AMPHIBIAN ARK SEED GRANT APPLICATION

CONSERVATION OF THE CUBAN LONG NOSED TOAD (*PELTOPHRYNE LONGINASUS*): CAPTIVE BREEDING AND *IN SITU* MONITORING.

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EXECUTIVE SUMARY

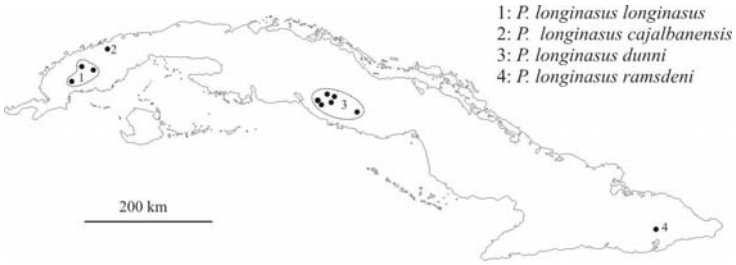
The Cuban Long Nosed Toad (*Peltophryne longinasus*) is the first anuran species in which the chytrid fungus have been found in Cuba (Díaz *et al*., 2007). This species is currently evaluated as endangered (EN), following the IUCN categories and criteria (Hedges and Díaz, 2004). Mean threats are the historical loss of suitable habitats and the very limited range of distribution. No further information exist about the impact of the chytrid fungus on this species and other frogs that co-occurs in the same habitats. Regarding the critical danger that chytrid fungus represent for amphibians, *ex situ* and *in situ* conservation efforts are strongly neccessary to avoid species extintion. Preliminary experiences on the captive breeding of *Peltophryne longinasus longinasus* exist (Díaz and Cádiz, 2007), and may represent a good starting point for a long term captive program. This AArk Seed Grant application is intended to obtain support for the following purposes: **1**) to develop a facility for *ex situ* conservation of *Peltophryne longinasus* in Cuba; and **2**) to monitor wild populations of this species and co-occurring frogs, in order to assess the impact and spread of the chytrid fungus, the habitat health and quality, and to gather basic information on the biology of species for a long term conservation. This project is expected to develop first actions to protect Cuban amphibians from extintion combining *ex situ* and *in situ* strategies, particularly in this species in which the chytrid fungus and habitat viability are critical threats for its survival in a period of less than 10 years. New experiences derived from this project will let the opportunity to complete a practical handbook about the biology and captive management of *P. longinasus*.

INTRODUCTION

The recent Global Amphibian Assessment (Stuart et al., 2004) concluded that 71% of West Indian amphibian species are threatened, being listed in the IUCN Redlist categories of vulnerable, endangered and critically endangered. This is the largest proportion of threatened species in any major amphibian fauna globally. The relatively small distributions of the species, small amount of remaining forests, and continuing threat from habitat destruction were major factors that led to the designation of such a large proportion of threatened species. In the West Indies, the chytrid fungus has been reported in Puerto Rico (Burrowes *et al*., 2004), Hispaniola (Joglar *et al*., 2007), Cuba (Díaz *et al*., 2007), and Dominica (Malhotra *et al*., 2007). In Cuba, the chytrid was discovered in October of 2006 in a dying individual of the endemic toad *Bufo longinasus dunni*.

The genus *Peltophryne* is a monophyletic lineage endemic to the West Indian (Pramuk, 2006). *Peltophryne longinasus* is one of eight Cuban toads (the largest Caribbean radiation), and has four major populations considered as subspecies (Díaz and Cádiz, 2008) (see the map below). Of these subspecies, *P. l. ramsdeni*, has not been found since the original description, suggesting it is very rare or extinct. Previous (Valdés de la Osa and Ruíz, 1980) and new evidence suggests that these subspecies may actually

represent full species and therefore the conservation status is even more critical in need of re-evaluation. Díaz and Cádiz (2006) presented information on preliminary experience with the captive breeding of *Peltophryne longinasus longinasus*. The only West Indian toad with a successful captive breeding program is the Puerto Rican Crested Toad (*Peltophryne lemur*) (see Lentini, 2000).



METHODOLOGY

Captive breeding:

The preliminary experience of Díaz and Cádiz (2006) will be followed, with corrections in the water quality; together with standard husbandry technics (Gagliardo and Pramuk, 2008). Mantainance of *Peltophryne longinasus longinasus* in water harder than in natural habitat (L. M. Díaz, personal observations) caused hypercalcemia in adults after three years. Two males and three females will be kept in aquaterraria (80 x 40 x 40 cm). In nature water has 2-3°dGH and Ph=6.4. Air temperature is 25-31°C, and water temperature is 25-26°C. In each vivarium water will be filtered by means of pump sending water to a home made external filter (for chemical, mecanical, and biological filtration). The vivarium bottom will be drilled out to drain water to the filter by means of a PVC pipe system. Water movement inside the vivarium seems to be essential to estimulating breeding in this toad. Room and water temperature will be controled by a central air conditioner. Because *Bufo longinasus* is a diurnal species, often seen basking in the wild, halogen lamps with proper UV emissions will be used (Eiko Supreme, 50W). Food includes small crickets (*Gryllodes sigillatus*), small cockroaches (*Blatta orientalis*), lesser mealworms (*Alphitobius diaperinus*), and springtails (for smaller toadlets). Toadlets will be kept in plastic illuminated containers with proper substrate (tree fern) and misted regularly.

Field work

Four expeditions of seven days each. Expeditions will cover the two western populations of *Peltophryne longinasus* (*P. l. cajalbanensis* and *P. longinasus longinasus*), and the central-eastern populations (*P. l. dunni* and *P. l. ramsdeni*, respectively). Toads will be examined for the chytrid by taking samples using standard procedures (Brem *et al*., 2007). Population estimates will follow transect methodologies along streams (Heyer *et al*. 1994, Thomas *et al*., 2002; Veith *et al*., 2004). Ecological parameters will be measured (water speed, air and water temperature, PH, conductivity, water composition, humidity, air velocity), and correlated with aspects of the toad's biology. Food composition will be qualitatively estimated by sampling feces. Tadpoles with damaged mouth parts will be tested for the chytrid. Parasites will be stored in 70% alcohol. Biosecurity standards (Speare *et al*., 2004) will be carefully followed. Males will be recorded with a Marantz PMD 222 and Senheisser Microphone. Photographs will be taken with a Nikon D300 digital camera and lens AF-S Micro Nikkor 105 mm. Live specimens will be collected in the wild with pertinent permits and transferred to the lab to start *ex situ* management. Specimen transportation will follow recommendations by Gagliardo and Pramuk (2008).

OUTCOMES

- Captive breeding facility at Museo Nacional de Historia Natural.
- Status of *P. l. ramsdeni* in the wild.
- Status of the chytrid fungus in four wild populations of *P. longinasus.*
- Observations on the natural history of *P. longinasus* both in the wild and in captivity.

TIMELINE OF WORK

Activities	Jul. to Sept. 2010	Oct. to Dec. 2010	Jan. to Mar. 2011	Apr. to Jun. 2011	Jul. to Sept. 2011	Oct. to Dec. 2011	Jan. to Mar. 2012	Apr. to Jun. 2012	Jul. to Sept. 2012
Creation of the captive breeding facility at Museo Nacional de Historia Natural	X	X							
Field work, first expedition (West Cuba)-one week	X								
Field work, second expedition (East Cuba)	X								
Field work, third expedition (Central Cuba)		X							
Field work, fourth expedition (West-central Cuba)				X					
Sample analyses for chytrid detection (with collaboration of the Hospital Hermanos Ameijeiras in Havana)					X				
Amphibian captive management and observations		X	X	X	X	X	X	X	X
Ecological data analyses						X			
Processing data from captivity								X	
Writing manuscripts								X	X
Papers submissions									X
Fundraising for program expansion									X

TEAM: Luis M. Díaz, head of project; Alejandro Silva, molecular biology; Agustín Chong, chytrid testing; Ariatna Linares, amphibian captive management and insect rearing.

BUDGET

Field vehicle rental: 50$/day, 30 days= 1500$
Diesel: 300$
Food 30 days, 2 persons: 300$
Bd testing supplies $5/swab, $20/test, 20 samples, $500
Field equipment, data logger, $206
Field equipment, 1 Oyster Portable pH/Conductivity/Temperature Kit $205.00

pH/Conductivity/Temperature Calibration Solutions $43.00
Field equipment, Stream Flow meter $146.00
Glass aquaria, $30, 20 units, $600
Water pumps, $30, 20 units, $600
10 liters plastic containers, $10, 20 units, $200
Halogen lamps, $8, 50 units, $400
Total: $5000

*Note: Cuban National Museum of Natural History will cover expenses for PVC pipes, plastic water reservoirs, and room for the captive breeding facility.

Literature Cited

Brem, F., J. R. Mendelson III, and K. R. Lips. 2007. Field-Sampling Protocol for *Batrachochytrium dendrobatidis* from Living Amphibians, using Alcohol Preserved Swabs. Version 1.0 (18 July 2007). Electronic document accessible at http://www.amphibians.org Conservation International, Arlington, Virginia, USA.

Burrowes, P.A., Joglar, R.L., & Green, D.E. 2004. Potential causes for amphibian declines in Puerto Rico. *Herpetologica* **60**: 141-154.

Díaz, L.M. & Cádiz, A. 2006. Pflege und Vermehrung von *Bufo longinasus* Stejneger, 1905: Ein Beitrag zur Erhaltung dieser Art. *Aquaristik Fachmagazin & Aquarium heute* 38: 18–21.

Díaz, L.M., & Cádiz, A. 2008. Guía taxonómica de los anfibios de Cuba. *Abc Taxa* **4**: 1-294 (+CD).

Díaz, L.M., Cádiz, A., Chong, A. & Silva, A. 2007. First report of chytridiomycosis in a dying toad (Anura: Bufonidae) from Cuba: a new conservation challenge for the island. *EcoHealth* 4: 172–175.

Gagliardo, R. & Pramuk, J. 2008. General Amphibian Husbandry. *In*: Poole, V. & Grow, S (eds.): Amphibian Husbandry Resource Guide, AZA. pp. 4-52.

Hedges, B. & Díaz, L. 2004. *In*: IUCN 2004. *2004 IUCN Red List of Threatened Species.* <www.redlist.org>.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster. 1994. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, D.C. Pp. 364.

Joglar, R.L., Álvarez, A.O., Aide, T.M., Barber, D., Burrowes, P.A., García, M.A., León-Cardona, A., Longo, A.V., Pérez-Buitrago, N., Puente, A., Rios-López, N., & Tolson, P.J. (2007): Conserving the Puerto Rican herpetofauna. *Appl. Herpetol.* **4**: 327-345.

Lentini, A. 2000. Puerto Rican Crested Toad (*Peltophryne lemur*) SSP Husbandry Manual. Keeper and Curator Edition. Toronto Zoo: Scarborough, Ontario, 48pp.

Malhotra, A., Thorpe, R.S., Hypolite, E., & James, A. (2007): A report on the status of the herpetofauna of the Commonwealth of Dominica, West Indies. *Appl. Herpetol.* **4**: 177-194.

Pramuk, J.B. 2006. Phylogeny of South American *Bufo* (Anura: Bufonidae) inferred from combined evidence. *Zoological Journal of the Linnean Society* 146: 407-452.

Speare, R., L. Berger, Skerratt, L. F., R. Alford, D. Mendez, S. Cashins, N. Kenyon, K. Hauselberger, and J. Rowley. 2004. Hygiene protocol for handling amphibians in field studies. Amphibian Diseases Group, James Cook University, Townsville 4811, Australia.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, S.L., Fischman, D.L., & Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. Science, 306: 1783-1786

Thomas, L., Buckland, S.T., Burnham, K.P., Anderson, D.R., Laake, J.L., Borchers, D.L. & Strindberg, S. 2002. Distance sampling. *In*: Shaarawi, A.H. & Piegorsch (eds), Encyclopedia of Environmetrics, vol. 1, pp 544-552.

Valdés de la Osa, A. & Ruíz García, F. 1980. Consideraciones sistemáticas sobre *Bufo longinasus* (Anura: Bufonidae) de Cuba y descripción de una nueva subespecie. *Poeyana* 206: 1–34.

Veith, M., Lötters, S., Andreone, F. & Rödel, M-O. 2004. Measuring and monitoring amphibian Diversity in tropical forests. II Estimating species richness from standarized transect censing. Ecotropica 10: 85-99.