

Ex situ management of five extant species of *Atelopus* in Ecuador: assisted reproduction essays for *A. sp.*, *A. elegans*, *A. spumarius*, *A. balios*, and *A. nanay*

Luis A. Coloma, Ph.D.; Centro Jambatu de Investigación y Conservación de Anfibios/Fundación Otonga
lcoloma@otonga.org

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Executive summary

This project aims to save from extinction five extant species of harlequin frogs *Atelopus* in Ecuador, through *ex situ* breeding and management. Most harlequin frogs went extinct and most of the extant are Critically Endangered (based on IUCN criteria) through all its distribution. Given the threats this genus faces, *in situ* management is not enough to save the species, and at this point, *ex situ* management is an urgently needed proactive solution to save extant species from extinction. Previous efforts to captive breed *Atelopus* have been relatively minor and unsuccessful, except for *A. zeteki*. Some of these efforts have failed because they relied on the spontaneous breeding of amplexant pairs kept under lab conditions. Previous experience shows that breeding under such circumstances rarely occurs. Thus, our objectives and activities are directed to find additional founders, adequately equip the *ex situ* facilities for the program Arca de los Sapos of Jambatu Center, and perform essays of assisted reproduction (using hormones) of *A. sp.*, *A. elegans*, *A. spumarius*, *A. balios*, and *A. nanay*, on the basis of successful previous essays with two of the species. With this project we will expect to produce first descendants of future genetically viable populations of these five species. We will keep them under lab conditions until the causes of their declines and disappearances are better understood and mitigated in nature, so their reintroduction would be feasible.

Introduction

Ecuador is one of the most important countries of the world for research and conservation of amphibians because of its exceptional diversity and high levels of endemism (496 species of amphibians described, plus about 200–250 species awaiting descriptions, 208 endemic species, 42% of endemism). Diversity of amphibians in Ecuador represents about 8% of the world amphibian diversity. Despite this high diversity, amphibians have been affected by numerous threats, which have produced the possible extinction of at least 14 species and drastic declines of no less than 136 species.

One of the most extreme examples of amphibian declines and extinctions is the harlequin frog clade *Atelopus*. Until the early 1980s, harlequin frogs were conspicuous components of the Neotropical fauna, but now, the genus is in critical condition. It is in decline throughout its geographical range and in all habitats and altitudinal zones it is known to inhabit. Of the species with adequate data, 81% show evidence of decline. Most important threats are climate change and the presence of the Bd, which specially affects species in higher altitudes; habitat fragmentation and destruction; pasture lands and agriculture; exotic predators such as introduced trouts in rivers (affecting eggs and tadpoles); water contamination; and changes in microhabitat condition (e.g. environmental temperature increase and decrease in humidity).

Although there are some studies related to *Atelopus* declines, little effort has been done in Latin America to conserve extant species. The alarming decline in moderate and high elevation areas is a call for more detailed actions where we know individuals persist. In Ecuador only ten out of 28 species of *Atelopus* (25 described) still are found, these are: *Atelopus elegans*, and *A. balios* in the western lowlands, *A. exiguus*, *A. nanay*, and *A. nepiozomus* in the southern high altitude Andes; *A. palmatus*, *A. sp.* (from Limón), *A. sp.* (from Cóndor), *A. sp.* (from 9 de Octubre) in the eastern versant of the Andes, and *A. spumarius* in the eastern Amazonian lowlands.

Ex situ programs are a viable alternative to keep extant individuals healthy and out of extinction risk until *in situ* conditions are suitable again for the species. Few captive breeding programs for *ex situ* conservation exist in Latin America. For example, *Atelopus zeteki* in Panama, which is being captive reared on the basis of sufficient founder individuals. However, complete long term *ex situ* conservation efforts are still missing. Because many species are already disappeared, *ex situ* programs will not save the entire genus, but these programs may be the only strategy currently available to conserve the few remaining alive populations.

On this basis, our main goal for *Atelopus* in Ecuador is to perform specific long term integrative activities to conserve the populations of the ten extant species. This strategic plan includes *in situ* and *ex situ* integrative approaches and activities, which will hopefully avoid their extinction. Within this context, the main goal for the current project is to perform essays on assisted reproduction of five species *A. sp.* 1. (from Limón), *A. elegans*, *A. spumarius*, *A. balios*, and *A. nanay*.

Given the urgency to breed these critically endangered species in the lab, induced oviposition through hormones is the only rapid way to ensure successful reproduction. Besides, several studies have shown that many species of *Atelopus* have reproduction site fidelity, making captive reproduction more difficult. Our current efforts will set a new era in *ex situ* reproduction for *Atelopus* because we already have the expertise and protocols for assisted reproduction of this genus. We will improve on the techniques we already tested in the past, at a level that we can guarantee the successful reproduction of *A. sp.*, *A. elegans*, and *A. spumarius* and attempt to reproduce *A. balios*, and *A. nanay* if we find reproductive females in the field. If funding is granted we will improve our equipment and levels of biosecurity. With those reproductive essays we expect to avoid extinction of these species, increase the number of individuals of the captive population, and keep the genetic variability until specimens can go back to their habitats.

Methodology

At the Centro Jambatu we currently keep individuals of *A. sp.* (*spumarius-pulcher* complex), *A. spumarius*, *A. elegans*, and *A. balios*:

1. Five adult individuals (four males and one female) of *A. sp.* (*spumarius-pulcher* complex) collected in 2010 in southeastern Ecuador.
2. One female of *A. spumarius* collected at the riverbank of Pucayacu river in Pastaza Province.

3. Four adult individuals of *A. elegans* (three males and one female) collected in Durango, Esmeraldas Province.
4. Five individuals of *A. balios*, all males collected close to Patul river, (Cañar-Azuay border of provinces)

Based on the number of males and females we currently have at the lab for each species, we will make field trips to Pucayacu river to collect males and females of *A. spumarius*, to Patul river to collect females of *A. balios*, to San Carlos de Limón to collect males and females of *A. sp.*, to Azuay province to collect males and females of *A. nanay*; and to Durango to collect additional *A. elegans* specimens. We will bring them back to the *ex situ* lab to start assisted reproduction. Individuals of *A. nanay* also might be provided by Centro de Conservación de Anfibios del Bosque Mazán (or ACC – Amphibian Conservation Center-Mazán. Frogs will be collected at sites where habitat destruction is currently occurring.

Centro Jambatu has the best qualified staff in Ecuador with the expertise and knowledge for *ex situ* management. The lab has been strictly designed to have the appropriate facilities for the maintenance of amphibians at risk of extinction.

All specimens of the five species mentioned before are placed individually in enclosures adapted to their natural conditions to ensure their wellness. Males are placed in Penn Plax SW 02, and females in SW 03. The enclosures are adapted with an open water system, letting the water to get in and out, and with four periods of spraying. A water reservoir protected with a false floor, natural plants, leaf litter, and shelter for the specimens are also adapted for each enclosure.

Once we get to have tadpoles, they will be fed twice a day with SAR Type I (Super Alimento de Renacuajos) in powder and plates. SAR is elaborated in the lab and is a mix of fiber, proteins, carbohydrates, and minerals. This food has been already tested in many tadpole species having successful results. We will also use brown algae, which have been used successfully with *Atelopus* tadpoles.

Frogs in early stages will be fed with collembola (*Folsomia candida*), then fruit flies will be added to the diet (*Drosophila melanogaster*), tiny grasshoppers (*Gryllus sp. assimilis* complex) and weevils (Curculionidae). Adults will have a diet based on small grasshoppers (between 1 and 5 days of hatched), fruit flies and weevils. Food will be supplied three times a week. All insects will be powdered with vitamin supplements (calcium and vitamin D) ones a week before placed inside the enclosures. This supplement helps the frogs to avoid nutritional problems. UV lighting will be provided to metamorphs and juveniles.

Assisted reproduction essays

Since 2007 we have been doing essays for the reproduction of *Atelopus* species. At the beginning we emphasized on *Atelopus sp. (spumarius-pulcher complex)* of Morona Santiago Province, but after several essays only one amplexus produced eggs, but infertile ones. In June and July 2010 we had two fertile egg masses; the tadpoles grew up and currently the juveniles are kept at the PUCE lab. In 2010 we kept doing reproductive essays with some *Atelopus* species. We worked

with assisted reproduction using the Chorionic Gonadotropin hormone. Nonetheless, the technique is still been improved for its complete success.

The procedure for assisted reproduction for the *Atelopus* species of this project is as follow:

1. Enclosure characteristics: controlled temperature and humidity (85 – 95%) pH (6.8), rainfall, constant water flux and appropriate volume, and vegetation. Size of enclosure 80cmx40cmx60cm. In the inside we will place medium size river stones on the floor; trunks or flat stones forming a small cave under the water; plants with large roots to filter the water and big leaves to serve as perchs; and a water pump to recirculate the water.
2. Assisted reproduction: two males and one female will be kept in one enclosure, once we get an amplexus, the single male will be removed from the enclosure. The couple will be kept without any disturbance for five days before the female is stimulated with the Chorionic Gonadotropin hormone. The exact dose will be tested in this Project; we will start with the baseline dose we have from past essays

Budget

The budget for our project will be distributed in two parts: 1) to search for specimens in the field and 2) to equip the labs and place them in captivity and start assisted reproduction. Details are in table 1:

Table 1. Budget description for the project “*Ex situ* management of extant species of *Atelopus* in Ecuador: assisted reproduction essays for *A. sp*, *A. elegans*, *A. spumarius*, *A. balios*, and *A. nanay*”.

	Item details	Saint Louis Zoo	Amphibian Ark	Total
	Electric automatization	4.000,00		4.000,00
	Racks	7.000,00		7.000,00
Equipment	Air system	1.500,00		1.500,00
	Spray system	2.700,00		2.700,00
	Filtration system	4.000,00		4.000,00
	10 Water pumps \$50 each		500,00	500,00
Materials	3 Ebottoms \$200 each		600,00	600,00
	Eiko lights	750,00		750,00

