Title: Rescuing the southernmost Marsupial Frog species (*Gastrotheca gracilis*) in Argentina

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Summary:
Gastrotheca gracilis is the southernmost species of Marsupial Frogs. This species was missing for twenty years, but the rediscovered populations are facing new threats. We will start the very first conservation actions on this threatened species with a reinforcement of the populations of La Banderita Marsupial Frog by means of the headstarting program with the establishment of a breeding facility combined with a monitoring of the ongoing threats to assess its current conservations status. This actions will be jointed with a proactive educational campaign and with the delivery of a technical report to environmental authorities for the implementation of measures prone to mitigate or eradicate the most severe threats on wild populations.

Introduction:
Yungas Andean forests represent the southernmost extension of the biodiversity hotspot in the tropical Andes (Myers *et al*., 2000), and is one of most biodiverse ecoregions of Argentina (Brown *et al*., 2006). Despite of its conservation relevance, the amphibian diversity of southern Yungas are being over-threatened in comparison with other ecoregions of the country (Vaira *et al*., 2017). The main threats are a consequence of human activities such as clear-cutting of primary forest, introduction of exotic species, extensive cattle raising, oil prospection and exploitation, and the development of large scale engineering projects (Lavilla & Heatwole, 2010).
La Banderita Marsupial Frog (*Gastrotheca gracilis* Laurent, 1969) was rediscovered in 2011 after twenty years without registries in the wild (Akmentins *et al*., 2012). This species is the southernmost Marsupial Frog of family Hemiphractidae (Duellman, 2005), and is distributed in Yungas montane forests and montane grasslands in Tucumán and Catamarca provinces,

Argentina (Akmentins *et al*., 2014a). Furthermore, one of the three rediscovered populations of *G. gracilis* in Los Sosa Provincial Reserve are facing severe threats, being the most alarming the mass mortality caused by domestic pigs by direct tadpoles' predation and by the destruction of reproductive habitats (Akmentins *et al.*, 2014b). There are reports of the presence of *Batrachochytrium dendrobatidis* (*Bd*) in Andean amphibian species of northwestern Argentina (Barrionuevo & Ponssa, 2008), however there are no reports of infection of chytridiomycosis in Marsupials Frogs species in the country (Akmentins *et al*., 2012). *Gastrotheca gracilis* is listened as an endangered species in the last Argentinean conservation assessment (Vaira *et al.*, 2012), and as vulnerable by the IUCN red list with population decreasing trends. Only one population of *G. gracilis* that have effective habitat protection is in Campo de Los Alisos National Park, since the registry of this species in 2013 and the later incorporation of this endangered species as conservation priority in the management plan of this protected area (Akmentins *et al*., 2014a).
The recommended conservation actions of the 2010 Amphibian Conservation Need Assessment of Amphibian Ark for *Gastrotheca gracilis* where *in situ* research and *ex situ* breeding for conservation and education. In light of the threat recent evidence this species is facing in a very crucial life stage as tadpole, the *ex situ* actions must include a conservation component, to increase the population's numbers and help to bypass the risks in the reproductive habitats. Captive breeding and population supplementation programs have been focused on regions that have the most expertise, rather than the most biodiversity (Griffiths & Pavajeau, 2008). Reinforcement of frog's populations by means of the construction of breeding facilities, can be interpreted as a key tool for the recovery of some endangered species (Edmonds *et al.*, 2012). Also, the supplementation of significant numbers of metamorphic individuals to wild populations maximize the extinction probability reduction, minimizing the conservation efforts (Kissel *et al.*, 2014).

Methodology:

Ex situ component:

To make the supplementation of *Gastrotheca gracilis* populations we will establish a breeding facility and husbandry research center. This laboratory will be placed within the Horco Molle Nature Reserve facility, an institution managed by the Universidad Nacional de Tucumán (http://www.reservahorcomolle.com.ar/). Horco Molle Nature Reserve is located on Sierra de San Javier (26°47'35.63"S; 65°18'59.11"W), Tucumán province, Argentina. This institution has been working with the rescue and recovery of native and endemic species for the last three decades and has been designated as a field laboratory for students and scientists for the implementation of several research works. The breeding facility will be finished at the end of December 2018.
An estimated of 100 representative tadpoles of La Banderita Marsupial Frog will be collected from Los Sosa Provincial Park (27°0'23.75"S; 65°39'42.92"W) in January/February 2019 and raised in captivity in two aquariums in the *ex situ f*acility with a period of 50-60 days until the metamorphosis occurs, and later the individuals will be release on the same site after quarantine in an aquaterrarium in April/May 2019. Los Sosa Provincial Park is only 41 km away in straight line from Horco Molle Nature Reserve, that ensure similar microclimatic conditions and minimize the stress of captivity. This project will be continued by Horco Molle Nature Reserve administration, long as needed, until the threats in the natural habitat will be controlled or eradicated (mostly domestic pigs and reproductive habitats' pollution/destruction).

In addition, with the support of Horco Molle Natural Reserve administration, we will open a new education-research program focused on understanding the unique La Banderita Marsupial Frog's life traits and the importance of preserve this distinctive species of Yungas Andean Forests. The breeding facilities also could act with Conservation Education purposes, helping to sensitize to general public about the amphibian diversity crisis and the relevance of preserve Marsupial Frogs and their habitat in Yungas Andean forests.

This captive breeding program will be strictly supervised by our team in order to assure the correct biosecurity measures following the AZA Amphibian Husbandry Manual. Water source will be standard tap water, previously de-chlorinated. Tadpoles' will be fed with TetraFin Goldfish Food flakes, to ensure a correct food supply (this food source was successfully tried in *Gastrotheca gracilis* tadpoles), and minimize the risks of contaminants. Metamorphs individuals will be kept in quarantine in an aquaterrarium, fed with springtails (Collembola) and fruit flies (*Drosophila*) breed in the *ex situ* facility, until the re-introduction to his natural habitat. A random subset of ten metamorphic specimens will be tested for *Bd* infection prior the release in the wild. A Standard Operating Procedure Guide will be written by the team members and handled by the Natural Reserve staff. These guidelines will be previous reviewed by the General Coordinator of the Conservation Center for Amphibians CCA-AMARU, Biol. Fausto Siavichay Pesántez (http://www.zoobioparqueamaru.com/webs/nuestro_equipo.php).

We already count with the commitment of the Amphibian Conservation Specialist Dr. Carlos C. Martínez Rivera of Philadelphia Zoological Gardens for a budget of US$ 2,000 for the period 2019-2020 destined to continue the conservation program once established.

In situ component:

We will survey three reproductive sites in Los Sosa Provincial Park in order to determine the incidence of habitat alterations in the reproductive habitats of *Gastrotheca gracilis*. Tadpoles' densities in the reproductive habitats will be estimated employing a standardized sampling effort with dip net (Schaffer *et al.,* 1994). In Addition, we will record any habitat alteration detected in each field survey.

For *Bd* detection in La Banderita Marsupial Frog, by default, all adult specimens detected will be swabbed following the RLM Protocol proposed by Fisher *et al*. (2018). We will look for abnormalities in tadpole's mouth morphology as a preliminary indication of chytridiomycosis, in the case of abnormalities detection we will proceed to take *Bd* samples (Fischer *et al*., 2018). The obtained samples will be analyzed with the qPCR technique proposed by Kerby *et al*. (2013).

Conservation actions:

Once concluded field surveys, we will gather information of the ongoing threats and write a technical report for Tucumán province environmental authorities with recommendations to mitigate or eradicate the active threats on La Banderita Marsupial Frog population in Los Sosa Provincial Park.

Budget:

Budget category	Item/amount	**Requested from AArk**	Other sources/status
In situ activities	50 *Bd* samples: swabs/Eppendorf tubes/micropipette tips/ADN extraction kit/*Bd* primers $700	**700 US$**	0 US$
	Field equipment: Dip net/rubber boots/disposable gloves/Ziploc bags $100	**100 US$**	0 US$
	Fuel/vehicle maintenance $60/day, 12 days	**720 US$**	0 US$
	Food/lodging 2 people $90/day, 12 days	**540 US$**	540 US$ requested from INECOA
	2 Team members salary for field work $120/day, 12 days	**0 US$**	1440 US$ pledged from employer (CONICET/UNT)
	1 Team member salary for laboratory analysis $60/day, 14 days	**0 US$**	$840 pledged from employer (CONICET)
Ex situ facilities	Retrofit existing building/build a partially new facility	**1,000 US$**	0 US$
	Support tables/shelves	**200 US$**	0 US$
	2 Equipped aquariums of 200 lts, $350/each	**700 US$**	0 US$
	1 Aquaterrarium of 240 lts, $300/each	**300 US$**	0 US$
	6 TetraFIN Fish Food 200gr, $10/each	**60 US$**	0 US$
	Foot baths/solutions $50	**50 US$**	0 US$
	Four months keeper salary	**0 US$**	6000 US$ pledged from employer (UNT)
Field collection/release	Fuel/vehicle maintenance $60/day, 6 days	**360 US$**	0 US$
	Food/lodging 2 people $40/day, 6 days	**240 US$**	240 US$ requested from INECOA
	2 Team members salary $60/day, 6 days	**0 US$**	720 US$ pledged from employer (CONICET/UNT)
Unforeseen costs		**30 US$**	0 US$
Total		**5,000 US$**	9,780 US$
Percentage		**33.8%**	66.4%

Timeline of work:

Activity	Oct18	Nov18	Dec18	Jan19	Feb19	Mar19	Apr18	May19	Jun19	Jul19	Agu19	Sep19
Fieldwork activities				X	X		X	X				
qPCR analysis									X	X		
Results publication											X	X
ex situ facility	X	X	X	X	X	X	X	X	X	X	X	X
Field collection/release				X				X				
Conservation actions											X	X

Citations:

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