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Ranavirus is widespread in Costa Rica and co-occurs with threatened amphibians

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ABSTRACT: Amphibians are globally threatened by emerging infectious diseases, and ranaviruses are among the most concerning pathogens to threaten species in the wild. We sampled for ranaviruses in wild amphibians at 8 sites in Costa Rica, spanning broad climatic zones and taxonomic associations. Seven of these sites are inhabited by highly threatened amphibian species that persist at low global population sizes after population declines due to amphibian chytridiomycosis. One of the surveyed sites is occupied by an introduced amphibian species, which is relatively rare in Central America but may be an important pathway for long-distance transport of ranaviruses. We detected ranavirus using quantitative polymerase chain reaction in 16.3% of the 243 individuals and among 5 of our 8 sites, but not at the site with the introduced species. Infection prevalence varied among species and sites, but not with mean annual temperature or mean annual precipitation. Infection intensity did not vary with species, site, temperature, or precipitation. Our results show that ranavirus infection is spatially widespread in Costa Rica, affecting a broad range of host species, and occurs across climatic zones — though we encountered no mortality or morbidity in our sampled species. Ranaviruses are known to cause intermittent mass mortality in amphibian populations, and the threatened species sampled here are likely vulnerable to population impacts from emerging ranaviruses. Therefore, we believe the potential impacts of ranaviruses on amphibian populations in tropical regions have likely been underestimated, and that they should be viewed as a potential major stressor to threatened amphibians in tropical regions.

KEY WORDS: Iridovirus · Emerging infectious diseases · Frogs · Pathogen surveillance · Central America · Tropical forest

1. INTRODUCTION

Global amphibian biodiversity is threatened by emerging infectious diseases, which have driven widespread population declines, extreme range contractions, and global extinctions (Stuart et al. 2004, Lips et al. 2006, Rosenblum et al. 2013, Scheele et al. 2019). Most emerging infectious disease research concerning amphibians has focused on chytridiomycosis, a potentially lethal infection associated with chytrid-

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*Corresponding author: steven.whitfield@miamidade.gov iomycete fungi of the genus *Batrachochytrium* (Lips et al. 2006, Martel et al. 2013, 2014, Scheele et al. 2019). However, diseases caused by ranaviruses are also of growing concern for amphibian populations worldwide (Chinchar 2002, Gray et al. 2009, Schloegel et al. 2010, Lesbarrères et al. 2012, Price et al. 2014).

In contrast to the directional and sustained amphibian population declines driven by amphibian chytridiomycosis, ranaviruses have classically been viewed as regulating population cycles in amphibian populations rather than causing sustained directional population declines (Gray et al. 2009, Brunner et al. 2015). Recently however, ranaviral disease has been linked to mass mortality events and range contractions in European amphibians (Price et al. 2014), raising concern that diseases from ranaviruses could be important drivers of further decline in amphibian communities already devastated by chytridiomycosis (García-Rodríguez et al. 2012, Whitfield et al. 2017, Zumbado-Ulate et al. 2019, 2021). Ranaviruses can clearly cause high mortality in amphibians (Gray et al. 2009, Brunner et al. 2015), even when population declines are not sustained.

Central America is a global hotspot for amphibian biodiversity and endemism, as well as for amphibian species threatened by emerging infectious disease (Stuart et al. 2004, Lips et al. 2005, Whitfield et al. 2016, Scheele et al. 2019, Zumbado-Ulate et al. 2021). Ranaviruses are known to occur in Central America, though geographic distribution, host range, and impacts to amphibian populations remain unclear. Speare et al. (1991) detected a virus in the family *Iridioviridae* (which includes ranaviruses) in a wild giant toad *Rhinella horribilis* as early as 1991. Puschendorf et al. (2019) conducted a retrospective analysis of museum specimens and detected a ranavirus in 1 of 4 *Craugastor ranoides* from northwestern Costa Rica collected in 1987. They also showed contemporary infection in this species. Whitfield et al. (2012, 2013) detected ranavirus using quantitative polymerase chain reaction (qPCR) at a single site in the lowlands of Costa Rica, and detected co-infection with the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), but did not assess impacts to individuals or populations. Stark et al. (2014) reported a mass mortality event associated with ranavirus in southern Nicaragua — the first mortality event known from Central America. It remains unclear whether ranaviruses in Central America are enzootic pathogens with which hosts evolved, or whether they have been recently introduced to the region via trade in live amphibians (Picco & Collins 2008, Schloegel et al. 2009, 2010, Schock et al. 2009). Consequently, the conservation threat posed by ranaviruses in Central America (and similar tropical environments) is difficult to evaluate.

Following large scale population declines associated with *Bd*, many highly threatened amphibian species are known to persist in small, geographically isolated relict populations (Abarca et al. 2010, García-Rodríguez et al. 2012, González-Maya et al. 2013, Puschendorf et al. 2013, Chaves et al. 2014, Whitfield et al. 2016). These relict populations are of very high value to conservation as the last remaining individuals of their species. However, these populations are inherently vulnerable because of their small size, isolation, probable limited genetic diversity, and continued potential threat from *Bd*. These populations are likely vulnerable to any significant population disturbance — potentially including ranaviruses.

Here, we conduct pathogen surveillance for ranaviruses at 8 climatically and faunistically distinct sites in Costa Rica. Seven of these sites are occupied by relict populations of highly threatened amphibian species. Our eighth study site is occupied by an introduced amphibian species — a potential carrier for anthropogenic long-distance dispersal of ranaviruses. Our primary goals were to evaluate the distribution of ranaviruses with regard to climatic variables and across host species, and to evaluate 1 potential long-distance carrier as a source for ranaviruses. These data will be necessary to evaluate whether ranaviruses should be a management concern for relict amphibian species already decimated by chytridiomycosis and should improve our understanding of amphibian ranaviruses in tropical ecosystems.

2. MATERIALS AND METHODS

We sampled Costa Rican amphibian communities across 8 ecosystems representing a wide variety of climatic conditions (elevations, precipitation, and temperature regimes; Table 1, Fig. 1). Seven of our study sites are occupied by relict populations of threatened amphibian species that are collectively a regional conservation priority (Whitfield et al. 2016). These sites include Sector Murcielago within the Santa Elena Peninsula of the Guanacaste Conservation Area (occupied by the critically endangered *Craugastor ranoides*; Puschendorf et al. 2005, Zumbado-Ulate et al. 2011), Rancho Chutas within the Children's Eternal Rainforest (occupied by the vulnerable *Lithobates vibicarius*; Whitfield et al. 2017), Cerro Chompipe (occupied by the critically endangered toad *Incilius holdridgei*; Abarca et al. 2010),

Table 1. Field sites, focal species, and environmental characteristics of the study areas sampled in this study. Temperature and precipitation are mean annual measurements. *Focal species that was sampled directly for ranavirus infection

Location	Focal species	Sampling dates	Lat. (°N)	Long. (°W)	Altitude (m)	Temp. (°C)	Precip. (mm)
Sector Murciélago, Peninsula of Santa Elena, Guanacaste	*Craugastor ranoides*	2 Mar 2013	10.9370	85.6819	100	26.4	1639
Rancho Chutas, Children's Eternal Rainforest, Alajuela	*Lithobates vibicarius**	16–17 July 2012	10.3648	84.7285	1763	18.6	3365
Cerro Chompipe, Braulio Carrillo National Park, Heredia	*Incilius holdridgei*	21 Jul 2013, 20 Jul 2013	10.0869	84.0719	2100	15	3405
Costa Rican Amphibian Research Center (CRARC), Guayacán, Siquirres, Limón	*Agalychnis lemur**	16 Nov 2012	10.0575	83.5482	533	24.28	3572
Las Alturas de Coton, Las Tablas Protected Zone, San Vito de Coto Brus, Puntarenas	*Atelopus varius*	15–16 Jan 2016	8.9374	82.8265	1350	18.18	2908
Las Cruces Biological Station, San Vito, Puntarenas	*Duellmanohyla legleri**	10–11 Nov 2012	8.7863	82.9622	1200	20.3	2928
Pavones, Golfito, Puntarenas	*Craugastor taurus**	27 Jan 2013	8.3919	83.1192	20	25.8	3554
Barrio Jorge Debravo, Turrialba, Cartago	*Eleutherodactylus coqui**	2 Sept 2013, 11 Jan 2014	9.8990	83.6736	622	22.09	3165

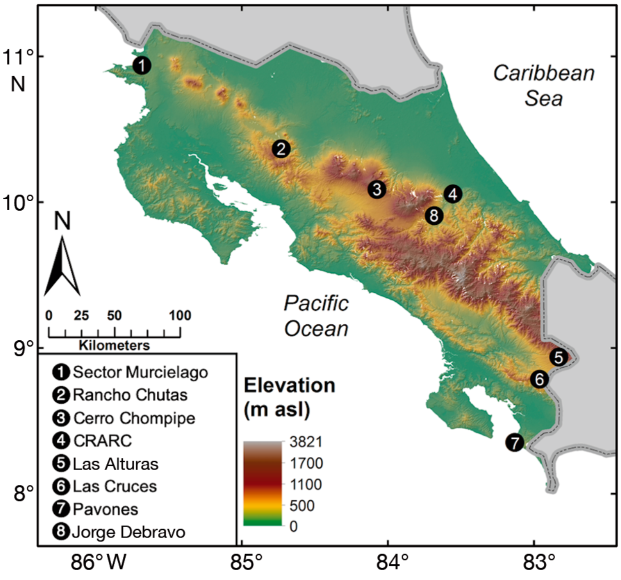

Fig. 1. Costa Rica including the 8 field sites examined in this study. Sites varied greatly in their elevation and climatic associations (see Table 1 for details)

The Costa Rican Amphibian Research Center ('CRARC') in Guayacan de Siquirres (occupied by the critically endangered lemur leaf frog *Agalychnis lemur*), Las Alturas de Coton in the Las Tablas Protected Zone (home to one of the last populations of the critically endangered variable harlequin toad *Atelopus varius*, Puschendorf 2003, González-Maya et al. 2013), Las Cruces Biological Station in San Vito de Coto Brus (occupied by the endangered Legler's stream frog *Duellmanohyla legleri*; Whitfield et al. 2017), and Pavones de Golfito (home to the last populations of the critically endangered Golfito robber frog *Craugastor taurus*; Chaves et al. 2014).

Our eighth site (Barrio Jorge Debravo in the town of Turrialba, Cartago province) harbors an established population of non-native Puerto Rican coquis *Eleutherodactylus coqui*. Coquis are 1 of only 4 known introduced amphibian species in Central America, and were deliberately introduced into Costa Rica. The only coqui populations within Costa Rica to our knowledge are within suburban areas of Turrialba and nearby towns (Barrantes-Madriga et al. 2019).

At each site, we searched for amphibians over a 1 to 3 d period, captured individuals by hand in unused plastic bags, and identified each amphibian to species. We sampled adult amphibians and juveniles of direct developing species, but did not sample any larval amphibians — although prevalence is typically higher in larval amphibians than in adults (Brunner et al. 2015). We sampled for ranaviruses by collecting and excising a single toe from the hind foot with stainless steel surgical scissors. To prevent contamination between sampling subsequent individuals, we flamed the scissors after each toe clip until they glowed red for 2 s. Stainless steel glows red at 490°C, which should be sufficient to destroy both live virus and any DNA. We preserved toes in 70% ethanol until laboratory analysis, following procedures widely used elsewhere (Garcia et al. 2007, St-Amour & Lesbarrères 2007, Whitfield et al. 2013).

2.1. Pathogen detection

We detected the presence of ranavirus using protocols described previously (Brunner et al. 2004, Kerby & Storfer 2009, Whitfield et al. 2012, 2013). We extracted DNA from toe clips using Qiagen DNeasy Blood and Tissue spin column kits. We used 18 µl reactions containing 2 µl of DNA template. We ran PCR reactions using 300 nmol forward primer, 900 nmol reverse primer, 200 nmol probe, and Taqman 2× Universal PCR master mix. We ran plates on an activating cycle of 95°C (10 min), and then 40 cycles at 95°C (20 s), 54°C (20 s), and 72°C (30 s) on a StepOne Plus qPCR Machine (Applied Biosystems). We ran each sample in triplicate and each plate contained negative controls and standards as internal positive controls. We estimated infection intensity (in viral copies) from a standard curve using calibrated gBlock (IDT) gene fragments of the targeted sequence. The amplification curves of all positive samples were visually inspected to determine if they were positive or an anomaly. Samples were only determined positive if at least 2 of 3 wells were positive. Any samples that were positive in only 1 well were run a second time; if the rerun samples were positive in 2 wells we classified them as positive and as negative if no wells were positive.

2.2. Statistical analysis

To test for main of site and species on infection prevalence, we used a generalized linear model (GLM) with binomial data for infection as a response variable, and specified species and site as predictor variables. It is common in diverse tropical ecosystems to have few common species and many uncommon ones, and this was inevitably reflected in our sampling (see Table 2). Because many of our sampled species were not common, we restricted this analysis to species sampled with ≥8 individuals. As few species occurred across multiple sites (see Table 2), we did not include an interaction term in this model. We specified binomial family and logit link, and used the glm function in the base package of R.

To test for main effects of site and species on infection load, we used a linear model, and specified log(ranavirus viral copies + 1) as a response variable and species and site as predictor variables. As with the GLM above, we restricted this analysis to species sampled with ≥8 individuals and only with individuals that were positive for ranavirus. Again, we did not include an interaction term in this model. We ran this analysis using the lm function in the base package of R.

To evaluate climatic associations with infection prevalence, we downloaded the mean annual temperature (°C) (BIO1) and mean annual precipitation (mm) (BIO12) BIOCLIM layers from the WorldClim 2 database (Beaumont et al. 2005, Fick & Hijmans 2017) at a spatial resolution of 30 arc-seconds. We cropped the climatic layers to the extent of Costa Rica (7.750 to 11.500° N, 82.150 to 86.250° W) and extracted data for each study site. We chose these variables as they are among the most fundamental climatic parameters and vary considerably among the study sites (Table 1). To test for climatic associations with infection prevalence, we constructed a binomial generalized linear mixed model with the glmer function of the lme4 package in R (Bates et al. 2014). We specified temperature and precipitation as fixed predictor variables, species identity as a random effect, and infection as a binomial response variable. We estimated p-values in glmer by comparing a model with a given effect to a model without the given effect (Bates et al. 2014).

To test for climatic associations with infection intensity, we used the subset of our data which only included infected individuals. We constructed a generalized linear mixed model with the lme function of the nlme package in R (Pinheiro et al. 2013). We specified temperature and precipitation as fixed predictor variables, and species identity and site as random effects. All analyses were conducted using R 4.0.2 (R Development Core Team 2015).

3. RESULTS

Across our 8 study sites, we sampled 245 individual amphibians spread across 40 species in 8 families (Table 2). Of these, 40 individuals (16.3%, 95% confidence intervals 11.9 to 21.6%) were ranavirus-positive on the basis of qPCR (Fig. 2). Ranavirus-positive individuals were detected across 17 of our 40 study species, 5 of our 8 study sites, and 6 of our 8 taxonomic families (Table 2, Fig. 2).

Infection prevalence varied among sites (deviance = 19.55, df = 5, p = 0.0015, Fig. 2A) and among species (deviance = 37.38, df = 10, p < 0.0001, Fig. 2B). Infection intensity did not vary among sites ($F_{2,21}$ = 0.7792,

Table 2. Study sites and species sampled in this study. Numbers in each cell indicate the number of sampled individuals, and numbers in parentheses indicate the number of individuals testing positive for ranavirus. (–) Species not found at this site

Taxon	Sector Murcielago	Rancho Chutas	Cerro Chompipe	CRARC	Las Alturas	Las Cruces	Pavones	Jorge Debravo
Plethodontidae								
Bolitoglossa colonnea	–	–	–	1	–	–	–	–
Hylidae								
Agalychnis callidryas	–	–	–	2	–	–	–	–
Agalychnis lemur	–	–	–	2 (1)	–	–	–	–
Agalychnis spurrelli	–	–	–	2	–	–	–	–
Dendropsophus ebraccatus	–	–	–	2	–	–	–	–
Dendropsophus phlebodes	–	–	–	1 (1)	–	–	–	–
Dendropsophus microcephalus	1	–	–	–	–	–	–	–
Duellmanohyla rufioculis	–	–	–	1	–	–	–	–
Duellmanohyla legleri	–	–	–	–	–	14 (1)	–	–
Hyloscirtus palmeri	–	–	–	1 (1)	–	–	–	–
Isthmohyla pseudopuma	–	–	4	–	–	–	–	–
Isthmohyla tica	–	1	–	–	–	–	–	–
Scinax elaeochroa	–	–	–	3 (1)	–	–	–	–
Smilisca baudinii	1	–	–	–	–	–	–	–
Smilisca sila	–	–	–	–	–	1	–	–
Tlalocohyla loquax	–	–	–	1	–	–	–	–
Centrolenidae								
Espadarana prosoblepon	–	3	–	–	2	21 (10)	–	–
Hyalinobatrachium colymbiphyllum	–	2 (1)	1	–	–	–	–	–
Teratohyla spinosa	–	–	–	1	–	–	–	–
Craugastoridae								
Craugastor bransfordii	–	–	–	1 (1)	–	–	–	–
Craugastor crassidigitus	–	–	–	6 (3)	17	5 (1)	–	–
Craugastor fitzingeri	7 (1)	–	–	1	5	–	6 (1)	–
Craugastor gabbi	–	–	–	–	7	–	–	–
Craugastor melanostictus	–	–	1	–	–	–	–	–
Craugastor noblei	–	–	–	1	–	–	–	–
Craugastor podiciferus	–	5 (1)	5	–	–	–	–	–
Craugastor stejnegerianus	–	–	–	–	–	–	12 (7)	–
Craugastor taurus	–	–	–	–	–	–	8 (3)	–
Strabomantidae								
Pristimantis cerasinus	–	–	–	1	–	–	–	–
Pristimantis cruentus	–	2	1	–	3	2	–	–
Pristimantis ridens	–	–	–	–	–	1	–	–
Eleutherodactylidae								
Diasporus diastema	–	–	–	1	–	–	–	–
Diasporus hylaeformis	–	–	3	–	–	–	–	–
Eleutherodactylus coqui	–	–	–	–	–	–	–	25
Leptodactylidae								
Leptodactylus melanonotus	2	–	–	–	–	–	–	–
Leptodactylus poecilochilus	1	–	–	–	–	–	–	–
Ranidae								
Lithobates forreri	2 (1)	–	–	–	–	–	–	–
Lithobates vibicarius	–	22 (3)	–	–	–	–	–	–
Lithobates warszewitschii	–	1	–	4 (1)	–	2	–	–
Bufonidae								
Rhinella horribilis	10	–	–	–	5	–	1 (1)	–

p = 0.485, Fig. 2A) or among species ($F_{5,21}$ = 0.9234, p = 0.4854, Fig. 2B). Prevalence increased with temperature (χ^2 = 6.00, df = 1, p = 0.0143, Fig. 3A), but did not vary with precipitation (χ^2 = 2.7423, df = 1, p = 0.9772, Fig. 3B). Infection intensity did not vary with either temperature (χ^2 = 0.019, df = 1, p = 0.890, Fig. 3C) or with precipitation (χ^2 = 0.521, df = 1, p = 0.470, Fig. 3D).

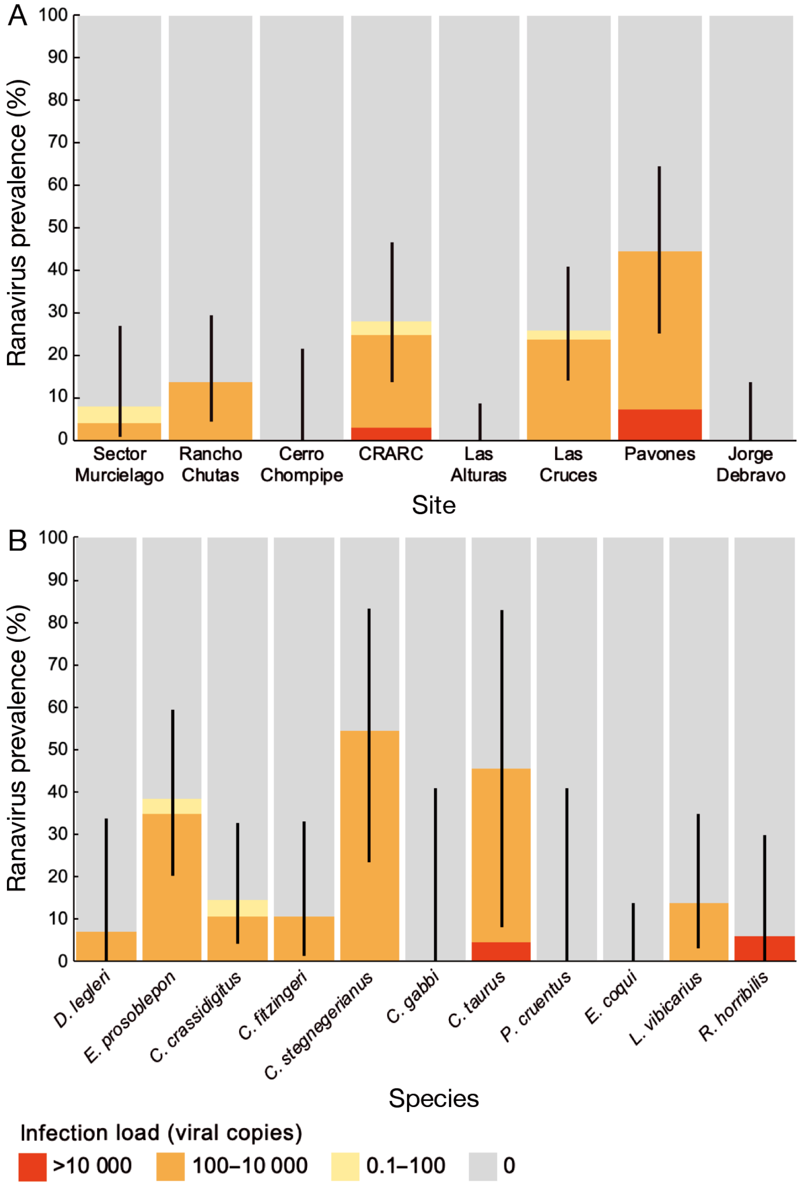

Fig. 2. Ranavirus infection prevalence and intensity by site (A) for 8 Costa Rican study sites and (B) for 11 species that were sampled ≥8 times. See Table 2 for full species names. Error bars indicate the 95% binomial confidence intervals for infection prevalence

4. DISCUSSION

We found that ranavirus infection was widespread in Costa Rica among geographic regions, bioclimatic zones, and across broad taxonomic groups of amphibians. However, none of the amphibians encountered in this study showed clinical signs of ranaviral disease, a result shared with other ranavirus studies from Costa Rica (Speare et al. 1991, Whitfield et al. 2012, 2013, Puschendorf et al. 2019). Prevalence varied among sites and among species, and increased weakly in response to temperature, but not precipitation. We found no patterns in infection intensity across sites, species, or in relation to climatic variables. Finally, we did not find evidence of infection in our potential mode for long-distance dispersal, Puerto Rican coquis.

We found that ranavirus infection is geographically widespread — from dry forests at approximately 100 m elevation to cloud forests at approximately 2300 m above sea level. We found that ranavirus was present in 5 of our 8 sites, but our only climatic association was a weak increase in prevalence in response to temperature. Ranaviruses have been found in broad climatic zones, ranging from high latitudes (Gahl & Calhoun 2010, Schock et al. 2010) to near the equator (Zupanovic et al. 1998, Warne et al. 2016). While most studies of ranavirus surveillance in wild amphibians have been from temperate regions, a growing body of literature indicates ranaviruses are widespread in tropical regions (Zupanovic et al. 1998, Whitfield et al. 2013, Stark et al. 2014, Warne et al. 2016, Ruggeri et al. 2019), where most amphibian species occur. Still, relatively few tropical regions have been sampled for ranaviruses, and pathogen surveillance programs in hyperdiverse tropical regions would be important for identifying pathogens of conservation concern. With the available data from several tropical regions, it now appears that ranaviruses are widespread in tropical amphibians, occur across life histories and taxonomic groups, but are not necessarily tied to clinical signs of disease.

We also found that ranavirus affects a wide range of tropical species, reflecting other studies from the Neotropics (Whitfield et al. 2012, 2013, Stark et al. 2014, Warne et al. 2016). Here, we found ranavirus-positive individuals in pond-breeding species (*Agalychnis spurelli*, *Dendropsophus phlebodes*, *Scinax eleaochroa*, *Lithobates forreri*, and *L. vibicarius*), stream-breeding species (*Duellmanohyla legleri*, *Hyloscirtus palmeri*, *Hyalinobatrachium colymbiphyllum*, *Espadarana prosoblepon*, and *L. warszewitschii*),

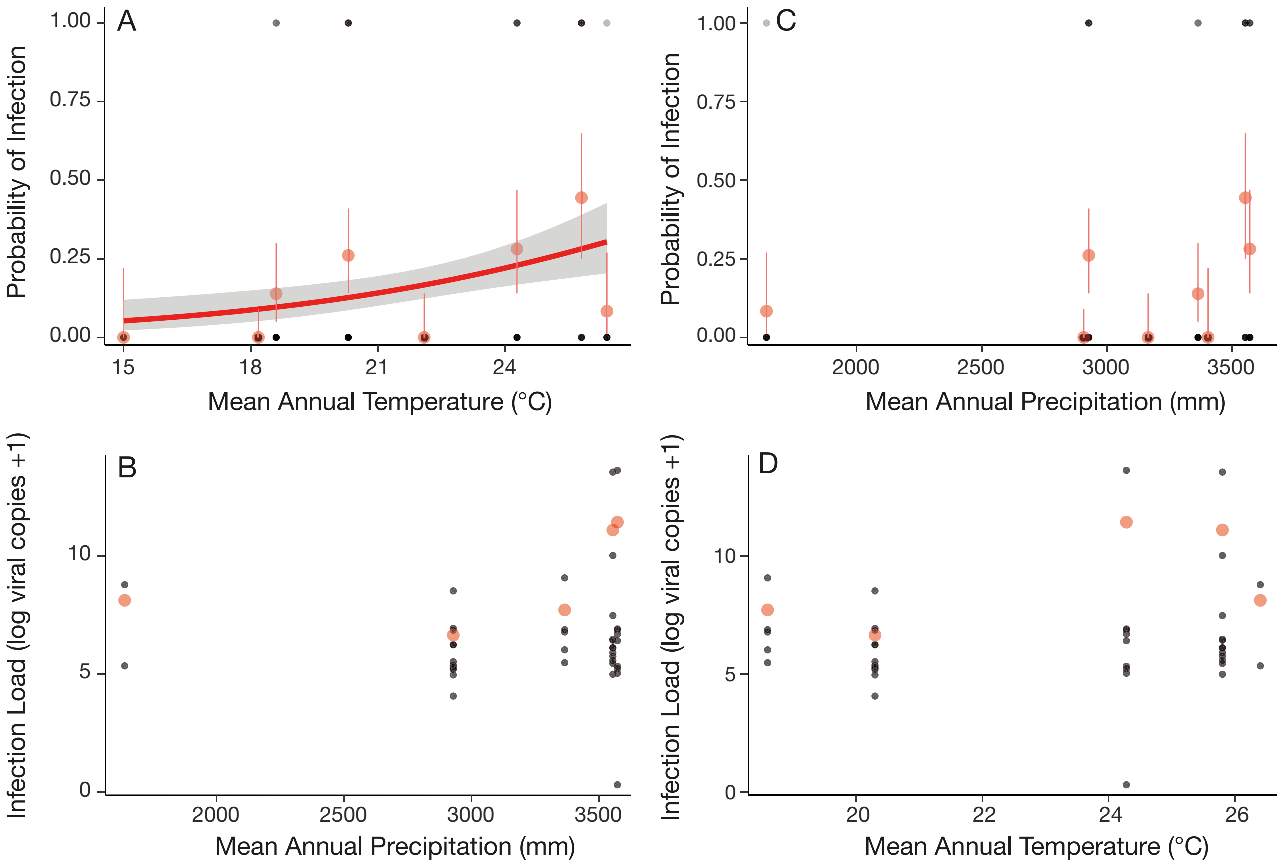

Fig. 3. Associations between infection (prevalence and infection intensity) and climatic variables (mean annual temperature and mean annual precipitation) shown for (●) each sampled individual and as (●) means per site (with 95 % confidence intervals). Trendline and 95 % confidence intervals are shown only for statistically significant associations

and direct developing species (several species of *Craugastor*). Infection across reproductive modes has similarly been detected in Andean Peru (Warne et al. 2016), and even in temperate North America, direct developing salamanders have shown infection with ranaviruses (Sutton et al. 2015). Well-studied impacts of ranaviruses have been documented primarily from pond-breeding amphibians in temperate regions (Brunner et al. 2004, 2007), but transmission dynamics are likely to be very different for species whose life histories are mostly or entirely terrestrial. Consequently, the disease ecology may be very different in tropical systems, where life history variation in amphibians is much greater than in temperate regions.

We did not find evidence of infection in Puerto Rican coquis. It is possible that true prevalence is below our detection threshold, as we only sampled 25 individuals (and our upper CI for infection prevalence for this species is still 13.7 %). It is also possible that this species is not a major mode of transport for long-distance dispersal of ranaviruses. Ranaviruses have not been sampled in Puerto Rico, or detected anywhere in the Caribbean — though to our knowledge, only 1 study has sampled for ranaviruses in the Caribbean (Garcia et al. 2007). Still, Costa Rica hosts a few introduced amphibian species that may represent an important long-distance mode of transport for dispersal of pathogens. Pet trade amphibians may be another important mode of transport that warrants study in tropical regions; while the magnitude of the pet trade is small relative to North America, this is probably an important pathway for long-distance transport.

The overall prevalence that we found in this study (16 %) is higher than that found in many studies outside of disease outbreaks (Brunner et al. 2015). Still, other studies from tropical regions have found similar infection prevalence. Whitfield et al. (2013) found a prevalence of 16.6 % at a single site in Costa Rica that was not included in the present study. Warne et al. (2016) found that 40 % of stream-breeding frogs and 35 % of terrestrial frogs were infected with ranavirus in a study from Peru — considerably higher rates than we report. Even within North America, where ranaviruses have received more research attention, several studies have found similarly high prevalence yet no clinical disease (Duffus et al. 2008, Greer et al. 2009, Horner et al. 2017). Such a pattern could result from interactions among host, pathogen, and environment; however, ultimately accounting for widespread differences in infection prevalence and disease rate is beyond the scope of this study.

In this study, we have no information on the species or strain of ranavirus detected in any of our target populations, yet ranaviruses are diverse and virulence may vary among species and strains (Schock et al. 2009, Chinchar et al. 2011). Whitfield et al. (2013) determined that a ranavirus sampled from a single site in the lowlands of Costa Rica was genetically consistent with frog virus 3. Currently, we cannot determine whether the ranaviruses sampled in our study are from a single or multiple species.

Further, it is not possible to determine whether ranaviruses are endemic to Central America or occur there as the result of long-distance transport. Speare et al. (1991) reported a likely ranavirus from *Rhinella horribilis* before 1991. Puschendorf et al. (2019) found a ranavirus in museum specimens dating to 1987. As retrospective sampling has detected ranavirus infection prior to the emergence of chytridiomycosis in Costa Rica, it is plausible that ranaviruses were a co-stressor in historic population declines. At a minimum, the presence of ranaviruses prior to *Bd*-associated declines indicates that amphibians did not decline in an environment free from other stressors. Further retrospective studies of museum specimens, tied to genetic profiling of ranaviruses present in Central America, will likely help determine whether amphibian hosts have co-evolved with local ranaviruses, are facing novel threats from recently established pathogens, or both.

Concerningly, we found that ranaviruses are not only widespread, but that they co-occur in at least 5 sites with highly threatened relict populations that are greatly imperiled after the emergence of *Bd*. Because these populations are small, often fragmented, and likely have limited genetic diversity, they may be inherently susceptible to mass mortality from ranaviruses. Further, the lack of climatic associations reduces the possibility of a climatic refuge from pathogens, a plausible strategy for mitigation of chytridiomycosis (Puschendorf et al. 2009, 2013). We highly encourage further surveillance of the distribution of ranaviruses in tropical regions worldwide, and argue that future research should investigate genetic associations and virulence of ranaviruses in tropical regions where amphibians are most diverse. This information will be critical for developing adequate conservation and management practices to protect amphibians.

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