

The Rufford Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Gayani Senevirathne
Project title	DNA-Barcoding and documenting the morphology of Sri Lankan tadpoles to species-level to advance amphibian ecology and conservation in the region
RSG reference	19272-1
Reporting period	12 months
Amount of grant	£4,979
Your email address	gayani88s@gmail.com
Date of this report	November 10 th 2018

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
1. Collections of larval stages across the country				Larval stages of anurans were collected from the major biogeoclimatic regions of Sri Lanka. Field work expanded over several months - scrutinising streams, rivers, lakes, ponds, reservoirs, phytotelmata, marshes, peat bogs, and other likely tadpole habitats. We found several undocumented tadpole species and locations which were not recorded before (explained in detail in a subsequent section). The sampling was done non-destructively, photographs of the tadpoles were taken at the captive site and released after 10-15 minutes. To validate the species' identity, our goal was to take tail tips from a tadpole from one population and carry out barcoding, as a method of identifying tadpoles across Sri Lanka. We found nearly 200+ populations; two new species of adults were discovered through the tadpoles we sampled (one has been published: Senevirathne <i>et al.</i> 2018 and one publication is being prepared). We sampled all the anuran species with a tadpole stage – a total of 36 species (and a new species) within our planned time.
2. Photograph all the tadpole species in Sri Lanka				Because of our extensive fieldwork, we were able to discover and photograph all the tadpole species in Sri Lanka. To date, only a few species have been documented and the remaining have received less attention. We believe that our work will enhance the importance of conserving the larval stages as much as the adults. We hope to publish this

				work as a monograph, including all the photographs and features that helps documenting and identifying different species in the wild. Also, if time permits, we plan to design a small leaflet with all the frog species in Sri Lanka and distribute it among locals, for them to get a better understanding of the different life history stages that also require conservation. This work is currently being planned.
DNA barcoding and validating species identity				For the samples that were collected through non-destructive sampling (by a tail tip), we did 16S rRNA barcoding, to validate species identity. Through this process, we were able to identify tadpole species that were not recorded or seen before (e.g., <i>Uperodon rohani</i> , <i>Uperodon palmatus</i> , <i>Uperodon variegatus</i> , <i>Uperodon taprobanicus</i> , <i>Uperodon systoma</i> , <i>Microhyla karunaratnei</i> , <i>Microhyla zeylanica</i>). Also to identify localities for some anuran species through the tadpoles that we barcoded (e.g., Garg et al., (2018) records a new population of <i>U. palmatus</i> , which we discovered only through tadpoles.)
Record morphological characteristics and measurements; making the finalized results available to the public				This work is still ongoing. We have measured and recorded all the characteristics that are unique to each species. Finalising this as a publication is being done.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

This project's main focus was to do a comprehensive survey of the life history stages of amphibians (mostly focusing on the species with a tadpole stage). The larvae, with partitioned habitats, face a wide variety of threats. Initial sampling was done focusing mostly on the areas that have been already reported to house extant amphibian species. However, there was a plethora of habitats, which were suitable for amphibian egg-laying, and we found several larvae present in tree holes, paddy fields, even in small water puddles on roads. Analysing and identifying larvae (tadpoles) took us a long time than we anticipated because of the lack of descriptive morphological studies or barcoding to confirm the larval identities.

However, with the help of the Rufford small grant we received, I am happy to say that we have achieved our goals, and this will immensely help in future conservation studies of the anuran species in Sri Lanka.

3. Briefly describe the three most important outcomes of your project.

This study helped in identifying some of the unidentified amphibian larvae and also some misidentified tadpole species in Sri Lanka - we have published several scientific articles and a conference presentation with the help of the grant money (Senevirathne *et al.*, 2017; Garg *et al.*, 2018; Senevirathne *et al.*, 2018). We thoroughly scrutinise "suspected" habitats (over 200+ localities) for tadpoles, did constructive morphological descriptions, and identified several tadpole species, which were not recorded/described previously. Tadpoles/larvae identification also led to discovering new populations of adult amphibians, which were not recorded before (Senevirathne *et al.*, 2018; Garg *et al.*, 2018) – this helped in identifying conservation of frog species, which were either data deficient or threatened according to the IUCN categories. We generated a phylogenetic tree, highlighting tadpole species throughout Sri Lanka, where we did extensive sampling in both wet and dry seasons. This work is currently being prepared for publication, as a monograph, with descriptions of all the frog/toad genera with a tadpole stage. We also identified species that were either undescribed or described inaccurately (e.g., *Uperodon rohani*, *Uperodon palmatus*, *Uperodon variegatus*, *Uperodon taprobanicus*, *Uperodon systoma*, *Microhyla karunaratnei*, *Microhyla zeylanica*, *Lankanectes pera*) without the support of molecular data. With the help of the grant money, we were able to obtain high quality photographs of all the tadpole species and videos, which will be published soon. We hope that this will enhance the quality of monitoring activities of tadpoles. This complete documentation of life histories of amphibians will help research and conservation efforts in the future.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

Amphibians (mostly frogs and toads) are a common sight in Sri Lanka. Sri Lanka, an island with a majority of people living in the countryside is somewhat oblivious to the importance of tadpoles in species conservation. Tadpoles represent the most susceptible life history stage of a frog and could go extinct if a tadpole habitat is not given equal importance as the adult frogs. Even though we published several publications with the help of the grant money, still educating the local communities require work. When we were on our field expeditions, we got help from several local communities in identifying populations of tadpoles, some welcomed us to their homes, and got involved in our search process. I am planning on publishing a small book and a leaflet with the tadpole photographs, habitat information, and morphological descriptions, so that local communities can be aware of the importance of tadpoles in our environment.

5. Are there any plans to continue this work?

Yes, the book on Sri Lankan tadpoles is currently being planned. This will help any person to identify and contribute to amphibian conservation in Sri Lanka, which is considered an amphibian hotspot and requires protection of this invaluable fauna.

6. How do you plan to share the results of your work with others?

I presented at the World Congress of Herpetology, in China, 2016 – a small part of our project. However, with the data that we gathered, I am planning on publishing a small book with the tadpole photographs, habitat information, and morphological descriptions, so that local communities can be aware of the importance of tadpoles in our environment. Sri Lanka, which is considered an amphibian hotspot, is rich in biodiversity. Hence, it is very much needed to make people aware of the tadpoles and their importance. To date there are very few valid descriptions of tadpole species in Sri Lanka. We have confirmed species identities of all the tadpole species in Sri Lanka; therefore, we hope to finalise our photographs and videos of the tadpole species as a way of disseminating knowledge.

7. Timescale: Over what period was The Rufford Foundation grant used? How does this compare to the anticipated or actual length of the project?

N/A

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
UV meter (portable)	25	25	0	-
Extech PH300 Waterproof pH/mV/Temperature Kit 3-in-1 Meter, Lab-Quality	0	187	-187	Already had this in the Meegaskumbura Lab.
Extech DO700 Portable Dissolved Oxygen Meter Kit	300	370	-70	Difference was funded through Meegaskumbura Lab
Underwater Camera – GoPro - HERO4 Black 4K	241	232	+9	
Canon EF 100mm f2.8/L Macro IS USM Lens (second-hand)	547	547	0	
Battery charger (eneloop) and Batteries	36	36	0	

Camping Tent (capacity: 6 people)	0	200	-200	
Accommodation for team members (6 people)	300	1000	-700	Money allocated for field work was not sufficient, and the difference was funded by Meegaskumbura lab funds and some of the money allocated for publication fees. Our field work took several trips than we anticipated. After we got our barcoding results, we went back to some locations again to confirm tadpole identities. This is what led to the discovery of tadpole species that were not previously recorded or photographed before.
Food during field work	300	800	-500	
Travel (Fuel for the field vehicle, public transportation by trains and buses)	200	500	-300	
Qiagen DNeasy extraction kit (250 reactions)	730	730	0	
Chemicals	300	500	-200	
Miscellaneous Items (nitrile gloves, sterile vials, tubes, jars)	500	500	0	
Sequencing charges (150 samples)	900	900	0	
Publication fees	600	350	+250	Three papers are published; the final publication is still being processed.
Total	4979	6877	-1898	

9. Looking ahead, what do you feel are the important next steps?

As I mentioned earlier, Sri Lanka has a rich amphibian diversity. However, much attention is given to the conservation of adult frogs. Our effort has been to show the wide plethora of habitats the tadpoles occupy, and to show that it is equally important to give attention to the larval stages in measures of conservation. There were species that were recognized under data deficient or Critically Endangered, for which we had no information about the larval stages. However, our work has documented all the species and will be an excellent source in conservation studies. We plan to publish this as soon as possible, so that the local communities can gain

access/knowledge about the wide range of species in their areas. It is important to make a small leaflet, which should be easily accessible to locals, which we will plan next.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Rufford Foundation receive any publicity during the course of your work?

Yes, when I presented my work at the World Congress of Herpetology, in China, 2016, I used the Rufford Foundation logo. Further, all the published work acknowledges the Rufford foundation in helping our work immensely.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Gayani Senevirathne: I was involved in all the field trips, and sampling. I did all the 16s rRNA barcoding, photographed the tadpoles, took measurements, and carried out all the phylogenetic analyses to validate species identities.

Prof. Madhava Meegaskumbura: Dr. Madhava Meegaskumbura (www.frogs.lk) advised and supported me throughout the project. With his wide expertise with amphibian systematics (having described over 25 species of frogs, including two new genera), barcoding, tadpole morphology, phylogenetics, population genetics, life-history studies, captive breeding, ecology, and amphibian conservation, he played an important role in this project. He is also a Tier-I member of the Amphibian-Red Listing Authority of the IUCN and a co-chair of the Amphibian Specialist Group-Sri Lanka. He was involved in field work and helped in all the phylogenetic analyses.

Dr. Nayana Wijayathilaka, Mr. Nuwan Karunarathne, Mr. Aravinda Jayanath and Mr. T. P Sylvester are graduate/undergraduate students in the lab who supported me in fieldwork and photography.

Mr. Kelum Manamendra-Arachchi, a well-known Sri Lankan amphibian taxonomist, who has also studied some of these tadpoles was also part of our team. He helped in taking morphological measurements of the tadpoles.

12. Any other comments?

We would like to thank the Rufford Foundation for supporting us in this work, which will be an important stepping stone in future amphibian conservation work in Sri Lanka.

References:

Senevirathne, G., Kerney, R. and Meegaskumbura, M., 2017. Comparative Postembryonic Skeletal Ontogeny in Two Sister Lineages of Old World Tree Frogs (Rhacophoridae: Taruga, Polypedates). *PLoS one*, 12(1), p.e0167939.

Garg, S., Senevirathne, G., Wijayathilaka, N., Phuge, S., Deuti, K., Manamendra-Arachchi, K., Meegaskumbura, M. and Biju, S.D., 2018. An integrative taxonomic review of the South Asian microhylid genus *Uperodon*. *Zootaxa*, 4384(1), pp.1-88.

Senevirathne, G., Samarawickrama, V.A.M.P.K., Wijayathilaka, N., Manamendra-Arachchi, K., Bowatte, G., Samarawickrama, D.R.N.S. and Meegaskumbura, M., 2018. A new frog species from rapidly dwindling cloud forest streams of Sri Lanka—*Lankanectes pera* (Anura, Nyctibatrachidae). *Zootaxa*, 4461(4), pp.519-538.