amphibian ark

Keeping threatened amphibian species afloat

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Ex situ conservation for the critically endangered tree-frog *Nyctimantis pomba*

Cybele Sabino Lisboa, Reserva Paulista – Zoológico de São Paulo; Cauê Monticelli, Fundação Parque Zoológico de São Paulo, Brazil; and Clodoaldo Lopes Assis, Universidade Federal de Viçosa, Brazil

Introduction

Nyctimantis pomba is a Critically Endangered tree-frog from Brazil, endemic to a small (1.36 km²) and non-protected fragment of Atlantic Forest in the municipality of Cataguases (Minas Gerais State), which faces legal problems and is highly impacted by farming activities. It is a very rare species and field researchers believe it will be extinct in a few years if nothing is done to protect and preserve it. Considering this scenario, the Instituto Chico Mendes para Conservação da Biodiversidade - ICMBio (a federal government agency responsible for biodiversity related issues), and the Association of Zoos and Aquariums of Brazil (AZAB), included *N. pomba* among the twenty-five Brazilian species which have the highest priority for developing captive breeding programs. So far, this is the only amphibian in this list. This species was evaluated using the Conservation Needs Assessments process (https://conservationneeds.org/Reports/SummaryReport?assessmentId=6780&speciesId=7253&countryId=101) and it is one of the highest priority amphibian species for conservation action in Brazil.

So, in 2019 we began a conservation program to develop protocols for captive management and reproduction for this species, and to establish an *ex situ* back-up population in case there is a need to supplement the wild population. To start the program, we were provided with a US $3,000 grant from a private donor (via Amphibian Ark), which allowed us to undertake three field trips to search for founder animals and to buy basic lab equipment for maintaining them. During those trips, we collected four males and two juveniles to start the *ex situ* population, but we didn't find any females, which are essential for the program.

As this species is very rare, seasonal and probably only consists of a small population, it is not easy to find individuals, so we needed to increase our efforts and to change the strategies we had used before. To help solve the challenges and continue the search for females, in 2021 we received another grant of US $3,000 provided by Amphibian Ark, and our actions and results are presented in this report.

Fieldwork

To increase search efforts, we hired three field assistants who spent nearly a month in the field daily, from November 16th to December 10th. In addition, during this time, we also accompanied them on a five-day field trip. We sampled three main sites, which are formed by bamboo and swamp with running water.

We realize that these increased efforts were essential to achieve our goals. For the first time, we recorded a large chorus of more than twenty males calling together in one of these sites. This event only occurred on one night and we could have missed it if the assistants didn't go to the field daily, as we arrived at the field a day later. In the days following this event, we found individuals walking around, but we did not experience the same calling activity, and we also found females for the first time.

During the field trips, we found twenty-five individuals, of which seventeen were males, four females and four juveniles (undetermined sexes). We didn't record any re-captures from previous trips. We performed a complete biometry and photo ID of

Field trips were made in November and December 2021 to collect founders of *Nyctimantis pomba*. Photo: Cybele Lisboa.

all captured individuals, and we swabbed the skin of some of the animals to investigate the presence of *Bathrachochytrium dendrobatidis* (chytrid fungus) in the wild population. We sent this material to the Amphibians Natural History Lab (LaHNAB), at the State University of Campinas (UNICAMP). They completed the testing for free and all the results were negative.

Of the individuals captured, all females and some undetermined sexes were collected to become part of the *ex situ* population, and all males were released in the encounter area. Including these new animals, the captive population at the Wildlife Conservation Center of the State of São Paulo (CECFAU) ended up with twelve individuals (five males, four females and three juveniles/undetermined sex).

Ex situ management

The laboratory for managing the species is set up at the CECFAU, located in Araçoiaba da Serra, in São Paulo. We equipped the lab in July 2019 with two terrariums and accessories, purchased with funds from the initial grant, where the first six individuals were already placed. With the most recent grant, we bought another terrarium with accessories (canister filter and rain chamber system). Sao Paulo Zoo also donated three smaller terrariums (without accessories) so we could house all twelve individuals, but they are temporary facilities and we need to acquire bigger terrariums.

We have continued with the same husbandry practices used since 2019. Terrariums are furnished with gravel, trunks, bamboo, plastic tubes and live plants, and circulating water treated with a canister filter. We installed an artificial rain system and fogger to raise enclosure humidity and to stimulate breeding. We provided UVB lighting with the use of a single 18 watt Exo Terra® Repti Glo 2.0 Compact Fluorescent bulb fixture in two enclosures. To feed the animals, we offered crickets, cockroaches and meal worms supplemented with Repashy Superfoods Calcium Plus ICB® vitamins. The food was provided in plastic pots inside the terrariums twice weekly.

One of the females was seen to have eggs in her abdominal cavity. As soon as we got to the lab, we decided to put her in a terrarium with two males, and the next day, she laid eggs in the water. Unfortunately, the eggs didn't develop and we are unsure if they weren't fertilized or if the water conditions were not suitable for their development.

The other individuals were distributed in the terrariums and monitored in relation to food intake and body score. After two months in captivity, one of the juveniles died and the diagnosis of the cause of death was inconclusive. It was the first death we recorded in captivity. The animal's carcass was sent to the Butantan Institute to be integrated into the doctoral thesis entitled "The biology and glands of the Casque-headed Tree Frogs: a successful adaptation". All the other individuals appear healthy, have good body scores and are eating regularly. We have started to collect nocturnal images through the camera, which will be used by a student from Federal University of Viçosa to obtain data about the behavior of the species. The current project is entitled "Behavior of the critically endangered species *Nyctimantis pomba* in captivity".

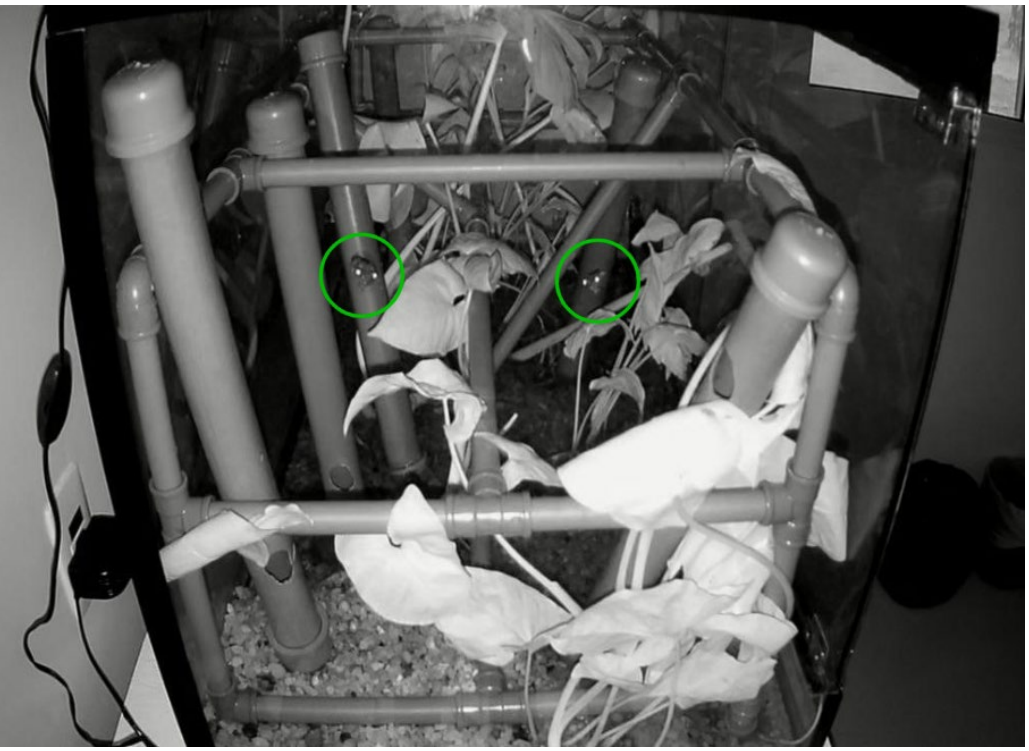
Final considerations and next steps

We successfully achieved our primary goal, which was to find females for the founder captive population of *Nyctimantis pomba*. The funding of this project provided by Amphibian Ark and the strategies used were crucial to find these females and to collect additional information about the reproductive behavior of the species in the wild.

Our husbandry seems to be adequate, and we will wait for the next breeding season to offer stimuli and move the pairs to try to reproduce the species in captivity, which is the next step in the program. We are also waiting for the juveniles to mature, so we know the final composition of sexes in our captive population. We also hope that the images collected with the night camera will provide us with important information about the behavior of the species and that they can contribute to its conservation. Now, as the *ex situ* population is larger and we hope to breed them, we will need new terrariums to expand the lab.

In relation to other actions involving the species, the Amphibian Specialist Group (ASG-Brasil) is organizing a workshop before the end of 2022 to develop a species action plan for *N. pomba*, which will involve all relevant stakeholders.

Nocturnal images from the *Nyctimantis pomba* terrarium. Photo: Cybele Lisboa.



Valcheta frog (*Pleurodema somuncurense*) in the Buenos Aires Ecopark. Photo: Tomas Cuesta.

Valcheta frogs at the Buenos Aires Ecopark

Borja Baguette Pereiro, Dolores Medina and Tomas Sciolla, Fauna Conservation and Management Operative Management, Interactive Ecopark Special Projects Unit, Secretary of the Environment, Government of the City of Buenos Aires, Argentina; and Federico Kacoliris, Somuncura Foundation, Faculty of Natural Sciences and Museum of the National University of La Plata, Buenos Aires, Argentina

In 2013, the Herpetology Laboratory at the Faculty of Natural Sciences and Museum of the National University of La Plata (FCNyM-UNLP) created the Wild Plateau Initiative (now known as the Somuncura Foundation), to work mainly towards the protection of three endemic and emblematic species of the province of Río Negro: the naked mojarra (*Gymnocharacinus bergii*, a small species of fish), the Valcheta frog (*Pleurodema somuncurense*) and the Somuncura frog (*Atelognathus reverberii*). The main objective of this initiative is to ensure the long-term survival of the populations of these species, and to use them as flagship species to promote the biological and socio-economic sustainability of the areas where they occur.

With only a handful of isolated populations remaining in the wild, the Valcheta frog is one of the most endangered frogs living in Patagonia. Its high degree of endemism and the existence of habitat threats have led to this species being listed in the highest threat category both nationally (Endangered, Vaira et al., 2012) and internationally (Critically Endangered, IUCN Red List). In addition to this, due to its evolutionary relevance, the Valcheta frog is the only amphibian in Argentina that is included in the Zoological Society of London's top 100 EDGE (Evolutionarily Distinct & Globally Endangered) amphibians.

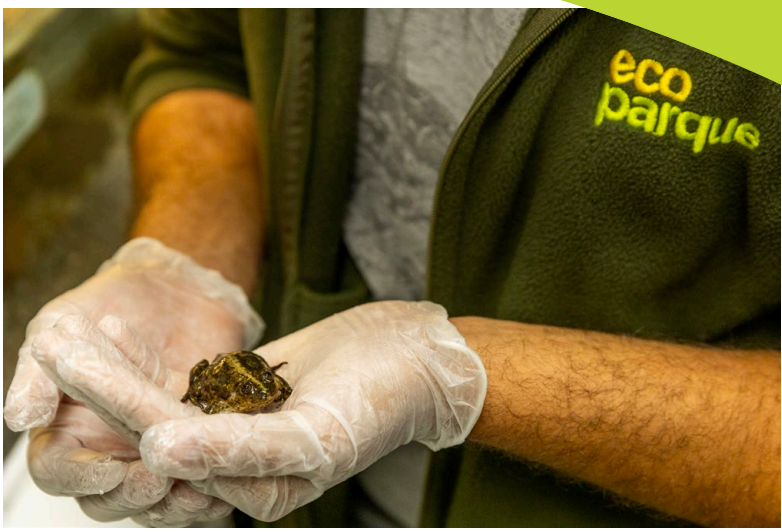
In 2014, with the help of a grant from Amphibian Ark, the Herpetology Section of the FCNyM-UNLP created the Cururú Program and successfully established an *ex situ* survival colony of this species. The primary objective of this colony is to support the reintroduction program of this species carried out by the Somuncura Foundation, with captive-bred individuals, to recover wild populations of this threatened amphibian. Within this framework, six reintroductions of captive-bred frogs have already been carried out in three previously restored and threat-free habitats. In at least one of these habitats, where a local population of this species was extinct, reintroductions have succeeded in re-establishing a new healthy population of this amphibian.

After the joint work of the FCNyM-UNLP Somuncura Foundation with the Buenos Aires Ecopark, we decided to establish a second backup population of Valcheta frogs at the Ecopark, with the support and authorization of both provincial and national organizations. The Buenos Aires Ecopark belongs to the Secretary of the Environment of the Government of the City of Buenos Aires and is the oldest zoological institution in Latin America. The objective of this second colony is to support a larger population of the species, to contribute to its repopulation. Likewise, a second reserve population can act as a guarantee against stochastic events, thus allowing greater support for the *ex situ* program for this species.

During the first half of 2022, a total of eighty-five Valcheta frogs were transferred from the FCNyM-UNLP facilities to the Buenos Aires Ecopark. It is noteworthy that the "Endangered Amphibian Research and Conservation Program" was approved by the Buenos Aires Ecopark, with the aim of contributing to the conservation of threatened amphibian species in Argentina.

The Buenos Aires Ecopark receives thousands of visitors throughout the year and the work carried out here in relation to the Valcheta frog is shared with visitors through posters and educational talks, with information provided on the species, its conservation status, and the role of amphibians in ecosystems, among other details.

This initiative is of great relevance in the field of biodiversity conservation on a national scale, being the first time that a zoological institution in Argentina has taken responsibility for the start-up and development of a breeding center for a threatened amphibian, primarily for management and conservation purposes.



Reference

Vaira M., M. Akmentins, M. Attademo, D. Baldo, D., D. Barrasso, S. Barrionuevo, N. Basso, B. Blotto, S. Cairo, R. Cajade, J. Céspedez, V. Corbalán, P. Chilote, M. Duré, C. Falcione, D. Ferraro, F. R. Gutierrez, M. R. Ingaramo, C. Junges, R. Lajmanovich, J. N. Lescano, F. Marangoni, L. Martinazzo, R. Marti, L. Moreno, G. S. Natale, J. M. Pérez Iglesias, R. Peltzer, P., L. Quiroga, S. Rosset, E. Sanabria, L. Sanchez, E. Schaefer, C. Úbeda and V. Zaracho. 2012. Categorización del estado de conservación de los anfibios de la República Argentina. *Cuadernos de Herpetología* 26 (1), 131–159.

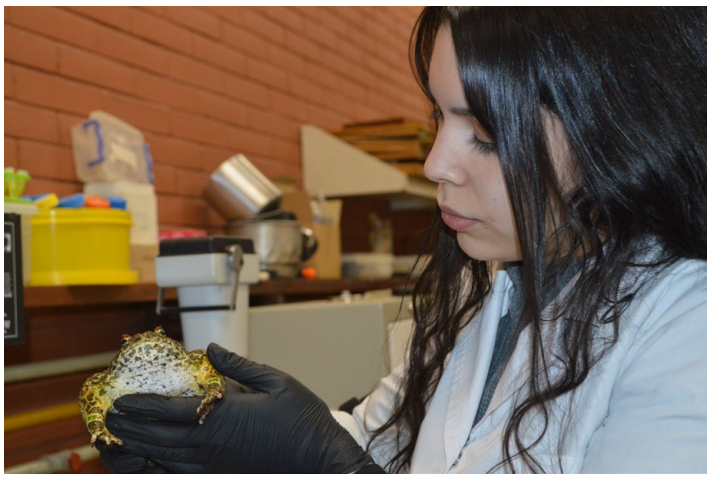
Additional conservation grant awarded

As mentioned in the June 2022 edition of the AArk Newsletter, three grant applications from this year were still under review. We are very pleased to announce that one of these, from Argentina, has subsequently been awarded a US $5,000 grant.

First *ex situ* management actions for the conservation of *Ceratophrys ornata* in Argentina

Sofía M. Perrone – Conservación de Anfibios en Argentina (COANA)

The Argentine horned frog (*Ceratophrys ornata*) is an amphibian which is endemic to the temperate grasslands of Argentina, Brazil and Uruguay. The last records for Brazil and Uruguay date back to 1982 and an intense field search conducted by our working group since 2018 confirmed that the species is extinct in both countries. In Argentina, the conservation status is Vulnerable, while the Conservation Needs Assessment (2021, https://conservationneeds.org/Reports/SummaryReport?assessmentId=6865&speciesId=5027&countryId=99) highlights the possibility of initiating *ex situ* management accompanied by research and education activities.



Sofía M. Perrone examining an Argentine horned frog (*Ceratophrys ornata*).
Photo: Gabriela Agostini.

In 2017, our working group identified priority areas for the conservation of *Ceratophrys ornata* in Argentina in which we conducted studies of population dynamics, reproductive phenology and habitat requirements in parallel with a communication strategy to link communities and key stakeholders. The main threats facing the species in these areas are the increasing urbanization that brings with it a profound modification of the wetland systems where *Ceratophrys ornata* reproduces, and the elimination of individuals by aversion. Additionally, we have identified and mapped areas feasible to be included in planning of management actions that allow increased survival of the species and ensure the viability of the populations.

The main objective of this project is to implement an *Ex Situ* Management Plan in relict populations of *Ceratophrys ornata*. This plan will include the rescue and translocation of adult specimens from urbanized areas to sites identified with habitats which are suitable for the species. In addition, eggs and larvae from fragmented wetlands will be collected, and raised to juvenile stages in a reception center, for the subsequent release of nearby populations. In the long term and in synergy with *in situ* activities, we hope to reduce the impact of habitat loss on relict populations of *Ceratophrys ornata*.

A pair of Argentine horned frogs in amplexus. Photo: Camila Deutsch.

New *ex situ* and *in situ* efforts for the long-lasting conservation of the El Rincon stream frog in Argentina

Federico Kacoliris and Melina Velasco, Wild Plateau Initiative, La Plata Museum, Argentina

The El Rincon stream frog (*Pleurodema somuncurense*) is microendemic to the Valcheta Stream, Somuncura Plateau, in Patagonia, Argentina. This almost wholly aquatic species is highly adapted to live in the thermal headwaters of this stream, however, the introduction of predatory trout in the stream has cornered the frogs to a few hot springs still protected by waterfalls that trout are unable to pass. But even in these remnants of habitat where the frogs were expected to thrive, they are declining because of habitat destruction by livestock (sheep, cattle, and horses) that trample on the watercourse and feed on the native vegetation, leaving the frogs without shelter and reproductive habitat. Nowadays, the entire population of El Rincon stream frog only includes seven extant local populations, isolated from each other by high frequencies of trout in intermediate habitats. Additionally, field records indicate the extinction of at least three of these local populations in recent decades. For these reasons, this species was categorized as Critically Endangered in the IUCN Red List (www.iucnredlist.org/species/20372/85948443) and is the only frog in Argentina listed among the Top 100 Evolutionarily Distinct and Globally Endangered (EDGE) amphibians worldwide (www.edgeofexistence.org/species/el-rincon-stream-frog/).

Since 2013, the Amphibian Ark, the Amphibian Survival Alliance, La Plata Museum, and Fundación Somuncura have been combining efforts towards the recovery of the El Rincon stream frog. Our vision is aimed at doubling the distributional range and the population size of this species by 2030 to ensure its long-lasting viability. To achieve this, we are working on three main objectives, (i) alleviation of the main threats, (ii) increasing suitable habitat, and (iii) recovery of local populations. Our main activities to achieve these objectives are based on the management of invasive predatory fishes, habitat restoration and creation of new habitats, and reintroduction of frogs in suitable habitats which are free of threats. For this last objective, we are combining our *ex situ* and *in situ* efforts.

In 2015 we created the first *ex situ* center for endangered amphibians in Argentina and established there a survival colony of El Rincon stream frogs. This center was a key component of the Action Plan (www.amphibianark.org/wp-content/uploads/2021/02/Plan-de-Accion-para-Pleurodema-somuncurense.pdf, in Spanish) for this frog, allowing the reintroduction of captive-bred animals in habitats where three local populations had previously gone

Suitable habitat, known as a “wild mesocosm”, has been created next to the Biological Station to establish a new local population of El Rincon stream frogs. Photo: Sofía Quiroga.

The wild mesocosm will also serve to host a breeding pool to house frogs for translocation to other restored habitats within the range of the species. Photo: Sofía Quiroga.

extinct. Our actions ensured these habitats were free of threats and fully restored prior to each reintroduction attempt, acting as wild sanctuaries for the species. Further monitoring showed the successful establishment of frogs in at least one of these habitats (i.e., wild reproduction and recruitment of reintroduced frogs). We are still monitoring the frogs in the other two habitats and hope to have good news to share soon.

During 2021 and 2022 we expanded our *ex situ* efforts by creating a new survival colony of El Rincon stream frog. This new *ex situ* colony was established at Eco Parque Zoo, one of the main zoos in Argentina. The combined efforts of both survival centers of this species, La Plata Museum and Eco Parque Zoo, will ensure not only the long-lasting survival of the *ex situ* colony of El Rincon stream frogs but also the production of enough individuals for reintroduction purposes. Additionally, we have also been working *in situ* during this time period.

In 2020 Fundación Somuncura was able to obtain international support to acquire some land in the Somuncura Plateau, with the aim of protecting the El Rincon stream frog as well as other endangered species which live there. This is the first protected area in Argentina primarily created with the aim of protecting an amphibian species. Having the land allowed the creation of a Biological Station to booster conservation actions for this frog. We are now creating *ex situ* facilities within this Biological Station to act as a quarantine to receive frogs intended for reintroduction and coming from the *ex situ* centers of La Plata Museum and Eco Parque Zoo. We are also creating new suitable habitat next to the Biological Station to establish a new local population of El Rincon stream frogs. This sanctuary will act as a "wild mesocosm", which means, a habitat which is free of threats and predators, full of shelter and resources, but with wild water conditions for frogs, and which is fully monitored. This mesocosm will help us to better assess the status of reintroduced frogs, which in turn will help us to improve reintroduction attempts for this species. The wild mesocosm will also serve to host a breeding pool to house frogs for translocation to other restored habitats within the range of the species.

The El Rincon stream frog (*Pleurodema somuncurense*) is microendemic to the Valcheta Stream, Somuncura Plateau, in Patagonia, Argentina. Photo: Hernán Povedano.

In October 2022, the first group of frogs from the *ex situ* center of La Plata Museum will be translocated to this sanctuary. We are enthusiastic and confident that these combined efforts will serve to ensure the conservation of the El Rincon stream frog and promote other amphibian conservation initiatives in Argentina and beyond.

Amphibian nutrition and feeding webinar series

Understanding the nutritional needs of the different orders and groups of species and their different life stages is essential for their well-being. Thus, providing proper nutrition to amphibians kept under human care is necessary to ensure they remain healthy.

Amphibian Ark is pleased to invite you to our *ex situ* amphibian nutrition and feeding webinar series, where renowned professionals will share with us their experience and expertise on this very important aspect of the *ex situ* management of amphibians. The target audience is amphibian conservation program managers, curators, keepers, veterinarians and nutritionists. There is no cost to attend the webinars.

Dates: October 10th, 12th and 14th, 2022

Registration: Please register at www.amphibianark.org/nutrition-webinars/ – the deadline for registration is October 3rd, 2022. The link to the Zoom meetings will be emailed to you after your registration has been received.

For any further information please contact Luis Carrillo – luis@amphibianark.org

Monday October 10, 2022. Times shown are (EDST/GMT -4)
The importance of nutrition on amphibians in captivity - Andrea Fidget (San Diego Zoo Wildlife Alliance) - 10:00 am
Amphibian nutrition and feeding - basic principles - Chris Michaels (ZSL-London Zoo) - 11:00 am
Feeding ecology and applications in *ex situ* populations - Arturo Muñoz (Ghent University) - 12:00 pm

Wednesday October 12, 2022. Times shown are (EDST/GMT -4)
Amphibian energetics and metabolism - Andrea Fidgett (San Diego Zoo Wildlife Alliance) - 10:00 am
Carotenoids and vitamins in amphibian nutrition - Richard Preziosi (University of Plymouth) - 11:00 am
Nutrient composition of insects and gut loading - Dennis Oonincx (Wageningen University and Research Centre) - 12:00 pm
Establishing live food colonies using native species - Ben Tapley (ZSL-London Zoo) - 1:00 pm

Friday October 14, 2022. Times shown are (EDST/GMT -4)
The importance of UV light for amphibians - Arturo Muñoz (Ghent University) - 10:00 am
Nutritional diseases in amphibians - Allan Pessier (Washington State University) - 11:00 am
Applied nutrition research for Mountain Chicken - Amanda Ferguson (ZSL-London Zoo) - 12:00 pm

AArk Husbandry Document library

The Husbandry Document library on the AArk web site (www.amphibianark.org/husbandry-documents) currently has over 295 documents in it, with additional documents being added regularly.

Three new documents have been added recently:

Wyoming toad *Bufo hemiophrys baxteri* now known as *Anaxyrus baxteri* Revised Recovery Plan, May 2015 (English)

This recovery plan's structure articulates both short and long-term strategies that together comprise the conditions under which the Wyoming toad may be delisted. An adaptive management approach, which allows for the continual inclusion of updated research and information, will be the main strategy guiding the management of the species. The captive program maximizes genetic diversity in its annual breeding and continuously develops husbandry strategies to maximize the health of captive populations.

Author: U.S. Fish and Wildlife Service
Publication: 2015
www.researchgate.net/publication/312372803_First_Revised_Recovery_Plan_for_Wyoming_Toad

Husbandry Guidelines for Pickersgill's Reed Frog (*Hyperolius pickersgilli*) (English)

The Amphibian Research Project (ARP) of the Johannesburg City Parks and Zoo (JCPZ) was initiated in 2006 with the objective of conserving selected South African endangered amphibian species by creating and establishing sustainable insurance populations. The project's other objectives were to compile detailed husbandry manuals and protocols to successfully breed and maintain these endangered species *ex situ*, for introduction, reintroduction, or reinforcement in the wild. The first Endangered species bred in this project was the Pickersgill's reed frog (*Hyperolius pickersgilli*).

Authors: Johannesburg City Parks and Zoo Amphibian Research Project Team
Publication: 2022
www.amphibianark.org/wp-content/uploads/2022/05/Hyperolius-pickergilli-Husbandry-manual-2022.pdf

Amphibian Monitoring in Latin America: A Protocol Manual (English)

We wrote this manual to coincide with a series of workshops sponsored by the United States National Science Foundation. The workshops were inspired by the need to coordinate efforts among herpetologists, government agencies, and environmental organizations in light of recent, widespread reports of population declines and extinctions of amphibians in Latin America. Home to the world's richest amphibian fauna, Latin America is a critical region for amphibian conservation. We hope that by promoting inventory and monitoring efforts across the region, scientists will be quicker to find causes for the recent declines and extinctions and recommend management actions that can reverse the population trend and avert an ecological disaster (Wake 1998).

Authors: Karen R. Lips, Jamie K. Reaser, Bruce E. Young, Roberto Ibáñez
Publication: Ver. 2.1 July 2020
https://bit.ly/2WK5HGq

AArk's new Workshop Grants

Since 2009, Amphibian Ark has awarded forty-eight conservation grants totalling over US $222,000 to programs in twenty-two countries. While these have predominantly been for *ex situ* start-up programs for threatened amphibian species, we have also offered program extension, workshop attendance, mentorship and emergency grants. More information about our grants is available on our web site at www.amphibianark.org/conservation-grants/.

From next year, we are pleased to announce that we will also be seeking applications for workshop support grants, which will provide support for organizations which are planning an in-person amphibian conservation-related workshop or symposium, especially those which focus on amphibian husbandry, planning and reintroduction. Applicants must have already secured partial funding for the workshop, and the dates and location for the workshop should have been publicly announced. Grants up to US$2,500 will be available.

Applicants will be required to provide the following information:

- The name, dates and location of the workshop, along with the details of the workshop organizer(s).
- A list of the instructors or presenters, and their institutional affiliations.
- Information about the number of expected participants.
- The scope, format, goals and benefits of the workshop, and how the knowledge gained by participants will be used.
- What the workshop hopes to achieve, and the proposed outcomes or products of the workshop.
- How the effectiveness of the workshop in reaching the goals or producing the desired outcomes will be evaluated.
- Funding received or committed to date to support the workshop, along with any other funding which has been requested from other organizations. A brief income and expenditure budget which shows the breakdown of the workshop costs, including the items for which funding is being requested from AArk.

We will be calling for applications for our next round of grants in March 2023.

Establishing an *ex situ* conservation strategy for Persian mountain salamanders in Iran

Dr. Haji Gholi Kami, Department of Biology, Golestan University, Iran; and Dr. Seyyed Saeed Hosseinian Yousefkhani, Institute of Biological Science, Damghan University, Iran

During the past six months of this project, we carried out fieldwork to determine the presence of salamander habitats and continued with our public awareness campaign. The fieldwork stations were at Ramian, Ziarat waterfall (visited in August and December 2021), and Fazel Abad in the Iranian mountains. These habitats were surveyed, and the springs and water resources investigated to search for populations of Persian mountain salamanders (*Paradactylodon persicus gorganensis*). Unfortunately, we only found one population in Ramian. They were all larvae and no adult animals were seen. Tissue samples were collected from them and they were taken to the laboratory. We also carried out public awareness activities to increase knowledge of the local people about the importance of biodiversity conservation.

Close to Ramyan we reached Pashmeki Spring and to the west of Paqaleah village, is Pashmaki Spring or Ab Qulu Spring, where the water from the spring has flowed over the road. Subadult salamanders have been observed under car wheels there, and they have disappeared. Water has collected on the south side of the road, where there are stones, and we found about ten salamanders there, all of which were larvae. We went to this area again in July 2022 and found a population of about twelve subadult individuals at the top of the spring.

Ziarat village is located in the south of Gorgan. In August 2021 we crossed the iron bridge over the Ziarat River and changed direction to the west and into the forest. In the local language, the area is known as Sorkh Cheshmeh (Red Spring in English). We moved through the forest for about an hour and a half and reached the Khush Darreh area, which is the habitat of salamanders. The path has many small springs. Near the small waterfall, which has a relatively vertical tree next to it, we found salamander larvae. The depth of the springs is shallow and is only about 20-30 cm. The path is relatively difficult to walk along and the place where the salamanders were observed is about 3 km away from the side of the road. This station is the second locality of salamander presence in Golestan province and it will be important to sample for adult specimens for captive breeding. In late December 2021 we visited Ziarat village again. The forest floor was full of dry leaves at this time, the weather was very cold and the path was shady. A small amount of snow could be seen in the shadows and the temperature on this day varied from 2-4ºC. The water temperature at 10 am was 4ºC. No larvae or adult salamanders were observed on this date, possibly because of the cold water in the habitat and the air. Among the habitat plants, we saw *Hedera helix*, *Alnus*, *Urtica*, ferns, *Parrotia persica* and *Viscum album*.

In the east of Gorgan is Fazel Abad. In March 2022, we headed towards the south from the middle of the city to the mountains and the forest, towards Kholindereh and then Mahian. The weather was relatively rainy, and unfortunately, no salamanders were found. The air and water temperature was also very low.

The life cycle of this salamander means they will be adults in the Autumn, and we hope to find adult specimens in October or November. If we manage to find adults, we will bring them to the lab and focus on captive breeding of this species. This is the main focus during the next part of the project. Once we have a population established in the lab, we hope to have developed a species action plan and husbandry guidelines at the end of the first year of the project.

Subadult Persian mountain salamanders (*Paradactylodon persicus gorganensis*) from the Ziarat village. Photo: Dr. Haji Gholi Kami.

Within the facility center at Golestan University, we setup equipment including aquaria, tubes, air pump, a refrigerator motor, rocks, etc. that are essential for captive breeding of the salamanders. We received an Amphibian Ark Conservation Grant in 2021 to help establish the *ex situ* component of our program. The education activities and fieldwork are continuing and we hope to find suitable populations, adult specimens, and suitable ponds within the forest, along with egg batches which we can use to start the *in situ* and *ex situ* program.

We applied for and received additional funding from the People's Trust for Endangered Species (PTES) at the beginning of this project of about US $6,100 and we are also providing an interim report for them.

The aquaria and other equipment for the breeding part of the study at the Golestan University zoology lab. Photo: Najmeh Okhli.

Indigenous women lead defence of Lake Titicaca's dying life

Sally Jabiel, Peru

Indigenous community leaders carry out water monitoring on the shores of Lake Titicaca. In recent years, there have been mass die-offs of species in the lake, linked to increasing pollution. Photo: Mujeres Unidas en Defensa del Agua.

With pollution endangering fish, frogs and birds in the lake that unites Peru and Bolivia, indigenous women are fighting to heal its waters. The year 2015 was a warning for Titicaca, the lake that straddles the border between Peru and Bolivia at 3,800 metres above sea level. Over the course of the year, thousands of giant Titicaca water frogs (*Telmatobius culeus*), fish and birds washed up dead on the Bolivian side of the lake, South America's second largest.

"It was a wake-up call of what could also happen to us," recalls Vilma Paye, 48, a women who lives in Sampaya, an Aymara indigenous community on Titicaca's Bolivian shores.

That same year, Lake Poopó, which is connected to Lake Titicaca by the Desaguadero River, dried up completely. Disaster followed months later, when another 10,000 giant frogs died on the Coata River, a tributary which flows into the Peruvian part of the lake.

It was a crisis foretold. "Our grandparents said the climate was going to change and also that some species would become extinct, but we never believed it," says Paye, one of fifty indigenous leaders in the Mujeres Unidas en Defensa del Agua (Women United in Defence of Water), a network created in 2016 to save Titicaca from pollution.

"It is alarming that we ourselves are polluting it, because this is going to cause us to become extinct – just like the giant frogs," she adds.

A symptom of the crisis

What is happening to this endangered aquatic amphibian is a symptom of the degradation of the world's highest navigable lake. Wastewater discharges and waste from homes, hospitals and mining operations are polluting Titicaca and its entire basin, from the Desaguadero River to Lake Poopó. Several studies have already confirmed the presence of heavy metals in its waters, such as arsenic, cadmium, mercury and lead, among others.

"We can feel what this is causing, because we [women] are the heads of the household and we use water for everything," says Paye.

Paye joined Mujeres Unidas in 2020. Together with Aymara and Quechua women defenders, she collects plastic bottles and bags from Titicaca's shores, raises awareness among young people in schools, talks to the authorities and even measures the quality of the water in order to heal her ancestral lake.

A refuge in captivity

After the mass die-offs of frogs in 2015, an emergency team rescued the survivors in Wiñaymarka, the southern portion of Lake Titicaca, to keep them at the K'ayra Centre, the only facility in Bolivia dedicated to protecting threatened amphibian species.

"Because they have such permeable skin, frogs are sentinels of our environment," says biologist Teresa Camacho Badani, who is in charge of the research and conservation centre in Cochabamba, some 430km from the Wiñaymarka Lake.

"Nobody realises that what is happening to these frogs, to some extent, can happen to us. We must take notice of this signal that nature is giving us before it is too late," the herpetologist adds.

During their rescue, the Titicaca frogs went through something of an odyssey once they left the lake. The team evacuated thirty-five amphibians from the polluted waters by plane, with support from the International Union for Conservation of Nature (IUCN) and Amphibian Ark, among other organisations. They then undertook an overland journey to the K'ayra Centre and finally settled in a conditioned container where no pollutants enter and a control system simulates the temperature and daylight hours of their natural habitat.

This refuge is part of the Alcide d'Orbigny Natural History Museum in Cochabamba, which was declared a National Heritage Site in 2014. To date, it houses 450 individuals belonging to five species of the genus *Telmatobius* – native to the Andes – such as the giant Titicaca frog, as well as two terrestrial species, including the glass frog (*Nymphargus bejaranoi*), which had not been seen in seventeen years.

In this environment, the rescued Titicaca frogs reproduced in mid-2020, bringing their population to more than 290 individuals. For Camacho, this is the result of a great efforts to help these amphibians overcome the stress they have undergone due to the deterioration of their habitat.

Guardians of the species

In the community of Perka Norte, located on Peru's Titicaca coast, there is still hope for the giant frogs. In 2017, a scientific mission from Denver Zoo in the US, the Cayetano Heredia University in Lima and Peruvian non-profit organisation Natural Way surveyed thirteen spots in the lake. In most, there were no frogs. At some sites, only one to four frogs were found in 100m transects; at Perka Norte, however, the number was as high as twenty frogs.

A Titicaca water frog (*Telmatobius culeus*). Photo: D Alarcón and D Grunbaum, Alcide d'Orbigny Natural History Museum.

"We weren't aware that frogs were disappearing in other communities," says Elvira Chicani, a forty-five-year-old leader and artisan who used to fish with her grandparents in this Aymara village. "Sometimes they got caught in our nets, but we have always thrown them back into the lake so that they can grow bigger. Now we are looking after them much more because people want to take them to the markets."

Installation of underwater transects for research and monitoring of the Titicaca giant frog, in the community of Perka Norte. Photo: Renzo Turpo.

In 2020, rescued Titicaca water frogs successfully reproduced in captivity. Photo: Teresa Camacho.

In addition to pollution, the biggest threat facing this Titicaca amphibian is its trafficking for its use and sale as the main ingredient in green "frog juices" in cities, such as Lima, due to popular beliefs about its supposed healing properties. In fact, it is the most trafficked species in Peru, according to an investigation by InSight Crime, accounting for more than 50% of the 20,000 animals seized by authorities between 2015 and 2020.

For this reason, after the findings of 2017, Natural Way continued with a population density study of the Titicaca frog, in collaboration with the Perka Norte community.

According to Jhazel Quispe, a biologist in charge of the organisation, during those months of research it was discovered that the traffickers had not yet arrived in this community, located an hour by boat from the city of Puno. It was also confirmed that its inhabitants had no connection with the frog other than its infrequent involvement in a ritual to call for rain. Moreover, the animal was considered unpleasant looking, with children said to be afraid of it.

"We came to understand that if at some point they were offered money to extract frogs, they were going to do it because they didn't feel much affection for them," says the researcher.

In this respect, community leader Chicani is firm: "We don't allow that here. Environmental education with the community – and especially with children and young people – was a central strategy to conserve this aquatic amphibian."

Chicani reports that traffickers have arrived in cars asking where they could find frogs. "Sometimes we see boats coming, we take pictures of them and pass the word to the authorities," she says. "But we have chased them away."

Other endangered species

The ecological disaster at Lake Titicaca is wiping out other native species, such as the Titicaca grebe (*Rollandia microptera*). In 2019, 119 of these flightless waterbirds were found dead in Suchipujo, on the Peruvian side. "The species that are dying are endemic and, therefore, indicators of any change in the quality of the lake," says biologist Quispe.

For Bolivian community leader Elizabeth Zenteno, the lake is approaching a point of no return. "Titicaca has lost its capacity for self-recovery, because the pollution is such that the lake is not resilient – it is now a polluted body," says the environmental engineer, who has been involved with the Mujeres Unidas for a little over a year.

In the last three decades, Titicaca, with a total area of over 8,200 square kilometres, has lost 90% of native fish species mainly due to overfishing and pollution, according to a recent diagnosis by the Binational Autonomous Authority of Lake Titicaca. An estimated twenty species of Orestias, a genus of fish, have become extinct over the past six decades, while another six are on the brink of extinction.

A future in the lake

For the frogs at the K'ayra Centre to return to Titicaca, the ecosystem conditions must be made safe again. To release them now "would be to condemn them to die", laments the herpetologist Camacho.

Since 2019, the conservation centre has launched a scientific mission – once more with Natural Way, Cayetano Heredia University and Denver Zoo, alongside the Pontifical Catholic University of Ecuador – to study the habitat of the Titicaca giant frog, its threats and the genetics of this species. This research, supported by the United Nations Development Programme, aims to improve its care so that this amphibian can have a long-term future.

In this vein, its defenders are seeking to have Lake Titicaca declared a subject with legal rights in Peru. This recognition was granted last year in Bolivia, due to the risk of "the disappearance of all forms of animal and plant life that live in its bed and banks".

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Reproductive Technologies and Biobanking for the Conservation of Amphibians

Edited by: Aimee Silla, Andy Kouba, Harold Heatwole

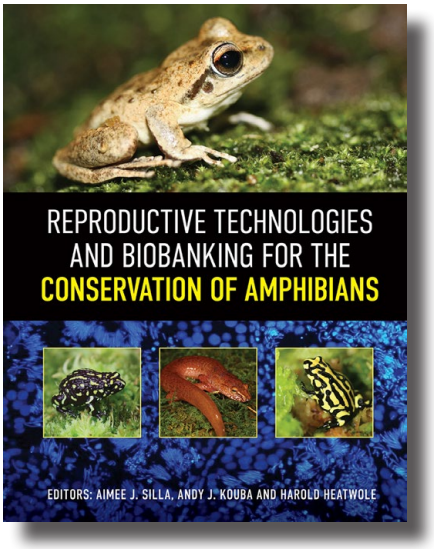
Hardback - October 2022 - AU $180.00

Integrating reproductive technologies into amphibian conservation breeding programs.

How to decelerate loss of global biodiversity is one of the greatest challenges of our generation. Reproductive technologies have enormous potential to assist the recovery of species by enhancing reproductive output, facilitating genetic management, and supporting reintroduction of threatened species. Of particular value are cryopreservation technologies coupled with the establishment of global gene banks to conserve, in perpetuity, the remaining extant genetic diversity of threatened amphibians.

Reproductive Technologies and Biobanking for the Conservation of Amphibians brings together leading experts in the field to provide a comprehensive overview of current best practices, summarise technological advancements, and present a framework for facilitating the integration of reproductive technologies and biobanking into conservation breeding programs for threatened amphibians. It is an invaluable reference for the next generation of conservation practitioners: captive breeding facilities, researchers, and policy-makers involved with biodiversity conservation.

The book will be produced in two editions – one with a CSIRO Publishing ISBN/logo and the other with a CRC Press ISBN/logo. The two editions will be identical internally. CSIRO will retain rights to sell their printed edition in Australia and New Zealand, as well as the global rights for the eBook editions. CRC Press will be purchasing 300 copies of the book to sell in their territories.



The estimated date of publication is October 2022 for both editions of the book. The estimated RRP is AU $180 for the CSIRO edition, and US $130 for the CRC Press edition. Both versions are available for pre-order:

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Approach to the cultivable microbiota of the skin of amphibians from the south of the Andes mountain range and the eastern foothills of Ecuador

Jazmín Salazar and Adriana Mogrovejo, Center for Research Innovation and Technology Transfer Microbiology Laboratory of the Catholic University of Cuenca, Ecuador; and Fausto Siavichay, AMARU Amphibian Conservation Center, Ecuador

The appearance of emerging diseases caused by fungi and bacteria is one of the main threats facing amphibians. The first response to a pathogen is given by the innate immune system that is activated immediately during the interaction with the pathogen and is mediated by the production of antimicrobial peptides synthesized in the glands present in the skin of amphibians (Apponyi et al, 2004; Rollins et al, 2011; Varga et al, 2019).

The skin of these animals constitutes the ideal environment for the development and maintenance of a great diversity of microorganisms that play a fundamental role in protection against infectious agents. Several genera of bacteria have been reported as controllers of these pathogens, including *Janthinobacterium*, *Pseudomonas*, *Bacillus*, *Lysobacter*, *Pedobacter*, *Serratia* and *Streptomyces* (Lauer et al., 2007; Lam et al., 2011; Woodhams et al., 2020). However, the microbiota varies depending on the ontogenetic development of the species (larva, metamorph, adult) and its habit. Thus, changes in the composition of the skin microbiota of amphibians under human care have been reported. A lower microbial diversity has been reported in *ex situ* management conditions, probably due to the lack of substrates, which constitute a microbial supply for the skin of amphibians and can cause greater susceptibility to pathogens (Kueneman et al., 2016; Loudon et al., 2014).

In this sense, the study of the role that these microorganisms play in the skin of amphibians, allows an approach to the understanding of the ecophysiology of their environment and the role that they may be playing in the protection against these emerging diseases, climate change and disturbance of ecosystems as threats responsible for the decline of amphibian populations. This approach will allow us to understand the diversity of the microbiota, and how it is related and varies depending on the habits of the species to identify possible species susceptible to changes in the ecosystem. Finally, these results will be used in the search for microorganisms with potential antimicrobial and antifungal effect through antagonism tests to formulate probiotics that can be inoculated into the skin of amphibians to create a biofilm, thus avoiding the decline of populations, and proposing possible strategies for both *in situ* and *ex situ* conservation programs.

This project was carried out with the Amaru Amphibian Conservation Center and the Microbiology Laboratory of the Center for Research Innovation and Technology Transfer of the Catholic University of Cuenca. *In situ* sampling included a total of twenty-two species including: *Ctenophryne aequatorialis*, *Hyloxalus vertebralis*, *Prismitantis erythros*, *Pristimantis lutzae*, *Gatrotheca pseustes*, *Scinax cruentommus*, *Dendropsophus bifurcus*, *Pristimantis matildae*, *Prismimantis diadematus*, *Epipedobates anthonyi*, *Lithobates catesbeianus*, *Pristimantidmus andinognos*, *Gatrotheca cuencana*, *Trachycephalus jordani*, *Hyloxalus toachi*, *Chiasmocleis bassleri*, *Adenomera hylaedactyla*, *Pristimantis conspicillatus*, *Pristimantis versicolor*, *Trachycepalus macrotys* and *Ctenophryne aequatorialis*.

The amphibians were swabbed on the skin of the belly, back, groin, mouth and interdigital membranes, the swabs were placed in TGhL medium (tryptone, hydrolyzed gelatin and lactose) and brain heart infusion (BHI) medium that allow the sample to be preserved until it is transferred to the laboratory. Once in the laboratory, the samples were seeded in culture media specific for fungi and bacteria, PDA (potato dextrose agar) and NA (nutrient agar),

Swabbing *Prismitantis erythros* in the field.
Photo: Jaime Culebras.

respectively, and incubated at 24ºC for fungi and 20ºC for bacteria. Growths were evaluated at 16, 24 and 48 hours after inoculation. Once the cultures were isolated and purified, the microorganisms were identified by MALDI-TOF mass spectrometry, a Gold Standard technique assisted by time of flight (TOF), which analyzes the distribution of intact thermolabile molecules based on their mass and compares them with a database identifying the microorganism of interest.

We identified species of bacteria belonging to the genera: *Pseudomonas*, *Aeromonas*, *Bacillus*, *Lactobacillus*, *Comamonas*, *Acinetobacter*, *Serratia*, *Chryseobacterium*, *Providencia*, *Crnobacterium*, *Lelliottia*, *Pantoea*, *Proteus*, *Kluyvera*, *Pseudarthrobacter*, *Corynebacterium*, *Rahnella*, *Delftia*, *Staphylococcus*, *Citrobacter*, *Hafnia*, *Morganella*, *Ewingella* and *Raoultella*. While the genera of fungi identified included: *Fusarium*, *Penicillium*, *Aspergillus*, *Geotrichum*, *Bipolaris*, *Hortaea*, *Synchephalastrum*, *Rhizopus*, *Mucor*, *Kosakonia* and *Exophiala*. Several genera of both bacteria and fungi have been reported as controllers of pathogens, while others have been reported in the normal microbiota of amphibians both in captivity and in the wild.

Fungus grown in Potato Dextrose Agar medium. Photo: Gustavo Morejón.

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Ditch guardians - A step forward for the conservation of the southernmost marsupial frog species in Argentina

Mauricio Sebastián Akmentins and Martín Boullhesen, INECOA, CONICET-UNJu, Argentina; Elena Correa, Reserva Experimental Horco Molle-UNT, Argentina; and Paula Daniela Cano, Administración de Parques Nacionales, Argentina

La Banderita marsupial frog (*Gastrotheca gracilis*) is the southernmost species of marsupial frog included in the family Hemiphractidae. This Endangered species is endemic to the Yungas Andean forests of Northwestern Argentina, and the main threats for this frog are habitat loss, climate change, and chytrid fungus.

Most of the extent of occurrence of this threatened species is now effectively protected by Aconquija National Park. but one population outside the boundaries of this protected area, at the type locality of *Gastrotheca gracilis* in “La Banderita”, is still under threat. The main threats are related to the natural biology of this marsupial frog because the females lay their tadpoles in temporary ponds formed in the roadside ditches of the unpaved National Road N° 65. During the reproductive season of La Banderita marsupial frog, from December to April, tadpoles are jeopardized by high cattle density, road maintenance works, and mudslides due to extreme weather events. We identified tadpoles as the most vulnerable life stage of the La Banderita marsupial frog.

Considering this situation, in August we coordinated a one-day workshop to begin a conservation program for La Banderita marsupial frog in its type locality with the National Park Administration, National Road Administration (Vialidad Nacional), Reserva Experimental Horco Molle (Universidad Nacional de Tucumán) and Instituto de Ecorregiones Andinas (CONICET - UNJu). During the workshop we discussed the importance of preserving this threatened and unique marsupial frog and the possible strategies to prevent or mitigate the potential harm to tadpoles during the necessary road maintenance, and we shared the knowledge and experiences of park rangers, Vialidad Nacional personnel, and researchers.

As a result of this workshop, we developed a roadmap of direct actions to preserve *Gastrotheca gracilis* in the roadside ditches of National Road 65, considering the dates for scheduled maintenance works and establishing a quick response task force for translocation of tadpoles if we need to. This joint action between multiple institutions is a new way of thinking for conserving amphibian diversity in Northwestern Argentina.

Reproductive habitat of La Banderita marsupial frog in the type locality of the species in “La Banderita”, National Road N° 65 in Tucumán province, Argentina. Photos: Mauricio Akmentins.

Workshop for the conservation of La Banderita marsupial frog (*Gastrotheca gracilis*) in its type locality developed in the Aconquija National Park with the participation of National Park Administration, National Road Administration, Reserva Experimental Horco Molle and Instituto de Ecorregiones Andinas. Photo: Mauricio Akmentins and Daniela Cano.

The Rancho Grande striped toad will soon have a safe home to breed at the Leslie Pantin Zoo

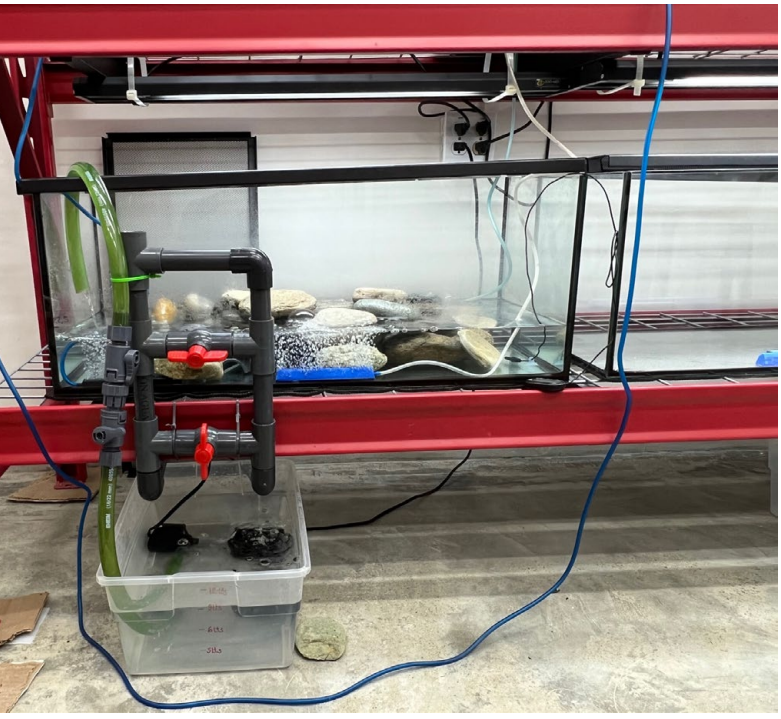
Margarita Lampo, Leslie Pantin Zoo, Venezuela

The Rancho Grande striped toad (*Atelopus cruciger*) will soon have a safe home to breed at the Leslie Pantin Zoo in Venezuela. Populations of this harlequin toad were decimated by a fungal disease called chytridiomycosis in the late 1980s, and currently only two viable populations are known. Although these two populations have coexisted with the disease-causing fungus for decades, climate scenarios that increase transmission could cause their collapse, and possibly the extinction of this species, if backup colonies are not available.

The Leslie Pantin Zoo is a private non-profit center founded in 1963 that has been dedicated to rescuing wildlife and raising awareness among school children about the need to preserve biodiversity. Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales (FUDECI, Foundation for the Development of Physical, Mathematical and Natural Sciences in English), an organization of the Academy of Physical, Mathematical and Natural Sciences, in partnership with this zoo recently began a program to establish a backup colony to reinforce wild populations of the Rancho Grande striped toad.

Since receiving an AArk start-up grant in June this year, we have been refurbishing a small building at the Zoo and acquiring the necessary equipment to develop a suitable, biosecure environment to house this Critically Endangered species. With the advice of Roberto Ibáñez and Jorge Guerrel from the Panama Amphibian Rescue Center (PARC), our facilities have been specifically designed for the breeding of harlequin toads, with environments that simulate streams and that allow water level control, nebulization, irrigation and automated UVB lighting regimes. It will have an initial capacity for two amplexus pairs or tadpole/morph cohorts in two breeding tanks, twenty-five adults in five maintenance tanks, and 150 juveniles in fifteen small plastic cages. This capacity can be doubled in later stages with a modest investment. It will also have treatment systems to adapt the incoming water to the requirements of the species and the wastewater to minimize the risk of contamination of the environment with pathogens. Most of the equipment has already been purchased and we are currently completing the construction, assembling and testing the misting and water level control systems.

Center for the rescue of Rancho Grande striped toads (*Atelopus cruciger*) at the Leslie Pantin Zoo in Venezuela. Photo: Federico Pantin.



Tank for the reproduction of harlequin toads with water level control. Design adapted from the Panama Amphibian Rescue Center. Photo: Margarita Lampo.

Our *ex situ* facility will be ready to receive the first six FUDECI specimens in October 2022. Additionally, eight adults will be collected in January 2023, during the next breeding season, to complete a total of fourteen founders. We have also established colonies of fruit flies, crickets and springtails that will be moved to the Zoo when the building is complete. The females currently in the FUDECI laboratory have increased their weight by 10-15%, some already have large masses of oocytes inside their abdomens and are waiting to be mated with the males in their new home at the Leslie Pantin Zoo.

Leslie Pantin Paya's *ex situ* facility has been conceived so that visitors, mostly school children, can view this endangered species in a display tank and observe laboratory activities through a window. We believe that sensitive children make responsible adults.

The mountain stream salamander, a little-known inhabitant of Mexico City

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An adult mountain stream salamander (*Ambystoma altamirani*). Photo: Alejandro Rafael Calzada Arciniega.

When talking about axolotls, the first thing that comes to mind is the very popular Mexican axolotl (*Ambystoma mexicanum*), a worldwide reference for hundreds of studies related to its genetic code and its amazing capacity for regeneration. It is also closely related to popular culture in Mexico and the worldwide efforts to protect it from extinction and conserve its habitat, which are the wetlands and canals of Xochimilco, located in the center of Mexico City. But few people know the other axolotl that also lives in this huge city, perhaps because they do not live in the center or in such a popular tourist area. It is a more discreet inhabitant, it lives in the mountains, in crystal clear streams, surrounded by forests. It is the mountain stream salamander (*Ambystoma altamirani*). This small axolotl, which reaches a maximum size of 17cm, has a distribution that is restricted to some localities of the Sierra de las Cruces, Sierra del Ajusco and Sierra de Zempoala in the states of Estado de México, Ciudad de México and Morelos. It inhabits high mountain streams, between 2,500 and 3,600 meters above sea level. An endemic Mexican species, it is listed in NOM-059-SEMARNAT-2010 (DOF, 2019) as a threatened species and in the IUCN Red List as an Endangered species.

Despite its small size, this species of axolotl was chosen as a flagship species and indicator of habitat quality. Through resources provided by the Secretariat of Education, Science, Technology and Innovation of Mexico City (SECTEI) and joint work with the Secretariat of the Environment of Mexico City (SEDEMA) and the Center for Biological and Aquacultural Research of Cuemanco (CIBAC), belonging to the Autonomous Metropolitan University - Xochimilco, we began the project “Studies for the conservation of biodiversity in Mexico City: mountain stream axolotl (*Ambystoma altamirani*) as an umbrella species for the protection of ecosystems and the benefits they provide”.

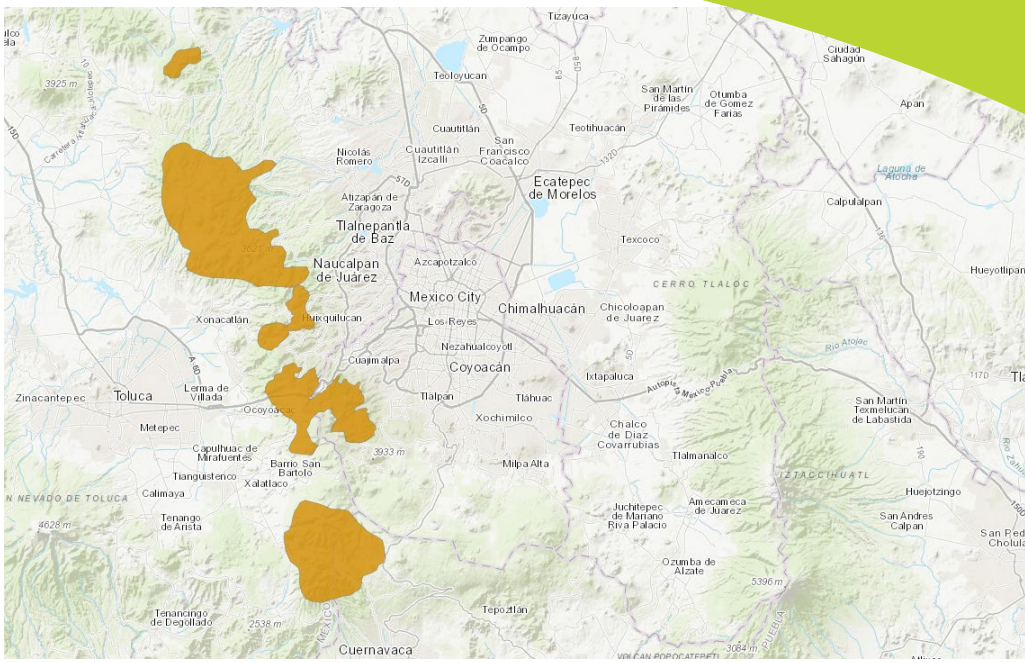
This project seeks to combine the efforts of the government, academia and civil organizations to conserve this species of axolotl, which is threatened by climate change, change in land use, invasive alien species and habitat loss in the streams which it inhabits. Some populations of the mountain stream salamander are located within two protected natural areas to the west of Mexico City: in the Cumbres del Ajusco and Desierto de los

Training of technical-operational personnel of the Desierto de los Leones National Park for monitoring activities of the mountain stream axolotl. Photo: Alejandro Rafael Calzada Arciniega.

Leones National Parks (Shaffer 2008). It is also reported in the Magdalena River, a community of La Magdalena Atlitic, and in the community-owned San Nicolás Totolapan, while in the state of Morelos it is reported in Lagunas de Zempoala National Park (Castro-Franco 2006).

The first stage of our conservation work is being done *in situ*. Population monitoring has begun to determine the size and assess the genetic variability of the subpopulations. Periodic sampling is carried out to establish environmental and water quality parameters in the streams. Likewise, aquatic invertebrates are monitored to determine the abundance, richness and diversity of species that could be potential food for the mountain stream salamander. The main factors that threaten the species have also been identified, highlighting among them the presence of the rainbow trout (*Oncorhynchus mykiss*), a species considered exotic and invasive. In the second stage, work has begun with the creation of an environmental management unit, which will allow the management of the species under human care. Among the main objectives of this is the formation of a reproductive colony which will allow us to develop reintroduction programs and an environmental education program aimed at users of the Desierto de los Leones National Park, in the medium and long term.

One very important part of this project has been the collaboration of the local communities where the project is being developed. Through working groups and participatory workshops, people have been joining the project to promote the protection of this species and the ecosystem which it shares with other species that are also in some category of risk, such as the ridged tree frog (*Dryophytes plicatus*). By sharing with the communities which live close to the distribution areas of this species and sharing information to the inhabitants of Mexico City through different media, we aim to make this beautiful species of amphibian better known, as much as the Xochimilco axolotl, and ensure the conservation not only of the mountain stream axolotl, but of one of the most beautiful ecosystems that surround this huge city and which offers a large number of ecosystem services.



Distribution area of the mountain stream salamander. Image taken from The IUCN Red List of Threatened Species. Version 2022-1. www.iucnredlist.org/species/59049/53973139#geographic-range.

Monitoring works in the Las Regaderas-Monte Alegre stream, San Nicolas Totolapan. Photo: Alejandro Rafael Calzada Arciniega.

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Captive reproduction of *Atelopus nanay* at the AMARU Amphibian Conservation Center

Jackeline Arpi, Fausto Siavichay and Nataly Aguilar, AMARU Amphibian Conservation Center, Ecuador

In its Action Plan, the Atelopus Survival Initiative (ASI) has included the *ex situ* management and maintenance of viable rescue colonies of these species as a conservation strategy for harlequin frogs. The management of *ex situ* programs for *Atelopus* species in the AMARU Amphibian Conservation Center, has allowed us to implement new reproduction trials. A pair of Mazán jambato toad (*Atelopus exiguus*) is currently reproducing and there is a clutch of Tres Cruces stubfoot toad (*Atelopus nanay*). The first species is listed as Endangered according to the IUCN global Red List and the latter is listed as Critically Endangered. Both are listed as Critically Endangered in the Red List of Amphibians of Ecuador.

A pair of *Atelopus nanay* in amplexus at the AMARU Amphibian Conservation Center in Ecuador. Photo: Jaime Culebras.

Part of this process was to improve our technical capacities, as well as incorporating equipment which was acquired with the support of a grant from the Amphibian Ark (AArk). Amongst other items specific UV lamps and heat emitters for amphibians and a chiller that regulates water temperature were used, to simulate the conditions in which the species are found in the Andean moors of southern Ecuador.

The pair of *Atelopus nanay* began to reproduce on August 18 this year, spending a period of fourteen days in amplexus, exploring the terrestrial and aquatic environments of the terrarium. Then approximately 150 eggs were laid, and these are still developing. The temperature of the water is regulated at 11°C with a humidity of 80% in the terrarium. UV lamps are used for a period of five hours daily so that individuals can assimilate vitamin D.

Food for the frogs is based on a diet of various invertebrates including moths, crickets, tenebrios and fruit flies, which are maintained and reproduced under biosecure conditions in the center's laboratories. The invertebrates reproduce at a temperature of 24°C.

The health conditions and the physical controls that are carried out on the individuals prior to attempts at reproduction are thoroughly evaluated by the management technician and include coproparasitic examinations and general posture inspections such as displacement, mental status, body condition (weight and size), and breathing.

The *Atelopus* conservation project at the AMARU Conservation Center links *ex situ* management with *in situ* monitoring of populations, and building community awareness about the protection and care of this species.

The female *Atelopus nanay* laid approximately 150 eggs and these are still developing. Photo: Fausto Siavichay.

Amphibian Ark donors, January-August 2022

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