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Authors: Goodman, Rachel M., González-Galvis, Leidy, Lamadrid-Feris, Faride, Cepeda-Quilindo, Belisario, and Crawford, Andrew J.

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# Survey of ranavirus in amphibians and reptiles of Colombia

Rachel M. Goodman<sup>1,\*</sup>, Leidy González-Galvis<sup>2</sup>, Faride Lamadrid-Feris<sup>3</sup>,  
Belisario Cepeda-Quilindo<sup>2,4</sup>, Andrew J. Crawford<sup>4</sup>

<sup>1</sup> Department of Biology, Hampden-Sydney College, Virginia, USA.

<sup>2</sup> Departamento de Biología, Universidad de Nariño, Pasto, Colombia.

<sup>3</sup> Grupo GICMARA, Programa de Biología, Facultad de Ciencias Básicas, Universidad del Atlántico, Barranquilla, Colombia.

<sup>4</sup> Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia.

\* Corresponding author. Email: [rgoodman@hsc.edu](mailto:rgoodman@hsc.edu)

**Abstract.** Ranaviruses are emerging pathogens of ectothermic vertebrates that have been linked to mass mortality events globally in amphibians, reptiles, and fishes. In South America, ranavirus has been identified in multiple countries, but its distribution, host range, and epidemiology remain poorly characterized relative to other continents. Most infections have been documented in amphibians, particularly the invasive *Aquarana catesbeiana*, although detections have also included native species across Brazil, Chile, Argentina, Venezuela, Ecuador, Peru, and Colombia. To date, no studies have surveyed wild reptiles for ranavirus infection in South America, despite reptile mortality events linked to this virus in other regions. Here, we surveyed ranavirus in amphibians and reptiles of Colombia using quantitative polymerase chain reaction (qPCR) with genomic DNA extracted from toe and tail tips. We did not detect ranavirus in reptiles from the departments of Nariño ( $n = 30$ ) or Atlántico ( $n = 30$ ). We report the first detection of ranavirus in the striped robber frog (*Pristimantis unistrigatus*) in Nariño in southern Colombia, with a prevalence of 26.7% (8 of 30). Ranavirus was not present in amphibians from a lowland site in Atlántico on the northern Caribbean coast ( $n = 30$ ). This study expands the known geographic and host range of ranavirus in Colombia and is the first to survey reptiles in South America. The lack of ranavirus detection in reptiles is notable but does not preclude the possibility of infection, as sample sizes, study sites, and temporal coverage were limited. Our findings highlight the potential role of direct-developing frogs of the genus *Pristimantis* as valuable sentinels for pathogen monitoring, given their high species diversity, local abundance, and wide elevational distribution. Previous surveys have demonstrated low-level but geographically broad ranavirus prevalence in other *Pristimantis* species across the Andes, though sampling has often been haphazard and limited by small sample sizes. Given the growing threats to amphibian and reptile species in Colombia, further research is needed to assess the full scope of ranavirus dynamics. We recommend integrating pathogen surveillance into ongoing ecological and conservation programs, especially in biodiverse regions where invasive species and anthropogenic pressures may amplify disease emergence.

**Keywords.** Anura; Colombia; Emerging infectious disease; *Iridoviridae*; Pathogen; *Pristimantis unistrigatus*; Ranavirus; Squamata.

## INTRODUCTION

Ranaviruses are significant pathogens of wildlife, which in the past two decades have been documented to cause mass mortality events in reptiles, amphibians, and fishes (Chinchar, 2002; Jancovich et al., 2005; Marschang et al., 2025). Globally, 36% of amphibian and 18% of reptile species are considered threatened or endangered, and emerging infectious diseases in these groups are therefore worthy of study (Cox et al., 2022; Luedtke et al., 2023; IUCN, 2025a). Ranaviruses include seven species of double-stranded DNA viruses that replicate in temperatures of 12–32°C and may survive for several months outside of any host in aquatic environments (Chinchar, 2002; Nazir et al., 2012; Waltzek et al., 2025). Among these, *Frog Virus 3* (FV3; *Ranavirus rana1*) is the most commonly occurring ranavirus in reptiles and amphibians in the western hemisphere and the only species documented thus far in South America (Rodrigues et al., 2024; Marschang et al., 2025; Waltzek et al., 2025). Research on and surveillance of ranaviruses has been prolific in North America, but studies in South America have lagged significantly, with the majority

of studies occurring in Brazil (Wirth et al., 2021; Azat et al., 2024; Rodrigues et al., 2024; Marschang et al., 2025).

The earliest detection of ranavirus in South America occurred in Venezuela, where antibodies were found in *Rhinella marina* (Linnaeus, 1758) (Zupanovic et al., 1998), and isolates were recovered from *Aquarana catesbeiana* (Shaw, 1802), which is nonnative but widespread in South America (Barrasso et al., 2009; Oliveira et al., 2020). Three mass mortality events have been attributed to ranavirus infections in South America: one in northern Patagonia, Argentina, impacting frogs and tadpoles of *Atelognathus patagonicus* (Gallardo, 1962) (Fox et al., 2006), one in frog farms in central Brazil impacting tadpoles of *A. catesbeiana* (Mazzoni et al., 2009), and one in southern Brazil impacting tadpoles of native species (families Bufonidae Gray, 1825 and Hylidae Rafinesque, 1815; not identified to species) and nonnative *A. catesbeiana* (Ruggeri et al., 2019). Other studies document ranavirus presence without evident mortality; for example, Neves et al. (2016) found ranavirus in 90% of tadpoles and adult *A. catesbeiana* from one frog farm in southeastern Brazil; two other farms had no ranavirus-positive tadpoles but

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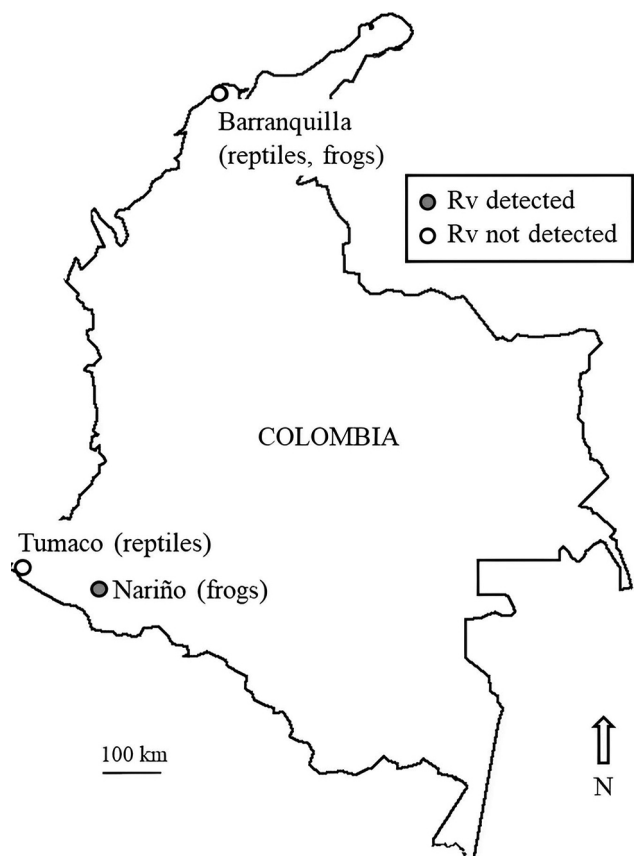
yielded ranavirus prevalence of 65% and 15% for adult *A. catesbeiana*. Another ecological survey in southern Brazil revealed ranavirus prevalence of 38% among tadpoles of *A. catesbeiana* and four native anuran families (Bufonidae, Hylidae, Leptodactylidae Werner, 1896, and Odontophrynidae Lynch, 1969; Ruggeri et al., 2024). Ash et al. (2024) focused on *A. catesbeiana* and detected ranavirus in 27% of 65 individuals sampled from nine sites across southern Brazil. Landscape-scale research in Brazil's southern Atlantic Forest failed to detect ranavirus in tadpoles of several native species from 26 water bodies despite extensive *Batrachochytrium dendrobatidis* Longcore et al. (1999) (Bd) presence (Coelho dos Santos et al., 2024). Similarly, Galli et al. (2006) detected ranavirus in tadpoles of *A. catesbeiana* from a frog farm in Brazil and another farm in Uruguay. Soto-Azat et al. (2016) sampled dead, native adult frogs taken from mortality events in central Chile and also euthanized frogs of the nonnative species *Xenopus laevis* (Daudin, 1802a). They found ranavirus present in 4.3% of these individuals, including seven *X. laevis* and one giant Chilean frog (*Calyptocephalella gayi* [Duméril and Bibron, 1841]). However, other pathogens were present and cause of death was not established for these frogs. A more recent survey of 1,752 amphibians across a 2,500 km transect in Chile detected low-level FV3 only in the nonnative species *X. laevis* with no detections in native species, suggesting an incipient incursion via introduced *X. laevis* (Peñafiel-Ricaurte et al., 2023). Ranavirus was detected in Peru in wild-harvested live frogs (*Telmatobius marmoratus* [Duméril and Bibron, 1841]; prevalence = 53%) from Cusco markets and in wild populations of *Boana gladiator* (Köhler et al., 2010) (prevalence = 40%), *Pristimantis* spp. (prevalence = 38%), and *Rhinella manu* Chaparro et al., 2007 (prevalence = 75%) in nearby montane forests (Warne et al., 2016). In Ecuador, Urgiles et al. (2021) detected ranavirus in three out of 213 adult frogs from 17 sites in the southern Andes; specifically, prevalence was  $\leq 1\%$  in two species of *Pristimantis* Jiménez de la Espada, 1870 frogs. In Colombia, Flechas et al. (2023) detected ranavirus in 14 individuals (5% of 274 individuals total) from 8 different sites and in 6 amphibian species, including native frogs and *A. catesbeiana*.

Ranavirus presence in South America is now well documented, and existing studies highlight high local prevalence, sporadic epizootic events, and anthropogenic drivers such as trade and invasive species as key epidemiological factors (Azat et al., 2024). Surveys across multiple native species and sites are rare and much needed to better understand the dynamics and potential impacts of ranavirus on the native fauna of South America. Importantly, reptiles are absent entirely from these studies in South America, although ranavirus infections and high prevalence have been documented in wild lizards (20% in North American terrestrial lizards; Goodman et al., 2018; 78.6% in Australian semi-aquatic lizards; Maclaine et al., 2020), and several mortality events have been attributed to ranavirus in North American turtles (De Voe et al., 2004; Sim et al., 2016; Kimble et al., 2017). In the current study, we expanded the geographic sampling of ranavirus in

Colombia by including study sites in departments further south (Nariño) and further north (Atlántico) than in the previous Colombian survey of ranavirus (Flechas et al., 2023). We also expanded the taxonomic sampling of this pathogen in Colombia by including reptiles, specifically squamates, thereby presenting the first survey of this pathogen in South American reptiles.

## MATERIALS AND METHODS

Frogs ( $n = 30$ ; species in Table 1) were captured from the Universidad de Nariño Granja Experimental Botana near Pasto (Dept. Nariño;  $1^{\circ}09'30.84''N$ ,  $77^{\circ}16'13.42''W$ ; elevation = 2,810 m; Figure 1) during the hours of 19:00–20:30 on three nights during 30 September–6 October 2023. Few reptiles were active at this site during a brief daytime reconnaissance, so reptiles ( $n = 30$ ; Table 1) were instead surveyed at the Granja Mar Agrícola near Tumaco (also Dept. Nariño;  $1^{\circ}41'38.4''N$ ,  $78^{\circ}45'16.5''W$ ; elevation = 4 m) during the hours of 8:00–19:00 on six days during 15–24 November of 2023. Frogs and reptiles ( $n = 30$  each; Table 1) were captured from a private farm outside of Barranquilla (Dept. Atlántico,  $10^{\circ}55'24.5''N$ ,



**Figure 1.** Map of three study sites in Colombia, including Pasto: Universidad de Nariño Granja Experimental Botana near Pasto, Dept. Nariño; Tumaco: Granja Mar Agrícola near Tumaco, Dept. Nariño; and Barranquilla: a private farm outside of Barranquilla, Dept. Atlántico. Tissue samples were collected from frogs ( $n = 30$ ) and reptiles ( $n = 30$ ) where noted in parentheses during October and November 2023 and tested for presence of ranavirus DNA using quantitative polymerase chain reaction (qPCR). Rv = ranavirus.

**Table 1.** Amphibian and reptile species sampled and tested for ranavirus in three study sites in two departments in Colombia. N is sample size, and RV+ refers to the number of individuals who tested positive for ranavirus DNA in tissue samples (tail or toe tips).

Site	Taxon	Species	Common name	N	RV+
Department of Atlántico (near Barranquilla)	Anura	<i>Boana pugnax</i> (Schmidt, 1857)	Chiriqui-flusse treefrog	3	0
		<i>Dendropsophus microcephalus</i> (Cope, 1886)	yellow treefrog	10	0
		<i>Engystomops pustulosus</i> (Cope, 1864)	túngara frog	7	0
		<i>Leptodactylus insularum</i> Barbour, 1906	spotted foam-nest frog	2	0
		<i>Rhinella horribilis</i> (Wiegmann, 1833)	Mesoamerican cane toad	1	0
		<i>Rhinella humboldti</i> (Gallardo, 1965)	Rivero's toad	3	0
		<i>Scinax ruber</i> (Laurenti, 1768)	red-snouted treefrog	3	0
		<i>Scarthyla vigilans</i> (Solano, 1971)	Maracaibo Basin treefrog	1	0
	Reptilia	<i>Anolis auratus</i> Daudin, 1802b	grass anole	11	0
		<i>Dryophylax gambotensis</i> (Pérez-Santos and Moreno, 1989)	n/a (endemic to Colombia)	2	0
		<i>Helicops danieli</i> Amaral, 1938	Daniel's keelback	1	0
		<i>Hemidactylus frenatus</i> Duméril and Bibron, 1836	common house gecko	9	0
		<i>Iguana iguana</i> Linnaeus, 1758	green iguana	6	0
	Mabuyinae sp.	unidentified scincid lizard	1	0	
Department of Nariño (near Pasto)	Anura	<i>Pristimantis unistrigatus</i>	striped robber frog	30	8
Department of Nariño (near Tumaco)	Reptilia	<i>Anolis auratus</i>	grass anole	18	0
		<i>Anolis festae</i> Peracca, 1904	Veronica's anole	1	0
		<i>Anolis lyra</i> Poe et al., 2009	lyre anole	1	0
		<i>Anolis</i> sp.	unidentified anole lizard	3	0
		<i>Basiliscus galeritus</i> Duméril, 1851	western or red-headed basilisk	2	0
		<i>Dipsas gracilis</i> (Boulenger, 1902)	graceful snail-eater	1	0
		<i>Dipsas</i> sp.	n/a (snail-eater snake genus)	1	0
		<i>Imantodes cenchoa</i> (Linnaeus, 1758)	blunthead tree snake	1	0
		<i>Rhinoclemmys melanosterna</i> (Gray, 1861)	Colombian wood turtle	1	0
<i>Sibon nebulatus</i> (Linnaeus, 1758)	cloudy snail-eating snake	1	0		

74°56'23.0"W; elevation = 83 m) during the hours of 17:00–22:30 on three nights during 18–24 November of 2023. Amphibians were hand-captured. Reptiles were captured by hand or with the use of a lasso attached to a collapsible fishing pole.

To prevent possible contamination between individuals, all animals were kept in separate disposable bags or plastic containers that were washed and disinfected between field sessions. Disinfection consisted of saturation for at least one minute with a 1% solution of Virkon® S Disinfectant and Virucide (Lanxess, PA, USA), which has been shown to be effective for inactivating ranavirus (Bryan et al., 2009). Nitrile gloves were worn and changed between handling different individuals. Any equipment that touched animals was disinfected between individuals, and footwear was washed and disinfected between different field sites.

We used sterile disposable scalpel blades to remove tail tips from reptiles and toe tips from frogs totaling 3–5 mm in length (depending on thickness of tissue) from each animal. The wound site was then disinfected with 10% povidone-iodine and, in reptiles, sealed with KwikStop Styptic Powder containing benzocaine anesthetic. Animals were released at their exact capture location within 5 hours of capture. Tissue samples were stored in 100% ethanol for preservation, transported to Universidad de los Andes in Bogotá, and frozen within one week at -70°C.

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), and

DNA concentrations were subsequently quantified using a Nanodrop 2000c (Thermo Scientific, Abingdon, UK). Detection of ranavirus was performed via quantitative PCR (qPCR), utilizing 75 ng of template DNA per reaction and targeting a 70 base pair (bp) fragment of the major capsid protein (MCP) gene, a region known to detect FV3 and FV3-like ranaviruses, following the protocol described by Gray et al. (2012). All samples were run in duplicate on a BIORAD CFX96 Real-Time qPCR System and scored using Bio-Rad CFX Maestro software (Hercules, CA, USA). Analysis included nuclease-free water for a negative control and serial dilutions (1 to 10<sup>6</sup> copies/μL) of artificial ranavirus DNA (gBlocks, Integrated DNA Technologies, Inc., IA, USA) for positive controls. Samples were considered ranavirus-positive if Cq values from both wells exceeded were below or equal to that of the positive control with a concentration of 1 viral copy/μL. Among those, samples with estimated viral copies of < 10/μL were run two additional times for confirmation. A 95% confidence interval around the estimated prevalence was obtained using the command `binom.test()` in base R (R Core Team, 2025).

## RESULTS

Ranavirus was detected in 8 out of 30 frogs (26.7% prevalence; 95% CI = 12.3–45.9%) from the montane study site near Pasto (Dept. Nariño, elevation = 2,810 m). All frogs at this site were striped robber frogs, *Pristimantis unistrigatus* (Günther, 1859). Viral loads were 1–10 copies/

μL in four individuals, 11–100 copies/μL in two individuals, and 176 and 245 copies/μL in two individuals. No external symptoms of disease were noted in any of these frogs during examinations in the field. We did not detect ranavirus in any reptiles from Tumaco (Dept. Nariño, elevation = 4 m) or in any amphibians or reptiles sampled near Barranquilla (Dept. Atlántico, elevation = 83 m).

## DISCUSSION

Our study represents the first detection of ranavirus in *Pristimantis unistrigatus*, which had not previously been surveyed for this pathogen with an adequate sample size. This was the only species found to carry ranavirus in our study, possibly because the sample size was largest for this species and much smaller for other species that we surveyed; this was a product of opportunistic sampling and not intentional. *Pristimantis unistrigatus* was the only active frog species during our brief survey period in our study site near Pasto, whereas our other study sites had multiple species of active frogs and reptiles during their survey periods. A previous study in Ecuador detected ranavirus in *P. orestes* (Lynch, 1979) (2 of 162 frogs pooled over multiple sites) and *P. phoxocephalus* (Lynch, 1979) (1 of 30 frogs pooled over multiple sites) but not in *P. unistrigatus* (Urgiles et al., 2021). However, only 3 individuals of *P. unistrigatus* were sampled in that study. In the first and still only previous report for Colombia, Flechas et al. (2023) detected ranavirus in museum tissue samples of *P. anolirex* (Lynch, 1983), *P. bogotensis* (Peters, 1863), *P. elegans* (Peters, 1863), and *P. peraticus* (Lynch, 1980). The pathogen was present in 29% of 28 frogs among these species pooled over study sites. Sample sizes in that survey were too small ( $n = 1–10$  from each species and site) to estimate prevalence for comparison with the current study. Also, the known range limit of *P. unistrigatus* is further south than the southernmost collection site in the Flechas et al. (2023) study, such that no samples were available for *P. unistrigatus*. Warne et al. (2016) detected ranavirus in the congeners *P. lindae* (Duellman, 1978a), *P. pharangobates* (Duellman, 1978a), *P. platydactylus* (Boulenger, 1903), and *P. toftae* (Duellman, 1978b) (38% of 68 frogs, combined across species and sites) in the Peruvian Andes. Whitfield et al. (2012, 2021) sampled frogs in three species of *Pristimantis* in Costa Rica and did not detect ranavirus. However, small sample sizes of 1–3 frogs per species and site ( $n = 4$  at one site, Whitfield et al., 2012;  $n = 10$  among five sites, Whitfield et al., 2021) prohibit drawing any conclusions from those surveys about the suitability of these particular species as hosts for the virus.

The aforementioned studies suggest that *Pristimantis* frogs are well-suited as hosts for ranavirus and may be useful, convenient targets for new surveys of this pathogen. In several Central and South American surveys for ranavirus and the pathogenic fungus, *Batrachochytrium dendrobatidis* (Bd), *Pristimantis* species are among those with individuals testing positive for the pathogen under

study (Acevedo et al., 2016; Flechas et al., 2017). This may reflect greater abundance and therefore sample size, rather than host suitability per se. *Pristimantis* is the most species-rich genus of terrestrial vertebrates, and members of the 633 currently delineated species occur from central Panama and some Caribbean islands in the north to Bolivia and Brazil in the south (Frost, 2024). Frogs in this genus are terrestrial, small-bodied, direct developing, and occur from sea level to 4,000 m in elevation (Hedges et al., 2008). The highest species diversity within *Pristimantis* occurs in the Andes of Colombia, Ecuador and Peru, with 222, 274, and 156 species in each country, respectively, whereas Costa Rica and Bolivia, countries at the edges of the range, harbor 11 and 17 species, respectively (Frost, 2024).

The previous survey of ranavirus in Colombia found infected specimens in varied ecosystems such as high-elevation paramos and lowland savannas, aquatic and terrestrial habitats, and in direct-developing frogs as well as aquatic species that undergo metamorphosis (Flechas et al., 2023). We note that study used liver tissue samples, whereas our study used toe and tail tip samples. We did not detect ranavirus in reptiles from lowland study sites near the Pacific or Atlantic coasts (Tumaco and Barranquilla) or in amphibians from the latter site; however, our survey periods were brief and our study sites limited. We therefore encourage more surveys in reptiles, especially in areas where the presence of ranavirus has already been detected through sampling of amphibians. Investigators already working on turtles, which require more dedicated effort for trapping, could incorporate swab or tissue sampling to be used for pathogen surveys as well as genetic analyses. Of the 33 native turtle species in Colombia, Páez et al. (2022) estimated that over 43% are threatened. According to the IUCN Red List, 78% of Colombian turtle species that have been assessed ( $n = 18$ ) are listed as threatened (vulnerable, endangered, or critically endangered), including three out of four endemic species (the remaining species has not been assessed, IUCN, 2025b). Continued surveys of ranavirus in these and other species can have important conservation and economic implications, because this wildlife pathogen can be transmitted among wild and captive fishes, amphibians, and reptiles (Brenes et al., 2014a,b).

The studies reviewed herein demonstrate that ranavirus is geographically widespread though under-sampled in South America and infects both endemic and invasive amphibians. The current study presents the first detection of ranavirus in the Department of Nariño and in the native frog *Pristimantis unistrigatus*. Although we failed to detect ranavirus in frogs in the Department of Atlántico, or in reptiles in either location, we encourage continued sampling and collaboration among Colombian scientists to incorporate pathogen sampling into other existing research programs. Given that reptile disease ecology, especially regarding microparasites such as ranavirus, remains poorly studied in South America (Arguedas and Troiano, 2024), this underscores the need for expanded and systematic pathogen surveillance. It is therefore imperative

that Colombian herpetologists integrate ranavirus screening protocols into ongoing biodiversity and ecological monitoring programs in reptiles as well as amphibians. Such enhanced, geographically broad sampling, coupled with multidisciplinary collaboration, will facilitate a more comprehensive understanding of pathogen prevalence, transmission dynamics, and potential conservation risks across diverse vertebrate taxa.

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