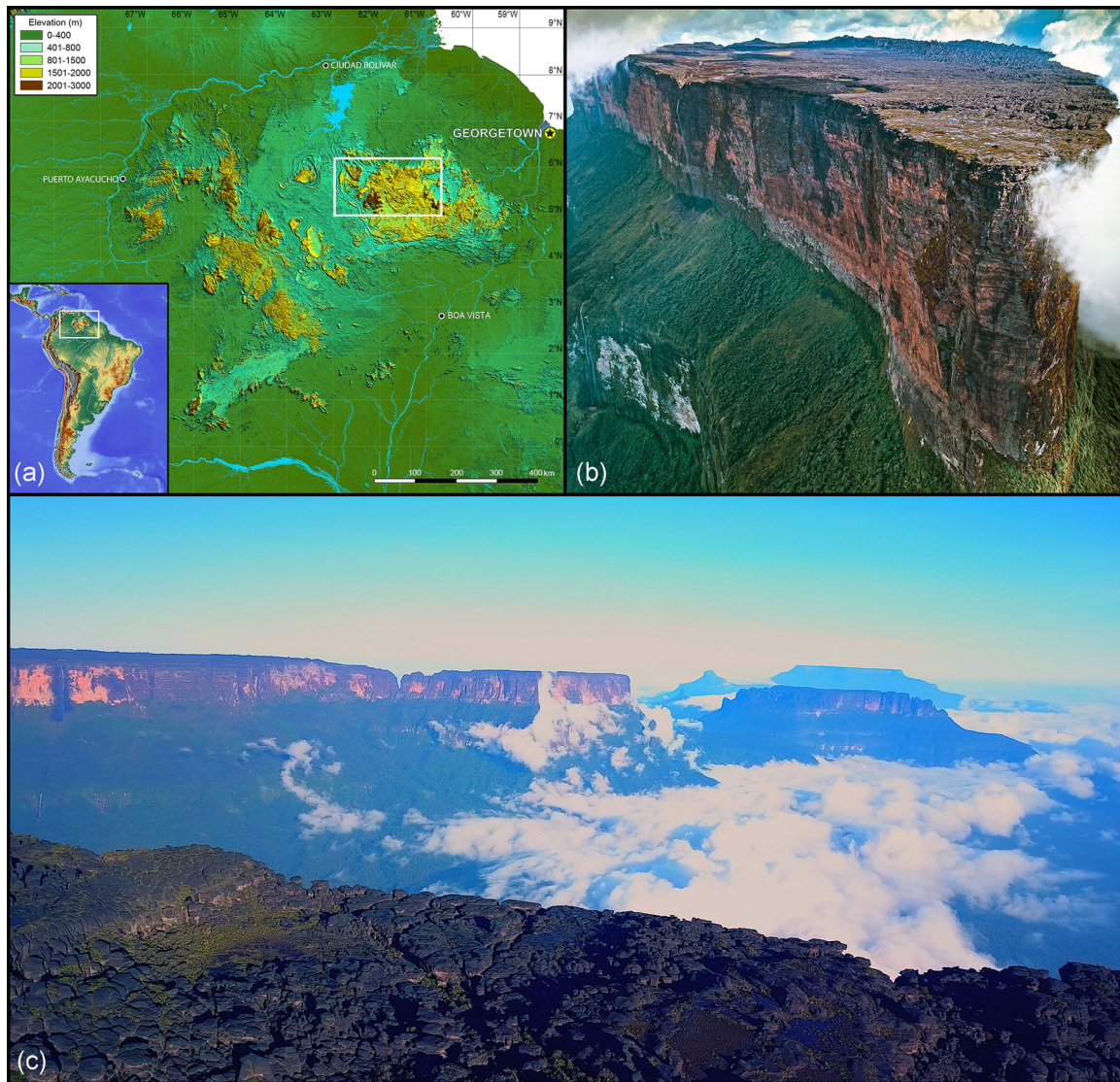
## 2 | METHODS

### 2.1 | Sample collection and processing

To determine the prevalence of *Bd* in amphibians, noninvasive skin swabs were collected using standard swabbing protocols (Hyatt et al., 2007; Van Rooij et al., 2011) over a period spanning 28 months (2016–2019). To test if tourist incursions correlated with *Bd* infections in the region, we explored/defined four types of areas in the Eastern Pantepui District, based on the extent of tourism development: (1) areas mostly devoid of regular tourist incursions, here represented by the slope and summit of Kukenántepui (Site 1), and the rainforest at the northern foot of the Aprada massif (Site 2); (2) areas subjected to moderate tourism impacts, here represented by the village of Kamarata and the southern slope and summit of Auyántepui (Site 3; 100–400 visitors per year, Arturo Berti, pers. obs.); (3) areas subjected to strong tourism impacts, here represented by the southern slope and summit of Roraimatepui (Site 4; up to 600 visitors in a single month, da Silva et al., 2011); and (4) areas subjected to recently increasing tourism impacts, here represented by the trail

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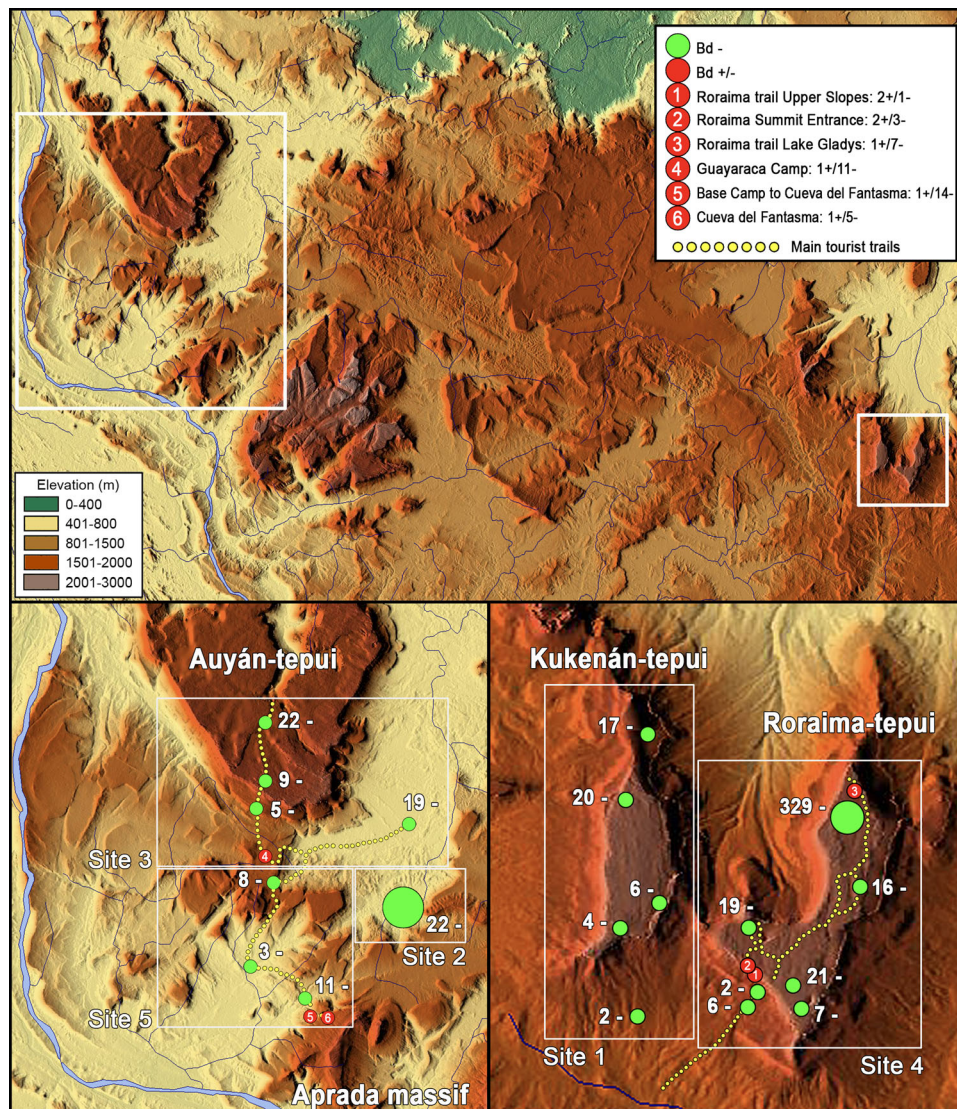
**FIGURE 1** Studied area. (a) Map of Pantepui and its location in South America (lower left inset). (b) Drone photograph of the “Prow” of the summit of Roraima-tepui, showing the summit’s physiographical and ecological isolation from the surrounding uplands and neighboring tepuis. (c) Drone photograph of tepuis in the Eastern Pantepui District, taken over the summit of Roraima-tepui, Kukenán-tepui is on the left in the background. Photos by P.J.R.K

leading to Cueva del Fantasma along the western slope of Aprada-tepui, a site only revealed in February 2001 (Barrio-Amorós, 2006) and offered as a tourism destination since 2014 (Site 5; Freddy Vergara, pers. comm.). We swabbed 576 individuals of 35 amphibian species (nine families, Table S1) from these five sampling sites (Figure 2). Field campaigns were conducted as follows: November 2016 (Sites 2, 3), December 2017 (Sites 2, 5), June 2017 (Site 1), July–August 2018, and February–March 2019 (Site 4). Whenever possible we sampled along trails and at main camp sites (93 swabs), but we also intensively sampled (483 swabs) in areas that are not accessible to the public, either because trails are absent (e.g., the western portion of “the Prow” on Roraima-tepui) or because the area is exclusively acces-

sible by helicopter (e.g., the isolated northernmost part of the summit of Kukenán-tepui). The criterion for assigning a sample to ON or OFF trail/camp site was as follows: ON, specimen either collected directly on a trail or maximum 0.5 m from it, or within a camp site area; OFF, specimen collected more than 50 m away from a trail/camp site.

DNA was extracted from the swabs using 100  $\mu$ l of PrepMan Ultra DNA extraction buffer. The extracted DNA samples were diluted 1/10 with HPLC water to reduce PCR inhibition (Hyatt et al., 2007). The samples were then stored at  $-20^{\circ}\text{C}$  until processing. All the samples were prepared blind to the analyst to eliminate any possible interpretative bias. Samples were processed using the *B. dendrobatidis* real-time PCR procedure as





**FIGURE 2** Maps of the studied area in the Eastern Pantepui District, depicting the five main sites, main tourist trails, sampling localities and presence of *Bd* (see upper right inset and main text for details)

described by Boyle et al. (2004) on a CFX96 real-time system (Bio-Rad Laboratories, Hercules, CA). The real-time PCR results (genomic equivalents [GE] of *B. dendrobatidis* zoospores) were corrected for the applied dilution factor.

## 2.2 | Statistical analyses

We performed a Fisher's exact test to evaluate the null hypothesis that the probability of infection is independent of the effect of tourist infrastructure. The test was performed on a  $2 \times 2$  contingency table in which the rows represented *Bd* positive and *Bd* negative and the columns were ON and OFF tourist infrastructure. The result was considered significant at  $p < 0.05$ .

The occurrence of *Bd* as a function of elevation, proximity to a trail or campsite, and the interaction of these two variables was modeled in a Bayesian framework using a phylogenetically corrected Bernoulli Generalized Linear Mixed Model (GLMM). To correct for potential taxonomic differences in susceptibility to infection, genetic distances among species were estimated based on 16S rRNA that excluded ambiguous regions (see Section 2.3). A NEXUS file generated from these data was used to conduct phylogenetic regression with the *phyr* package (Li et al., 2020). In this package, we implemented the model while accounting for phylogenetic relationships using a phylogenetic tree, which was pruned and then converted to a covariance matrix assuming Brownian motion evolution. The model was fitted with collection site elevation (m) and proximity to a trail/camp site as fixed effects; elevation was a

continuous variable, and proximity a factor with two levels (ON/OFF trail/camp site). Sample location was included as a random term in the model and default penalized complexity priors were placed on model parameters.

All analyses were performed using the R Statistical Language Environment.

### 2.3 | DNA extraction, PCR, sequencing, and sequence alignment

To correct for potential taxonomic differences in susceptibility to infection (see Section 2.2), we estimated genetic distances among species by using: (1) newly generated sequences of individuals from our study sites (66% of the sequences, often specimens we swabbed), (2) sequences from our study sites that were already available on GenBank (14%), and (3) sequences for the same taxa but from different localities (20%). Taxa included in our analysis and GenBank accession numbers are in Table S3. Novel sequences have been catalogued in GenBank under the accession numbers ON468483-ON468505. Genomic DNA was isolated from liver or muscle samples (preserved in 99% ethanol in the field) using the Qiagen DNeasy<sup>®</sup> Tissue Kit following manufacturer's instructions. A fragment of the mitochondrial 16S rRNA gene was amplified using polymerase chain reaction (for primers and PCR conditions, see Van Bocxlaer et al., 2010). PCR amplifications were confirmed on a 1% agarose gel and negative controls were run on all amplifications to exclude contamination. PCR products were sent to BaseClear BV (Leiden, The Netherlands) for subsequent purification and sequencing (along both strands using the same primers used for PCR). Chromatograms were assembled and edited in Codon-Code Aligner 7.1.2 (Codon Code Cooperation, Dedham, USA). Consensus sequences were aligned in Mesquite 3.61 (Maddison & Maddison, 2018) using MAFFT 7 (Katoh & Standley, 2013) with the G-INS-i algorithm and default parameters.

### 2.4 | Decontamination protocols

Footwear, tent floors, and other equipment with potential to spread disease were thoroughly cleaned, dried, and disinfected with either 70% ethanol or bleach prior to the beginning of each field work campaign, except when they were brand new. High efficacy of both chemicals against *Bd* has been reported (Van Rooij et al., 2017).

## 3 | RESULTS

We detected *Bd* at three of the five selected sites, albeit with low prevalence (1.39%,  $N = 8$ , samples tested positive) and

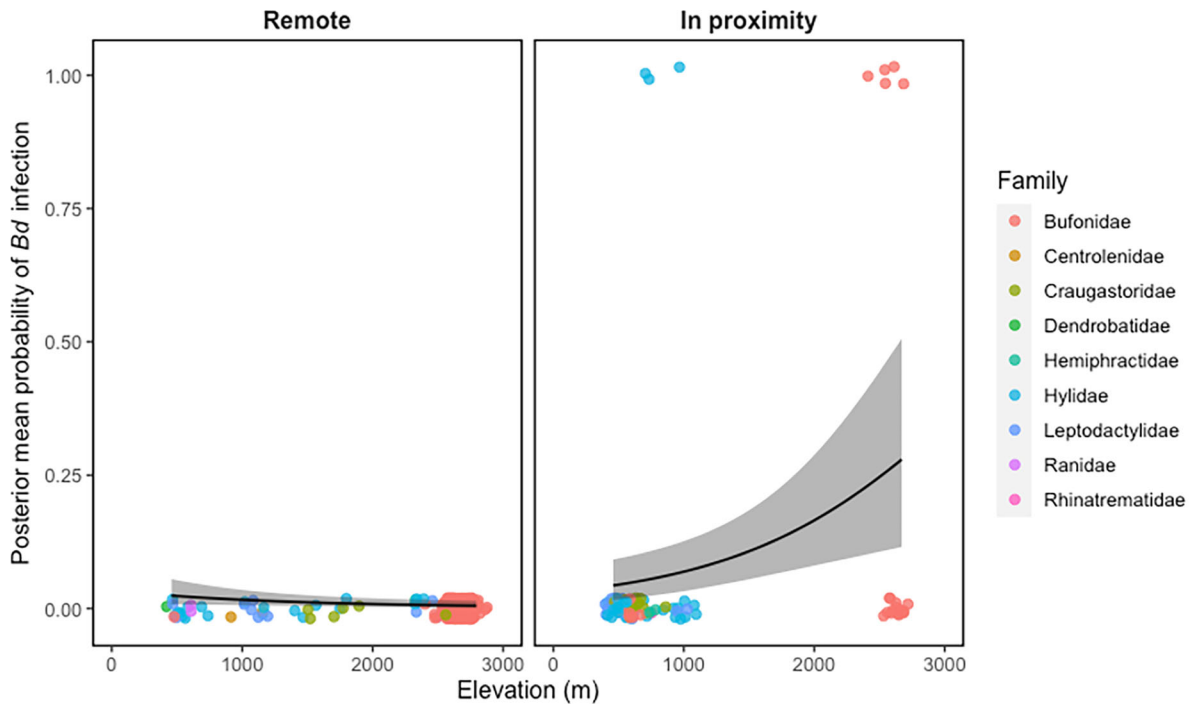
without observed mortality or obvious clinical infection. However, our results indicated that the probability of *Bd* detection is a function of proximity to the nearest infrastructure used by tourists visiting tepui summits and their slopes and elevation (100% of samples tested positive are from trails/camp sites;  $p < 0.001$ , Fisher's exact test; see Table S1). Likewise, the results of the phylogenetically corrected Bernoulli GLMM showed a statistically important interaction of elevation and proximity to a trail/camp site (Table S2), with a greater probability of infection associated with proximity to a trail/camp site that increased with site elevation (Figure 3). Most positive samples (5/8) were from trails on the upper slope and summit of Roraima-tepui (Figures 4 and 5), the site that is also the most impacted by tourism (see above, Rull et al., 2016; Safont et al., 2014). Lower genomic equivalents (GE) were detected on tepui summits and in the lowlands, and highest GE on tepui slopes (Table S1). Conversely, despite intensive sampling (84% of all swabs), *Bd* was not detected in any of the sites that are largely devoid of tourist incursions. Positive samples from the upper slope and summit of Roraima-tepui were exclusively found during the wet season in 2018; no positive samples were found during the dry season in 2019 (Table S1).

Positive samples were found in four species only (out of 35 species sampled, 11.4%; Figure 5): the Pantepui endemic hylids *Scinax exiguus* (five individuals swabbed, one positive sample), *Boana angelica* (five individuals swabbed, one positive sample), and *Boana tepuiana* (nine individuals swabbed, one positive sample), all sampled on tepui slopes, and the Pantepui endemic bufonid *Oreophrynella quelchii* (414 individuals swabbed, five positive samples) from the upper slope and summit of Roraima-tepui. The latter is listed as Vulnerable (VU) by the IUCN Red List of Threatened Species (IUCN, 2021) due to an alarming climatic threat and highly restricted range, and of special concern since *Oreophrynella* is closely related to *Atelopus* (sister genus; Kok et al., 2018), a taxon that has experienced catastrophic species loss linked to *Bd* infection in recent history (La Marca et al., 2005).

## 4 | DISCUSSION

We failed to detect *Bd* outside trails and camp sites, and this unexpected result suggests that nonhuman dispersal mechanisms might not play a significant role in the range expansion of *Bd* in Pantepui. Further sampling is needed to determine the cause of the apparent absence of *Bd* from areas devoid of tourism, especially on tepui top (e.g., inability to successfully colonize following arrival). To the best of our knowledge, this is the first time that prevalence of *Bd* has been unambiguously correlated with human recreation intensity.

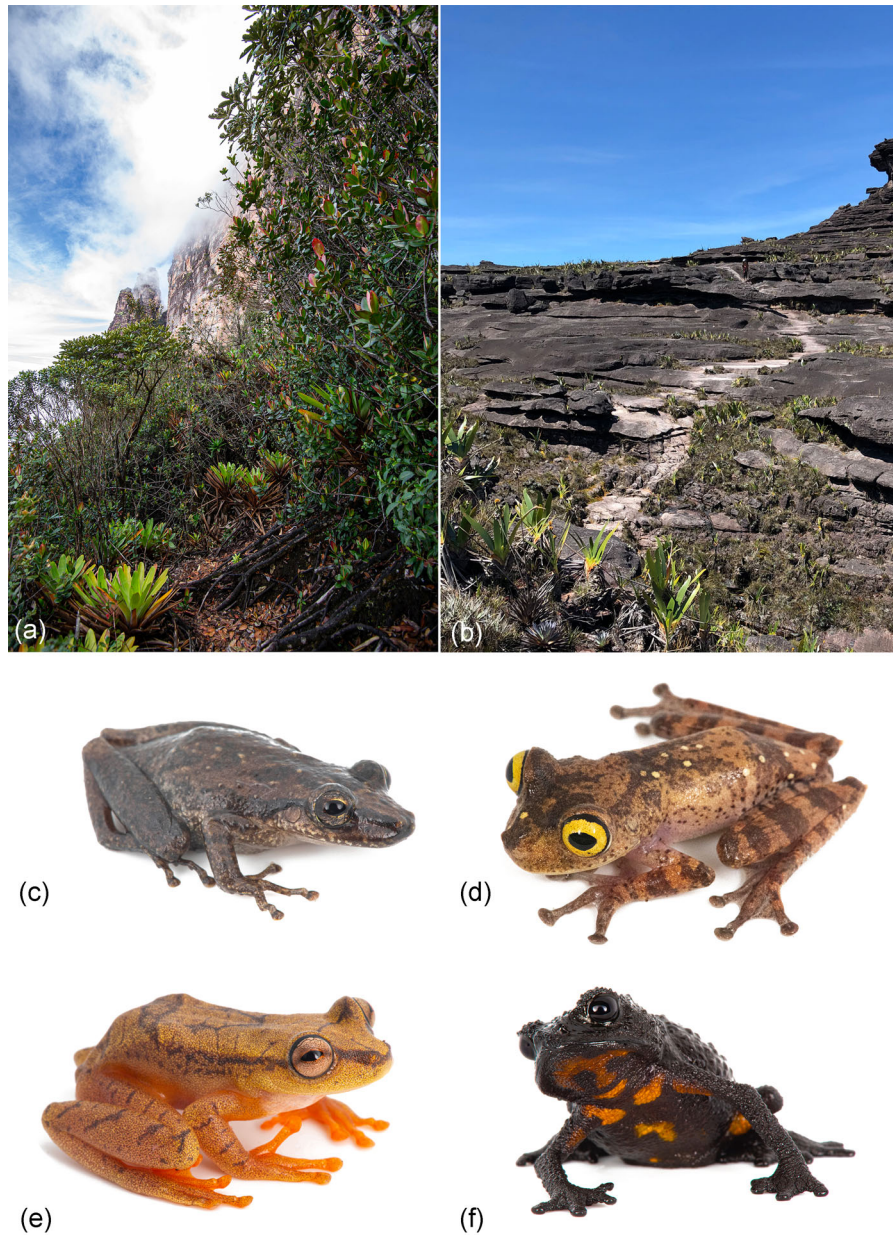




**FIGURE 3** Probability of infection by *Bd* as a function of elevation (m) at sampling sites in proximity or remote from camp sites/trails. Each point represents a sampled individual ( $N = 576$ ), different colors represent families. Black lines are posterior mean probabilities of infection modeled using a Bernoulli mixed-effects model with phylogenetic correction. Shaded gray areas are 95% Bayesian credible intervals



**FIGURE 4** Pantepui localities found to be affected by *Bd*. (a) Cueva del Fantasma, a highly picturesque locality on the western slope of Aprada-tepui, subjected to increasing tourism impact since 2014 (see text for details). (b) Guayaraca camp, on the southern slope of Auyán-tepui, a popular camp site on the way to the summit of Auyán-tepui. (c) Summit entrance of Roraima-tepui, an area accessed by thousands of international tourists visiting the summit of Roraima every year. Photos by P.J.R.K



**FIGURE 5** Touristic trails and *Bd* positive endemic species. (a) Trail leading to the summit of Auyán-tepui. (b) Trail on the summit of Roraima-tepui. (c) *Scinax exiguus*. (d) *Boana angelica*. (e) *Boana tepuiana*. (f) *Oreophrynella quelchii*. Note: Species not at scale. Photos by P.J.R.K

Based on the full data set, the low prevalence of *Bd* seems to indicate a modest risk of infection, though one could argue that our data are too limited and/or that tourist incursions have not generated a strong pattern of infection. It must be highlighted that 84% of our swabs were from pristine areas, which are not, or only weakly, affected by human incursions. Once the focus is set on the samples from trails and camp sites only (i.e., sites regularly affected by tourism), prevalence reaches ca. 9% (8/93), which is substantial. Courtois et al. (2015) reported a prevalence of 6% in French Guiana (eastern Guiana Shield) where human disturbance is widespread across the studied sites, while *Bd*

prevalence in the Venezuelan Andes has been estimated to be as high as 49.8% (Li et al., 2021 based on Sánchez et al., 2008). Since infection load needs time to build up, the overall low prevalence in Pantepui may also reflect a recent introduction.

A criticism could be made concerning possible resistance to infection in some taxa, potentially giving a misleading picture of where infections are the most abundant. We agree that this hypothesis cannot be ruled out. *Bd* resistance is difficult to assess, especially for areas where sampling size is inadequate. However, when the focus is set on a single species with high sample size (*Oreophrynella*



*quelchii*), the pattern is compelling. Among the 414 *O. quelchii* individuals swabbed, 395 individuals were “OFF trail” with 0 positive cases (0%), while 19 individuals were “ON trail” with 5 positive cases (26%).

Our study highlights circumstantial evidence for the potential role of fomites as vehicles of *Bd* introduction, bringing new data to an old debate. It should be noted that in most parts of the studied areas, trails are primarily designed for outdoor recreation. In addition, these trails are generally poorly differentiated from the surrounding environment (Figure 5). Therefore, although it may be tempting to limit their role as environments conducive to retaining *Bd* infections and/or merely as potential dispersal corridors for nonhuman vectors, thus refuting the role of fomites, such an assumption is likely reductive. In any case, this would not invalidate our hypothesis for tourist incursions predicting wildlife disease introduction (i.e., no tourism, no trail).

Our results indicate that conditions for *Bd* are more favorable on tepui slopes, where the GE are the highest (Table S1, Figure 3), putting some endemic species/populations at higher risk. Tepui summit taxa live in highly isolated, resource-limited, and strongly competitive ecosystems (Kok et al., 2019), which might increase their vulnerability to introduced disease. Although the present data suggest that the atypical conditions on tepui summits (i.e., high ultraviolet/solar radiation coupled with highly acidic soils and severe seasonal droughts) might have a negative impact on the development of the pathogen, the close phylogenetic relationship of *Oreophrynella* and *Atelopus* (Kok et al., 2018), one of the anuran taxa the most affected by *Bd* (La Marca et al., 2005; Lampo et al., 2017), is quite alarming and warrants follow up. All tepui summit *Oreophrynella* species have very restricted geographical ranges, and their thermal and elevational specialization are likely suitable for a clinical course of infection. Indeed, a small geographic range and a narrow elevational range increase the probability of severe declines (Scheele et al., 2019). Likewise, high elevation species have been reported to have a lower probability of recovery after epidemic (Scheele et al., 2019).

## 5 | CONCLUSION

Our findings are concerning as they imply that even limited human infrastructure that only allows foot-traffic represents a significant risk factor for disease introduction in isolated ecosystems. The principle of precaution should be applied, and we advocate for urgent control of anthropogenic pressure in Pantepui (and elsewhere), along with

implementation of awareness campaigns and disinfection protocols at major tourist entry points (i.e., Amerindian communities such as Paraitepui de Roraima and Canaima in Venezuela).

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## AUTHOR CONTRIBUTIONS

Conceptualization: PJRK, AL, FP. Methodology: PJRK, AL, FP. Investigation: PJRK, AB, AL. Visualization: PJRK, CS. Funding acquisition: PJRK. Project administration: PJRK. Supervision: PJRK. Writing—original draft: PJRK. Writing—review & editing: PJRK, FP, AM, AL, CS.

Philippe J. R. Kok<sup>1,2</sup>  
 Alexandra Laking<sup>3</sup>  
 Carl Smith<sup>1,4</sup>  
 Arturo Berti<sup>5</sup>  
 An Martel<sup>3</sup>  
 Frank Pasmans<sup>3</sup>

<sup>1</sup>Department of Ecology and Vertebrate Zoology, University of Łódź, Łódź, Poland

<sup>2</sup>Department of Life Sciences, The Natural History Museum, London, UK

<sup>3</sup>Department of Pathology, Bacteriology and Poultry Diseases, Ghent University, Merelbeke, Belgium

<sup>4</sup>Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

<sup>5</sup>Community of Kamarata, Canaima National Park, Bolívar State, Venezuela

## Correspondence

Philippe J. R. Kok, Department of Ecology and Vertebrate Zoology, University of Łódź, 12/16 Banacha Str., Łódź 90–237, Poland.

Email: [philippe.kok@biol.uni.lodz.pl](mailto:philippe.kok@biol.uni.lodz.pl); [philippe.kok@nhm.ac.uk](mailto:philippe.kok@nhm.ac.uk); [pjrkok@gmail.com](mailto:pjrkok@gmail.com)

## KEYWORDS

*Batrachochytrium dendrobatidis*, *Boana angelica*, *Boana tepuiana*, chytridiomycosis, human impact, *Oreophrynella quelchii*, Pantepui, prevalence, *Scinax exiguus*, tepui

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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