



## Review



# Harlequin frog rediscoveries provide insights into species persistence in the face of drastic amphibian declines

Kyle E. Jaynes<sup>a,b,\*</sup>, Mónica I. Páez-Vacas<sup>c</sup>, David Salazar-Valenzuela<sup>c</sup>, Juan M. Guayasamin<sup>d</sup>, Andrea Terán-Valdez<sup>e</sup>, Fausto R. Siavichay<sup>f</sup>, Sarah W. Fitzpatrick<sup>a,b</sup>, Luis A. Coloma<sup>e</sup>

<sup>a</sup> Department of Integrative Biology, W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, USA

<sup>b</sup> Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, MI, USA

<sup>c</sup> Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb), Ingeniería en Biodiversidad y Cambio Climático, Facultad de Ciencias del Medio Ambiente, Universidad Tecnológica Indoamérica, Calle Machala y Sabanilla, Quito, Ecuador

<sup>d</sup> Laboratorio de Biología Evolutiva, Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito USFQ, Quito, Ecuador

<sup>e</sup> Centro Jambatu de Investigación y Conservación de Anfibios, San Rafael, Quito, Ecuador

<sup>f</sup> Centro de Conservación de Anfibios, Bioparque Amaru, Cuenca, Ecuador

## ARTICLE INFO

## Keywords:

Amphibian declines  
Conservation  
Extinction  
Heterozygosity  
Historical samples  
Rediscovery

## ABSTRACT

Amphibians face global declines, and it remains unclear the extent to which species have responded, and through what mechanisms, to persist in the face of emerging diseases and climate change. In recent years, the rediscovery of species considered possibly extinct has sparked public and scientific attention. These are hopeful cases in an otherwise bleak story. Yet, we know little about the population status of these rediscovered species, or the biology underlying their persistence. Here, we highlight the iconic Harlequin frogs (Bufonidae: *Atelopus*) as a system that was devastated by declines but now encompasses between 18 and 32 rediscoveries (25–37 % of possible extinctions) in the last two decades. Geographic distributions of rediscoveries closely match regional described species abundance, and rediscoveries are documented at elevations from 100 m to >3500 m, with no significant differences between mean historical and contemporary elevations. We also provide genomic data on six decimated species, with historical sample comparisons for two of the species and find a pattern of decreasing genetic variation the longer a species had been missing. Further, we document marked decrease in heterozygosity in one species, but not the other, indicating potential idiosyncratic consequences of declines. Finally, we discuss research priorities to guide the potential transition from amphibian declines to recoveries and to maximize conservation efforts.

## 1. Introduction

Taxonomic extinction estimates are often reached with varying lines of evidence, such as ‘time missing’ thresholds or specified search efforts (Mace et al., 2008). The ‘Extinct’ designation can be declared by expert opinions through an extinction risk assessment using the International Union for Conservation of Nature (IUCN) Red List Categories and Criteria (<https://www.iucnredlist.org/resources/categories-and-criteria>); however, it is difficult to know with certainty if a species is ever truly extinct (Roberts, 2006; Scott et al., 2008; Collen et al., 2010). Some species persist on the brink of extinction for some time, embodied in rediscoveries, where a species’ detectability in the field has decreased to the point of presumed extinction, but is found again. Rediscoveries are

difficult to study because they are infrequent and hard to characterize across Critically Endangered, and often understudied species (Scheffers et al., 2011). However, rediscoveries provide important conservation opportunities to understand the intrinsic and/or extrinsic features that underlie population persistence in the face of widespread extirpation (e.g., Chaves et al., 2014; Voyles et al., 2018; Jiménez-Monge et al., 2019; Byrne et al., 2021). They also provide hope for scientists doing conservation research, and for non-scientists supporting conservation initiatives in an otherwise devastating chapter of the biodiversity crisis. These cases typically represent urgent conservation priorities, as rediscovered species may still be on the brink of extinction and likely benefit from active management to ensure that rediscovery leads to recovery (e.g., Mendelson et al., 2019; Scheele et al., 2021). For example, Scheffers

\* Corresponding author.

E-mail address: [jaynesky@msu.edu](mailto:jaynesky@msu.edu) (K.E. Jaynes).

<https://doi.org/10.1016/j.biocon.2022.109784>

Received 8 April 2022; Received in revised form 6 October 2022; Accepted 18 October 2022

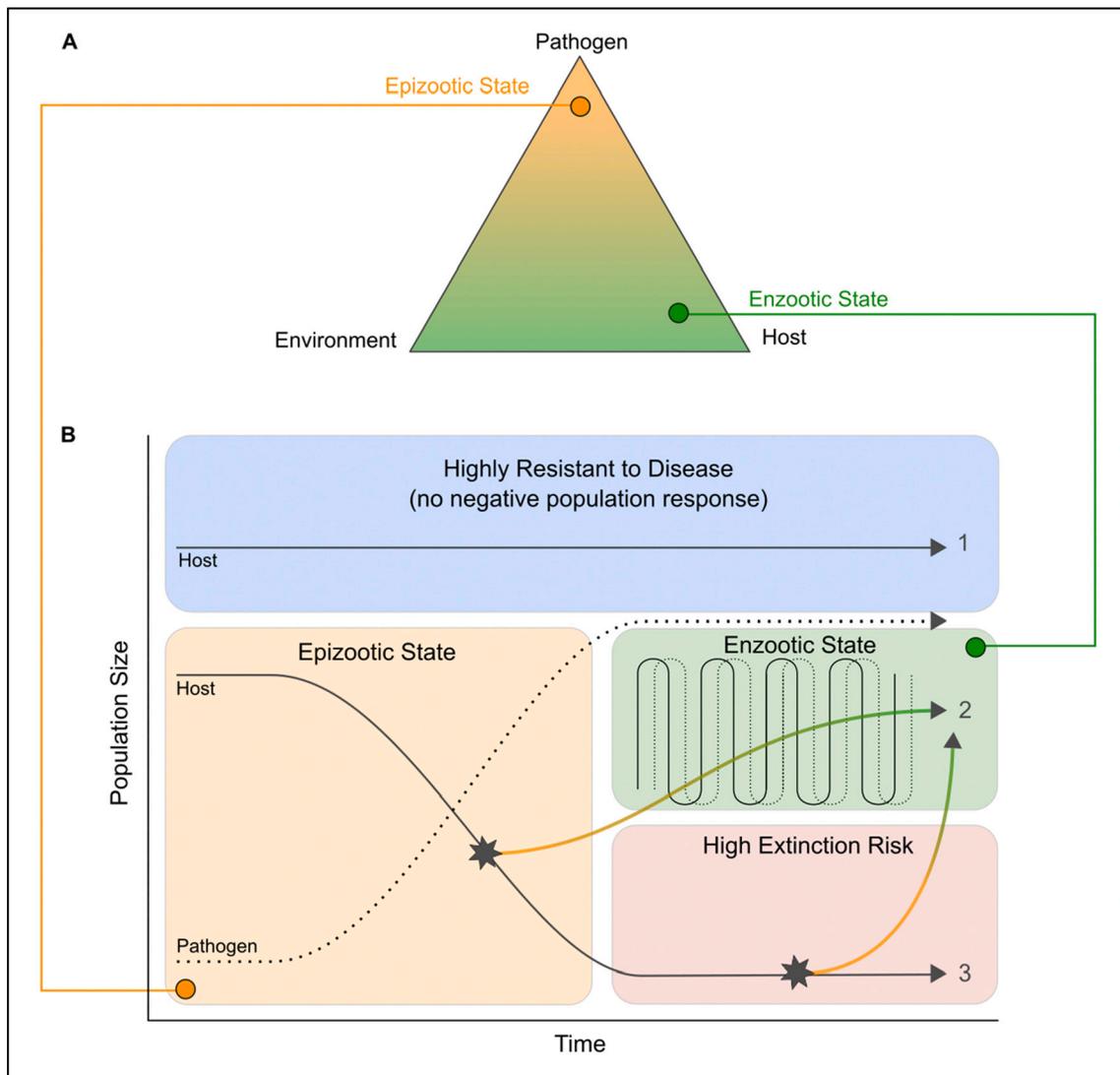
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et al. (2011) found that 88 % of rediscovered amphibians, birds, and mammals remained threatened after their rediscovery. Ideally, scientists, conservationists, and other stakeholders will be poised to take advantage of the urgent opportunities provided by species rediscoveries. After four decades of amphibian declines, a recent trend of species rediscoveries provides a compelling system to understand persistence and promote recovery.

1.1. Global amphibian decline crisis

Amphibians have faced some of the most severe declines of any taxonomic group, with between 40 and 60 % of species under concern globally (IUCN, 2021). The magnitude and speed of these declines is unprecedented (i.e., four decades), even considering generally accelerated extinction rates in most taxonomic groups driven by anthropogenic changes (McCallum, 2007; Catenazzi, 2015). Global drivers of

amphibian population declines include habitat loss and fragmentation (Cushman, 2006), climate change (Pounds et al., 2006), and disease (Berger et al., 1998; Lips et al., 2006; Scheele et al., 2019). Physiological traits such as permeable skin and low vagility make amphibians especially vulnerable to rapid environmental changes in climate and disease. A particularly well-studied pathogen of amphibians is *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al., 1998; Lips, 1998; Longcore et al., 1999; Scheele et al., 2019). *Bd* is a species that consists of multiple lineages, which have different geographic extents and virulence (Byrne et al., 2019). The emergence of the hyper-virulent and widespread Global Pandemic Lineage (GPL) of *Bd* was largely thought to have occurred in the late twentieth century through anthropogenic spread (O’Hanlon et al., 2018). Some studies have documented the collapse of entire species, often in seemingly pristine areas, presumably due to the arrival of a highly virulent strain of *Bd* (e.g., Lips, 1998; La Marca et al., 2005; Scheele et al., 2019). Despite the breadth of factors amphibians face in a



**Fig. 1.** Conceptual model of epizootic and enzootic transitions. (a) Host-Pathogen-Environment disease triangle representing epizootic (orange) and enzootic states (green). (b) Theoretical species trajectories following initial responses to novel *Bd* pathogen emergence. Black solid (host) and black dotted (pathogen) lines represent population size/growth, respectively. Host population size decreases rapidly with the arrival of a new pathogen and eventually stabilizes. Low host population sizes decrease infection/transmission of *Bd*, which stabilizes pathogen population size. Solid and dotted population cycles represent enzootic host-pathogen dynamics. Numbered solid lines represent theoretical host population responses in species with varying susceptibility. Outcome #1 represents a highly resistant/tolerant host population. Outcome #2 (orange and green gradient lines) represent target populations of interest in our paper that have transitioned to enzootic states (e.g., species rediscoveries). Outcome #3 represents populations that declined and are either teetering in near extinction risk or have gone extinct. Black stars represent potential points of host mechanism/response that changes trajectory to avoid extinction (currently unknown mechanisms). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

changing world, *Bd* is considered one of the leading drivers in ongoing extinctions for many amphibian taxa (Scheele et al., 2019).

### 1.2. *Bd* epizootic-enzootic transition period

Wildlife diseases are often studied in a disease triangle framework, where eco-evolutionary dynamics among host susceptibility, pathogen virulence, and environment alter interactions and change through time (Scholthof, 2007; James et al., 2015; Fig. 1). In the epizootic period, initial emergence of a disease in a region might cause mass mortality and rapid population declines due to naivety of the host population. In contrast, the post-epizootic period or enzootic period, is the phase in which a disease has previously established and may remain at high, yet relatively constant prevalence. In this phase, host populations have undergone an initial disease response and are persisting at more stable population sizes with infection loads that support host-pathogen coexistence (Briggs et al., 2010). In this paper, we use ‘epizootic’ and ‘enzootic’ as terms to describe the naivety of the host populations to a novel disease emergence of chytridiomycosis, whether through recent

mutation or environmental change of an endemic strain or arrival of a global strain to a new region.

The epizootic phase of the disease chytridiomycosis, caused by *Bd*, is well documented from cases across the globe, such as highland lake systems in the Sierra Nevada Mountain range in North America (Vredenburg et al., 2010), tropical rainforests in Queensland, Australia (Richards et al., 1994), and montane rainforests in Central America (Lips, 1998). Although *Bd* is still present and causing active infection in many places throughout the world, most of the vivid descriptions of the onset of *Bd* followed by mass mortality occurred in the 1980s–90s. The severity of these early mass declines following emergence of *Bd* have decreased since the 1990s (Fey et al., 2015). The epizootic-enzootic transition is not well-understood for *Bd*, but theory suggests a transition mediated by a change in pathogen virulence, host tolerance, or environment (James et al., 2015; Scheele et al., 2017b). Some areas where *Bd* has already spread and established, such as throughout regions of Central and South America, are now proposed to be in the enzootic period based on stable host-pathogen dynamics where hosts persist in the presence of disease (Guayasamin et al., 2014; Perez et al., 2014;

#### Box 1

*Atelopus ignescens*: A case study of persistence and rediscovery.



*Atelopus ignescens* in the wild, 2019 (photo: Kyle Jaynes).

The Jambato Harlequin frog, *Atelopus ignescens*, was once widespread and common through the entire range of the Ecuadorian Andes (Ron et al., 2003). The Spanish naturalist M. Jiménez de la Espada observed “thousands of individuals” in 1864 (Jiménez de la Espada, 1875) on the Antisana Páramo, while Black (1982) counted up to 50 individuals per square meter in 1981 in the above-mentioned páramo. Population data leading up to 1986 found thousands of individuals on roads during rain events (Peters, 1973; L.A. Coloma field notes). Populations suddenly declined in the late 1980s, and the last individual was seen in 1989, albeit not for the lack of searching. Surveys were conducted across Ecuador from 1999 to 2003 (Bustamante et al., 2005; Ron et al., 2003) with no avail. Ron et al. (2003) reported zero individuals per square meter in a monitoring study in the Páramos de Antisana and Guamaní in 2000, where Black originally reported up to 50 individuals per square meter in 1981, despite search efforts being nine times greater in 2000. Anecdotal stories talk about pastors from churches and other community leaders offering rewards in hopes of uncovering persisting populations.

On 21 April 2016, one relict population was rediscovered in a small valley in Northern Ecuador (Coloma, 2016). Individuals from this population were collected in 2016 to form the basis of an ex-situ breeding program driven to conserve the potentially last remaining individuals of the species. Nine out of 35 individuals were positive for *Bd*. A brief field survey of this site by authors KEJ, MIP, DS, and SWF in November 2019 found three additional individuals, reinforcing that the only known wild population of this species is persisting at this location, even in the presence of *Bd*. We provide the first data on the contemporary rediscovered population (see Sections 3.2, 4.2; Fig. 3). Photo: Wild-caught individual *Atelopus ignescens*; 26 November 2019 by KEJ.

Catenazzi et al., 2017; Whitfield et al., 2017; Voyles et al., 2018; Bolom-Huet et al., 2019; Zumbado-Ulate et al., 2019; LaBumbard et al., 2020). However, proposed enzootic systems remain ambiguous for understanding species persistence, with different species showing high mortality and extirpation, stability, or range expansions in the same communities (Catenazzi et al., 2017; Scheele et al., 2017b; Zumbado-Ulate et al., 2021).

The possible shift from epizootic to enzootic disease states calls for increased scientific attention because of the continued threat of global fungal pathogens to the biodiversity crisis (Fisher et al., 2012), and largely unknown mechanisms of persistence in natural populations. In most cases, it remains unclear the extent to which species have responded and through what mechanisms, to persist in the face of rapid, novel changes (Voyles et al., 2018). In an encouraging turn of events, there have been recent rediscoveries of amphibian species previously considered to have gone extinct, such as the Variable Harlequin frog (González-Maya et al., 2013), the Jambato Harlequin frog (Box 1), and more than a dozen others (Supplemental Table ST1). These rediscovered species provide key opportunities as natural experiments for understanding the mechanisms that have allowed certain populations and species to persist, while many others went extinct (Fig. 1). Here, we highlight an iconic group, the Harlequin frogs of the genus *Atelopus*, for investigation of amphibian rediscoveries.

### 1.3. *Atelopus*: an iconic genus in the amphibian decline crisis

Harlequin frogs (genus *Atelopus*) are a Neotropical species-rich genus of Bufonidae, with 94 species currently recognized, and an estimated 126–166 putative species in the genus (Löters, 1996; Coloma et al., 2010; Supplemental Table ST1). Harlequin frogs experienced some of the most severe declines of any group throughout the past two decades, with 70 % of species evaluated by the IUCN considered Critically Endangered or Extinct (IUCN, 2021). Many sudden declines were associated with *Bd* outbreaks (La Marca et al., 2005; Scheele et al., 2019), often in combination with climate change (Ron et al., 2003; Pounds et al., 2006). Early work attempting to understand drivers of decline suggest that characteristics of *Atelopus*, which are diurnal and often occur in mid to high elevations and depend on streams for reproduction, may have played a role in their demise (Löters, 1996; Stuart et al., 2004; La Marca et al., 2005; Pounds et al., 2006). While the genus remains in peril, recent rediscoveries of several *Atelopus* species previously thought to be extinct has sparked public and scientific attention. Although some of these cases of presumed extinction followed by rediscovery may be the result of no or insufficient sampling during the missing years (e.g., indigenous communities at Sierra Nevada de Santa Marta in Colombia were always aware of *A. arsyecue*, prior to its ‘scientific rediscovery’), many cases of rediscovery have come from species in which the decline was unquestionable (La Marca et al., 2005; Scheele et al., 2019) and where there has been consistent effort to search for extant populations (e.g., see Ron et al., 2003). However, the extent of these rediscoveries, and what environmental, demographic and/or evolutionary responses have allowed certain populations to persist remains unclear.

To date, the most well-studied case of *Bd*-related *Atelopus* declines and rediscoveries are the Panamanian Golden frogs (*A. zeteki*, *A. varius*). Research on these species has shown physiological and behavioral shifts to higher body temperatures in infected individuals (Richards-Zawacki, 2010), demographic shifts to higher female densities after *Bd* arrival (McCaffery et al., 2015), and similar survival and recruitment rates between infected and uninfected hosts following declines (DiRenzo et al., 2018). A lack of environmental or pathogen change implicates a host response that underlies species persistence and recovery (Voyles et al., 2018). *Bd* infection assays showed high variability in response to *Bd* infections between species (*A. glyphus*, moderate infection; *A. zeteki*, high infection) despite considerable overlap in gene expression patterns (Ellison et al., 2015). Further, contrasts exist within species, as *A. zeteki* shows evidence of both innate and acquired immunity, as well as host

immune suppression with *Bd* infections (Ellison et al., 2014). Finally, a study that sequenced whole exomes from pre- and post-decline *A. zeteki* and *A. varius* revealed higher genetic diversity and evidence of recent admixture in some contemporary populations compared to historic samples from extirpated localities, but low sample sizes made it difficult to make associations with specific loci across the genome (Byrne et al., 2021). Despite the large body of literature addressing patterns and mechanisms of persistence in Central American *Atelopus*, it remains unclear how generalizable these outcomes are across regions and species.

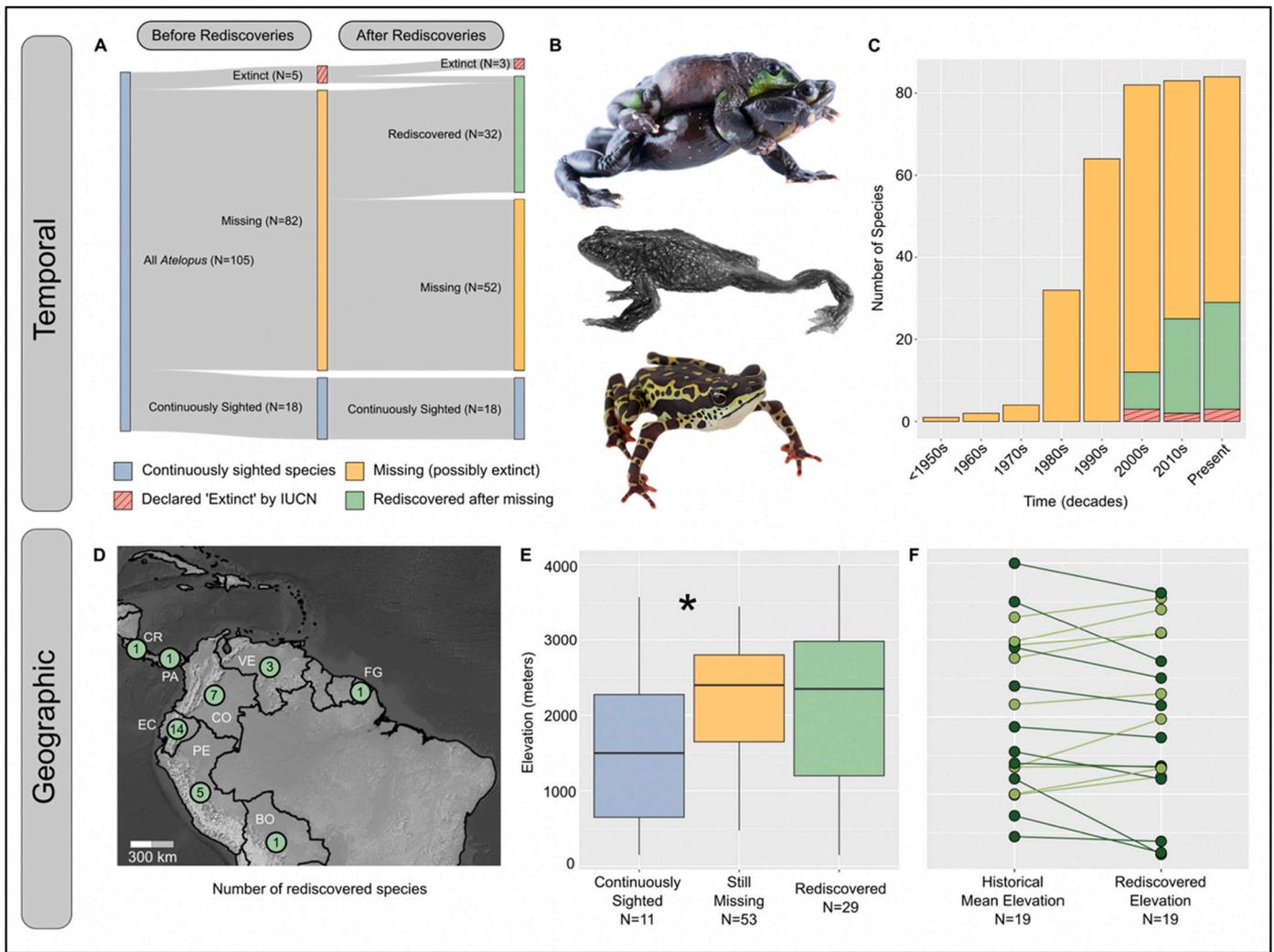
In contrast to the literature on Central American *Atelopus*, we know little about *Atelopus* rediscoveries in South America, which harbors the majority of *Atelopus* diversity and rediscoveries (Fig. 2). Like Central America, the bulk of research on South American *Atelopus* persistence has thus far focused on only a few species. For example, studies of *A. cruciger* in Venezuela have documented a population crash and high mortality rates due to *Bd*, but population stability maintained by rapid recruitment of new adults each year (Bonaccorso et al., 2003; Lampo et al., 2012). Lampo et al. (2017) suggest that this lowland species contradicts thermal refuge hypothesis patterns (i.e., that warmer lowland regions serve as refugia from *Bd*, which prefers cooler temperatures; Piotrowski et al., 2004; Woodhams et al., 2008), and may be able to persist because of decreased disease transmission. Most other studies (including in Central America) are short reports that have arisen in the past decade documenting the presence of *Bd* or first sighting of long-lost species or populations (e.g., González-Maya et al., 2013; Tapia et al., 2017; Jiménez-Monge et al., 2019; Barrio Amorós et al., 2020). An analysis focused on the 32 putative species of *Atelopus* found in Ecuador suggested that more than half of the species not seen after abrupt declines were found in recent years (Tapia et al., 2017). While the documentation of rediscovered species has developed greatly in the past decade, further investigation is needed across species, environments, and decline histories to gain a full understanding of mechanisms of persistence in *Atelopus*, and to apply this understanding more broadly to other amphibians.

Given that many populations and species may have transitioned to an enzootic phase of disease response, and the promising trend of accumulating species rediscoveries, we aimed to characterize the geographic and temporal patterns of rediscoveries in the genus *Atelopus*. Additionally, we provided the first genomic data on six species of *Atelopus* with varying decline histories to explore genetic diversity patterns in persisting populations. Finally, we discussed ideas for prioritizing efforts around *Atelopus* rediscoveries in future research and conservation. These recent case studies have enormous value in providing a comparative framework to investigate multiple closely related species that vary in population ranges, sizes, decline histories, and environments.

## 2. Material and methods

### 2.1. Species information and status categorization

We evaluated all *Atelopus* species for which information was available ( $N = 105$ ) to determine the number of species in the genus that have ever been considered missing. We first compiled all species information from the IUCN Red List (2021), including previous published reports. Next, we gathered information from the literature on species either not recognized by the IUCN (e.g., newly described species) or filled in information that was not included in the IUCN listing since the last report (e.g., published research articles documenting a recent rediscovery). We recorded basic temporal and geographic information available from those sources, including year of disappearance or rediscovery, and location or elevation of rediscovered population (see Supplemental Table ST1 for all species information). We used the above-collected information and expert knowledge of unpublished search efforts when possible to assign each species one of four categories: ‘Still Missing’ (i.e.,



**Fig. 2.** Temporal and geographic patterns of Harlequin frog extinctions and rediscoveries (a) Sangey plot of species trajectories for all species analyzed in the study (N = 105). (b) Photos of rediscovered (*A. nanay*; Jaime Culebras), rendering of an extinct *Atelopus* sp., and continuously sighted species (*Atelopus* sp. Otoyacu; Centro Jambatu). (c) Stacked Bar Chart representing species missing or possibly extinct (orange), species officially declared extinct by the IUCN (red hatched), and rediscovered species, including ‘Possible Rediscoveries’ in our criteria (green) across decades. For species with ambiguous dates and/or different dates across countries, the most recent date was chosen as the date for ‘extinction period’ and the first date of their rediscovery as their ‘rediscovery period’. Species that had only partial extinction/rediscovery dates were excluded. (d) Map of *Atelopus* rediscoveries in Central and South America with number of rediscovered species in green circles for each country: Costa Rica (CR), Panama (PA), French Guiana (FG), Venezuela (VE), Colombia (CO), Ecuador (EC), Peru (PE) and Bolivia (BO). (e) Boxplots of mean historical elevation for species categories in (a). Boxes signify first quartile, median, and third quartile. Comparisons significant at an adjusted  $p < 0.05$  with a Tukey Honest Significant Difference test indicated with asterisk. (f) Plot of elevation for historical and contemporary populations. Left column contains a species’ mean historical elevation. Right column is the elevation of the rediscovered population. Line is connecting a species’ mean historical elevation with the rediscovered population’s elevation. Colors signify a qualitative upward (light green) and downward (dark green) difference from the historical mean elevation. See Supplemental Table ST1 for species information in all plots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

species has still not been sighted after disappearance), ‘True Rediscovery’ (i.e., species was missing and subsequently rediscovered, with search efforts occurring at known historical localities during the missing period), ‘Possible Rediscovery’ (i.e., same as true rediscovery, but where no known search efforts at historical localities occurred during the missing period), and ‘Continuously Sighted’ (i.e., the species was never missing or deemed to be continuously sighted during the missing period). We excluded all species that were ‘Continuously Sighted’ in further analyses because we do not consider these species to represent rediscoveries. We calculated a rigorous estimate of rediscoveries, which is the number of species in the ‘True Rediscovery’ category divided by the total number of species missing at one point in time:  $[\text{True Rediscovery} / (\text{True Rediscovery} + \text{Still Missing})]$ ; and a less-rigorous estimate, with the inclusion of species categorized as ‘Possible Rediscovery’:

$$[(\text{True Rediscovery} + \text{Possible Rediscovery}) / (\text{True Rediscovery} + \text{Possible Rediscovery} + \text{Still Missing})].$$

## 2.2. Genomic data collection and analyses

We extracted DNA from opportunistically sampled contemporary tissues of six species (Table 1) collected from deceased specimens in ex-situ programs (wild-caught individuals) using a DNeasy Blood and Tissue Kit, following manufacturer protocols (Qiagen). We received DNA extract from two museum samples collected in 1990 (flash-frozen tissues; see supplementary material for discussion on species identification) from University of Kansas Biodiversity Institute (KU217429 from Pachancho river, Provincia Bolívar, hereafter ‘Pachancho’, and KU217433 from ca. Chaucán, Provincia Azuay, hereafter ‘Chaucán’),

**Table 1**

Summary of genomic samples for each species. Individual heterozygosity estimates represent the mean (minimum-maximum).

Species	Genomic samples (N)	Years missing	Individual heterozygosity
<i>Atelopus balios</i>	5	15	0.2583 (0.2445–0.2957)
<i>Atelopus elegans</i>	5	12	0.2523 (0.2315–0.2782)
<i>Atelopus ignescens</i>	8	27	0.1951 (0.1668–0.2170)
<i>Atelopus nanay</i>	2	18	0.2174 (0.1998–0.2350)
<i>Atelopus</i> sp. Limón ( <i>spumarius</i> complex)	3	0	0.3289 (0.3126–0.3582)
<i>Atelopus</i> sp. Otoyacu ( <i>spumarius</i> complex)	4	0	0.3265 (0.3193–0.3367)
Historical (Chaucán and Pachanchó)	2	NA	0.2445 (0.2174–0.2718)

extracted under the same protocols. We prepared Restriction Site-Associated DNA Sequencing (RADseq) libraries using a modified BestRad protocol (Ali et al., 2016). We sequenced our library in one lane with paired-end 150 base pair reads on an Illumina HiSeq 4000 at the Genomics Core at Michigan State University. We processed raw Illumina reads through the STACKS v2.0 (Rochette et al., 2019) and filtered SNPs using custom scripts. Due to few polymorphic loci shared between our six species, we performed the final steps of the STACKS pipeline (i.e., the *populations* module) separately for each species, generating seven separate datasets (one for each species, plus one for historical specimens). Lastly, we randomly subsampled 5000 SNPs from all datasets to make comparisons across a similar number of SNPs using custom scripts in *bcftools* (Li et al., 2009). We found no differences between subsampled results and results including all SNPs across datasets (Supplemental Fig. S1D). Raw reads are archived in the NCBI SRA (BioProject: PRJNA870111; accession numbers: SAMN30351361-SAMN30351399). See supplemental methods for library preparation and bioinformatic details.

We estimated individual heterozygosity using the *-het* flag in *VCFTools* v0.1.15 (Danecek et al., 2011), which estimates heterozygosity on a per-individual basis. Therefore, values are averaged individual estimates for each species, not population-level estimates. To quantify differences in heterozygosity among species and time points, we fit an ANOVA grouped by species, and used a Tukey Honest Significant Differences test to calculate adjusted P values for group mean comparisons. All statistical analyses were performed in R v3.3.0 (R Development Core Team, 2021) and data were visualized using the *ggplot2* package (Wickham, 2016).

### 3. Results

#### 3.1. Temporal and geographic patterns of *Atelopus* rediscoveries

We estimated that at least 18 species (rigorous), and up to 32 species (less rigorous) have been rediscovered to date (Fig. 2; Supplemental Table ST1). The first extinction was presumed in the 1950s, with rapid increases to >70 species presumably extinct in the 2000s. Subsequently, species disappearances became less common and there has been a rapid and steady increase in rediscoveries. The first rediscovery was reported in the 2000s (von May et al., 2008), and this number has increased to 32 rediscoveries by 2022, despite only a few additional species being categorized as extinct in the wild in those decades. Rediscoveries are documented from all regions in which *Atelopus* spp. occur, with 88 % occurring in four countries (Ecuador, Colombia, Peru, Venezuela; Fig. 2D).

Rediscovered populations have been found from throughout 120 m to >3600 m in elevation. We found that ‘Continuously Sighted’ species

had a lower historical mean elevation (1503 m) than ‘Still Missing’ (2232 m) and ‘Rediscovered’ (2116 m) species, although only ‘Continuously Sighted’ and ‘Still Missing’ comparisons were significantly different (Fig. 2E;  $p < 0.05$ ). Out of 32 rediscovered species, there were 19 rediscoveries with documented historical and contemporary elevations. The mean elevation for contemporary rediscovered populations was 1971 m, which was not statistically significant from historical mean elevations (2043 m, overall downward shift of 72 m; Fig. 2F).

#### 3.2. Genomic diversity patterns in persisting populations

Individual heterozygosity estimates ranged from 0.1668 to 0.3582 and were significantly different among the six species (Table 1). We also found a significant relationship between years missing and heterozygosity across rediscovered species (Fig. 3A;  $p < 0.001$ ; adjusted  $R^2 = 0.88$ ). We found three significant groups in our analyses corresponding to descending heterozygosity, which closely matches phylogenetic groups: Group A (lowland: *A. sp. Limón* (*spumarius* complex), *A. sp. Otoyacu* (*spumarius* complex)), Group B (lowland: *A. balios*, *A. elegans*; highland: *A. nanay*), and Group D (highland; *A. ignescens*, *A. nanay*;  $p < 0.001$ ), with the exception that *A. nanay* (highland) falls within both groups B and D (Fig. 3A;  $p > 0.05$ ). Historical sample comparisons between *A. ignescens* and *A. nanay* showed a similar trend, although *A. ignescens* is the only species between the two species that was significantly different from historical samples (Fig. 3B;  $p < 0.05$ ). See Supplemental Table ST2 for bioinformatic outputs for each dataset prior to subsampling.

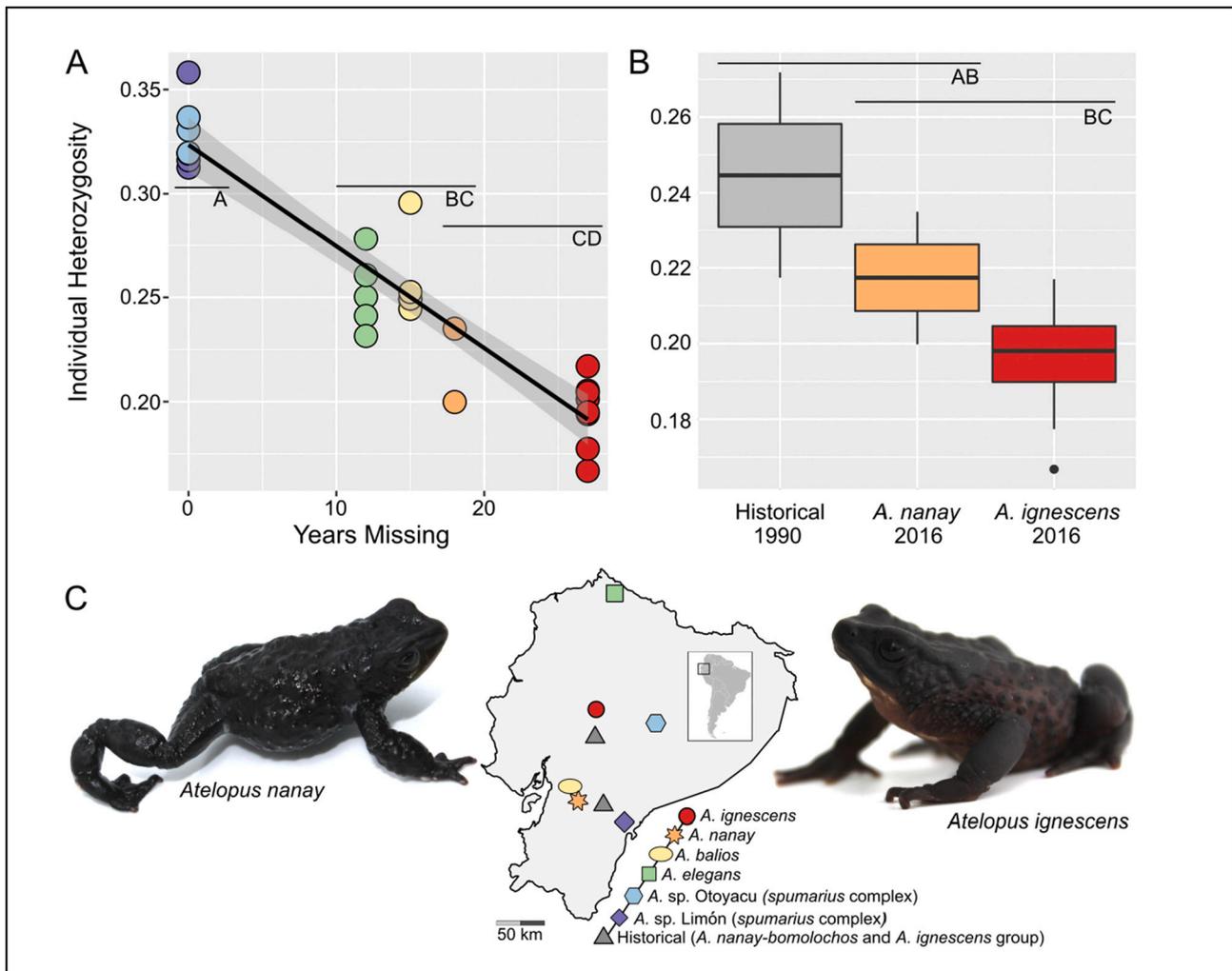
## 4. Discussion

#### 4.1. Temporal and geographic patterns of *Atelopus* rediscoveries

Attempts to understand the vulnerability of *Atelopus* to threats like emerging pathogens (e.g., *Bd*) and climate change have yet to find a unified pattern across all species, but prior assessments suggest their elevation, habitat preference, and breeding behavior are likely factors (La Marca et al., 2005). Our evaluation of *Atelopus* rediscoveries shows that relict populations span the latitudinal and longitudinal distribution of the genus across Central and South America (Fig. 2). Further, the geographic density of rediscoveries closely matches species diversity across the distribution of the genus. For example, 80 % of described *Atelopus* species, and 88 % of rediscoveries, occur in four countries: Ecuador, Colombia, Peru, and Venezuela (Fig. 2D). This suggests broadly similar patterns of occasional population persistence throughout the range of the genus, possibly reflective of large-scale enzootic disease state shifts across Central-South America. However, we also found that Ecuador harbors a particularly high number of rediscovered species (i.e., twice as many as the next country, Colombia), perhaps due to non-biological explanations (e.g., geo-political differences in search effort or scientific funding between countries) or persistence being driven by regional differences in environmental factors and/or host responses.

Rediscoveries span the elevational range of *Atelopus*, from 120 m to above 3600 m (Fig. 2E). Scheffers et al. (2011) found that most rediscovered amphibians ( $N = 104$ ) were endemic to high elevations (mean elevation of 1199 m). Although *Atelopus* rediscoveries are not restricted to certain elevations, species that went missing at some point (i.e., ‘Rediscovered’ and ‘Still Missing’) are documented at higher historical mean elevations than species that have been continuously sighted. This finding corroborates prior work that suggests *Atelopus* species restricted to high elevations are especially vulnerable (La Marca et al., 2005), which is thought to be largely driven by higher *Bd* growth in cooler montane temperatures at high elevations (Woodhams et al., 2008).

At present, persistence does not seem to be caused by a single common variable across rediscovered *Atelopus* (e.g., reduced disease transmission at lower elevations). Of the 19 rediscovered species with



**Fig. 3.** Trends of genetic diversity in historical and contemporary rediscovered species. Estimated individual heterozygosity for Harlequin frog species with varying decline histories from genomic data. (a) Scatter plot of individual heterozygosity plotted against years missing for six species (from left to right): *Atelopus* sp. Limón (*spumarius* complex; purple), *A. sp. Otoyacu* (*spumarius* complex; blue), *A. elegans* (green), *A. balios* (yellow), *A. nanay* (orange), and *A. ignescens* (red). Linear trend line represents 0.95 CI (grey). Comparisons significant at an adjusted  $p < 0.001$  with a Tukey Honest Significant Difference test are indicated by letters in a, and  $p < 0.05$  in b. (b) Boxplot of individual heterozygosity for historical *A. nanay-bomolochos* and *A. ignescens* species group (grey) and contemporary *A. nanay* (orange) and *A. ignescens* (red). (c) Sampling map of Ecuador for the six species with matching colors in (a) and historical samples (grey triangles) and photos of *Atelopus ignescens* and *A. nanay* by KEJ. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

documented historical and contemporary elevations, we did not find a significant shift between historical and contemporary mean elevations (Fig. 2F; overall downward shift of 72 m), as we might expect if populations are persisting at elevations absent of threats. Reduction of niche breadth in amphibians impacted by *Bd* has been proposed by geographic refugia contractions to areas where *Bd* is absent (Puschendorf et al., 2009; Puschendorf et al., 2011; Scheele et al., 2017a). However, contractions might not be clearly detectable due to high recruitment from lower elevations to higher elevations where extirpation is more rapidly occurring (Muths et al., 2011; Lampo et al., 2012). Future studies that account for other variables that may be confounded here (e.g., upward shifts from habitat loss in some species, downward shifts from environmental refugia in others) may be able to uncover potential mechanisms underlying elevational patterns.

#### 4.2. Genomic diversity patterns in persisting populations

Neutral genetic variation has long been considered important for population persistence and viability (Lande and Shannon, 1996; Kardos et al., 2021) and can provide insights into a population's demographic

and evolutionary history, such as estimates of genetic drift, inbreeding, and genetic bottlenecks. Genomic data from a subset of *Atelopus* species with varying decline histories, elevations, and relatedness in the geographic epicenter of *Atelopus* rediscoveries (Ecuador), reveal a pattern of decreased heterozygosity the longer a species was considered missing/extinct (Fig. 3A; Table 1). Although correlative, 'time-missing' may serve as a useful proxy for the severity of small population problems (e.g., drift or inbreeding) in disappeared populations, which results in lower genomic diversity the longer they remain presumably extinct. The documentation of disappearances, such as years missing, may be a predictor of a population's loss of genomic diversity and a crucial asset for determining potential for recovery and/or future adaptive potential. Notably, the three significant species groups in our data in descending order of genomic variation also correspond to Vulnerable (Fig. 3A 'A'), Endangered/Critically Endangered (Fig. 3A 'BC'), and Critically Endangered/Extinct (before *A. ignescens* was rediscovered; Fig. 3A 'CD') IUCN Red List categories. Thus, rediscoveries help document the relationship between threat status and genomic variation, which has been empirically limited due to the lack of studies pairing genomic data with known decline histories.

Historical and contemporary comparisons for two species (*A. ignescens* and *A. nanay*) showed an overall decrease in heterozygosity in contemporary samples, with a significant difference in one species (*A. ignescens*) but not the other (*A. nanay*) ( $p < 0.05$ ; Fig. 3B). This work corroborates similar genomic diversity patterns found in relictual *A. varius* and *A. zeteki* populations in Central America (Byrne et al., 2021). We note our historical samples are limited to one sample per species and represent species group- versus population-level comparisons (see supplementary materials). Therefore, it is possible the contemporary population of *A. nanay* has also lost variation over time, which may have been detectable with larger historical sample sizes. However, this pattern could also reflect the time missing-heterozygosity relationship proposed above, as *A. ignescens* was missing for a decade longer than *A. nanay*.

Alternatively, observed heterozygosity patterns could also be due to idiosyncratic responses to *Bd* at the species-level. For example, the historical ranges (and therefore range contractions) of these species differed drastically (121 km<sup>2</sup> for *A. nanay*; 7790 km<sup>2</sup> for *A. ignescens*), and so their decline histories are likely not equal. *A. nanay* also occurs in parapatry with other closely related *Atelopus* (*A. bomolochos*, *A. exiguus*, *A. onorei*) and cryptic species diversity within *A. nanay* also likely exists (see supplementary materials), whereas contemporary *A. ignescens* is known from a single isolated locality from other known closely related highland species. Therefore, higher rates of historical and/or recent gene flow in one species could have influenced idiosyncratic responses to *Bd*, which has been proposed in *A. varius* and *A. zeteki* (Byrne et al., 2021). Future studies exploring species relationships and susceptibilities to *Bd* by combining neutral and functional genomic data on more populations and species within this group may help delineate the intra- and interspecific demographic and adaptive contributions towards our observed genetic diversity patterns.

We note two important caveats of our genomic data: small sample sizes, which are often a common problem in opportunistically sampled conservation studies, and especially so in recently rediscovered populations, and the lack of a causal link between time missing and heterozygosity. It is possible the correlation between heterozygosity and time missing is confounded by other variables we could not collect, such as pre-decline population size or shared historical demography from phylogenetic relatedness. Definitive conclusions about the cause of these patterns are not possible in our study. However, the association we found between disappearances and genomic diversity across contemporary populations of multiple species is an intriguing pattern worthy of future investigation and could inform management strategies needed for population recovery.

## 5. Recommendations for leveraging rediscoveries

### 5.1. Small population size dynamics in amphibians

Despite widespread amphibian declines, we still know relatively little about small population dynamics (demographic, genetic, and behavioral) and effective population sizes of anurans (Schmeller and Merila, 2007). An extreme example is the Hula Painted frog (*Latonina nigriventer*), which has persisted for >60 years in a single pond surrounded by drastic habitat loss and fragmentation (Biton et al., 2013). While some amphibian populations seem to be able to survive in isolated regions with little gene flow, it is unclear how such isolation will affect their ability to respond to novel changes in their environment. For example, it is unknown the extent to which adaptive potential differs between naturally small populations restricted by geographic range, to those with historically larger geographic ranges that have recently experienced habitat fragmentation or population bottlenecks. Some relictual populations may be more resilient to novel stressors and/or environmental change; however, we typically lack the ability to predict variation in response and rarely understand what variables contribute to these patterns. We suggest comparative studies across closely related

species that occupy different geographic ranges and decline histories. This framework has the power to elucidate population dynamics important to understanding declines and responses, such as demographic and genetic mechanisms that help or hinder responses. Harlequin frogs in the Andes provide an excellent opportunity to perform these studies, with species of varying geographic range sizes (Lötters, 2007; Catenazzi, 2015), drivers of declines (La Marca et al., 2005), and now, decline and persistence histories.

### 5.2. Host immune response

Applying genomic tools to rediscovered amphibians (reviewed in Funk et al., 2021) will help inform mechanisms underlying population persistence. For example, an outstanding question is the extent to which selection on a few important gene regions involved in immune response vs. high levels of standing genetic variation across the genome, or both, are characteristics of persisting populations. Distantly related species across the amphibian tree show varying immunological responses (Zamudio et al., 2020), such as MHC genotype associations with *Bd* susceptibility in *Lithobates yavapaiensis* (Savage and Zamudio, 2011), or host immune response suppression by *Bd* in other species, such as *Atelopus zeteki* (Ellison et al., 2014). These studies provide important foundations in understanding genomic responses to *Bd* across amphibians broadly, but with such mixed results across major amphibian clades, and so few clades explored, it remains difficult to identify genomic regions that are predictors of persistence in any given species (Storfer et al., 2021). Temporal studies that combine functional and neutral genomic data in a single group (e.g., *Atelopus*) will be particularly useful for elucidating variation in evolutionary history, ecology, and the environment for species that largely share the same ‘genomic machinery’. Byrne et al. (2021) serves as an ideal example for the potential insights to be gained from both research and conservation perspectives (e.g., the largest contemporary population had the highest gene flow and most recent admixture, potentially suggesting a rescue event underlying their persistence). Understanding how these genomic mechanisms influence population dynamics and stress responses will provide powerful insights into conservation applications to assist current and future rediscoveries (see Table 2 for recommended conservation priorities).

### 5.3. Host skin microbiome response

The amphibian skin microbiome plays an important role in immune defense (Rebollar et al., 2020; Rollins-Smith, 2020). Skin microbiome communities can vary between epizootic and enzootic host populations (Jani et al., 2017), and in the latter, across distantly related species with different life-history strategies (e.g., terrestrial vs aquatic breeding strategies; Jervis et al., 2021). Rediscovered populations provide an opportunity to understand how broad patterns that shape amphibian skin microbiomes (e.g., microhabitats, biogeography, phylogenetic distance, host genetic variation, environmental reservoirs, and *Bd* infection) influence microbiome structure in imperiled populations. For example, do skin microbiomes of closely related species show parallel shifts in microbiome communities due to selection imposed by a shared disease? How do factors that shape microbiomes (e.g., geographic distribution, host genetic variation, skin secretions, microhabitat sources, temperature, and habitat quality) influence the ability for microbiome shifts in small populations, which are likely experiencing these other pressures? *Atelopus* show some level of immune defense mediated by the microbiome (e.g., Flechas et al., 2012; Becker et al., 2015) and the ability for reintroduced individuals in outdoor mesocosms to shift and mimic a wild-type microbiome profile (Estrada et al., 2022). A recent study explicitly surveying several rediscovered species (non-*Atelopus*) suggests that these species potentially have a *Bd*-protective microbiome based on previous microbial taxa identified in other studies (Jiménez et al., 2019). The *Atelopus* rediscovery system provides a unique

**Table 2**  
Recommended conservation priorities to leverage rediscoveries.

Conservation priorities	Areas of interest	Examples
Exploration of missing populations/species and monitoring of known population for threats and defense mechanisms	<ul style="list-style-type: none"> <li>○ Innovative approaches to uncover additional populations of relict species</li> <li>○ Understanding population variation, size, and stability of rediscoveries to assess ongoing threats</li> </ul>	<ul style="list-style-type: none"> <li>○ Murray et al. (2009)</li> <li>○ García-Rodríguez et al. (2012)</li> <li>○ Lopes et al. (2021)</li> <li>○ Zumbado-Ulate et al. (2021)</li> </ul>
Ex-situ breeding programs for selective breeding and reintroduction efforts	<ul style="list-style-type: none"> <li>○ Increase genetic diversity of source population with goals of reintroduction</li> <li>○ Optimizing protocols for ex-situ breeding e.g., reproduction assays, food regimens, naturally mimicking mesocosm terraria</li> <li>○ Communicating importance of amphibian conservation to the public</li> </ul>	<ul style="list-style-type: none"> <li>○ Lewis et al. (2019)</li> <li>○ Naranjo et al. (2021)</li> <li>○ Amaru Bioparque (Cuenca, Ecuador) <a href="https://www.zoobioparqueamaru.com/">https://www.zoobioparqueamaru.com/</a></li> <li>○ Centro Jambatu (Quito, Ecuador) <a href="http://www.anfibiosecuador.ec">www.anfibiosecuador.ec</a></li> </ul>
Habitat protection	<ul style="list-style-type: none"> <li>○ Ensure long-term persistence from rediscovery to recovery</li> <li>○ Publicize cases of emblematic species to preserve habitat of local and indigenous communities</li> </ul>	<ul style="list-style-type: none"> <li>○ <i>Atelopus longirostris</i> lawsuit case against the Ministry of Environment of Ecuador for failed protection of the species in the presence of large-scale mining operations. <a href="https://es.mongabay.com/2020/10/ranas-le-ganan-la-mineria-en-ecuador/">https://es.mongabay.com/2020/10/ranas-le-ganan-la-mineria-en-ecuador/</a></li> <li>○ Valencia and da Fonte (2022)</li> </ul>
Developing community-led conservation and ecotourism programs	<ul style="list-style-type: none"> <li>○ Economic alternatives to habitat destruction for sources of income</li> <li>○ Scientific training and opportunities for members of local communities</li> </ul>	<ul style="list-style-type: none"> <li>○ Intag Santuario de Vida (Intag Life Sanctuary) <a href="https://rainforestactiongroup.org/es/santuario-for-life-in-ecuador/">https://rainforestactiongroup.org/es/santuario-for-life-in-ecuador/</a></li> <li>○ Alianza Jambato (<a href="http://www.alianzajambato.org">www.alianzajambato.org</a>)</li> </ul>

opportunity to understand the replicability of host-microbiome relationships under global change by investigating microbiome shifts across species, environments, and decline histories. Importantly, these studies will have direct benefits to studies considering “skin probiotic” applications to wild populations.

#### 5.4. *Bd* virulence and community disease dynamics

While the impacts of *Bd* have been documented across the globe for nearly three decades, we know relatively little about the continued impacts of *Bd* in persisting host populations. There has been wide variability in population decline and response rates to *Bd* in enzootic systems, with some species increasing in population size and others remaining low (Catenazzi et al., 2017; Lampo et al., 2017). Decreased pathogen virulence has not been demonstrated in natural systems (Scheele et al., 2017b). Yet, some *Atelopus* populations in Central America are persisting in regions where *Bd* is detected (Perez et al., 2014). There are many strains of *Bd* (Byrne et al., 2019), which have different levels of virulence (Greenspan et al., 2018) and hybridization between two strains can cause hyper-virulence (Farrer et al., 2011). Although *Bd* is considered a global pathogen, some regions remain

unaffected or have one specific strain. The related pathogen, *Batrachochytrium salamandrivorans* (*Bsal*; Martel et al., 2013) has not yet arrived in the Americas, but *BdxBsal* infections cause worse infections in salamanders than *Bd* or *Bsal* alone (McDonald et al., 2020). Altogether, understanding the eco-evolutionary dynamics of this disease in collapsed and persisting populations will lend insights into future conservation relevant to population responses in unexposed regions (e.g., salamanders in North and Central America; García-Rodríguez et al., 2022), as well as arrival of new strains in other regions (e.g., new global pandemic lineages or hybridization events).

The impacts of *Bd*-driven declines in amphibian populations include community dynamics beyond amphibians. In Central America, snake (predator) populations declined, while malaria incidence in humans from mosquitos (prey) increased following the collapse of amphibian communities after *Bd* arrival (Zipkin et al., 2020; Springborn et al., 2022). In the disease triangle framework, shifting disease dynamics has effects on host and communities, which in turn affect host response through infection success, predation changes, and so forth. Future work will benefit by simultaneously increasing resolution of the disease (strain-specific sampling) and broadening the targets affected by it (amphibian and non-amphibian communities) in these systems.

## 6. Conclusions

Understanding the mechanisms that allow vulnerable species to persist in environments where threats occur requires novel approaches across different axes of investigation. Species rediscoveries serve as ‘natural experiments’ to uncover mechanisms underlying responses to novel changes in natural populations. Despite these promising examples of species persistence in the genus *Atelopus*, rediscovery does not mean recovery, and many rediscovered species are likely still hovering on the brink of extinction. Although we reported observed differences in genomic variation, it remains broadly unknown if the differences are linked to an adaptive response, or how any of these rediscovered species are positioned to continue persisting in the wild. Future research in this system will be important for understanding mechanisms and consequences of persistence through novel change, with potential widespread impacts in empirical global change biology research.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2022.109784>.

### CRediT authorship contribution statement

KEJ designed data and review synthesis. KEJ, LAC, AT-V, and JMG collated species information. LAC provided contemporary samples for genomic analysis. KEJ performed lab work, bioinformatic, and data analysis. KEJ wrote the initial manuscript with input from SWF and MIP and all authors made contributions to the final manuscript.

### Ethics statement

All animals were collected and analyzed following Ecuadorian legislation (MAE-DNB-CM-2019-0115). All work involving animals was approved by IACUC (PROTO201900341) at Michigan State University.

### Declaration of competing interest

The authors declare no conflict of interest and confirm that submitted work adheres to all ethical guidelines of the journal *Biological Conservation*. The authors appropriately acknowledge all funding sources and other contributions to the work, which adheres to ethical standards and necessary approvals for all work involving animals (IACUC-PROTO201900341 at MSU).

## Data availability

Raw sequences are archived in the NCBI SRA (BioProject: PRJNA870111; accession numbers: SAMN30351361-SAMN30351399).

## Acknowledgements

Museum samples were provided by the Herpetology Division of the University of Kansas Biodiversity Institute. We thank Madison Miller for help with laboratory work. KEJ was funded by National Geographic Society Early Career Grant EC-60150R-19. Sequencing was funded by an Illumina-Michigan State University Partnership grant awarded to KEJ and SWF and Universidad Tecnológica Indoamérica funds awarded to MIP. Research by JMG was supported by the USFQ Collaboration Grant HUBI 16871, SENESCYT (INEDITA PIC-20-INE-USFQ-001), and Amphibian Survival Alliance Conservation Grant. Centro Jambatu researchers thank Jeff Bonner, Lisa Kelley, Lauren Augustine, and Mark Wanner (Saint Louis Zoo), and María Dolores Guarderas (Wikiri), for their commitment and sustained support for amphibian research. We thank multiple anonymous reviewers for thoughtful suggestions on earlier versions of this manuscript. This is Kellogg Biological Station contribution #2331.

## References

- Ali, O.A., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., Miller, M.R., 2016. RAD capture (Rapture): flexible and efficient sequence-based genotyping. *Genetics* 202 (2), 389–400. <https://doi.org/10.1534/genetics.115.183665>.
- Barrio Amorós, C.L., Costales, M., Vieira, J., Osterman, E., Kaiser, H., Arteaga, A., 2020. Back from extinction: rediscovery of the Harlequin toad *Atelopus mindoensis* Peters, 1973 in Ecuador. *Herpetol. Notes* 13, 325–328.
- Becker, M.H., Walke, J.B., Cikanek, S., Savage, A.E., Mattheus, N., Santiago, C.N., Gratwicke, B., 2015. Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. *Proc. R. Soc. B Biol. Sci.* 282, 20142881. <https://doi.org/10.1098/rspb.2014.2881>.
- Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Louise Goggin, C., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proc. Natl. Acad. Sci.* 95 (15), 9031–9036. <https://doi.org/10.1073/pnas.95.15.9031>.
- Biton, R., Geffen, E., Vences, M., Cohen, O., Bailon, S., Rabinovich, R., Gafny, S., 2013. The rediscovered Hula painted frog is a living fossil. *Nat. Commun.* 4, 1959. <https://doi.org/10.1038/ncomms2959>.
- Black, J., 1982. Los páramos de Antisana. Instituto Geográfico Militar. *Rev. Geogr.* 17, 25–52.
- Bolom-Huet, R., Pineda, E., Díaz-Fleischer, F., Muñoz-Alonso, A.L., Galindo-González, J., 2019. Known and estimated distribution in Mexico of *Batrachochytrium dendrobatidis*, a pathogenic fungus of amphibians. *Biotropica* 51 (5), 731–746. <https://doi.org/10.1111/btp.12697>.
- Bonaccorso, E., Guayasamin, J.M., Mendez, D., Speare, R., 2003. Chytridiomycosis as a possible cause of population declines in *Atelopus cruciger* (Anura: Bufonidae). *Herpetol. Rev.* 34 (4), 331–333.
- Briggs, C.J., Knapp, R.A., Vredenburg, V.T., 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Natl. Acad. Sci.* 107 (21), 9695–9700. <https://doi.org/10.1073/pnas.0912886107>.
- Bustamante, M.R., Ron, S.R., Coloma, L.A., 2005. Cambios en la diversidad en siete comunidades de anuros en los Andes de Ecuador. *Biotropica* 37 (2), 180–189. <https://doi.org/10.1111/j.1744-7429.2005.00025.x>.
- Byrne, A.Q., Vredenburg, V.T., Martel, A., Pasmans, F., Bell, R.C., Blackburn, D.C., Rosenblum, E.B., 2019. Cryptic diversity of a widespread global pathogen reveals expanded threats to amphibian conservation. *Proc. Natl. Acad. Sci.* 116 (41), 20382–20387. <https://doi.org/10.1073/pnas.1908289116>.
- Byrne, A.Q., Richards-Zawacki, C.L., Voyles, J., Bi, K., Ibáñez, R., Rosenblum, E.B., 2021. Whole exome sequencing identifies the potential for genetic rescue in iconic and critically endangered Panamanian harlequin frogs. *Glob. Chang. Biol.* 27 (1), 50–70. <https://doi.org/10.1111/gcb.15405>.
- Catenazzi, A., 2015. State of the world's amphibians. *Annu. Rev. Environ. Resour.* 40, 91–119. <https://doi.org/10.1146/annurev-environ-102014-021358>.
- Catenazzi, A., Swei, A., Finkle, J., Foreyt, E., Wyman, L., Vredenburg, V.T., 2017. Epizootic to enzootic transition of a fungal disease in tropical Andean frogs: are surviving species still susceptible? *PLoS One* 12 (10), e0186478. <https://doi.org/10.1371/journal.pone.0186478>.
- Chaves, G., Zumbado-Ulate, H., García-Rodríguez, A., Gómez, E., Vredenburg, V.T., Ryan, M.J., 2014. Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica. *Trop. Conserv. Sci.* 7 (4), 628–638. <https://doi.org/10.1177/19400829140070404>.
- Collen, B., Purvis, A., Mace, G.M., 2010. When is a species really extinct? Testing extinction inference from a sighting record to inform conservation assessment.

- Divers. Distrib.* 16 (5), 755–764. <https://doi.org/10.1111/j.1472-4642.2010.00689.x>.
- Coloma, L.A., 2016. El Jambato negro del páramo, *Atelopus ignescens*, resucitó. *IMCienca*. [https://issuu.com/centrojambatu/docs/coloma\\_2016\\_el\\_jambato\\_negro\\_del\\_p](https://issuu.com/centrojambatu/docs/coloma_2016_el_jambato_negro_del_p).
- Coloma, L.A., Duellman, W.E., Almandáriz, C., Ron, S.R., Terán-Valdez, A., Guayasamin, J.M., 2010. Five new (extinct?) species of *Atelopus* (Anura:Bufonidae) from Andean Colombia, Ecuador, and Peru. *Zootaxa* 2574, 1–54. <https://doi.org/10.11646/zootaxa.2574.1.1>.
- Cushman, S.A., 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biol. Conserv.* 128 (2), 231–240. <https://doi.org/10.1016/j.biocon.2005.09.031>.
- Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Durbin, R., 2011. The variant call format and VCFtools. *Bioinformatics* 27 (15), 2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>.
- DiRenzo, G.V., Zipkin, E.F., Campbell Grant, E.H., Royle, A.J., Longo, A.V., Zamudio, K.R., Lips, K.R., 2018. Eco-evolutionary rescue promotes host-pathogen coexistence. *Ecol. Appl.* 28 (8), 1948–1962. <https://doi.org/10.1002/eap.1792>.
- Ellison, A.R., Savage, A.E., DiRenzo, G.V., Langhammer, P., Lips, K.R., Zamudio, K.R., 2014. Fighting a losing battle: vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. *G3: Genes, Genomes, Genetics* 4 (7), 1275–1289. <https://doi.org/10.1534/g3.114.010744>.
- Ellison, A.R., Tunstall, T., DiRenzo, G.V., Hughey, M.C., Rebolgar, E.A., Belden, L.K., Zamudio, K.R., 2015. More than skin deep: functional genomic basis for resistance to amphibian chytridiomycosis. *Genome Biol. Evol.* 7 (1), 286–298. <https://doi.org/10.1093/gbe/evu285>.
- Estrada, A., Medina, D., Gratwicke, B., Ibáñez, R., Belden, L.K., 2022. Body condition, skin bacterial communities and disease status: insights from the first release trial of the Limosa harlequin frog, *Atelopus limosus*. *Proc. R. Soc. B Biol. Sci.* 289, 20220586. <https://doi.org/10.1098/rspb.2022.0586>.
- Farrer, R.A., Weinert, L.A., Bielby, J., Garner, T.W.J., Ballou, F., Clare, F., Fisher, M.C., 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proc. Natl. Acad. Sci.* 108 (46), 18732–18736. <https://doi.org/10.1073/pnas.1111915108>.
- Fey, S.B., Siepelski, A.M., Nussle, S., Cervantes-Yoshida, K., Hwan, J.L., Huber, E.R., Carlson, S.M., 2015. Recent shifts in the occurrence, cause, and magnitude of animal mass mortality events. *Proc. Natl. Acad. Sci.* 112 (4), 1083–1088. <https://doi.org/10.1073/pnas.1414894112>.
- Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484, 186–194. <https://doi.org/10.1038/nature10947>.
- Flechas, S.V., Sarmiento, C., Cárdenas, M.E., Medina, E.M., Restrepo, S., Amézquita, A., 2012. Surviving chytridiomycosis: differential anti-batrachochytrium dendrobatidis activity in bacterial isolates from three lowland species of *Atelopus*. *PLoS ONE* 7 (9), e44832. <https://doi.org/10.1371/journal.pone.0044832>.
- Funk, C.W., Zamudio, K.R., Crawford, A.J., 2021. Advancing understanding of amphibian evolution, ecology, behavior, and conservation with massively parallel sequencing. In: Hohenlohe, P.A., Rajora, O.P. (Eds.), *Population Genomics: Wildlife*. Springer, pp. 211–254.
- García-Rodríguez, A., Chaves, G., Benavides-Varela, C., Puschendorf, R., 2012. Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Divers. Distrib.* 18 (2), 204–212. <https://doi.org/10.1111/j.1472-4642.2011.00862.x>.
- García-Rodríguez, A., Basanta, M.D., García-Castillo, M.G., Zumbado-Ulate, H., Neam, K., Rovito, S., Parra-Olea, G., 2022. Anticipating the potential impacts of *Batrachochytrium salamandrivorans* on neotropical salamander diversity. *Biotropica* 54 (1), 157–169. <https://doi.org/10.1111/btp.13042>.
- González-Maya, J.F., Belant, J.L., Wyatt, S.A., Schipper, J., Cardenal, J., Corrales, D., Fischer, A., 2013. Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica. *Amphibia-Reptilia* 34, 573–578. <https://doi.org/10.1163/15685381-00002910>.
- Greenspan, S.E., Lambertini, C., Carvalho, T., James, T.Y., Toledo, L.F., Haddad, C.F.B., Becker, C.G., 2018. Hybrids of amphibian chytrid show high virulence in native hosts. *Sci. Rep.* 8, 9600. <https://doi.org/10.1038/s41598-018-27828-w>.
- Guayasamin, J.M., Mendoza, A.M., Longo, A.V., Zamudio, K.R., Bonaccorso, E., 2014. High prevalence of *Batrachochytrium dendrobatidis* in an Andean frog community (Reserva las Galarias, Ecuador). *Amphib. Reptile Conserv.* 8 (1), 33–44.
- IUCN, 2021. The IUCN Red List of Threatened Species. Version 2021-1. <https://www.iucnredlist.org>. Downloaded on [13 August 2021].
- James, T.Y., Toledo, L.F., Rödder, D., da Silva Leite, D., Belasen, A.M., Betancourt-Román, C.M., Longcore, J.E., 2015. Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. *Ecol. Evol.* 5 (18), 4079–4097. <https://doi.org/10.1002/ece3.1672>.
- Jani, A.J., Knapp, R.A., Briggs, C.J., 2017. Epidemic and endemic pathogen dynamics correspond to distinct host population microbiomes at a landscape scale. *Proc. R. Soc. B Biol. Sci.* 284, 20170944. <https://doi.org/10.1098/rspb.2017.0944>.
- Jervis, P., Pintanel, P., Hopkins, K., Wierzbicki, C., Shelton, J.M.G., Skelly, J., Fisher, M.C., 2021. Post-epizootic microbiome associations across communities of neotropical amphibians. *Mol. Ecol.* 30 (5), 1322–1335. <https://doi.org/10.1111/mec.15789>.
- Jiménez de la Espada, M., 1875. Vertebrados del viaje al Pacífico verificado de 1862–1865 por una comisión de naturalistas enviada por el Gobierno Español. *Batracios*. Imprenta Miguel Ginesta, Madrid, Spain.
- Jiménez, R.R., Alvarado, G., Estrella, J., Sommer, S., 2019. Moving beyond the host: unraveling the skin microbiome of endangered Costa Rican amphibians. *Front. Microbiol.* 10, 2060. <https://doi.org/10.3389/fmicb.2019.02060>.

- Jiménez-Monge, A., Montoya-Greenheck, F., Bolaños, F., Alvarado, G., 2019. From incidental findings to systematic discovery: locating and monitoring a new population of the endangered Harlequin toad. *Amphib. Reptile Conserv.* 13 (2), 115–125.
- Kardos, M., Armstrong, E.E., Fitzpatrick, S.W., Hauser, S., Hedrick, P.W., Miller, J.M., Funk, C.W., 2021. The crucial role of genome-wide genetic variation in conservation. *Proc. Natl. Acad. Sci.* 118 (48), e2104642118 <https://doi.org/10.1073/pnas.2104642118>.
- La Marca, E., Lips, K.R., Lötters, S., Puschendorf, R., Ibañez, R., Rueda-Almonacid, J.V., Young, B.E., 2005. Catastrophic population declines and extinctions in neotropical Harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37 (2), 190–201. <https://doi.org/10.1111/j.1744-7429.2005.00026.x>.
- LaBumbard, B.C., Shepack, A., Catenazzi, A., 2020. After the epizootic: host-pathogen dynamics in montane tropical amphibian communities with the high prevalence of chytridiomycosis. *Biotropica* 52 (6), 1194–1205. <https://doi.org/10.1111/btp.12824>.
- Lampo, M., Celsa, S.J., Rodríguez-Contreras, A., Rojas-Runjaic, F., García, C.Z., 2012. High turnover rates in remnant populations of the Harlequin frog *Atelopus cruciger* (Bufonidae): low risk of extinction? *Biotropica* 44 (3), 420–426. <https://doi.org/10.1111/j.1744-7429.2011.00830.x>.
- Lampo, M., Señaris, C., García, C.Z., 2017. Population dynamics of the critically endangered toad *Atelopus cruciger* and the fungal disease chytridiomycosis. *PLoS ONE* 12 (6), e0179007. <https://doi.org/10.1371/journal.pone.0179007>.
- Lande, R., Shannon, S., 1996. The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* 50 (1), 434–437. <https://doi.org/10.2307/2410812>.
- Lewis, C.H.R., Richards-Zawacki, C.L., Ibañez, R., Luedtke, J., Voyles, J., Houser, P., Gratwicke, B., 2019. Conserving Panamanian harlequin frogs by integrating captive-breeding and research programs. *Biol. Conserv.* 236, 180–187. <https://doi.org/10.1016/j.biocon.2019.05.029>.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., 1000 Genome Project Data Processing Subgroup, 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25 (6), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
- Lips, K.R., 1998. Decline of a tropical montane amphibian fauna. *Conserv. Biol.* 12 (1), 106–117. <https://doi.org/10.1111/j.1523-1739.1998.96359.x>.
- Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proc. Natl. Acad. Sci.* 103 (9), 3165–3170. <https://doi.org/10.1073/pnas.0506889103>.
- Longcore, J.E., Pessier, A.P., Nichols, D.K., 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91 (2), 219–227. <https://doi.org/10.1080/00275514.1999.12061011>.
- Lopes, C.M., Baêta, D., Valentini, A., Lyra, M.L., Sabbag, A.F., Gasparini, J.L., Zamudio, K.R., 2021. Lost and found: frogs in a biodiversity hotspot rediscovered with environmental DNA. *Mol. Ecol.* 30 (13), 3289–3298. <https://doi.org/10.1111/mec.15594>.
- Lötters, S., 1996. In: Vences, M., Glaw, F. (Eds.), *The Neotropical Toad Genus Atelopus*. Verlags GBR, Cologne, Germany.
- Lötters, S., 2007. The fate of the harlequin toads – help through a synchronous multidisciplinary approach and the IUCN ‘amphibian conservation action plan’? *Zoosyst. Evol.* 83 (S1), 69–73. <https://doi.org/10.1002/mmnz.200600028>.
- Mace, G.M., Collar, N.J., Gaston, K.J., Hilton-Taylor, C., Akcakaya, H.R., Leader-Williams, N., Stuart, S.N., 2008. Quantification of extinction risk: IUCN’s system for classifying threatened species. *Conserv. Biol.* 22, 1424–1442. <https://doi.org/10.1111/j.1523-1739.2008.01044.x>.
- Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M.C., Pasmans, F., 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences* 110 (38), 15325–15329. <https://doi.org/10.1073/pnas.1307356110>.
- McCaffery, R., Richards-Zawacki, C.L., Lips, K.R., 2015. The demography of *Atelopus* decline: harlequin frog survival and abundance in Central Panama prior to and during a disease outbreak. *Glob. Ecol. Conserv.* 4, 232–242. <https://doi.org/10.1016/j.gecco.2015.07.003>.
- McCallum, M.L., 2007. Amphibian decline or extinction? Current declines dwarf background extinction rate. *J. Herpetol.* 41 (3), 483–491. [https://doi.org/10.1670/0022-1511\(2007\)41\[483:ADOECD\]2.0.CO;2](https://doi.org/10.1670/0022-1511(2007)41[483:ADOECD]2.0.CO;2).
- McDonald, C.A., Longo, A.V., Lips, K.R., Zamudio, K.R., 2020. Incapacitating effects of fungal coinfection in a novel pathogen system. *Mol. Ecol.* 29 (17), 3173–3186. <https://doi.org/10.1111/mec.15452>.
- Mendelson, J.R., Whitfield, S.M., Sredl, M.J., 2019. A recovery engine strategy for amphibian conservation in the context of disease. *Biol. Conserv.* 236, 188–191. <https://doi.org/10.1016/j.biocon.2019.05.025>.
- Murray, K.A., Skerratt, L.F., Speare, R., McCallum, H., 2009. Impact and dynamics of disease in species threatened by the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *Conserv. Biol.* 23 (5), 1242–1252. <https://doi.org/10.1111/j.1523-1739.2009.01211.x>.
- Muths, E., Scherer, R.D., Pilliod, D.S., 2011. Compensatory effects of recruitment and survival when amphibian populations are perturbed by disease. *J. Appl. Ecol.* 48 (4), 873–879. <https://doi.org/10.1111/j.1365-2664.2011.02005.x>.
- Naranjo, R.E., Naydenova, E., Proaño-Bolaños, C., Vizuete, K., Debut, A., Arias, M.T., Coloma, L.A., 2021. Development of assisted reproductive technologies for the conservation of *Atelopus* sp. (*spumarius* complex). *Cryobiology* 105, 20–31. <https://doi.org/10.1016/j.cryobiol.2021.12.005>.
- O’Hanlon, S.J., Rieux, A., Farrer, R.A., Rosa, G.M., Waldman, B., Bataille, A., Fisher, M.C., 2018. Recent asian origin of chytrid fungi causing global amphibian declines. *Science* 360 (6389), 621–627. <https://doi.org/10.1126/science.aar1965>.
- Perez, R., Richards-Zawacki, C.L., Krohn, A.R., Robak, M., Griffith, E.J., Ross, H., Voyles, J., 2014. Field surveys in Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. *Amphib. Reptile Conserv.* 8 (2), 30–35.
- Peters, J.A., 1973. The frog genus *Atelopus* in Ecuador (Anura: Bufonidae). *Smithsonian Contrib. Zool.* 145, 1–49.
- Piotrowski, J.S., Annis, S.L., Longcore, J.E., 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96, 9–15. <https://doi.org/10.1080/15572536.2005.11832990>.
- Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., Young, B.E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439, 161–167. <https://doi.org/10.1038/nature04246>.
- Puschendorf, R., Carnaval, A.C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F., Alford, R.A., 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Divers. Distrib.* 15 (3), 401–408. <https://doi.org/10.1111/j.1472-4642.2008.00548.x>.
- Puschendorf, R., Hoskin, C.J., Cashins, S.D., McDonald, K., Skerratt, L.F., Vanderwal, J., Alford, R.A., 2011. Environmental refuge from disease-driven amphibian extinction. *Conserv. Biol.* 25 (5), 956–964. <https://doi.org/10.1111/j.1523-1739.2011.01728.x>.
- R Development Core Team, 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, R Foundation for Statistical Computing <https://www.R-project.org>.
- Rebollar, E.A., Martínez-Ugalde, E., Orta, A.H., 2020. The amphibian skin microbiome and its protective role against chytridiomycosis. *Herpetologica* 76 (2), 167–177. <https://doi.org/10.1655/0018-0831-76.2.167>.
- Richards, S.J., McDonald, K.R., Alford, R.A., 1994. Declines in populations of Australia’s endemic tropical rainforest frogs. *Pac. Conserv. Biol.* 1 (1), 66–77. <https://doi.org/10.1071/PC930066>.
- Richards-Zawacki, C.L., 2010. Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proc. R. Soc. B* 277, 519–528. <https://doi.org/10.1098/rspb.2009.1656>.
- Roberts, D.L., 2006. Extinct or possibly extinct? *Science* 312 (5776), 997–998. <https://doi.org/10.1126/science.312.5776.997c>.
- Rochette, N.C., Rivera-Colón, A.G., Catchen, J.M., 2019. Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. *Mol. Ecol.* 28 (21), 4737–4754. <https://doi.org/10.1111/mec.15253>.
- Rollins-Smith, L.A., 2020. Global amphibian declines, disease, and the ongoing battle between *Batrachochytrium* fungi and the immune system. *Herpetologica* 76 (2), 178–188. <https://doi.org/10.1655/0018-0831-76.2.178>.
- Ron, S.R., Duellman, W.E., Coloma, L.A., Bustamante, M., 2003. Population decline of the Jambato toad *Atelopus ignescens* (Anura: Bufonidae) in the Andes of Ecuador. *J. Herpetol.* 37 (1), 116–126.
- Savage, A.E., Zamudio, K.R., 2011. MHC genotypes associate with resistance to a frog-killing fungus. *Proc. Natl. Acad. Sci.* 108 (40), 16705–16710. <https://doi.org/10.1073/pnas.1106893108>.
- Scheele, B.C., Foster, C.N., Banks, S.C., Lindenmayer, D.B., 2017a. Niche contractions in declining species: mechanisms and consequences. *Trends Ecol. Evol.* 32 (5), 346–355. <https://doi.org/10.1016/j.tree.2017.02.013>.
- Scheele, B.C., Skerratt, L.F., Grogan, L.F., Hunter, D.A., Clemann, N., McFadden, M., Berger, L., 2017b. After the epidemic: ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis. *Biol. Conserv.* 206, 37–46. <https://doi.org/10.1016/j.biocon.2016.12.010>.
- Scheele, B.C., Pasmans, F., Berger, L., Skerratt, L.F., Martel, A., Beukema, W., Canessa, S., 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363 (6434), 1459–1463. <https://doi.org/10.1126/science.aav0379>.
- Scheele, B.C., Hollanders, M., Hoffmann, E.P., Newell, D.A., Lindenmayer, D.B., McFadden, M., Grogan, L.F., 2021. Conservation translocations for amphibian species threatened by chytrid fungus: a review, conceptual framework, and recommendations. *Conserv. Sci. Pract.* 3 (11), e534 <https://doi.org/10.1111/csp2.524>.
- Scheffers, B.R., Yong, D.L., Harris, J.B.C., Giam, X., Sodhi, N.S., 2011. The world’s rediscovered species: back from the brink? *PLoS ONE* 6 (7), e22531. <https://doi.org/10.1371/journal.pone.0022531>.
- Schmeller, D.S., Merila, J., 2007. Demographic and genetic estimates of effective population and breeding size in the amphibian *Rana temporaria*. *Conserv. Biol.* 21 (1), 142–151. <https://doi.org/10.1111/j.1523-1739.2006.00554.x>.
- Scholthof, K.B., 2007. The disease triangle: pathogens, the environment and society. *Nat. Rev. Microbiol.* 5, 152–156. <https://doi.org/10.1038/nrmicro1596>.
- Scott, J.M., Ramsey, F.L., Lammertink, M., Rosenberg, K.V., Rohrbaugh, R., Wiens, J.A., Reed, J.M., 2008. When is an ‘extinct’ species really extinct? Gauging the search efforts for Hawaiian forest birds and the Ivory-billed woodpecker. *Avian Conserv. Ecol.* 3 (2), 3.
- Springborn, M.R., Weill, J.A., Lips, K.R., Ibañez, R., Ghosh, A., 2022. Amphibian collapses increased malaria incidence in Central America. *Environ. Res. Lett.* 17 (10), 104012 <https://doi.org/10.1088/1748-9326/ac8e1d>.
- Storfer, A., Kozakiewicz, C.P., Beer, M.A., Savage, A.E., 2021. Applications of population genomics for understanding and mitigating wildlife disease. In: Hohenlohe, P.A., Rajara, O.P. (Eds.), *Population Genomics: Wildlife*. Springer, pp. 357–383.

- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306 (5702), 1783–1786. <https://doi.org/10.1126/science.1103538>.
- Tapia, E.E., Coloma, L.A., Pazmiño-Otamendi, G., Peñafiel, N., 2017. Rediscovery of the nearly extinct Longnose harlequin frog *Atelopus longirostris* (Bufonidae) in Junín, Ecuador. *Neotropical Biodiversity* 3 (1), 157–167. <https://doi.org/10.1080/23766808.2017.1327000>.
- Valencia, L., da Fonte, M., 2022. Collaborative work brings hope for threatened harlequin toads. *Oryx* 56 (1), 12. <https://doi.org/10.1017/S0030605321001319>.
- Von May, R., Catenazzi, A., Angulo, A., Brown, J.L., Carrillo, J., Chávez, G., Twomey, E., 2008. Current state of conservation knowledge on threatened amphibian species in Peru. *Trop. Conserv. Sci.* 1 (4), 376–396. <https://doi.org/10.1177/194008290800100406>.
- Voyles, J., Woodhams, D.C., Saenz, V., Byrne, A.Q., Perez, R., Rios-Sotelo, G., Richards-Zawacki, C.L., 2018. Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. *Science* 359 (6383), 1517–1519. <https://doi.org/10.1126/science.aao4806>.
- Vredenburg, V.T., Knapp, R.A., Tunstall, T.S., Briggs, C.J., 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proc. Natl. Acad. Sci.* 107 (21), 9689–9694. <https://doi.org/10.1073/pnas.0914111107>.
- Whitfield, S.M., Alvarado, G., Abarca, J., Zumbado-Ulate, H., Zuniga, I., Wainwright, M., Kerby, J., 2017. Differential patterns of *Batrachochytrium dendrobatidis* infection in relict amphibian populations following severe disease-associated declines. *Dis. Aquat. Org.* 126 (1), 33–41. <https://doi.org/10.3354/dao03154>.
- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. ISBN 978-3-319-24277-4. Springer-Verlag, New York. <https://ggplot2.tidyverse.org>.
- Woodhams, D.C., Alford, R.A., Briggs, C.J., Johnson, M., Rollins-Smith, L.A., 2008. Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. *Ecology* 89 (6), 1627–1639. <https://doi.org/10.1890/06-1842.1>.
- Zamudio, K.R., McDonald, C.A., Belasen, A.M., 2020. High variability in infection mechanisms and host responses: a review of functional genomic studies of amphibian chytridiomycosis. *Herpetologica* 76 (2), 189–200. <https://doi.org/10.1655/0018-0831-76.2.189>.
- Zipkin, E.F., DiRenzo, G.V., Ray, J.M., Rossman, S., Lips, K.R., 2020. Tropical snake diversity collapses after widespread amphibian loss. *Science* 367 (6479), 814–816. <https://doi.org/10.1126/science.aay5733>.
- Zumbado-Ulate, H., Nelson, K.N., García-Rodríguez, A., Chaves, G., Arias, E., Bolaños, F., Searle, C.L., 2019. Endemic infection of *Batrachochytrium dendrobatidis* in Costa Rica: implications for amphibian conservation at regional and species level. *Diversity* 11 (8), 129. <https://doi.org/10.3390/d11080129>.
- Zumbado-Ulate, H., Searle, C.L., Chaves, G., Acosta-Chaves, V., Shepack, A., Salazar, S., García-Rodríguez, A., 2021. Assessing suitable habitats for treefrog species after previous declines in Costa Rica. *Diversity* 13 (11). <https://doi.org/10.3390/d13110577>.