Final Evaluation Report

Your Details	
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Project Title	Investigation of Kenyan cheetah subspecies and their potential involvement in the Illegal Wildlife Trade
Application ID	32264-1
Date of this Report	27 April 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
Finalize MSc			■	The MSc thesis was finalised and submitted to the university as planned. However due to Covid-19 challenges, the review process was significantly delayed. I received and addressed the reviewers' comments in March 2022, and the *viva voce* took place in May 2022, which I passed without corrections to be made. The printing of the thesis happened in June 2022 which allowed us to finalise the budget.
Selection of 50 samples from Kenya and 50 from Somaliland rescue cheetahs.			■	We identified 57 relevant Kenyan samples with an *Acinonyx jubatus raineyi* (or *jubatus* if merging of subspecies applied as per Kitchener et al., 2017) haplotype and selected 55 samples with a *A. j. soemmeringii* haplotype from Somaliland. In addition, there were four Kenyan samples which were selected as they had an *A. j. soemmeringii* haplotype.
Microsatellite-based genotyping with up to 17 markers.			■	Out of the 116 selected samples, we were able to finalise all 57 Kenyan samples with *raineyi* (or *jubatus* if merging of subspecies applied) haplotype (48 for all 17 loci, 2 for 16 loci, and 7 for 11-14 loci), all 55 samples from Somaliland (52 for 17 loci, two for 16 loci, and one for 11 loci) and three of the four Kenyan samples with a *soemmeringii* haplotype.
Assess whether individuals are related. Perform phylogenetic analysis.			■	A total of eight Kenyan individuals were of known relatedness to other individuals in the Kenyan dataset (pedigree analysis); and 18 Somaliland individuals were flagged as full sibling to another individual in the dataset (analysis with programme Colony). We therefore included 89 samples in the phylogenetic analysis: 49 Kenyan

				samples with *raineyi* (or *jubatus* if merging of subspecies applied) haplotype, 37 Somaliland samples with *soemmeringii* haplotype, and the three Kenyan samples with *soemmeringii* (vs *raineyi*) haplotype.
Write report and draft manuscript				The final report for Rufford is completed and submitted here, as per agreed extended deadline. My MSc was printed and published later than planned due to the university delays; this was the last outstanding point from this proposal and approved by the University in June 2022. The draft manuscript was not yet started as we need to verify our findings with additional samples prior to publication; so, we are waiting for another batch of samples, that we would like to use to verify our findings prior to putting together a manuscript for publication.

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

a). We established a reference dataset of 17 microsatellite loci for both Kenyan and confiscated Somaliland cheetahs. Such a dataset can assist with assigning cheetahs of unknown origin to a given region.

b). We identified/verified a total of four (out of 61) Kenyan samples with an *A. j. soemmeringii* haplotype (instead of the expected *A. j. raineyi* haplotype). This confirms the preliminary data from my MSc thesis, which first identified the presence of the *A. j. soemmeringii* haplotype in Kenya, and points to the importance of including microsatellite (or other nuclear) data from confiscated animals to verify their origin. Indeed, since mitochondrial haplotype data alone only provides information on the female lineage, detecting an individual with a" neighbouring" mitochondrial haplotype in a region does not allow to distinguish between a migrant from the neighbouring region, a subspecies hybrid, or a local individual with the remnant of an ancestral mitochondrial genotype that was shared between the regions in the past (via incomplete lineage sorting or introgression). Including nuclear data allows us to understand the ancestries of the individual relative to the population.

c). We determined that all three Kenyan samples with a *soemmeringii* haplotype for which we were able to obtain a microsatellite profile, were fully or partially (2/3rd) consistent with Kenyan (vs confiscated Somaliland) individuals (analysis with program Structure). This indicates that there are some very interesting population dynamics occurring at the Kenya-Somali border, where the two subspecies *A. j. raineyi* (or *jubatus* if merging of subspecies applied) and *A. j. soemmeringii* meet. It suggests some subspecies hybridisation (if recent) or incomplete lineage sorting (if

ancestral) between the two subspecies. The one individual that has a combined microsatellite genotype suggests that recent subspecies hybridisation occurs; however, this is based on one individual, which is of uncertain origin, therefore we cannot exclude that hybridisation event may have originated in unreported captive breeding.

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

Several unforeseen difficulties arose due to Covid-19:

1. My MSc was delayed by about a year and a half due to the issues the university was facing. We did not let this slow us down with the actual work and I have now submitted my thesis for printing.

2. We had several delays in the supply chain for laboratory items (tips, PCR plates amongst others), leading to delays of several months.

3. The most stressful event was the analyser breaking down leading to a slowdown of the data production and then a full stand still of close to half a year as the installation of the new analyser was postponed for months.

Thankfully Rufford was very understanding and allowed us to extend our report deadline.

We are glad to have overcome the challenges that we were facing and are looking forward to smooth progress in the future.

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

This multi-national project has a significant involvement of local entities. The sample collection in Kenya is performed by local NGOs (Action for Cheetahs Kenya, Mara Meru Cheetah Project) and the Kenyan Wildlife Services. The sample collection in Somaliland results from confiscations by the Ministry of Environment and Climate Change in collaboration with CCF. All CCF's projects also include capacity building. As part of the involvement in the rescue efforts CCF has held training courses for local veterinarians in Somaliland. In Namibia we host and train students and interns from Namibia and other African countries at our genetics laboratory and at other CCF departments. And of course, I, a Namibian national, am benefitting from this project specifically, as I get the opportunity to be a part of an important conservation project and am obtaining my MSc.

5. Are there any plans to continue this work?

Yes! Our results indicate that: a) based on mitochondrial haplotype alone we cannot exclude that Kenyan cheetah are involved in illegal wildlife trade if a *soemmeringii* mitochondrial haplotype is obtained; and b) subspecies hybridisation

may occur between *A. j. raineyi* (or *jubatus* if merging of subspecies applied) and *A. j. soemmeringii*. Therefore:

A. we need to genotype all confiscated individuals for both mitochondrial and microsatellite (or other nuclear) markers, to ensure that subspecies, and thus source populations, are not wrongly assigned, and

B. we need to obtain additional samples from the Kenya-Somali trans frontier cheetah populations at the distribution edge of *A. j. raineyi* (or *jubatus* if merging of subspecies applied) and *A. j. soemmeringii*, to verify whether the one subspecies hybridisation event detected by our analysis is a natural occurrence in the wild or whether it may be an exception and possibly attributable to unreported captive breeding. This is a very important systematics question as it may have implications on the way the cheetah subspecies should be managed in the future.

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

My thesis is about to be published, and as soon as I graduate, I will write a blog to share the good news. We want to include a couple of additional samples from the trans-frontier region to the north-east of Kenya to verify our results; as soon as we have those, we will write a manuscript for submission to a peer-reviewed journal.

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

This project has shown the need for additional research, both in terms of exploring population and subspecies dynamics and also in terms of whether Kenyan cheetahs may be involved in the illegal wildlife trade after all. While our data did not identify any individual with Kenyan genetic profile amongst the confiscated animals, despite having included genomic (microsatellite data), going forward, we will routinely include microsatellite genotyping for confiscated individuals in addition to mitochondrial haplotype analysis, as we have shown that mitochondrial haplotype data alone is insufficient to infer subspecies/region of origin. We will especially focus our attention on the Kenyan cheetah population to resolve the subspecies question of the Kenya-Somali trans frontier region, as it has implications for subspecies nomenclature and our international conservation strategies. I will be looking for funding opportunities to continue this important research.

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?

I posted a blog about my MSc on the CCF global website and highlighted the support of Rufford as well as including the Rufford logo (https://cheetah.org/ccf-blog/science-and-research/kenyan-cheetahs-in-namibia-genetic-analysis/). In addition, we have a link to the Rufford website and logo placement on the CCF UK website (https://cheetah.org/uk/partnerships-2/). Rufford also features in the acknowledgements of my MSc thesis. Going forward, I am planning to put together another blog when I finally graduate, which will include the highlights of the project

and of course also mention the Rufford support. And we will acknowledge Rufford on any publication that comes out of this work.

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

Myself, Hafeni Hamalwa: I performed most of the laboratory work as well as the initial steps of the analyses and wrote up my MSc thesis.

Dr Anne Schmidt-Küntzel, my MSc supervisor and head of the CCF genetics laboratory: helped lay out the project design, guided me in my progress and for analyses.

Dr Laurie Marker, Founder and Executive Director of CCF: overall coordination and access to resources.

The team in Kenya: Action for Cheetahs Kenya, the Mara Meru project, and the Kenyan Wildlife Services for sample collection and information. They also extracted the samples as only DNA received approval for export.

The team in Somaliland: CCF Somaliland, the Ministry of Environment and Rural Development for animal confiscation, rescue and care.

10. Any other comments?

My main comment would be that from my side and on behalf of the team we are very thankful to The Rufford Foundation for supporting me for the last stretch of my MSc and the continuation of the project. This was particularly valuable as the last two years were very challenging due to the Covid-19 complications. I hope that I will be able to continue this important work, ideally in collaboration with Rufford.