Official journal website:
amphibian-reptile-conservation.org

Amphibian & Reptile Conservation
14(2) [General Section]: 157–164 (e243).

Disease reservoirs threaten the recently rediscovered Podocarpus Stubfoot Toad (*Atelopus podocarpus*)

[1,2,3,4]Phillip Jervis, [5]Berglind Karlsdóttir, [6]Robert Jehle, [3,7]Diego Almeida-Reinoso, [3,8]Freddy Almeida-Reinoso, [3]Santiago Ron, [2]Matthew C. Fisher, and [3,8,]*Andrés Merino-Viteri

[1]Institute of Zoology, Zoological Society of London, Regent's Park, London, NW1 4RY, UNITED KINGDOM [2]MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, UNITED KINGDOM [3]Museo de Zoología (QCAZ), Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre, 1076 y Roca, Quito, ECUADOR [4]Department of Chemistry, University College London, London, UNITED KINGDOM [5]Durrell Wildlife Conservation Trust, Les Augrès Manor, La Profonde Rue, Trinity, Jersey JE3 5BP, Channel Islands, UNITED KINGDOM [6]School of Science, Engineering and Environment, University of Salford, UNITED KINGDOM [7]SARgrillo: Ex-situ Management Program of Endangered Amphibians and Insect Breeding Program, Quito, ECUADOR [8]Iniciativa de Conservación "Balsa de los Sapos", Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, ECUADOR

***Abstract.*—The Andes have experienced an unprecedented wave of amphibian declines and extinctions that are linked to a combination of habitat reduction and the spread of the fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*). In the present study, a range of high-altitude habitats in Southern Ecuador were surveyed for the presence of *Bd*. With a particular focus on Yacuri National Park, infection data are presented from across the resident amphibian community. This community contains a once putatively extinct species which was rediscovered in 2016, the Podocarpus Stubfoot Toad (*Atelopus podocarpus*). Across species, local *Bd* prevalence was 73% in tadpoles (*n* = 41 individuals from three species) and 14% in adults (*n* = 43 individuals from 14 species). Strikingly, 93% (14/15) of tested tadpoles of the recently described local endemic, *Gastrotheca yacuri*, were infected with a high pathogen load, suggesting that this species likely acts as a reservoir of infection in Yacuri. These findings show that the threat of disease for *A. podocarpus* still exists, and that this species requires urgent action to ensure its survival.**

Keywords. Amphibian, Anura, chytrid, conservation, Ecuador, emerging infectious disease, *Gastrotheca*

***Resumen.*—Los Andes han experimentado una ola sin precedentes de declinaciones y extinciones de anfibios que están vinculadas a una combinación de factores como la reducción de hábitat y la dispersión del hongo patógeno *Batrachochytrium dendrobatidis (Bd*). En el presente estudio, muestreamos la presencia de *Bd en un rango de hábitats de altura en el sur de Ecuador. Nos enfocamos, particularmente, en el Parque Nacional Yacuri, de donde presentamos datos de infección* a través de la comunidad residente de anfibios. Esta comunidad incluye una especie anteriormente considerada como extinta la cual fue redescubierta en 2016, la Rana Arlequín o Jambato de Podocarpus (*Atelopus podocarpus*). La prevalencia local de *Bd* fue 73% en renacuajos (*n* = 41 individuos de tres especies) and 14% en adultos (*n* = 43 individuos de 14 especies). Sorprendentemente, el 93% (14/15) de los renacuajos examinados de la especie endémica, recientemente descrita, *Gastrotheca yacuri*, estuvieron infectados con una alta carga del patógeno, sugiriendo que esta especie, probablemente, actúa como un reservorio de infección en Yacuri. Nuestros hallazgos muestran que la amenaza de la enfermedad para *A. podocarpus* aún existe, y que esta especie requiere acción urgente para asegurar su supervivenciae.**

Palabras clave. Anfibios, Anura, quitridio, conservación, Ecuador, enfermedad infecciosa emergente, *Gastrotheca*

Citation: Jervis P, Karlsdóttir B, Jehle R, Almeida-Reinoso D, Almeida-Reinoso F, Ron S, Fisher MC, Merino-Viteri A. 2020. Disease reservoirs threaten the recently rediscovered Podocarpus Stubfoot Toad (*Atelopus podocarpus*). *Amphibian & Reptile Conservation* 14(2) [General Section]: 157–164 (e243).

Accepted: 5 June 2020; **Published:** 7 July 2020

Introduction

Amphibians have experienced marked population declines and extinctions across all continents where they occur, and represent a particularly prominent example of the current biodiversity crisis (Hoffmann et al. 2010; Wake and Vredenburg 2008). A main driver for these declines is chytridiomycosis, an infectious disease caused by the fungal pathogen, *Batrachochytrium dendrobatidis* (hereafter *Bd*), which may act synergistically with other

Correspondence. **armerino@puce.edu.ec*

Fig. 1. The Podocarpus Stubfoot Toad (*Atelopus podocarpus*) was rediscovered along a single stream in Yacuri National Park (Ecuador) in 2016, after having been presumed extinct in the years following the last previous sighting in 1994. *Photo by Phil Jervis*.

threats such as habitat destruction and climate change (e.g., Hof et al. 2011). *Bd* has affected hundreds of amphibian species globally, and is regarded as the most devastating vertebrate disease ever recorded (Scheele et al. 2019).

The invasion and rapid spread of *Bd* (global panzootic lineage, *Bd*GPL; Farrer et al. 2011) throughout the Neotropics during the 1980s led to severe population declines in susceptible amphibian species (Pounds et al. 2006; Lips et al. 2006). Amphibian communities at high-elevation sites in the Neotropics, such as the Ecuadorian Andes, occur in the global ecoregions which have been most severely affected by chytridiomycosis (Catenazzi et al. 2011; O'Hanlon et al. 2018). Severe declines have led to range contractions and extinctions of many species, particularly those confined to breeding in high-elevation streams and lakes (Merino et al. 2005; Ron et al. 2003). However, despite the scale of these declines, the epidemiology of chytridiomycosis in the Ecuadorian Andes is not yet well understood. *Bd* is thought to occur throughout much of the country, and it is generally assumed to currently display an enzootic persistence characterized by low prevalence following the epizootic phase nearly 30 years ago (Catenazzi et al. 2011). Enzootic conditions are, amongst other factors, facilitated by infected reservoir host species which tolerate *Bd* and maintain infection within local amphibian communities after the functional extinction of more susceptible populations (Brannelly et al. 2018; Haydon et al. 2002).

With 64 species currently listed as either Critically Endangered or Extinct on the IUCN Red Lists, the genus *Atelopus* has been disproportionately affected by *Bd* (La Marca et al. 2005; IUCN 2020). In Ecuador, the Podocarpus Stubfoot Toad, *Atelopus podocarpus,* was described from museum specimens after the last known individual died shortly after capture in 1994 (Loetters et al. 2011). In line with many other Ecuadorian *Atelopus* species, anecdotal evidence indicates that before the onset of catastrophic declines, *A. podocarpus* was relatively common within its range (Ron et al. 2003). In 2016, three individuals of *A. podocarpus* were rediscovered along a single stream in Yacuri National Park by a field team from the Museum of Zoology (QCAZ), of the Pontificia Universidad Católica del Ecuador (e.g., Fig. 1). However, searches in the surrounding habitat were unsuccessful in finding additional individuals or new populations. The specific aims of the present study are to: (1) survey a range of high-altitude sites in southern Ecuador for *Bd* presence and prevalence in both tadpoles and adults of all encountered species; (2) revisit the last known site of *A. podocarpus* in Yacuri National Park, to survey for additional individuals of this species and to establish the *Bd* infection status of the local amphibian community; and (3) identify potential *Bd* reservoir species which maintain local infection.

Methods

Fieldwork was conducted at six sites during 13–21 June 2018, which is in the dry season. Locations were selected at a range of elevations encompassing habitats of previous *Atelopus* occurrences, covering an elevation range of 1,014–3,423 m from eastern foothill forest (Zamora) and eastern montane forest (San Francisco and Loja) to Paramo/Subparamo (Urdaneta, Madrigal del Podocarpus Reserve and Yacuri National Park, see Fig. 2). The ecology of *A. podocarpus* is unknown. However,

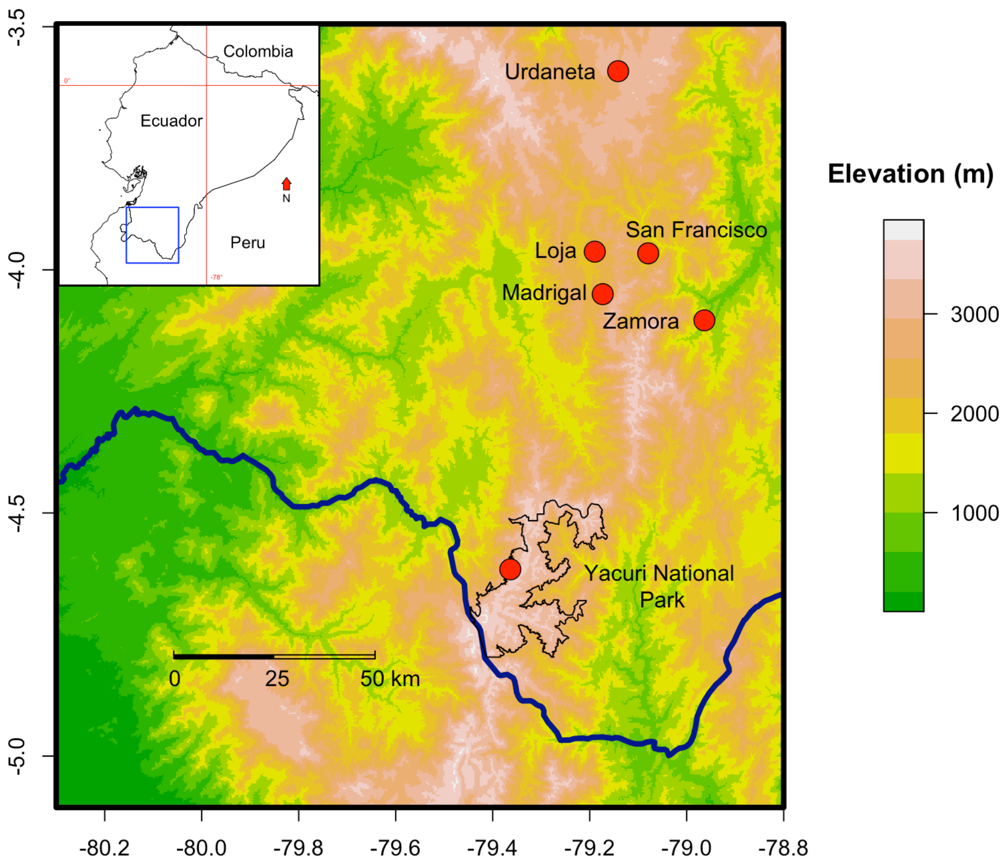


Fig. 2. Map of the study area showing the six field sites.

the closely related *A. ignescens* has been observed breeding in December–January (Peters 1973), so it is likely that sampling occurred outside the breeding season of *A. podocarpus*.

All individuals in this study were captured by hand or with a small fishing net, swabbed with a sterile cotton swab (Medical Wire 100), and immediately released at the site of capture. Tadpoles had their mouthparts swabbed (only the mouthparts of tadpoles can be infected by *Bd,* Hyatt et al. 2007). Post-metamorphic individuals were swabbed by taking five strokes at the center of the underside, on each flank, the inside of the legs, and the bottom of each of the rear feet. Animals contaminated with soil were washed before swabbing to remove debris, using water purified through mechanical, active carbon, and UV filtration. Swabs were stored in an icebox in the field when possible, until they were returned to the lab and refrigerated below 4 °C until analysis. The coordinates and elevation of each sampled animal was georeferenced using a Garmin GPS. All equipment used in environmental sampling or for handling animals was sterilized in 5% chlorhexidine solution between sites to prevent contamination of potentially disease-naïve sites. Each individual was contained in a new plastic bag and handled with a fresh pair of nitrile gloves to prevent cross-infection. Gloves and bags were disposed of following return from the field.

DNA extractions of swabs were performed using Prep-Man extraction kits (Hyatt et al. 2007), followed by a qPCR-based standard protocol for the quantification of *Bd* prevalence and infection burden (Boyle et al. 2004). Standard curves were generated using 0.1, 1, 10, and 100 *Bd* zoospore standards of BdGPL isolate IA042. To reduce PCR inhibition, samples were diluted 1:10 and the infection burden was multiplied by 10. Infection burden was defined as the number of zoospore genomic equivalents (GE) per swab following Clare et al. (2016). The sample was considered *Bd* positive if both replicates amplified above 0.1 GE.

Differences of infection intensities (GE) between sites were tested using ANOVA with a post-hoc pair-wise Tukey HSD test using R version 3.6.2 (R Development Core Team 2013).

Results

A total of 41 pre-metamorphic and 43 post-metamorphic individuals representing 18 species from seven sites were swabbed (Table 1). A total of 36 *Bd* positive animals were recorded (30 tadpoles of *Gastrotheca* sp. and six adults from four species in five genera), equating to a total infection rate of 43% (73% for tadpoles and 14% for adults). *Bd* was detected at five of the six sampling sites.

A single male *A. podocarpus* was found along the same stream as the previous expedition at Yacuri National Park. However, further searches along 3.5 km of the

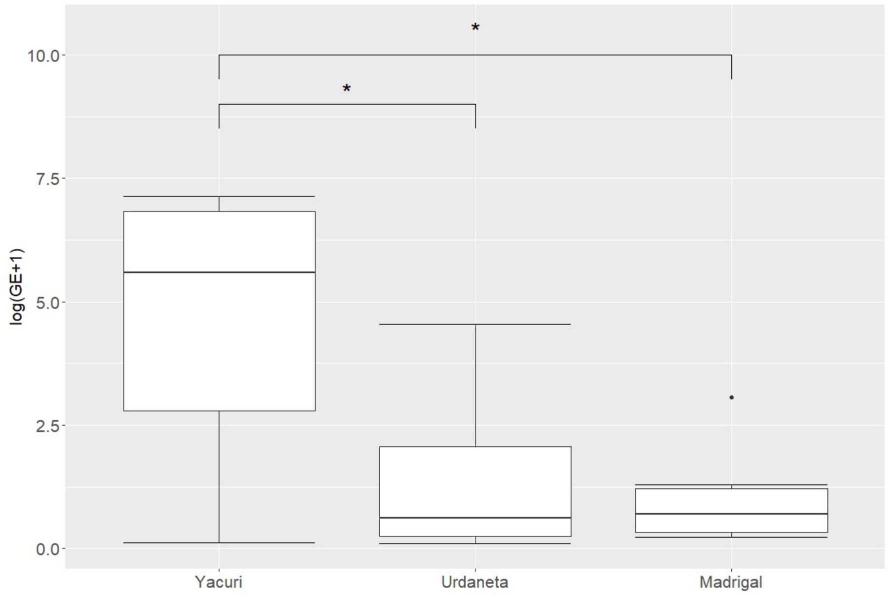


Fig. 3. Comparison of infection burdens in *Gastrotheca* tadpoles between three of the six sites. The asterisks denote significant differences. The boxplot was produced in ggplot 2 (Ginestet 2011).

streams surrounding the lake system were unsuccessful at locating any more individuals. Tadpoles of *G. yacuri*, a recently described, locally endemic species (Carvajal-Endara et al. 2019), were characterized by particularly high *Bd* prevalence (93%, $n = 15$) and infection loads. For *Gastrotheca* species in general, Yacuri had a significantly higher infection burden ($P < 0.05$) than Madrigal and Urdaneta, the other two sites for which tadpoles were analyzed (Fig. 3).

Discussion

This survey found that *Bd* is widespread in Southern Ecuador, but unevenly distributed between species and sites. The lower prevalence of *Bd* amongst adult amphibians would correspond well with an enzootic disease system (Catenazzi et al. 2017), although the sample size here does not allow unambiguous discrimination between sampling biases related to taxon and life stage. Moreover, an enzootic state does not necessarily equate to stable populations, and further declines are also possible when populations have been affected by *Bd* over significant periods of time (Longo and Burrowes 2010). *Gastrotheca* sp. tadpoles were particularly highly infected by *Bd* (Table 1), suggesting that this genus acts as a reservoir for local disease presence. *Gastrotheca* is a widely-distributed and locally common genus throughout the Andes which can often

Table 1. *Bd* prevalence and mean infection burden by species at each site. The *Atelopus podocarpus* swab was lost in transit from the field site. GE indicates the number of zoospore genomic equivalents as a measure of infection intensity.

Species	*n*	Life stage	Site	*Bd* positives	Prevalence	GE (mean)
Boana fasciata	8	Adult	Zamora	1	13% (1–53%)	3.17
Dendropsophus rhodopeplus	6	Adult	Zamora	2	33% (4–78%)	2.52
Dendropsophus sarayacuensis	2	Adult	Zamora	2	100% (16–100%)	1.87
Gastrotheca elicioi	9	Adult	Loja	0	0 (0–34%)	0
Gastrotheca pseustes	10	Tadpole	Urdaneta	6	60% (26–88%)	17.71
Gastrotheca aff. *pseustes*	16	Tadpole	Madrigal	10	63% (35–85%)	3.08
Gastrotheca yacuri	15	Tadpole	Yacuri	14	93% (68–100%)	465.00
Pristimantis atratus	1	Adult	San Francisco	0	0 (0–97.5%)	0
Pristimantis cf. *cajamarcensis*	1	Adult	Madrigal	0	0 (0–97.5%)	0
Pristimantis (*Huicundomantis*) sp.	1	Adult	Madrigal	0	0 (0–97.5%)	0
Pristimantis lymani	1	Adult	San Francisco	1	100% (2.5–100%)	6.22
Pristimantis multicolor	4	Adult	Yacuri	0	0 (0–60%)	0
Pristimantis orestes	1	Adult	Urdaneta	0	0 (0–97.5%)	0
Pristimantis sp. 1	2	Adult	Zamora	0	0 (0–84%)	0
Pristimantis sp. 2	1	Adult	San Francisco	0	0 (0–97.5%)	0
Pristimantis tiktik	4	Adult	Urdaneta	0	0 (0–60%)	0
Rhinella marina	2	Adult	Zamora	0	0 (0–84%)	0

tolerate human modified landscapes. Larval anurans, including *Gastrotheca* tadpoles, are generally tolerant to *Bd* infection (Grogan et al. 2018) as their lack of keratinized skin prevents disease progression until metamorphosis.

As a hypothesis, this situation allows for the proliferation of the pathogen in the habitats under investigation, following the declines and extinctions of more susceptible potential hosts such as *Atelopus* sp. (Haydon et al. 2002; La Marca et al. 2005; Woodhams et al. 2006). The life history of tadpole-producing *Gastrotheca* species in high-elevation habitats could allow for the continuous persistence of *Bd* in breeding pools. Mating takes place on land, and eggs hatch into larvae in the pouch of females which deposit advanced tadpoles into the breeding pool. Many *Gastrotheca* species do not have fixed reproductive periods and will breed whenever conditions are favorable (Del Pino 1989). Therefore, the combination of slow development and overlapping generations of tadpoles in permanent pools at high elevations creates an ecological system which has previously been characterized by high infection prevalence combined with *Bd* transmissions to tadpoles of other species (e.g., *Alytes obstetricans*: Bates et al. 2018 and *Rana muscosa*: Clare et al. 2016; Rachowicz and Briggs 2007). Although this study represents the first detection of *Bd* in *G. yacuri*, tadpoles of the closely related *G. riobambae* have previously been shown to be capable of maintaining infection across multiple cohorts in a breeding pond monitored over a 9-month period (S. Ron, unpub. data). Future research is needed to determine the potential for *Gastrotheca* species to act as *Bd* reservoirs in high-altitude habitats over an extended monitoring period.

Rediscoveries of *Atelopus* sp. in *Bd* positive sites are not unusual (Perez et al. 2014; Tapia et al. 2017; Lampo et al. 2011, 2017). However, many of these sites are at lower elevations than Yacuri and possess more diverse amphibian communities, leading to a wider array of options for cross-species infection dynamics. Being home to a smaller number of species, Yacuri National Park could thus be used as an accessible model system to infer the processes which allow for the coexistence of susceptible amphibians within a *Bd* positive community. An extremely high infection burden in breeding pools was discovered within 50 m of the locality where the four remaining *A. podocarpus* individuals were found (one individual during this study in 2018, and three individuals discovered in 2016). This suggests that *A. podocarpus* is still perennially exposed to *Bd* and therefore at risk of chytridiomycosis, although infection data are unavailable due to the loss of the skin swab from the individual sampled on the second expedition. The high-prevalence, high-intensity infection pattern found here is often seen in epizootic systems, and quantitative population data are required to assess the impact of *Bd* on both *G. yacuri* and *A. podocarpus*.

A high prevalence of *Bd* in Yacuri could inhibit the proliferation of relic populations of *A. podocarpus*, and is a major cause for concern for future conservation initiatives. For possible future *ex-situ* breeding, investigations into the availability of founder individuals are seen as a priority for the species (Conservation Needs Assessment 2012). However, more information is needed on the infection status and size of the population and, until such data are available, we do not recommend the collection of individuals for *ex-situ* conservation. All recent discoveries of this species have been in Yacuri National Park, and all visitors and guides must register in the park office. Hence, dissemination for a citizen-science monitoring project would be relatively straightforward. We also recommend further investigation into the possibilities of improving the habitat for this species, for example through the removal of introduced trout (Martín-Torrijos et al. 2016; Mouillet et al. 2018).

***Acknowledgements*.—**This research was primarily supported by Mohammed bin Zayed Species Conservation Fund project number 182518074. Field work was partially funded by “Balsa de los Sapos” Amphibian Conservation Initiative of the Pontificia Universidad Católica del Ecuador (PUCE) through Project QINV0132-IINV529010100 research fund granted to AMV by Dirección General Académica, PUCE. MCF is a fellow of the Canadian CIFAR ‘Fungal Kingdom’ program and is funded by the UK NERC grant NE/S000844/1 and MRC grant MR/R015600/1. Samples were obtained under Framework Contract of Access to Genetic Resources Nro. MAE-DNB-CM-2015–0039, and exported to the United Kingdom under material transfer agreement 96-2018-EXP-CM-MBI-DNB/MA granted by the Ministry of Environment of Ecuador to Dr. María Eugenia Ordoñez, who we thank for her support. For field assistance on the initial expedition to Yacuri National Park, we thank Leonardo Cedeño, Darwin Núñez, Kunam Nusirquia, and Fernando Ayala. Finally, we would like to thank Dr. Paul Szekely (Universidad Particular Técnica de Loja) for sharing field sites and field assistance, and for confirming the identifications of species sampled.

Literature Cited

Bates KA, Clare FC, O'Hanlon S, Bosch J, Brookes L, Hopkins K, McLaughlin EJ, Daniel O, Garner TWJ, Fisher MC, et al. 2018. Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial community structure. *Nature Communications* 9: 693.

Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases* *of Aquatic Organisms* 60(2): 141–148.

Brannelly LA, Webb RJ, Hunter DA, Clemann N, Howard K, Skerratt LF, Berger L, Scheele BC. 2018. Non-declining amphibians can be important reservoir hosts for amphibian chytrid fungus. *Animal Conservation* 21(2): 91–101.

Carvajal-Endara S, Coloma LA, Morales-Mite MA, Guayasamin JM, Szekely P, Duellman WE. 2019. Phylogenetic systematics, ecology, and conservation of Marsupial Frogs (Anura: Hemiphractidae) from the Andes of southern Ecuador, with descriptions of four new biphasic species. *Zootaxa* 4562(1): 1–102.

Catenazzi A, Lehr E, Rodriguez LO, Vredenburg VT. 2011. *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the Upper Manu National Park, Southeastern Peru. *Conservation Biology* 25(2): 382–391.

Catenazzi A, Swei A, Finkle J, Foreyt E, Wyman L, Vredenburg VT. 2017. Epizootic to enzootic transition of a fungal disease in tropical Andean frogs: Are surviving species still susceptible? *PLoS ONE* 12(10): e0186478.

Clare FC, Halder JB, Daniel O, Bielby J, Semenov MA, Jombart T, Loyau A, Schmeller DS, Cunningham AA, Rowcliffe M, et al. 2016. Climate forcing of an emerging pathogenic fungus across a montane multi-host community. *Philosophical Transactions of the Royal Society B-Biological Sciences* 371(1709): 20150454.

Conservation Needs Assessment. 2012. Assessment results: *Atelopus podocarpus*. Available: https://www.conservationneeds.org/AssessmentResults.aspx?AssessmentID=3170&SpeciesID=2588&CountryID=104 [Accessed: 5 June 2020].

Del Pino E. 1989. Marsupial Frogs. *Scientific American* 260(5): 110–119.

Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, du Preez LH, et al. 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proceedings of the National Academy of Sciences of the United States of America* 108(46): 18,732–18,736.

Ginestet C. 2011. *ggplot2*: elegant graphics for data analysis. *Journal of the Royal Statistical Society Series A. Statistics in Society* 174: 245.

Grogan LF, Jacques R, Berger L, Skerratt LF, Scheele BC, Castley JG, Newell DA, McCallum HI. 2018. Review of the amphibian immune response to chytridiomycosis, and future directions. *Frontiers in Immunology* 9: 2536.

Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. 2002. Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases* 8(12): 1,468–1,473.

Hoffmann M, Hilton-Taylor C, Angulo A, Boehm M, Brooks TM, Butchart SHM, Carpenter KE, Chanson J, Collen B, Cox N, et al. 2010. The impact of conservation on the status of the world's vertebrates. *Science* 330(6010): 1,503–1,509.

Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, Dalton A, Kriger K, Hero M, Hines H, et al. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73(3): 175–192.

IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-1. Available: https://www.iucnredlist.org [Accessed: 7 July 2020].

La Marca E, Lips KR, Lotters S, Puschendorf R, Ibanez R, Rueda-Almonacid JV, Schulte R, Marty C, Castro F, Manzanilla-Puppo J, et al. 2005. Catastrophic population declines and extinctions in neotropical Harlequin Frogs (Bufonidae: *Atelopus*). *Biotropica* 37(2): 190–201.

Lampo M, Celsa SJ, Rodríguez-Contreras A, Rojas-Runjaic F, García CZ. 2012. High turnover rates in remnant populations of the Harlequin Frog, *Atelopus cruciger* (Bufonidae): low risk of extinction? *Biotropica* 44: 420–426.

Lampo M, Señaris C, García CZ. 2017. Population dynamics of the Critically Endangered toad, *Atelopus cruciger,* and the fungal disease chytridiomycosis. *PLoS ONE* 12(6): e0179007.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America* 103(9): 3,165–3,170.

Loetters S, Van der Meijden A, Coloma LA, Boistel R, Cloetens P, Ernst R, Lehr E, Veith M. 2011. Assessing the molecular phylogeny of a near extinct group of vertebrates: the Neotropical Harlequin Frogs (Bufonidae; *Atelopus*). *Systematics and Biodiversity* 9(1): 45–57.

Longo AV, Burrowes PA. 2010. Persistence with chytridiomycosis does not assure survival of direct-developing frogs. *EcoHealth* 7(2): 185–195.

Martín-Torrijos L, Sandoval-Sierra JV, Muñoz J, Diéguez-Uribeondo J, Bosch J, Guayasamin JM. 2016. Rainbow Trout (*Oncorhynchus mykiss*) threaten Andean amphibians. *Neotropical Biodiversity* 2(1): 26–36.

Merino-Viteri A, Coloma L, Almendáriz A. 2005. Los *Telmatobius* de los Andes de Ecuador y su disminución poblacional. Pp. 9–37 In: *Estudios sobre las Ranas Andinas de los Géneros Telmatobius y Batrachophrynus (Anura: Leptodactylidae). Monografías de Herpetología 7*. Editors, Lavilla EO, De la Riva I. Asociación Herpetológica Española, Valencia, Spain. 349 p.

Mouillet C, Barta B, Espinosa R, Andino P, Christoffersen KS, Jacobsen D. 2018. Ecological effects of introduced Rainbow Trout (*Oncorhynchus mykiss*) in

pristine Ecuadorian high Andean lakes. *Fundamental and Applied Limnology* 191(4): 323–337.
O'Hanlon SJ, Rieux A, Farrer RA, Rosa GM, Waldman B, Bataille A, Kosch TA, Murray KA, Brankovics B, Fumagalli M, et al. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. *Science* 360(6389): 621–627.
Perez R, Richards-Zawacki CL, Krohn AR, Robak M, Griffith EJ, Ross H, Gratwicke B, Ibanez R, Voyles J. 2014. Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphibian & Reptile Conservation* 8(2) [General Section]: 30–35 (e85).
Peters JA. 1973. The frog genus *Atelopus* in Ecuador (Anura: Bufonidae). *Smithsonian Contributions to Zoology* 145: 1–49.
Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439(7073): 161–167.
R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Rachowicz LJ, Briggs CJ. 2007. Quantifying the disease transmission function: effects of density on *Batrachochytrium dendrobatidis* transmission in the Mountain Yellow-legged Frog, *Rana muscosa*. *Journal of Animal Ecology* 76(4): 711–721.
Ron SR, Duellman WE, Coloma LA, Bustamante MR. 2003. Population decline of the Jambato Toad, *Atelopus ignescens* (Anura: Bufonidae), in the Andes of Ecuador. *Journal of Herpetology* 37(1): 116–126.
Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A, et al. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363(6434): 1,459–1,463.
Tapia EE, Coloma LA, Pazmiño-Otamendi G, Peñafiel N. 2017. Rediscovery of the nearly extinct Longnose Harlequin Frog, *Atelopus longirostris* (Bufonidae), in Junín, Imbabura, Ecuador. *Neotropical Biodiversity* 3(1): 157–167.
Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11,466–11,473.
Woodhams DC, Voyles J, Lips KR, Carey C, Rollins-Smith LA. 2006. Predicted disease susceptibility in a Panamanian amphibian assemblage based on skin peptide defences. *Journal of Wildlife Diseases* 42(2): 207–218.

Phillip Jervis is currently a Ph.D. candidate at the Zoological Society of London (ZSL) Institute of Zoology and Imperial College London. His current research centers around using the chemical ecology of model systems in Panama and the Pyrenees to investigate discrepancies in the resilience of *Bd* susceptible amphibians. Phillip holds a Master's degree in Tropical Forest Ecology from Imperial College London and a Master's degree in Chemistry for Drug Discovery from the University of Bath (United Kingdom).

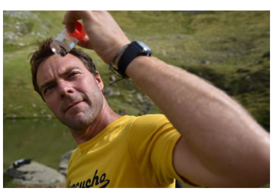
Berglind Karlsdóttir is currently a Social Scientist with the Forestry Commission. At the time of this study, Berglind was an intern with Durrell Wildlife Conservation Trust's *Saving Amphibians from Extinction* team, where she produced a set of *General Guidelines for Managers and Supporters of Amphibian Captive Breeding Programmes*, in collaboration with the Amphibian Ark (https://www.amphibianark.org/). This program was based on her Master's thesis in Conservation Science at Imperial College London, entitled: *Barriers to Amphibian Captive Breeding Programmes in Latin America, Africa, and Asia*. Berglind also holds a Bachelor's degree in Wildlife Conservation from the University of the West of England (Bristol, United Kingdom).

Robert Jehle had a childhood interest in amphibians and their habitats, and feels very fortunate that he could translate that passion into a professional career. Robert is currently a Reader in Population Biology at the University of Salford (United Kingdom), where he teaches in a range of undergraduate and postgraduate programs in Zoology and Wildlife Conservation. His main research area revolves around the ecology, evolution, and behavior of amphibians at the level of populations, often combining evidence from genetic markers with life-history inferences. Robert is a former (2009–2015) Editor of *Herpetological Journal*, and a current (2009–date) Associate Editor of the journal *Animal Conservation*.

Diego Almeida-Reinoso has a doctorate in Biology from Universidad Central del Ecuador. Diego is currently the manager of insect breeding at Farm SARGRILLO and director of the *ex-situ* conservation program for two endangered species of Ecuadorian amphibians, the Tiger Treefrog (*Hyloscirtus tigrinus*) and Stella de la Torre's Rocket Frog (*Hyloxalus delatorreae*).

Freddy Almeida-Reinoso has a degree in Biological Sciences from Universidad Central del Ecuador. Most of his professional career has involved the management, conservation, and research of *ex-situ* amphibian populations. Freddy is currently working as Administrator of the Amphibian Conservation Initiative *Balsa de los Sapos* (Life-raft for frogs) at Pontificia Universidad Católica del Ecuador.

Santiago Ron is an Ecuadorian evolutionary biologist, principal professor, and Curator of Amphibians at the Museum of Zoology (QCAZ), Pontificia Universidad Católica del Ecuador (PUCE). Santiago has a Ph.D. on Evolution, Ecology, and Behavior from the University of Texas at Austin and an M.A. degree in Systematics and Ecology from the University of Kansas (Lawrence, Kansas, USA). His research focuses on the evolution and diversity of the amphibians of the Neotropical Region, with special attention to Ecuador. Santiago leads the development of BIOWEB Ecuador, an on-line platform for managing and publishing information about the Ecuadorian biodiversity. He is member of The World Academy of Sciences (TWAS) and founding member of the Ecuadorian Academy of Sciences.



Matthew Fisher in a fungal biologist working at Imperial College London. His approach melds genomic epidemiology, modelling, macroecological analysis, and experimentation to understand the biology underpinning the global emergence of fungal diseases. Matthew leads the 'Fungal Pathogens' theme in the MRC Centre for Global Infectious Disease Analysis (United Kingdom) and is a fellow of the Canadian CIFAR program.

Andres Merino-Viteri is an Ecuadorian herpetologist with a Ph.D. in Tropical Ecology from James Cook University in Australia. Andres is currently working as a lecturer and researcher at the Biological Sciences School of the Pontificia Universidad Católica del Ecuador (PUCE) in Quito, Ecuador. He has been in charge of the Amphibian Conservation Initiative *Balsa de los Sapos* (Life-raft for frogs) at PUCE since 2011. Andres also focuses his research on the gathering of ecophysiological data for different species of Ecuadorian amphibians in order to assess their vulnerability to different climate change scenarios.