

Project Lemur Frog Project Final report, July 2017



Establishing captive breeding and rearing facilities

Specific *ex-situ* breeding and rearing facilities for lemur leaf frogs, including rearing facilities for the prey insects, have been completed at Manchester Museum, Bristol Zoo and Nordens Ark. The facility at Nordens Ark was completed in 2015. At the beginning of 2016 an assurance *ex-situ* population of lemur leaf frogs, representing 3 different initial bloodlines, was transferred to Sweden from Manchester Museum and Bristol Zoo. The aim of the transfer was to maintain a further assurance population in a bio-secure manner, while maintaining the genetic integrity of the captive population overall. The facilities are custom made to meet requirements of the species.

Managing distinct blood-lines in captivity

Genetic work at Salford University has focused on the sequencing of hereditary genes so as to allow for the identification of specific individuals and parentage following on from the genotyping of the species with primers described and characterised in 2013. (<https://link.springer.com/article/10.1007/s12686-014-0261-1>)

Building on genetic work, breeding of founders has started. With the support of the genetic evaluations conducted by Dr Jim Labisko, and overseen by Dr Robert Jehle at Salford University, Tim Skelton at Bristol Zoo has during the project period established a new studbook for the species. He proposed the species to European Association of Zoo and Aquaria on behalf of the project and the European Stud Book was formally approved by the Amphibian Taxon Advisory Group and European Endangered Species Programme committee in July 2016. The new studbook includes individuals from Manchester Museum, Bristol Zoo and Nordens Ark. All specimens have individual studbook numbers, and all have photograph ID's held at their holding institutions to allow for individual recognition. These specimens are all part of the official captive breeding program for the lemur leaf frog established as part of the project.

Staff training on lemur leaf frog captive husbandry and management

During the project period several staff exchanges have taken place between Manchester Museum and Nordens Ark in order to share knowledge between the two institutions and to increase the capacity of the staff at Nordens Ark in the care, management, and husbandry of the lemur leaf frogs. This has been essential since the species is delicate and has very specific husbandry needs. It has proved reliant on the skill set of the keepers at each institution that the species has been able to thrive in captivity.

Raising awareness of the conservation needs of the species

Over the duration of the project the project manager Andrew Gray and staff from Nordens Ark have conducted lectures, at both local and international meetings, to highlight the project and to also promote the species' conservation needs to the widest influential audience as possible (<https://frogblogmanchester.com/2016/11/14/amphibian-ambassadors/>). In 2015, Sir David Attenborough visited Manchester Museum to record and highlight the project in the BBC's TV documentary 'Fabulous Frogs', which was then screened worldwide. (<http://www.bbc.co.uk/programmes/p02503rc>). Nordens Ark and Bristol Zoo have also each created a special page about the project on their websites: (<http://nordensark.se/vara-projekt/lemurlovgroda/>) and (<http://www.bristolzoo.org.uk/explore-the-zoo/lemur-leaf-frogs>). A separate website is dedicated to the project (<https://lemurfrog.org/>).

***In-situ* habitat restoration and facilitating future research**

The Costa Rican Amphibian Centre owns and operates two biological reserves, the Guayacán Rainforest Reserve, and the Río Vereh Cloud Forest Reserve. Areas within the CRARC reserve forest have been actively managed by Brian Kubicki to support the population of Lemur Leaf frogs and other threatened amphibians. Manchester Museum, through Project Lemur Frog, has provided funds for the clearing of ponds as well as clearing large areas of understory dominated by undesirable fern species and vines within the current forest of the CRARC reserve. As part of the project, Nordens Ark, through funding from the Segre foundation, have provided the CRARC with a small research station that has been built in the in Rio Vereh Cloud Forest Reserve. The cabin was finished in April 2015. This cabin, which can accommodate several people, may play a vital role in facilitating research efforts with the amphibians in the area. The Rio Vereh Cloud Forest Reserve is located in one of the most unstudied, yet biologically diverse regions in Costa Rica, the upper margin of the premontane rainforest life zone of the Caribbean slopes of the Talamancan mountains.

Genetically identifying individuals

Genetic fingerprinting has been done on the assurance *ex-situ* population and the result has provided the first genetically-informed studbook for a critically endangered amphibian. The aim of this aspect of the project was to genetically characterise 30 individual lemur leaf frogs from collections held at Manchester Museum and Bristol Zoo, to inform the captive breeding programme and to compile a studbook based on genetic information. Individuals were genotyped with the use of nine microsatellite markers previously developed by Salford University (Petchy, *et al*, 2013). DNA was retrieved non-invasively using mouth swabs (27 samples) or was based on eggs (3 samples). A freelance post-doctoral scientist, Dr Jim Labisko, conducted the practical work. Tim Skelton at Bristol Zoo is now using the result from the study for the continued development of the official new studbook.

International Environmental Education programme

A new environmental education initiative, which provides resources for primary schools, three information films, and related children's activity booklets, has been created. The Ambassador of Costa Rica, His Excellency Enrique Castillo, launched this key educational aspect to the project in January 2016, at Manchester Museum (<https://frogblogmanchester.com/2016/02/02/lucys-launch/>). The related education material and films were translated into Spanish for use throughout Latin America. The project also funded the delivery of associated environmental education work in Costa Rica, in collaboration with the CRARC (<https://lemurfrog.org/in-situ/environmental-education-in-costa-rica/>). The production of Swedish versions of the Lemur frog films, with Swedish voice overs, will help bring the lemur frog related environmental education campaign to Swedish schoolchildren in the future. <http://www.manchester.ac.uk/discover/news/lucy-9-fronts-global-fight-to-save-frog-from-extinction> & (<https://phys.org/news/2015-12-lucy-global-frog-extinction.html>) (<http://mancunion.com/2016/03/09/uom-supports-campaign-save-endangered-amphibian/>).

International student opportunities

To support undergraduate student development and international academic collaboration in both Sweden and the UK, Andrew Gray and Professor Amanda Bamford from Manchester University visited Nordens Ark in January 2017 to discuss placement student opportunities. This has resulted in a placement student from University of Manchester Faculty of Biology, Medicine and Health conducting a 9 month project from November 2017 to support the lemur frog care and the development of related environmental educational aspects at both institutions.

Future conservation of the species

The Lemur Leaf frog, *Agalychnis lemur*, has also recently been nominated and accepted as an EDGE species. We hope this new status will encourage and provide opportunities for other conservationists to work with the species and through paid fellowships help actively support the species' future. Manchester Museum has supported the development of recognition software for the non-invasive identification of individual animals. The software has proved to be an efficient and reliable application that would be particularly appropriate for accurately assessing wild lemur frog populations and facilitates non-invasive survey methods for future monitoring (<https://lemurfrog.files.wordpress.com/2017/05/lemur-frog-recognition.pdf>).

We will fully support future proposals by our existing *ex-situ* collaborators for developing in-country initiatives relating the monitoring of lemur frog populations throughout the species' known range. Whilst supporting the conservation of wild populations is a priority, full commitment to all *ex-situ* aspects will continue by the partners concerned, so as to provide a secure safety net to ensuring the species never becomes extinct in the future.