



Research Networking Programmes

Short Visit Grant or Exchange Visit Grant

(please tick the relevant box)

Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: Ecology and conservation of Critically Endangered forest amphibians within an Eastern Arc centre of hyper-endemism.

Application Reference N°: 6760

1) Purpose of the visit

The main purpose of the visit was to extract DNA from historical samples collected in the Udzungwa Scarp Forest Reserve (USFR, Eastern Arc Mountains, Tanzania), conduct a preliminary phylogenetic analysis of sequence data under the supervision of experienced group members and start building a reliable database of the amphibian species occurring in this remarkable area. Given the occurrence of morphologically similar anuran species in the EAM range and that Eastern Arc herpetofauna shows distinctive changes in assemblages with small increases in altitude, molecular analysis is crucial to enable correct taxonomic classification, detection of potential sibling species and proper estimation of distribution ranges. The tissues to be analysed were loaned from the Muse – Museo delle Scienze (Trento, Italy).

2) Description of the work carried out during the visit

During my stay at the Institute of Biogeography (University of Basel, Basel, CH) I analysed 71 tissues from various anuran species collected in four sites within the USFR by colleagues of the Museo delle Scienze.

Samples were first lysed using proteinase K solution in ATL buffer, then DNA extraction was performed by the QIAGEN QIAcube following the DNeasy Mini spin column protocol for blood and tissues. Extracted DNA will remain within the storage facility of the University of Basel.

Each DNA sample was subsequently amplified in a Mastercycler machine using universal PCR primers to obtain 600 base pairs from the 16S region of the mitochondria. DNA presence/quality was verified by viewing the obtained PCR products on agarose gel with UV light after electrophoresis. Eleven samples failed to amplify, indicating extremely poor DNA quality; all successful PCR products were sent for sequencing. Species identification for each sample was done with a sequence BLAST search of the Genbank and NCBI databases in Geneious software.

3) Description of the main results obtained

According to the sequences of the target locus, one third of the samples do not match database sequences at a reliable level (percentage of identical sites < 97%; highlighted in red in **Tab.1**), which highlights the highly divergent nature of some of the USFR amphibian populations. Some of these specimens are morphologically similar and would be currently treated as a single species (*Arthroleptis cf. reichei*).

Tag	On-field ID	Sequence ID	% Identical Sites	Site
MTSN 5611	<i>Petropedetes yakusini</i>	<i>Petropedetes yakusini</i>	100,00%	Chita
MTSN 5613	<i>Petropedetes yakusini</i>	<i>Petropedetes yakusini</i>	99,80%	Chita
MTSN 5614	<i>Leptopelis flavomaculatus</i>	<i>Leptopelis vermiculatus</i>	99,60%	Chita
MTSN 5615	<i>Leptopelis flavomaculatus</i>	<i>Leptopelis vermiculatus</i>	99,60%	Chita
MTSN 5616	<i>Nectophrynoides cf. tornieri</i>	<i>Nectophrynoides tornieri</i>	99,00%	Chita
MTSN 5618	<i>Nectophrynoides cf. tornieri</i>	<i>Nectophrynoides tornieri</i>	99,00%	Chita
MTSN 5619	<i>Nectophrynoides cf. tornieri</i>	<i>Nectophrynoides tornieri</i>	99,20%	Chita
MTSN 5656	<i>Arthroleptis sp.</i>	<i>Arthroleptis stenodactylus</i>	98,30%	Chita
MTSN 5662	<i>Arthroleptis sp.</i>	<i>Arthroleptis affinis</i>	99,50%	Chita
MTSN 5004	<i>Arthroleptis cf. affinis</i>	<i>Arthroleptis reichei</i>	97,80%	Mkalazi
MTSN 5028	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	92,50%	Mkalazi
MTSN 5029	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	93,00%	Mkalazi
MTSN 5328	<i>Nectophrynoides sp.</i>	<i>Nectophrynoides tornieri</i>	98,50%	Mkalazi
MTSN 5331	<i>Nectophrynoides sp.</i>	<i>Nectophrynoides tornieri</i>	98,30%	Mkalazi
MTSN 5468	<i>Nectophrynoides sp.</i>	<i>Nectophrynoides tornieri</i>	98,60%	Mkalazi
MTSN 5469	<i>Petropedetes yakusini</i>	<i>Petropedetes yakusini</i>	100,00%	Mkalazi
MTSN 5494	<i>Phrynobatrachus uzungwensis</i>	<i>Phrynobatrachus rungwensis</i>	100,00%	Mkalazi
MTSN 5499	<i>Phrynobatrachus uzungwensis</i>	<i>Phrynobatrachus rungwensis</i>	99,80%	Mkalazi
MTSN 5533	<i>Phrynobatrachus sp.</i>	<i>Phrynobatrachus calcaratus</i>	97,40%	Mkalazi
MTSN 5837	<i>Amietophrynus brauni</i>	<i>Bufo brauni</i>	100,00%	Mkalazi
MTSN 5885	<i>Nectophrynoides poyntoni</i>	<i>Nectophrynoides viviparus</i>	94,10%	Mkalazi
MTSN 5900	<i>Arthroleptis xenodactyloides</i>	<i>Arthroleptis xenodactyloides</i>	98,30%	Mkalazi
MTSN 5644	<i>Nectophrynoides wendyae</i>	<i>Nectophrynoides tornieri</i>	90,70%	Ihimbo
MTSN 5648	<i>Nectophrynoides wendyae</i>	<i>Nectophrynoides tornieri</i>	90,90%	Ihimbo
MTSN 5649	<i>Afrixalus sp.</i>	<i>Afrixalus sp.</i>	99,10%	Ihimbo
MTSN 5030	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	90,50%	Kihanga
MTSN 5031	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis reichei</i>	97,90%	Kihanga
MTSN 5247	<i>Nectophrynoides viviparus</i>	<i>Nectophrynoides viviparus</i>	94,90%	Kihanga
MTSN 5252	<i>Nectophrynoides viviparus</i>	<i>Nectophrynoides viviparus</i>	94,30%	Kihanga
MTSN 5259	<i>Amietophrynus brauni</i>	<i>Bufo brauni</i>	100,00%	Kihanga
MTSN 5280	<i>Hyperolius substriatus</i>	<i>Hyperolius puncticulatus</i>	95,00%	Kihanga
MTSN 5474	<i>Afrixalus cf. uluguruensis</i>	<i>Afrixalus sp.</i>	99,40%	Kihanga
MTSN 5475	<i>Afrixalus cf. uluguruensis</i>	<i>Afrixalus sp.</i>	99,10%	Kihanga
MTSN 5519	<i>Arthroleptis cf. reichei/affinis</i>	<i>Arthroleptis reichei</i>	97,90%	Kihanga
MTSN 5520	<i>Arthroleptis cf. reichei/affinis</i>	<i>Arthroleptis nikeae</i>	90,40%	Kihanga
MTSN 5521	<i>Arthroleptis cf. reichei/affinis</i>	<i>Arthroleptis nikeae</i>	92,90%	Kihanga
MTSN 5522	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	93,30%	Kihanga
MTSN 5523	<i>Arthroleptis sp.</i>	<i>Arthroleptis reichei</i>	97,90%	Kihanga
MTSN 5525	<i>Arthroleptis cf reichei/affinis</i>	<i>Arthroleptis nikeae</i>	90,00%	Kihanga
MTSN 5681	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	90,20%	Kihanga
MTSN 5683	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis tanneri</i>	90,00%	Kihanga
MTSN 5689	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis reichei</i>	97,60%	Kihanga
MTSN 5701	<i>Arthroleptis cf reichei/affinis</i>	<i>Arthroleptis nikeae/tanneri</i>	90,60%	Kihanga
MTSN 5702	<i>Arthroleptis cf reichei/affinis</i>	<i>Arthroleptis nikeae</i>	92,90%	Kihanga
MTSN 5710	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	91,40%	Kihanga
MTSN 5717	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	93,20%	Kihanga
MTSN 5787	<i>Arthroleptis cf. affinis</i>	<i>Arthroleptis nikeae/tanneri</i>	90,70%	Kihanga

(continues)

MTSN 5789	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	93,00%	Kihanga
MTSN 5790	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae/stenodactylus</i>	90,70%	Kihanga
MTSN 5831	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	89,60%	Kihanga
MTSN 5832	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	91,10%	Kihanga
MTSN 5836	<i>Arthroleptis cf. affinis/reichei</i>	<i>Arthroleptis reichei</i>	97,80%	Kihanga
MTSN 5838	<i>Amietia angolensis</i>	<i>Amietia angolensis</i>	97,80%	Kihanga
MTSN 5841	<i>Amietia angolensis</i>	<i>Amietia angolensis</i>	97,60%	Kihanga
MTSN 5850	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis reichei</i>	92,10%	Kihanga
MTSN 5903	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	92,90%	Kihanga
MTSN 5908	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae</i>	92,90%	Kihanga
MTSN 5910	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis reichei</i>	97,30%	Kihanga
MTSN 5911	<i>Arthroleptis cf. reichei</i>	<i>Arthroleptis nikeae/tanneri</i>	90,60%	Kihanga
MTSN 5924	<i>Arthroleptis xenodactyloides</i>	<i>Arthroleptis xenodactyloides</i>	92,70%	Kihanga

Tab.1 – Identification of specimens on the basis of 16S sequences. Highlighted in red are the percentages of identical sites < 97%, representing potentially different species than they are currently classified as. Note that *Nectophrynooides poyntoni*, *N. wendyae* and *Hyperolius substriatus* (highlighted in light blue) have been described as species but their sequences are lacking from available databases.

A preliminary phylogenetic tree based on the *Arthroleptis* specimens showed a remarkable diversity within the genus (**Fig.1**), especially for a forest not larger than 200km². This suggests for the USFR an even higher biodiversity than previously known. Further data collection and analysis are needed to properly understand phylogenetic relationships and conservation value across the USFR. The preliminary analyses suggest a high cryptic diversity in certain lineages (e.g. *Arthroleptis*).

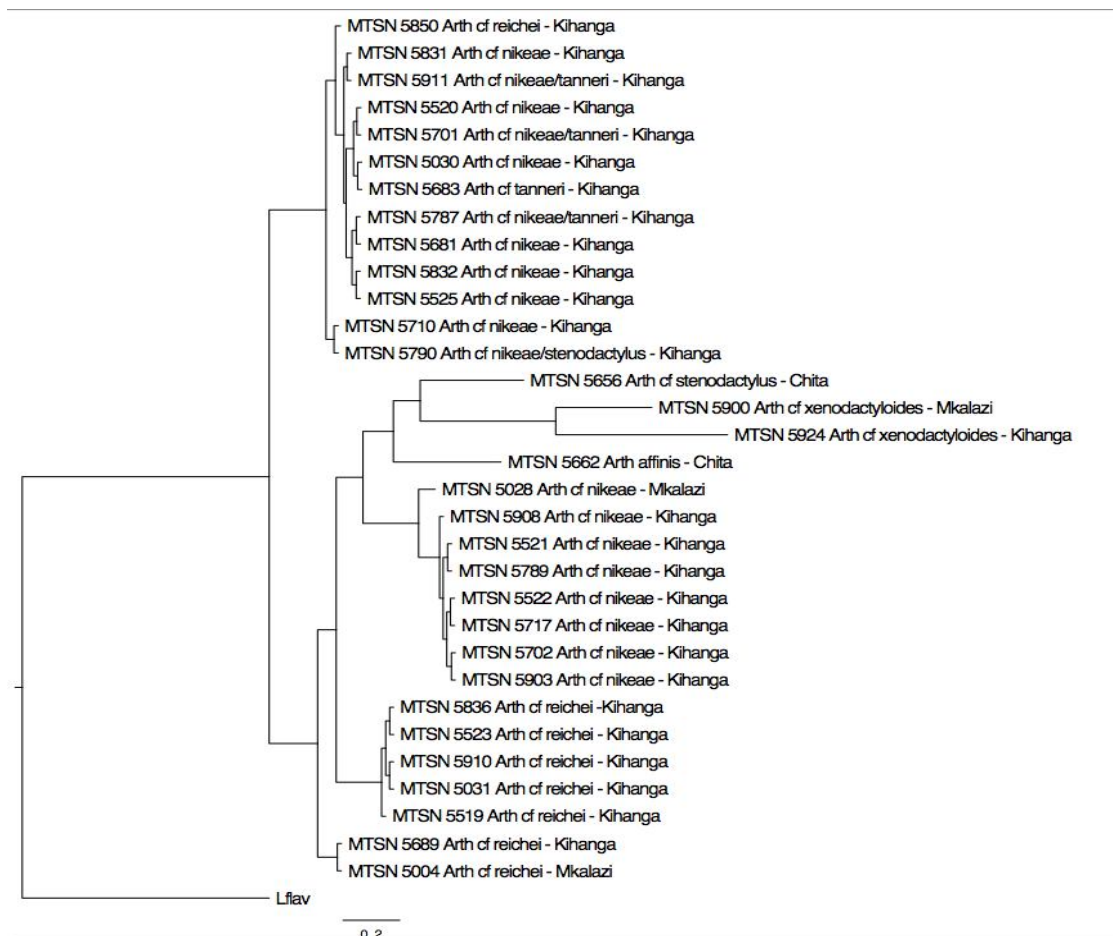


Fig. 1 – Phylogenetic tree for the genus *Arthroleptis* in USFR.

4) Future collaboration with host institution (if applicable)

This visit is part of a larger PhD project involving two field seasons of genetic sample collection in the USFR (2014-15 and 2015-16). We plan future collaborations for phylogenetic analysis and interpretation of the exciting but complex genetic data coming from the USFR.

5) Projected publications / articles resulting or to result from the grant (*ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant*)

For the time being results will be published on Dr. Marsden's research group blog <http://stuartmarsden.blogspot.co.uk> in the section describing the projects carried out in the Eastern Arc Mountains. ESF contribution to the project will be acknowledged on the website and in any future publication resulting from the work covered by this grant.

6) Other comments (if any)