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THE RANCHO GRANDE HARLEQUIN TOAD (*Atelopus cruciger*): CONSERVATION ACTION PLAN 2023-2033

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The Rancho Grande harlequin toad (*Atelopus cruciger*): Conservation Action Plan 2023–2033

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Executive Summary

Ranging from Bolivia to Costa Rica, harlequin toads (*Atelopus* spp.) are among the most diverse amphibians, but the majority of its species are threatened with extinction. According to the International Union for Conservation of Nature (IUCN), 83% of described species are listed as Endangered or Critically Endangered. In Venezuela, The Rancho Grande harlequin toad (*Atelopus cruciger*) is the only one of nine described species with known stable populations. However, because it currently exists in relictual form and has a very small range in the wild, this species is listed as Critically Endangered by the IUCN.

Rancho Grande harlequin toads disappeared from most of their former habitat, on the central portion of the Cordillera de La Costa in northern Venezuela, in the late 80's presumably due to chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*). This fungal pathogen continues to be highly virulent for this toad in remnant populations, but a high recruitment of juveniles and low *Bd* transmission allow this species to coexist endemically with the fungus in lowlands. Although most of its former habitats remain pristine and protected by national parks, environmental degradation due to agriculture related activities, urban expansion, fires and river pollution have been documented in some lowland habitats.

Following almost two decades of research on the Rancho Grande harlequin toad in relict populations, this document brings together the knowledge of a team of experts to identify priority conservation needs and design an action plan to rescue this species. This plan is based on the concept of "One Conservation", which integrates sustainability with *in-situ* and *ex-situ* conservation for the restoration of ecosystems, and was framed under the Amphibian Conservation Action Plan (ACAP) of the IUCN SSC Amphibian Specialist Group 2022 (https://www.iucn-amphibians.org/), and the Conservation Action Plan of the Atelopus Survival Initiative- HarleCAP (https://www.atelopus.org).

Contents

List of Figures

1 Background

1.1 The species

Atelopus cruciger (Lichtenstein & Martens, 1856). Sapito Rayado de Rancho Grande, Rancho Grande harlequin Toad, Veragua Stubfoot Toad.

1.2 Conservation status

Critically Endangered B1ab(iii)c(iv)+2ab(iii)c(iv) [1]

1.3 Distribution, population size, and trends

Until the 80's, the Rancho Grande harlequin toad was one of the most abundant and conspicuous amphibians in the montane forests and streams of the central portion of the Cordillera de La Costa in northern Venezuela [2, 3, 4]. Records of this harlequin toad exist from 77 localities distributed in most of the Cordillera de La Costa, from sea level to 2,400 meters of elevation [5, 6, 7, 8] **(Figure 1**). One individual was also sighted in Guatopo National Park, on the Serranía del Interior in 1984 [9]. Its range was estimated to be 16,500 km^2. During the late 1980's it disappeared from most of its range, a phenomenon that coincided with the appearance of the chytrid fungus *Batrachochytrium dendrobatidis* in museum specimens collected in the area [10].

In 2004–2005, 15 localities with past records of the species were searched (246 person/hour), but the species was found at only two localities between 120–322 meters of elevation on the Cata and Cuyagua rivers [8] (**Figure 1**). Subpopulations at Cata and Cuyagua appear to occupy areas less than 4 km^2 on the lower basin of the river, between 100–320 meters of elevation [11, 12, 13, 14]. However, we cannot rule out that they extend over a slightly larger area, since steep terrain and cliffs has limited explorations upstream. Based on a minimum cell size of 4 km^2, its area of occupancy (AOO) has been estimated as 8 km^2. The extent of occurrence (EOO) of its extant range is estimated to be 80 km^2 [1].

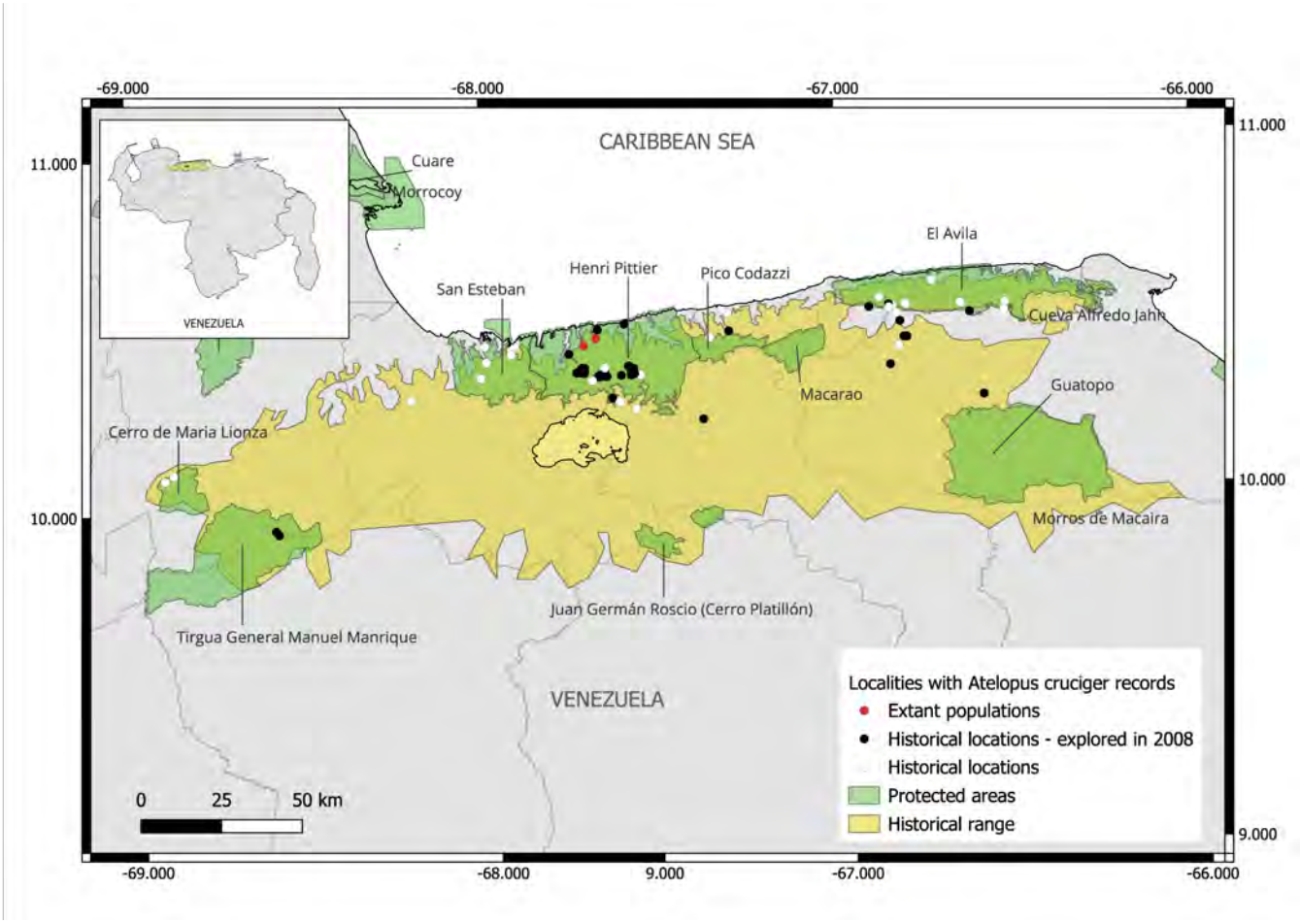

Figure 1: **Geographic distribution of the Rancho Grande harlequin toad (*Atelopus cruciger*).** Most known locations lie within protected areas.

Abundance of reproductive individuals in both subpopulations has been estimated based on capture histories and mark-recapture models. In Cata river, its abundance varied between 24 and 119 during 2005–2012, with no increasing or decreasing trend [12, 13]. The average abundance in this subpopulation was 77 reproductive individuals during 2005-2013. The subpopulation at Cuyagua appears to be larger. The number of reproductive individuals varied between 274 and 494 between December 2013 and May 2014, with an average of 356 reproductive individuals at this locality (capture histories from [14] reanalyzed). Based on the sizes of both subpopulations, we estimated the population to contain 433 reproductive individuals. However, a tenfold variation was observed between 2010 and 2013. The number of reproductive individuals increased from 35 to 350, due to a peak in recruitment [13].

Although systematic monitoring stopped in 2015, adults were sighted in 2016, 2017, 2018, 2020, 2022 and 2023. In 2023, 38 adults were counted during a six-hour session (5 persons) in a 350 m transect in Cata. Based on the number of detected adults and their detection probability estimated for the dry season from capture-recapture models [13], we approximated the total population size in Cata in 110 adults in 2023. In Cuyagua, more than one hundred of emerging juveniles were observed on the river beaches at the end of the dry season (April) in 2022 and 2023. We have no evidence indicating drastic changes in any of these subpopulations. However, as epidemic outbreaks can rapidly decimate entire populations and we do not fully understand what triggers them, precaution is warranted.

1.4 Habitat and ecology

The Rancho Grande harlequin toad is a terrestrial species with diurnal habits. Adults are found on the margins of the streams with clear neutral pH waters surrounded by gallery, cloud, deciduous and semi-

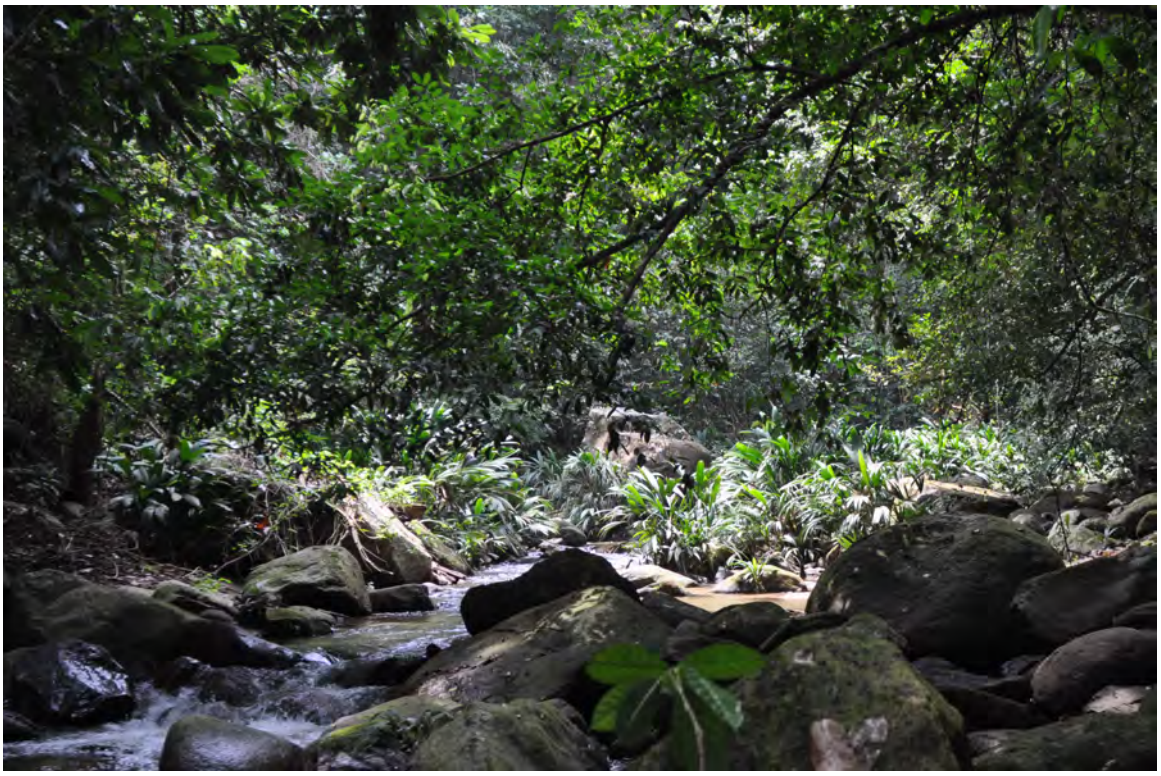

Figure 2: **Habitat of the Rancho Grande harlequin toad (*Atelopus cruciger*).** Lower section of Cata river, Aragua State, Venezuela. Foto: Margarita Lampo

deciduous montane forests (**Figure 2**). During the dry season, adults are commonly found on sand or gravel beaches, on rocks, or on *Cyclanthus bipartitus* plants that grow between rocks [15, 12]. Adults are occasionally seen on the vegetation climbing up to two meters above the ground. Males tend to occupy fixed territories that can partially overlap but no physical aggressive or territorial defence behaviour between adult males have been observed [16]. During the rainy season, the water level may rise two meters and most adults retreat into the forest [12].

This species shows indirect development with a larval stage represented by rheophilic benthic tadpoles. Between 400 and 1,200 oocytes have been counted in mature females. In captivity, females have laid 350–400 eggs in a single spawn. Tadpoles, although difficult to detect in the wild, can be found attached by their abdominal sucker underneath and on the side surfaces of the rocks in fast-flowing waters. Recently metamorphosed tadpoles, with snout-vent length of 0.5–0.7 cm, have been observed during April–May nearby the water. However, they disappear into the forest until they reach a size of 2.0–3.5 cm. Mature individuals congregate on the stream shoreline during the dry season to breed. Mark-recapture data suggests that few adults survive to the next dry season in natural population, therefore, generation time is estimated in 2–3 years. However, adults have been kept in captivity for >30 months. Adults feed on insects, with a predominance of ants and beetles [17]. Parasitology studies from museum specimens suggest adults are often infected with nematodes and cestodes [18]. A reduction in the average body size of reproductive individuals in post-epidemic populations compared to pre-epidemic populations suggest that Rancho Grande harlequin toads are breeding earlier to compensate for the high chytrid-induced mortality [19].

1.5 Primary threats

Chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*, is currently considered its major threat. The disappearance of *A. cruciger* from most historic localities during the late 80's and early 90's is attributed to an epidemic event caused by this pathogen [10]. *Batrachochytrium dendrobatidis* has been detected on other amphibian species in several localities on the northern and

southern slopes of the cordillera de La Costa [20]. The lower elevation of localities where *A. cruciger* still survives suggest that lowland subpopulations have better chances to recover from disease outbreaks [8]. Nonetheless, the fungus is highly virulent for this species. Infected adults have a life expectancy of few weeks [13]. Persistence of wild populations in the presence of this fungus has been attributed to a reduced transmission in lowland warm localities [13, 21]. The potential effects of global climate change on the epidemiology of chytridiomycosis remain uncertain. While an increase in temperature may reduce transmission rates and promote endemic coexistence of toads with the fungus, an increase in the severity of droughts can have the opposite effect on transmission triggering epidemic outbreaks and severe fluctuations in the population size. Rapid recruitment appears to play a key role in the persistence of *A. cruciger* populations. Therefore, climatic events that reduce recruitment could compromise the ability of population to persist with enzootic infection [21].

Although currently known subpopulations are located within seven protected areas (**Figure 1**), pressure from nearby inhabitants to use these habitats for recreational purposes continuously grow and local law enforcement capabilities are insufficient. Also, environmental degradation due to agriculture related activities in some of its former habitats on the Cordillera de la Costa may prevent the recovery of undetected populations. Satellite imagery analyses project a loss of 30% and 84% of semi-deciduous and deciduous forests, respectively, by 2036 [22]. In 2023, a crop smallholding was detected within one kilometre of the relict population at Cuyagua river.

2 Action plan

2.1 Rationale and goal

Chytridiomycosis is the major threat for Rancho Grande harlequin toads. *Bd* continues to be highly virulent for these amphibians in relict population, but high recruitment of juveniles and low transmission of the pathogen in lowland populations allow populations to coexist endemically with the fungus. However, scenarios that increase *Bd* transmission or reduce recruitment could rapidly push populations to a collapse and, with only a few relict populations, the species to extinction. This action plan aims to 1) increase the number of extant populations and the current geographic distribution of the species by reintroducing captive bred individuals into habitats within their former range, 2) mitigate threats by selecting lowland habitats for reintroduction and improving the management of protected areas to maximize juvenile recruitment, 3) explore both historic and new lowland localities, looking for other relict subpopulations in order to incorporate them to *ex-situ* breeding program and to increase the genetic diversity preserved, 4) engage with local communities and integrate them into harlequin toad conservation and 5) raise local, national, and global awareness of Rancho Grande harlequin toads and their major threats.

3 Current protection

Relict populations of Rancho Grande harlequin toads are located on the northern limit of the Henri Pittier National Park and most of its former habitats lie within this and four other protected areas, namely: Waraira Repano, Macarao, and Guatopo national parks, on the central portion of Cordillera de La Costa. While most habitats on this mountain range remain pristine, there is an increasing pressure to expand agricultural activities and urban settlements into protected areas. Satellite imagery suggests that 13% and 30% of semi-deciduous and deciduous forests were lost between 1986 and 2001 [22]. Semi-deciduous and deciduous forests are located at low elevation, where the Rancho Grande harlequin toads have thrive and appear to have a better chance to coexist with *Bd*. Nevertheless, current protection is insufficient to guarantee the preservation of these lowland habitats.

4 Previous and current conservation actions

4.1 *In-situ*

Since 2005, relict populations have been systematically monitored using mark-recapture methods (2005–2014) [12, 13, 14] or visual counts (2015–2023) [unpublished]. Estimates of detection rates based on mark-recapture data, have improved significantly our ability to approximate the abundance of reproductive individuals from visual counts. Regular visits to these population during the dry season every year are used to update the abundance of reproductive individuals. Also, the detection of juveniles at the end of the dry season is used as an indicator of breeding success. Habitat quality is also monitored yearly to detect potential threats to relict populations.

Since the systematic survey of 15 historical locations in 2008 [8], only few explorations have been conducted by trained field personnel. In 2023, the lower sections of Oricao river on the northern slope and Pacairigua river on the southern slope of Cordillera de la Costa were explored. A citizen science campaign was also initiated to aid with the location of undetected subpopulations.

4.2 *Ex-situ*

The first *ex-situ* program for Rancho Grande harlequin toads was launched in 2022, with the opening of the **Centro de Reproducción de Investigación de Arlequines (CRIA)**. CRIA consists of two facilities, one at Leslie Pantin Zoo in Turmero (Aragua state) and the other at FUDECI in Caracas, for holding a back-up captive colony and producing captive bred harlequin toads for future release into the wild [23]. CRIA currently holds 23 founders and one cohort of developing tadpoles [23].

4.3 Engaging communities and raising awareness

Cuyagua is a small town located in the central coast of Venezuela, flanking one of the two known *Atelopus cruciger* populations. The Government Statistics Bureau has not published demographic data in the last ten years, but local people estimate the resident population in less than 1,000 inhabitants. Some residents are already engaged in small community projects towards the management of local natural resources for the development of a ecologically friendly tourism. By incorporating some of these residents in field work activities, we are beginning to provide them with a toolkit for discovering, valuing, and managing their local biodiversity. Also, CRIA's exhibit of the Rancho Grande harlequin toad at the Leslie Pantin Zoo provides a unique opportunity to raise awareness about this species and its major threats.

5 Knowledge gaps

5.1 *In-situ*

Because tadpoles are rarely seen in the wild, little is known about their habitat, diet or behaviour. Also, the role of tadpoles in *Bd* transmission is unknown. We do not know if they get infected by *Bd* in the wild, and are vulnerable to developing the disease. Mathematical models suggest that tadpoles could act as a *Bd* reservoir for the infection of post-metamorphic individuals or as banks for recruitment of healthy juveniles [24]. Infection of tadpoles with *Bd* in rapid-water habitat where they live seems unlikely, but the distribution of free zoospores in the environment is unknown.

Little is known about environmental cues used by toads to move between habitats. Adults, for example, return to the same locations at each breeding season. The learning mechanisms involved in selecting locations or establishing routes are not well understood and may include interactions between juveniles and adults (*e.g.*, social learning). Understanding these mechanisms is crucial for designing reintroduction strategies [16].

The potential effect of climate change in populations of the Rancho Grande harlequin toads is still difficult to predict. Temperature is expected to increase and dry seasons will become extreme, but these

variables can have opposite effects on different demographic and epidemiological parameters. Mathematical models suggest that *Bd* transmission and juvenile recruitment are the two parameters with greater incidence in the probability of coexistence [21]. Therefore, we need to understand how extreme climate events will affect these two parameters.

5.2 *Ex-situ*

We are just beginning to understand how *Atelopus cruciger* adapts to captivity and its requirements to effectively breed outside its natural environment. Although four spawning events have occurred at CRIA, fertilization rates are still low: only one produced over 80 tadpoles. We do not fully understand what determines the fertilization success. Also, mechanisms of calcium incorporation and metabolism in developing tadpoles are not well understood. The incidence of spindly leg syndrome (SLS) in Rancho Grande harlequin toads at CRIA has been very high, despite tadpoles being raised in water with calcium hardness between 60–80 mg/L. We are currently exploring different nutrition protocols and their relation to calcium metabolism, to reduce the incidence of SLS.

Information on the genetics of the Rancho Grande harlequin toads is lacking. The genetic diversity in remnant populations, the genetic relatedness between individuals in our captive breeding program or the level of genetic divergence between subpopulations are unknown. Genetic characterization of both relict subpopulations and captive-bred specimens is required to address these questions and it is crucial for planning future population reinforcements and reintroduction to suitable historical areas of occupancy.

6 Challenges and obstacles

Obtaining funds for research or conservation is the greatest challenge in a country with an economy running on a three-digit inflation rate. Government funds are currently inexistent and local private donors are increasingly scarce. We are currently engaged in an aggressive fund seeking campaign to secure funds to sustain our conservation program.

7 Budget and funding sources

BUDGET CATEGORY	**ROUGH ESTIMATE**	**FUNDING SOURCES IDENTIFIED**	**FUNDS/TIME**
Captive propagation of species (5 years)	$50,000	**Private donors** **IVIC** **Amphibian Ark** Amphibian Ark	**$3,000 funded/year 1** **$3,600 funded/year 1** **$5,000 funded/year 1** **$4000 funded/year 2**
Identification of undetected populations and potential release sites (2 years)	$15,000	**Atelopus Survival Initiative** Bin-Zayed Conservation Fund	**$5,700 funded/1 year**
Engage local communities in the conservation of species (5 years)	$5,000	**Atelopus Survival Initiative**	**$1,300 funded/1 year**
Release captive bred individuals (5 years)	$30,000	Bin-Zayed Conservation Fund National Geographic	
TOTAL	**$100,000**		

8 Success indicators

	OBJECTIVES	**INDICATORS**
1	Established capacity in *ex-situ* facility	110 adults (Maintenance tanks) 150 juveniles (Small cages) 2 amplectant pairs (Breeding tanks) 240 tadpoles (Tadpole tanks)
2	Obtain founders from source populations	9 females 10 males 3 juveniles
3	Trigger spawning	4 spawning events
4	Obtain F1 tadpoles	~ 60 tadpoles (stage 25)
5	Raise F1 tadpoles to metamorphosis	~ 63% survival (to stage 44)
6	Raise F1 juveniles to adults	100% Spindly leg syndrome
7	Develop protocols	1 husbandry and breeding protocol
8	Identify potential release sites	# sites identified
9	Release captive bred toads	# captive bred individuals released
10	Establishment of new populations	# adults / juveniles
11	Community Awareness and engagement	# persons in local communities with some knowledge about the species and its threats and participating in conservation activities

9 Priority actions

The current plan is based on five priority actions described below:

OBJECTIVES	ACTIONS	TIME FRAME	RESPONSIBLE
Identification of undetected populations	Exploration of former and new habitats	2 years	Onil Ballestas Fernando Rojas-Runjaic
Regular assessment of the size and health of relict populations	Determine abundance and infection prevalence	10 years	Margarita Lampo Onil Ballestas
Establishment of a back-up colony and captive propagation of species	Establish an ex-situ captive breeding facility Build hubandry and breeding capacity	10 years	Margarita Lampo Onil Ballestas Ingrid Márquez
Raise public awareness and engage local communities in conservation actions	Create conservation awareness Build local capacity for monitoring species	5 years	Margarita Lampo Onil Ballestas Ingrid Márquez
Increase the geographic distribution of the species	Reintroduction of species into selected habitats	10 years	Margarita Lampo Onil Ballestas Ingrid Márquez

9.1 *In-situ*

The *in-situ* component of this action plan focuses in 1) monitoring the abundance and health of individuals in relict populations, 2) reintroducing captive bred toads in selected habitats within their historical range, 3) mitigating the main threats in relict and reintroduced populations, 3) explore both historic and new lowland localities, looking for other relict subpopulations in order to incorporate them to *ex-situ* breeding program and to increase the genetic diversity preserved, and 4) raising awareness and building capacity among local communities for the management of wild populations. Also, further research into the factors affecting *Bd* transmission and juvenile recruitment will be required to better understand the potential effects of climate change on harlequin toad populations and on the genetics of the species to warrant the preservation genetic variability.

9.2 *Ex-situ*

The primary goal of the *ex-situ* component is to establish a captive breeding colony as an insurance population to 1) reduce the extinction risks due to stochastic threatening processes associated with its small populations and reduced extent of occurrence, 2) buy time until an effective strategy for minimizing the impact of chytridiomycosis in wild populations is developed, and 3) restore wild populations through reintroduction of captive bred individuals into suitable habitats (*e.g.*, thermal refuges). The immediate goal is to maintain a genetically and demographically viable population in captivity and produce sufficient fit and healthy toads for release in the wild.

9.3 Capacity building for *ex-situ* management

Capacity building focuses on training individuals with skills for specific tasks, organizing teams for carrying on projects, developing institutional leadership, sharing knowledge, and experiences with other groups working in the conservation of harlequin toads. As part of the HarleCAP (https://www.atelopus.org/the-initiative), a broader action plan aimed at improving the range-wide conservation of harlequin toads, this

the Rancho Grande harlequin toad Action Plan benefits from the collaborative effort of 38 experts from eleven countries. Through training course, workshops, and a communication network for consulting on specific topics HarleCAP maximize the probability of success of the specific action plans. All research results, protocols or methodologies derived from the *ex-situ* and *in-situ* activities of this action plan will be freely available.

9.4 Develop husbandry guidelines

Husbandry and breeding guidelines for the The Rancho Grande harlequin toad developed at CRIA were modelled after the *ex-situ* operation of the Panama Amphibian Rescue (PARC) [25] for *Atelopus varius/zeteki* and of Center Jambatu in Ecuador [26] for several *Atelopus* species, the protocols developed by the Dallas World Aquarium for *Atelopus balios* [27], and the National Aquarium in Baltimore [28], with modifications according to in-house experience and information gathered during the last 17-years from two relict populations. As our program is in its initial phase and new knowledge is expected to emerge, this first version will be adjusted and updated.

9.5 *Ex-situ* research

9.6 Reintroduction strategy

Reducing the risk of extinction and restoring self-sustaining populations that will thrive naturally without the need for intensive conservation is the ultimate goal of this plan. Increasing its actual extent of occurrence will reduce the risk of extinction through local disappearance of subpopulations by locally driven causes. Rancho Grande harlequin toads are currently listed as Critically Endangered (CR) under criteria B1ab(iii)c(iv)+2ab(iii)c(iv) [1]. The species' estimated extent of occurrence (EOO) is only 80 km^2 and its area of occupancy (AO) is 8 km^2. Reintroduction strategy will aim to increase EOO and AO beyond the threshold for Critically endangered, 100 km^2 and 10 km^2 respectively. The selection of habitats for reintroduction would have to consider the elevation, as this species has only been rediscovered in lowlands (thermal refuges), and the proximity of semi-urban developments.

9.7 Raising awareness and engaging stakeholders

Raising awareness about the need to preserve the biodiversity a priority for this action plan. Research indicates that children's constructive engagement with the natural world contributes to the development of environmentally conscious and engaged individuals. It is crucial to involve not only children but also their families in this endeavour, as it fosters family support and cultivates a sense of community. While school workshops have been effective in reaching children, their experiences in extracurricular settings have demonstrated even greater efficacy. Interventions conducted within amphibian zoos have shown considerable success in enhancing students' scientific knowledge and motivation. The presence of the Rancho Grande harlequin toad exhibit at Leslie Pantin Zoo presents a remarkable opportunity to instill environmental awareness in children and promote the importance of conserving their local biodiversity.

The action plan aims to foster collaboration among a wide range of stakeholders, such as governments, NGOs, scientists, businesses, local communities, and other relevant parties, to collectively safeguard the Rancho Grande harlequin toad from the brink of extinction. The primary stakeholders for this action plan are:

- Leslie Pantin Zoo: Federico Pantin and Tuenade Hernández.
- Academic institutions - researchers and captive breeding specialists.
- Conservation groups: *Atelopus Survival Initiative*, Luis Fernando Marín.
- Communication organizations: Río Verde, Diego Bilbao.
- Education ONG organisations: AMBLEMA.
- Local communities close to sampling sites: Cuyagua and Cata.
- Local environmental and park authorities: Ministerio de Ecosocialismo and INPARQUES.

10 Exit strategy

The aim of this action plan is to improve the status risk of Rancho Grande harlequin toads from Critically endangered to Endangered in 10 years. This action plan will be considered terminated when the species is no longer in the Critically Endangered category. Below are listed the criterion that will trigger the termination of each action of this program.

	OBJECTIVES	**ACTIONS**	**EXIT TRIGGER CRITERIA**
1	Identification of undetected populations	Exploration of former and new habitats	Extent of occurrence > 100 km^2 threshold for CR Area occupancy > 10 km^2 threshold for CR
2	Regular assessment of the size and health of relict populations	Determine abundance and infection prevalence	Extent of occurrence > 100 km^2 threshold for CR Area occupancy > 10 km^2 threshold for CR Abundance of reproductive toads in each subpopulations > 250 threshold for CR
3	Captive propagation of species	Establish an ex-situ captive breeding facility Build hubandry and breeding capacity	Extent of occurrence > 100 km^2 threshold for CR Area occupancy > 10km^2 threshold for CR
4	Raise public awareness and engage local communities in conservation actions	Create conservation awareness Build local capacity for monitoring species	Local communities engaged conservation activities
5	Increase the geographic distribution of the species	Reintroduction of species into selected habitats	Extent of occurrence > 100km^2 threshold for CR Area occupancy > 10km^2 threshold for CR

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