Final Evaluation Report

Your Details	
Full Name	Emily Clare Patterson
Project Title	Combating illegal trade in animal products by rapid on-site DNA sequencing in Mongolia
Application ID	37568-1
Date of this Report	28/11/2022

1. 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
Carry out and demonstrate the simplified species identification methods developed to interested parties				I demonstrated the methods for the rapid identification of vertebrate species with Mongolian species which were of interest to both the National University of Mongolia (NUM) and the National Forensic Institute (NFI). I also sequenced plant samples which were of interest to the NUM and the Institute of Botany with regards to conservation projects.
Generate new reference date for Mongolian species of interest				Mitochondrial genome sequences were generated for several key mammal species.
Raise awareness of nanopore sequencing within Mongolia				I gave a talk on the technology and how I have used it for rapid on-site species identification. This talk was attended by members of the NUM, the NFI and members of the National Institute of Botany and was also recorded for future dissemination. I also contributed to two undergraduate seminars at the NUM.
Providing training in the developed species ID methods				I sent detailed protocols of the species ID methods prior to my arrival. However, during my first week in the country, a large case meant that members of the NFI were unavailable for training. Instead, I was shadowed by a DNA analyst at the NFI who recorded key steps of the process.

2. Describe the three most important outcomes of your project.

Background information was supplied in the original application and is not reproduced here.

a). I generated barcode sequences (used for species identification) for both mammal and plant species. These sequences will be used to create a database with the NFI in order to identify those illegally traded species. In addition, the plant

sequences generated from native and endemic plants will be used to answer conservation questions including clarifying the phylogenetic relationships and taxonomy of certain species. A total of 31 mammal samples (with up to four barcodes each) and 96 plant barcodes were sequenced.

b). Complete mitochondrial genome sequences were generated for both the Siberian and Altai marmots, which are some of the most commonly hunted and traded species in Mongolia. This data will aid in the correct identification of these species.

c). Demonstrating my species-ID methods in Mongolia showed how the technology could be used to rapidly obtain results, which was of interest to researchers within the NUM and the NFI. Currently, researchers need to send off their samples for sequencing, at a cost of approx. ~$30 per sample, and then wait some time before obtaining their results. Using nanopore sequencing would therefore allow them to overcome this time lag before obtaining a result. The portable components of the species ID set-up, namely the MinION and the BentoLab, were of particular interest to researchers, both as relatively inexpensive pieces of equipment which could be used in the laboratory but also as pieces of equipment which would allow them to conduct research within the Mongolian countryside.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

During my time in Mongolia, I used a MinION device connected to a laptop as well as a borrowed Mk1C MinION device (MinION device coupled with a touchscreen which allows for simple device control) to run up to two experiments at a time and therefore maximise the number of samples which could be sequenced while I was there. Regular power cuts at night meant that data generated with the Mk1C device was often lost. In an attempt to minimise this issue, small-scale experiments were instead run on the Mk1C device, with larger experiments (which used a flow-cell and could run for up to 72 hours) was run on the laptop. This meant that often it was not possible to analyse the data generated on the Mk1C when the laptop was in use.

4. Describe the involvement of local communities and how they have benefitted from the project.

A total of 127 mammal sample and plant barcode sequences were generated, and this sequence data will prove an important resource in aiding in the identification of illegally harvested and traded mammals and plants. This effort will be further aided by the sequencing of the three whole mitochondrial sequences of the Altai marmot (*Marmota baibacina)*, Siberian marmot (*Marmota sibirica)*, and Daurian hedgehog (*Mesechinus dauuricus*). This is of particular significance with regards to the marmot species where approximately 30% of Mongolians use some sort of marmot product on a regular basis (Wingard et al., 2018). The plant barcode sequences obtained will additionally allow for researchers to work towards answering their own conservation based research questions.

Two meat samples of interest from the NFI were sequenced based on the suspected presence of camel (a protected species). Using the simplified species identification methods, it was possible to complete the DNA extraction, amplification, and sequencing within a day. As a different experiment was running on the laptop, it was not possible to complete the database search until the following day. Nevertheless, this demonstrated the value of the methods developed in obtaining an answer very quickly particularly compared to the standard timeframe.

5. Are there any plans to continue this work?

We intend to continue collaborating with both the NUM and the NFI and are arranging online discussions as a first step.

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

We plan to share the sequences generated in the open-access database GenBank. The mammal sequences generated will also form part of a report to the Mongolian Ministry of Education and Science. We expect that this data will also be published in a scientific journal, following discussion with Mongolian collaborators.

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

We are planning follow-up meetings with the groups in Mongolia who kindly assisted me and will suggest to them collaborative applications for funding that could support their own use of portable sequence technology in the future, now that they have experienced its potential. We will also explore the opportunities for co-publication of data.

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

In my presentations about my work, I included the Rufford Foundation logo in the slides presented. The outcome of this trip, and The Rufford Foundation's role will also be included in a report to the Genetics Society.

9. Provide a full list of all the members of your team and their role in the project.

Professor Mark Jobling, Dr Jon Wetton, and Dr Celia May at the University of Leicester were all involved in supervising my project, and with providing technical advice when needed while I was abroad.

Dr Tungalag Ulambayar, the country director of the ZSL Mongolia Office, introduced us to interested parties at the NUM and the NFI before my arrival, and also provided support while I was there.
Dr Bayarmaa Gun-Ajav, an associate professor within the Department of Biology at NUM hosted me in her laboratory during my time in Mongolia.

Dr Davaa Bazarsad at the NFI held a meeting together with the NFI director to discuss potential future collaborations to help fight ecological crime within the country. All team members were kept up to date with my work in Mongolia and were interested in the technology and methods used. The mammal and plant samples which were sequenced were provided by both the NUM and the NFI.

10. Any other comments?

We want to thank The Rufford Foundation for their support with this project and express our appreciation for the thoroughness of the review process.