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	Monica Mwale
Project title	Characterisation and validation of STR loci and DNA markers for forensic identification of endangered vulture species in South Africa
RSG reference	18463-1
Reporting period	2016/17
Amount of grant	£5000
Your email address	monicam@nzg.ac.za
Date of this report	08 April 2017

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) Setting up genetic databases for three South African vulture species; Bearded vulture *Gypaetus barbatus* Cape vulture *Gyps coprotheres* and white-backed vulture *Gyps africanus*				The NZG had 253 samples for the three target species before initiation of this project; bearded vulture (5), white-backed vulture (30), Cape vulture (200) and other species (20) from several localities in southern Africa, Zoo collections and captive-release programmes. An additional 168 blood samples of the Cape (121) and white-backed (47) vulture were collected through collaborators and accessioned into the NZG biobank for species genetic databases during 2016-17. Other vultures species also collected during this period included the Egyptian Vulture (5), king vulture (2) and lappet faced vulture (12). We were unable to collect additional specimens of the bearded vulture as this species is critically is endangered locally with access to material being difficult. We have however been in contact with the Mr Andre Botha the manager of the Birds of Prey Programme of the Endangered Wildlife Trust (EWT) and a co-chair of the IUCN SSC Vulture Specialist Group to assist with sampling of all species including the bearded vulture. We prepared and handed over 50 sampling kits with forensic bags of collection of these samples in March 2016. As his sampling is ongoing, we have not yet received all the samples from his monitoring team who do routine

				sampling on birds of prey.
2) Identification and validation of mitochondrial genetic markers for the intra and interspecific variation				DNA barcodes have been validated for forensics using the mitochondrial DNA Cytochrome oxidase I gene (COI) and Cytochrome B genes for the 23 individuals from eight species of vultures namely; bearded vulture *Gypaetus barbatus* (1), Cape vulture *Gyps coprotheres* (5) and white-backed vulture *Gyps africanus* (5) (the target species) and the palm-nut vulture *Gypohierax angolensis* (2), hooded vulture *Necrosyrtes monachus* (1), Egyptian vulture *Neophron percnopterus* (1), Lappet-faced vulture *Torgos tracheliotus* (5) and white-headed vulture *Trigonoceps occipitalis* (5) other relevant look-alike species. These individuals have all been collected under chain of custody as for reliable and expertly identified reference material for forensic case work. All these DNA barcodes has been uploaded to GenBank under these accession numbers (KX012792, KX012818-19, KX012830-39, KX012844, KX012846, KX012864-65, KX012875 – 78). We were unable to collect the target of five specimens of the bearded vulture as this species is critically is endangered in South Africa with access to material being difficult.
3) Identification and validation of nuclear (STR) genetic markers for the intra and interspecific variation				The current and most efficient approach for the identification of STR loci for species and individual identification is using next-generation sequencing (NGS) technology. The NGS can generate genome wide data for the identification of polymorphic loci or SNPS in the genome. The NZG with collaborators have generated genome data reads for the Cape vulture on the Ion S5 Ion Torrent™ technology which is in the first 4 years since its introduction. The reads

				are being analysed through our bioinformatics platform (Dr Morne Du Plessis) for assembly to get the novel Cape vulture genome. The available data has a peak distribution in the 200 bp range for assembly. We have preliminarily cleaned up the data, in terms of quality and by removing the smaller reads to ensure accuracy. We are currently assembling / mapping this dataset at the Centre for High Performance Computing. This data together with some of the unassembled data will be used to design STR markers for profiling. The Cape vulture was prioritised for analysis as we have sufficient samples to evaluate the species for forensics. These markers will be tested on the other species for cross-species amplification based on their phylogenetic histories. The delay in the validation process was mainly due to access to high performance computing in South Africa. We have had to queue up on national platforms and have to ask for bioinformatics support. Currently, markers for gender assignment have been evaluated and validated for all vulture species. This is a priority for this project as all captive breeding programmes including VULPRO and the NZG send their samples to our genetics labs for this service. We will identify additional markers to ensure adequate loci are available. In conclusion, this research will therefore be highly novel and the first for African vultures. I have therefore now appointed a PhD student (Ms Bridget Nduna) who will process all the samples and validate these markers as her thesis dissertation. The aim is to complete and design this genetic technology for forensics. The NZG has also

				received funding for the purchasing a setting up a full the Ion S5 platform. This has been through the BID process and has been awarded to the supplier. This new platforms should assist with final completion of these analyses.
4) Forensic testing for monitoring illegal trade in traditional medicine				We have set-up collaboration with the Gauteng Department of Agriculture and Rural Development (GDARD) through Dr Craig Whittington-Jones from to do the legal sampling and analysis of the vulture muthi trade in Gauteng's big major markets such as Faraday Market. The aim of this proposed research is to verify the origins of vulture parts traded at muthi markets in Gauteng. We have had to proceed with caution and not approach the sampling ourselves as the negative, defensive and sometimes offensive attitude of traders to conservation officials or police has made this difficult. We also require permits to carry out sampling for vulture as Threatened and Protected Species (ToPS) irrespective of the current status of the traders at the muthi markets or the purpose for taking such possession as this activity is included as a "Restricted activity" with prohibitions that require a permit for: • Possession, conveying, gathering, picking or exercising physical control. • Selling, buying, receiving, import/ export etc., of any specimens of ToPS listed species. However, GDARD have initiated the process and are currently accumulating samples for us to analyse using permits. We will therefore be able to do this analysis once the sampling is complete. The Department of Environmental Affairs (DEA) is also aware of this concern and informed us that they have been in negotiations

				with traditional healers associations to assist with the sampling process from these markets. The nature of collections from these markets has therefore been treated with caution.

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

a) Bearded vulture samples: The species is very rare and difficult to sample. Although we have processes in place for sampling, our collaborators have not been able to get more samples. This remains part of our objective and should be achieved with time.
b) Access to high-performance computing and bioinformatics support: The major scope and timeframes of this work was underestimated in high sight as analysing and assembling "novel" genome data of vulture species will take time as there are no available published reference genome datasets online. The closest species or genome data available that was used for assembly was for the Turkey vulture *Cathartes aura* which has been suggested to be even more closely related to the chicken than to other old world vulture species. The ease of access to high-performance computing and bioinformatics support was also a drawback. Because the Ion S5 Ion Torrent™ technology is in the first 4 years since its introduction, there was limited support to process the data rapidly. This has therefore been a learning process for our team. However the new funding for the NGS platform comes with a budget for a computer server and training that should assist with this in the future.

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

a) Published DNA barcodes on GenBank: We have generated COI sequence data from chain of custody reference samples. This is very useful as it presents a new more reliable and accurate reference for species identification for the prosecution of wildlife crime. The data has been annotated as forensic chain of custody references on GenBank and should be readily available for use by the wildlife forensic community. (An image of part of the data generated is attached).

b) Generating novel genome data: This is important for South Africa and the African region as genome data for most threatened and endangered species from the illegal wildlife trade is unavailable. Through this research we will come up with protocols and procedures that should assist with development of other forensics technologies for identification and profiling of other species of concern. Using the

new Ion S5 Ion Torrent™ technology should also improve our bioinformatics capacity.

c) Setting up new strategic collaborations: The new collaboration with the Gauteng Department of Agriculture and Rural Development (GDARD) is critical to our local interactions with this department for assisting with wildlife crime and has developed into a major relationship for monitoring other legal (captive breeding) and illegal activities involving CITES and TopS bird species. For example, we are now also collaborating with GDARD and DEA in providing genetic profiles for all captive crane species for permitting purposes. GDARD have also invited us to assist with forensic genotyping of confiscations of tortoises from illegal possessions for reintroductions into the wild. These collaborations will all ensure that the forensic research developed by the NZG has relevance to local governing and management authorities.

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

N/A

5. Are there any plans to continue this work?

This work is not complete and is continuing as a PhD project (2017). I have a new student Ms Bridget Nduna under the NRF-DST PhD PDP programme who is registering with the University of Pretoria to complete the validating process of the STR markers for these three bird species. We will be also applying for funding support as the NGS cost estimates we provided were low and we will need extra funding to complete full analysis of all three species.

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

a) The DNA barcode reference data is already available online on the GenBank database. The cytochrome b data will be uploaded to the Barcode of Life Database system (BOLD) and will be available with other species data.

b) This work will be published in journals on completion. A PhD thesis will also be available on completion.

c) The research will also be submitted as an e-newsletter on the NZG website. We have already written an article for our website. See "*A bitter sweet tale of Tukollo the Cape Vulture*" by Clearance Mnisi and Monica Mwale (Researcher) - http://www.nzg.ac.za/newsletter/issues/October-2015/01.php

d) The research will also be presented at national and international conferences.

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

February2016 - May 2017; this period is longer than the project period due to some delays in sampling and data analysis.

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
NGS sequencing of vulture 3 samples	£1600	£1762.54 (R30000.00)	-£162.54	NGS cost from supplier. This was underestimated in the proposal.
Primers – fluorescent labelled and unlabelled	£2000	£ 935.18 (R15917.59)	£1064.82	We had contributions for others sources to cover cots. We however still have to spend some of this money with ordering new oligos for new loci for the PhD project.
DNA sequencing analyses	£1400	£ 1968.76 (R33510.00)	-£568.76	The amount for sequencing will cost more due to the numbers of runs needed for validation.
Field work - sampling	£1000	£691.74 (R11774.00)	£308.26	We have made some saving due to collaborators support. We have had to mainly pay for sampling kits to our biobank
TOTAL Spent	£6000	£5358.22		This remaining amount is the NZG contribution only. This will be spent on genome data marker validation as more loci oligos have to be ordered.

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

a) STR Marker validation – the generated Cape vulture genome data has to be validated for polymorphic loci for effective use. This process is a trial and error process as the selection of markers in the genome is random based on data. We therefore have to test the first 20 loci to verify their effectiveness. All monomorphic loci will be discarded if they provide no species level-signal. We will then have to go back to the genome data to identify an additional 20 loci for analysis. This process will work hand in hand with the validation analyses of these loci for all three species.

b) Sampling: We will continue to work with EWT and GDARD to complete sampling from Muthi markets and reserves for target species.

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. I have used the Rufford logo in my presentation to the 3rd biennial LAB (Learn About Birds) conference organised by Birdlife South Africa that took place on 10 and 11 March 2016 at Kruger National Park. My talk was entitled "A forensic DNA barcoding reference library for South African threatened and endangered bird species."

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

This is the current team that has been actively involved in the lab work and analysis of data.

a) Ms Bridget Nduna (Intern: 2016-2017) – DNA barcoding data and BOLD data reference database uploads. Bridget was an intern in 2016 and will continue as a PhD candidate from April this year.

b) Mr Morne Du Plessis (NZG Researcher) – Bioinformatics support for the genome assembly and marker identification.

c) Mr Clearance Mnisi (NZG Research assistant) – laboratory analysis, performs all species profile tests on vultures and gender determination assignments for the Vulture Programme and other captive facilities. He has also done the marker validation for the gender assignment tests.

12. Any other comments?

I'm very grateful for the financial support from the Rufford grant. While there have been some unforeseen hurdles and circumstances, this research will now contribute to the training of a student PhD candidate. The value and novel data obtained in this study has highlighted the need for more research on genomes of African bird species. It is also now clear that there is a need for more bioinformatics training and support in the region.