ACTION PLAN FOR *ATELOPUS CRUCIGER*

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COORDINATOR AND CONTACT DETAILS
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BACKGROUND

SPECIES
Sapito rayado or Rancho Grande harlequin frog, *Atelopus cruciger* (Lichtenstein & Martens, 1856).

CONSERVATION STATUS

Critically Endangered (CR) according to IUCN and Lista Roja de la Fauna Venezolana

DISTRIBUTION, POPULATION SIZE AND TRENDS
Until the 1980s, this species 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 (*1-4*). Records of this harlequin frog exist from 77 localities distributed in most of the Cordillera de La Costa, from sea level to 2,400 meters of altitude (*5-8*). One individual was also sighted in Guatopo National Park, on the Serranía del Interior, in 1984 (*9*). 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 hours), but the species was found at only two localities between 120-322 meters of altitude on the Cata and Cuyagua rivers (*8*). Subpopulations at Cata and Cuyagua appear to occupy areas less than 4 km² on the lower sections of the river, between 100-320 meters of altitude (*11, 12*), although the presence of steep terrain and cliffs has limited explorations upstream. Based on a minimum cell size of 4 km², its area of occupancy (AOO) has been estimated as 8 km². The extent of occurrence (EOO) of its extant range is estimated to be 80 km².
Abundance of reproductive individuals in both subpopulations has been estimated based on capture histories and mark-recapture models (POPAN). The abundance of reproductive individuals at Cata 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 average 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 (*11*) 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 and 2022. In Cuyagua, hundreds of 1-5 mm juveniles were observed on the river beaches in 2022. We have no evidence indicating drastic changes or future reductions 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.

HABITAT AND ECOLOGY

Atelopus cruciger is a terrestrial species with diurnal habits. Adults are found on the margins of the streams with clear neutral waters surrounded by gallery, cloud, deciduous and semi-deciduous montane forests. During the dry season, adults are commonly found on sand or gravel beaches, on rocks, or on the *Cyclanthus bipartitus* plants that grow between rocks. 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 and occasionally have been observed defending these areas (*14*). During the rainy season, the water level may rise two meters and adults retreat into the forest (*12*).

This species shows indirect development. Between 400 and 1,200 oocytes have been counted in mature females although we do not know how many are laid in a single spawn. Only one females has laid in cativity; the estimated number of eggs were 60-80. Although tadpoles raised in captivity were described (*15*), they have been seen in the wild just once attached to the bottom of rocks located on the flowing water. Recently metamorphosed tadpoles, with snout-vent length of 0.5—0.7 cm, have been observed during April—May. However, they disappear into the forest until they reach a size of 2.0—3.5 cm. Mature individuals (2.0—4.1) congregate on the stream shoreline during the dry season to breed. Only 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 (*16*). Parasitology studies

from museum specimens suggest adults are often infected with nematodes and cestodes (*17*).

PRIMARY THREATS

Chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis*, is currently considered its major threat. The disappearance of the species from most historic localities during the late 80's and early 90s is attributed to chytridiomycosis (*10*). *B. dendrobatidis* has been detected on other amphibian species in several localities on the northern and southern slopes of the cordillera de La Costa (*18*). The lower altitude of localities where this species is currently known suggest that lowland subpopulations have better chances to recover from disease outbreaks (*8*). Nonetheless, this 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, 19*). 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 and fungi, 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 populations. Therefore, climatic events that reduce recruitment could compromise the ability of population to persist with endemic infections (*19*).

Although subpopulations currently known are located within the Henri Pittier National Park, 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 (*20*).

CONSERVATION MEASURES REQUIRED

Research suggests species captive breeding and species re-introductions as the most effective strategies to prevent the species extinction. Mathematical models and data on demography, epidemiology, habitat use, and behavior are now available for the design of harvest plans, reintroduction and captive breeding programs. Also, local law enforcement in national should be reinforced to keep illegal activities that impact their ecosystems. Local communities could act as stewards for this species, but it is necessary to build up conservation awareness and local capacities in nearby communities.

CURRENT PROTECTION

Both known subpopulations are located on the northern limit of the Henri Pittier National Park and most of its former habitats lie within this park and El Ávila National Park, Macarao and Guatopo National Park. While most of its former habitats remain pristine, forest loss based on satellite imagery suggests that between 1986 and 2001 Cordillera de La Costa lost near 13% and 30% of semi-deciduous and deciduous forests, respectively (*20*). Most semi-deciduous and deciduous forests are located at low altitudes habitats (*i.e.* thermal refuges), where *A. cruciger* appears to have a better chance to coexist with *Bd*. Therefore, reintroductions in low altitude thermal refuges would have to consider the proximity of urban or semi-urban developments.

CURRENT AND PREVIOUS CONSERVATION ACTIONS

IN-SITU. During the last 15 years, both subpopulations have been systematically monitored using mark-recapture methods (*11-13*) Our ability to estimate the abundance of reproductive individuals from visual counts has significantly improved using detection rates estimated from mark-recapture data. Also, a public awareness program aiming to aid with the location of undetected subpopulations and the creation of awareness about the need to preserve relict subpopulations were initiated five years ago. Divulgative articles and pamphlets with the species basic information, photographs and the need for conservation have been produced and distributed [*e.g.* (*21*)]. We also began exploration of potential sites for the release of captive bred individulas in the future.

EX-SITU. A preliminary study to gain experience in husbandry and breeding of *A. cruciger* was undertaken in 2021. In 2022, with the supprot of Aark we initiated an *ex-situ* program with the opening of the Centro de Reproducción e Investigación de Alrelquines (CRIA), two facilities in Leslie Pantin Zoo in Turmero and FUDECI´s Lab in Caracas, respectively, dedicated to *Atelopus cruciger*. An aggressive fund seeking campaign is now advancing to secure funds to initiate an ex-situ conservation program.

KNOWLEDGE GAPS

IN-SITU: Although tadpoles raised in captivity have been described (*15*), they are rarely seen in the wild. We do not know if tadpoles are infected by *Bd* in the wild, or vulnerable to developing the disease. Mathematical models suggest that tadpoles can act as a *Bd* reservoir for the infection of post-metamorphic individuals or as banks for recruitment of healthy juveniles (*19, 22*). Infection of tadpoles with Bd on high current habitats, where they live, seems unlikely, but the role of tadpoles in *Bd* transmission is not fully understood. Mathematical models suggest that zoospore survival in biotic or abiotic reservoirs play a key role in the transmission rates and, therefore, in the likelihood of populations establishing endemic coexistence with *Bd* (*22*). On the other hand, the effect of extreme climate events on the recruitment of juveniles of *A. cruciger* needs to be explored to assess the ability of populations to compensate for losses due to chytridiomycosis.

EX-SITU: Although several species of *Atelopus* have successfully bred in captivity, we are just beginning to understand how *A. cruciger* adapts to captivity and its requirements to effectively breed outside its natural environment.

CHALLENGES AND OBSTACLES

Obtaining funds for research or conservation is the greatest challenge in a country with an economy running on a four-digit inflation rate. Government funds are currently inexistent and local private donors are increasingly scarce.

BUDGET AND FUNDING SOURCES

Because this is a long-term action plan, budgets and funding sources listed are only preliminary and will require constant updating.

BUDGET CATEGORY	ROUGH ESTIMATE	FUNDING SOURCES IDENTIFIED	FUNDS/TIME
Captive propagation of species (5 YEARS)	$40,000	**Private donors** Atelopus Survival Initiative Amphibian Ark Prince Bernard Nature Fund Whitley Award Rufford Foundation Private Donors	**$4,000 (secured) / 1 year** $5,000-25,000 (requested) / 1-3 years $5,000 funded £50,000 (requested) / 1 year $12,000 in-house / 1 year
Identification of undetected populations (2 YEARS)	$5,000	Bin-Zayed Conservation Fund Atelopus Survival Initiative	
Engage local communities in the conservation of the species	$5,000	Prince Bernard Nature Fund Atelopus Survival Initiative	
Release of captive bred individuals	$15,000	Bin-Zayed Conservation Fund Prince Bernard Nature Fund	
TOTAL	**$65,000**		

PROGRAM SUCCESS: INDICATORS

	OBJECTIVES	INDICATORS
1	Establish an ex-situ facility	Capacity of facility in terms of reproductive and maintenance units.
2	Obtain founders from wild populations.	# of founders obtained
3	Adapt adult pairs to captivity.	# of reproductive adults in good health and body condition
4	Obtain F1.	# batches and eggs per batch obtained
5	Raise tadpoles and juveniles	% survival of tadpoles and juveniles
6	Develop protocols	# of protocols and guidelines developed
7	Identify potential release site	# sites identified
8	Release of captive bred animals	# sites identified
9	Establishment of new population	# of adults recovered
10	Community engagement	# of persons in local communities with some knowledge about the species and its threats
11	Identification of undetected populations	# of locations visited / # of populations discovered

PRIORITY ACTIONS

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

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OBJECTIVES	ACTIONS	TIME FRAME	RESPONSIBLE
Identification of undetected populations	Exploration of former habitats	1-3 years	Onil Ballestas
Regular assessment of the size and health of relict populations	Determine abundance and infection prevalence	1-5 years	Margarita Lampo
Captive propagation of species	Establish an ex-situ captive breeding facility	1-3 years	Margarita Lampo Ingrid Márquez
Engage local communities in the conservation of the species	Create conservation awareness Build local capacity for monitoring species	3-5 years	Margarita Lampo Onil Ballestas Ingrid Márquez Omar Hernández
Increase the geographic distribution of the species	Reintroduction of species into selected habitats	>5 years	Margarita Lampo Omar Hernández Ingrid Márquez

IN-SITU

Relict populations of *A. cruciger* are located within the Henri Pittier National Park. The main actions to undertake are to continue to monitor relict populations to determine the abundance and health of individuals, regularly assess the threats and identify the factors affecting *Bd* transmission. Among potential threats are the expansion of small illegal farms from nearby communities and extreme climatic events that could reduce juvenile recruitment or increase *Bd* transmission. Aerial photographs are regularly taken to monitor illegal farming in the vicinities of relict populations.

EX-SITU

The primary goal is to establish a captive breeding colony as an insurance population to i) reduce the extinction risks due to stochastic threatening processes associated with its small populations and reduced extent of occurrence, ii) 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 adequate habitats (*e.g.* thermal refuges).

Capacity building for ex situ management

At the Genetics and Population Ecology Lab at IVIC, our team led several projects on experimental infections with *Bd* of several endemic amphibians. Biosecurity protocols were followed to avoid cross contamination between control and experimental groups. Survival of controls was 100%. A pilot study on the reproductive behavior of *Atelopus crucicer* was recently implemented at FUDECI to study the reproductive behavior of the species. Adults have been kept in captivity in good health and body condition and a first spawn was obtained. Experience gathered through these pilot studies have been used to train students and personnel on the husbandry of amphibian species. I am currently training two students and one assistant on the husbandry of *Atelopus cruciger*.

Develop husbandry guidelines

Although there are no husbandry or breeding protocols for *A. cruciger*, several have been developed and are available for other species in the genus [*e.g. Atelopus bolios* (*23*), *Atelopus zeteki* (*24*)]. These protocols and data collected *in situ* on habitat preferences and diet are currently being used to produce husbandry guidelines specifically for *A. cruciger* (*16*). Ingrid Márquez, a graduate student in zoology from Universidad Central de Venezuela, is adapting the existing protocols for *A. cruciger* and producing guidelines for the husbandry and breeding of this species in captivity.

Ex situ research

The *ex-situ* rearing of individuals from eggs to adults will provide valuable information on its cycle, development and reproduction. Although the eggs and early larval satges of *A. cruciger* were described, the development of larvae has not been fully described. Ingrid Márquez will be reponsible for collecting and publishing this information. Further research on the vulnerability of tadpoles to the chytrid fungus *Bd,* the genetics of Bd resistance will improve our understanding on the epidemiology of chytridiomycosis and provide tools for mitigating this disease in the field.

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. The selection of habitats for reintroduction would have to consider the altitude, as this species has only been rediscovered in lowlands (thermal refuges), and the proximity of urban or semi-urban developments.

Education and raising awareness: connecting children and their families

Literature suggests that it is the combination of multiple experiences, rather than one single life-changing experience, what helps to produce environmentally informed and active citizens. Effective environmental education programs need to be personally relevant to the children and directly related to their own context, that is, what is my own backyard. Children families need also to be included not only to encourage family approval and engagement, but also the sense of community tends to be nourished. Workshops in schools are an efficient way to target children, but printed material is necessary for the children to share the school experience with their parents at home. A recent local experience showed that short podcast for community radio stations are also an efficient way to spread information.

Community and stakeholder engagement

Cuyagua is a small town located in the northern coast of Venezuela, flanking one of the two known *A. cruciger* populations. Although the Government Statistics Bureau has not published demographic data in the last ten years, local people estimate the resident population in less than 1,000 inhabitants. There is one local school with 30-40 children under age 12. AMBLEMA a Venezuelan ONG dedicated to improving the reading, mathematics and environmental education programs in schools incorporated the Cuyagua school in their plan. We are jointly designing educational material to increase the awareness and engagement of children in the conservation of Atelopus cruciger. Also some residents in Cuyagua are already engaged in small community projects towards the management of local natural resources for the development of a ecologically friendly tourism. This project will build-up upon the work of these local leaders by providing a toolkit for discovering, valuing and managing their local biodiversity.

EXIT STRATEGY

The aim of this action plan is to improve the chances of long-term survival of *A. cruciger* by increasing the species population size and its extent of occurrence. This action plan will be considered terminated when the species is no longer in the Critically Endangered category. Below are described the criteria that will trigger the termination of each action of this program.

OBJECTIVES	ACTIONS	EXIT TRIGGER CRITERIA	RESPONSIBLE
1. Identification of undetected populations	Exploration of former habitats	Extent of occurrence > 100km2 threshold for CR	Onil Ballestas,
2. Captive propagation of species	Establish an ex-situ captive breeding facility	Goals 1 and 4 are achieved	Margarita Lampo, Omar Hernández, Ingrid Márquez.
3. Engage local communities in the conservation of the species	Create conservation awareness Build local capacity for monitoring species	Local communities engaged in monitoring of existing populations	Margarita Lampo Onil Ballestas
4. Increase the geographic distribution of the species	Reintroduction of species into selected habitats	Extent of occurrence > 100 km2 threshold for CR	Margarita Lampo. Omar Hernández. Ingrid Márquez.

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