Final Evaluation Report

Your Details	
Full Name	Davoud Fadakar
Project Title	Conservation genetics of Chinkara (*Gazella bennettii* Sykes, 1831) in Iran
Application ID	23344-1
Grant Amount	£4,976
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Date of this Report	

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
Clarification of the taxonomic status of Iranian Chinkara in regard to the Indian Chinkara, and adequate consideration in the Red List assessments of the IUCN and via national authorities				We collected and extracted 244 samples non-invasively from all Iranian habitats of Chinkara. 202 were successfully amplified and sequenced, resulting in 174 good quality sequences that will be submitted to GenBank. We used sequences from GenBank to compare theses sequences with Chinkara sequences from Pakistan and India. Preliminary analyses show a clear separation of the two Chinkara clades, but final analyses and publication of the results is not yet finished.
Identification of inbreeding or outbreeding problems in the breeding centers and individual advice for each of these centers.				We finished genetic analysis of gazelles from all available breeding centres (see details below) and will be able to provide detailed feedback and advice on how to increase the genetic diversity (which was in most cases very low) for each of them.
Identification of suitable populations for restocking of wild populations.				We found pure and hybrid populations in Iranian breeding centres, so we can now propose suitable populations for restocking wild populations.
Identification of suitable habitat for reintroduction programs within the historical range of the species.				This species is under severe reduction in population size and many parts of its historic range need conservation in first step and then reintroducing in next steps. We still need in-depth ecological modelling analyses to detect the most suitable areas for reintroduction of Chinkara. Data collection for these analyses is completed and we expect to get the modelling analyses done and published by the end of 2019.

Increased awareness for the vulnerability of these endemic gazelles and strengthening of the will to actively work for their protection, in the Iranian people in general and the policy makers in particular.				We designed a website to increase awareness of gazelles in Iran (www.persiangazelles.org). Project findings, progress and news have been regularly shared on the website and also on the project Instagram page (https://www.instagram.com/persiangazelles). In Sistan at south-eastern Iran, we increased awareness about illegal hunting and endangered population because of drought and illegal hunting in Moke Sorkh No Hunting Area and Sheile PA. Also, training about situation of endangered population was provided for local experts and game wardens during fieldwork. I talked about the importance of conservation genetics in Iran, especially for Persian gazelles including Chinkara in the seminar hosted by "Yarane Baran" (an environmental community group as a part of student Affairs Organization in Isfahan University of Technology, www.saorg.ir) and attended by students and experts of DoE.

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

The first unforeseen difficulties arose during fieldwork. Collecting the samples was more difficult than expected, because in many areas the numbers of Chinkara were very low. Faecal samples were therefore extremely rare, so we had to spend more days in the field to complete the sample collection (78 days compared to 60 days in the application).

Extracting and amplifying DNA sequences from faecal samples was unproblematic, but from some areas we only got tissue samples from animals that died several months or even years ago and were kept in unfavourable conditions, e.g., in a shed. Extracting DNA from these samples was difficult and for some we could only sequence short segments of the cyt *b* gene.

The biggest problem was getting a visa for Germany. When I submitted the grant application I did not expect that I had to wait 6x months just to get an appointment in the embassy, and then another four month for the visa. Therefore I could only start lab work in Bonn in September 2018, and not in early summer as I had planned.

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

a) Collecting and analysing Chinkara samples from all Iranian habitats.
We found that Iranian Chinkara from throughout the country form a monophyletic group and are distinct from the Indian Chinkara. This information will be forwarded to the IUCN Antelope Specialist Group and be included in the next species revision.

With the ecological parameters sampled in the Chinkara habitat we will be able to model the ecological niche of the species and identify the best areas for species protection *in situ*, while using the genetic data to select the best captive populations to be included in re-introduction programs.

b) For the first time, our genetic results show sign of hybridization between Chinkara and Persian gazelle.
This result was unexpected, as hybridisation between these two species was never hypothesised before, although they are sister species. The hybrids are phenotypically identical with Chinkara, and we are currently sequencing nuclear markers to see if the available intron markers show evidence of *G. subgutturosa* nuclear genome in these specimens. The hybrid populations are located in central Iran, in a contact zone between the two species. For future captive breeding programs the knowledge of these hybrid populations is very important, and we strongly advise against using these animals in Chinkara conservation programmes.

c) New insight into captive breeding based on the genetic results.
We obtained genetic data from almost all Iranian breeding centers of Chinkara. Unfortunately, in Naibandan WR the whole population was killed by a caracal (*Caracal caracal*) so we could only take samples from old dead remains. They could be identified as Persian gazelle (possibly hybrids), but this might stem from a natural hybridisation event in the founder population. From Shir Ahmad Center, all individuals were translocated to Salami Center and then they were reintroduced to natural habitats in north-eastern Iran, so we were not able to get samples from this centre. From the others breeding centres we were able to collect and sequence several samples. They show that in Semi Breeding Centre of Rahmatzehi (close to Shile PA and Moke Sorkh NH) and Semi Breeding Centre of Balouch (in Gandou PA) only one haplotype is present per centre which could reflect very high levels of inbreeding. In Naiband NP, we identified five haplotypes (in 10 samples), and this captive population was founded with individuals from various other places. However, we detected hybridisation with Persian gazelle in one of the founder populations, so it is possible that hybridization is also happening in Naiband NP. We advise to collect samples from every individual in the park (N=40) to check their genetic identity before the population is used for re-introduction programs.

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

Illegal hunting for meat and using skin of Chinkara for superstitions, capturing Chinkara as a pet, and livestock pressure were important matters for extinction in Iran, especially in southeast. So we had meetings with local people during fieldwork to talk about this endangered species. We also involved local people when collecting samples and monitoring habitats.

We designed a website to engage people in conservation of Iranian gazelles and with the Instagram page "persiangazelles" we increase awareness among local people. We found some populations are near extinction because of drought in south-eastern Iran and this awareness helped a local group to collect money to prepare dry hay and water for the Chinkara population in Mokesorkh No-Hunting Area and Sheile PA. On a national scale people are now involved in projects such as Nazr Tabiat campaign to collect money for water supplies for these areas and our findings help this campaign to focus on endangered populations.

5. Are there any plans to continue this work?

Yes, we received additional funding from the German Society for Mammalian Biology (DGS) to analyse the diversity of Iranian gazelle species on a larger scale. One of the most unexpected outcomes of our work was the apparent hybridization of Chinkara and Persian gazelle in one of their contact zones. Hybridisation is an interesting topic and we would like to investigate this further, as it might shed light on speciation and diversification in other gazelle species as well.

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

We are preparing two manuscripts to be published in peer-reviewed journals in the near future, i.e. in the second half of the year. One will deal with the population genetics and population history, as well as taxonomy of Chinkara (arguing that Iranian and Indian Chinkara should be separate species). This was the main focus of our study. Another manuscript will report on the hybridisation of Chinkara and Persian gazelle in central Iran which was a surprising side outcome of this study. We also want to share our results about populations of Chinkara with the Iranian Department of Environment.

Seminars and lectures have been organised for students, biologist and conservationists, and this will be continued when I am back in Iran to complete my PhD studies.

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

We started our fieldwork before we received the grant (more than 33 days) and collected samples in 14 areas, mainly in central Iran. During this project, a major part of the grant was used for fieldwork (area is almost 1 million km^2), collecting samples

in the remaining 40 areas, which were hard to access. DNA extraction was done at Isfahan University of Technology in Iran in between fieldwork trips, so extracted DNA and tissue samples could be brought to Germany for further analysis in September 2018. Amplification and sequencing of the samples was done from September 2018 to December 2018, and resequencing (including additional DNA extraction for tissue samples) in February and March 2019 in the laboratory of the Museum Koenig in Bonn, Germany. We are still working on the analysis and publication of the results, and fortunately we could secure additional funding so I can stay in Germany for three additional months which will facilitate completing the project, i.e. preparing the final scientific manuscripts and other paper work.

In total, our fieldwork cover 54 different sites, including 11 free areas, five islands in Persian Gulf, two museums of DOE, eight no-hunting areas, six national parks, 11 protected areas, three captive breeding and two semi-captive breeding centres, and six wildlife refuges and our team was more than 107 days in the field to collect all these samples.

8. Budget: 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. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

The exchange rate is 55772 Rials for 1 GBP; however, this rate is varying between 63000 and 52400, although fluctuation happens a lot in Iran because of economic sanction against Iran.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Research equipment	301		-301	We borrowed GPS from Isfahan University of Technology. Therefore, we saved the budgeted amount for other expenses.
Transportations (fuel)	750	1387	+637	We spent more days in the field collecting samples. Also, due to USA re-sanction against Iran, our external amount of budget was decreased sharply due to extremely high inflation rates.
Lab work in Bonn, incl. additional DNA Extraction (tissue samples), Cyt b primers, Sequencing of	2625	1400	-1225	DNA extraction and cyt b amplification only worked for ~200 samples (not 250 as calculated), so we spent less. Re-sequencing of successfully extracted DNA was

Cyt b, incl. PCR purification				rarely necessary which also saved money.
DNA Extraction (in Iran)	400	400		More faecal samples were already extracted in Iran due to the extra time that I spent there before coming to Bonn.
Subsistence payment for local team (food)	900	1875	+975	see transportation (inflation + more days)
TOTAL	**4976**	**5062**	**+86**	

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

The most important thing is to take the results from our project and use them to inform decision makers in Iranian conservation so they can improve the situation of Chinkara. When I am back in Iran I will contact the Department of Environment for a personal meeting to discuss these issues. I will also contact the breeding centers and give them a summary of the results, focussing on the relevant issues from their perspective. I really hope that I can establish a network of people who stay in contact about Chinkara conservation and work together whenever any relevant decisions are to be made.

One related issue is the situation of Asiatic cheetah (*Acinonyx jubatus venaticus*) in Iran. Their numbers are extremely low, but so far, the correlation of cheetah and Chinkara populations has not been considered properly. If the situation for Chinkara can be improved, the population of cheetah will also benefit. Interestingly, there are still a number of cheetahs living in the area where we detected Chinkara (*G. bennettii*) and Persian gazelle (*G. subgutturosa*) hybrids. This area should get more scientific attention which will be beneficial for species conservation as well as for understanding hybridization in gazelles which might be an important factor in speciation within this relatively young group.

10. 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?

We used Rufford Foundation logo during presentation entitled "Phylogeny and distribution of gazelles in Iran" in 92nd Annual Meeting German Society for Mammalian Biology, 17th to 20th September 2018-Bonn. Also, we used in our website (www.persiangazelles.org). The Rufford funding will further be acknowledged in the two manuscripts that we aim to publish by the end of this year.

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

Mansoureh Malekian, PhD., was supervisor for population genetics in Iran which brought her experiences of molecular systematics and conservation genetics to the project.

Mahmoud Reza Hemami, PhD., a member of Antelope Specialist Group, helped the project from the aspect of ecology and conservation plans of Chinkara in Iran and shared data about past and present distribution of Chinkara, and also collecting samples in Qatroeih NP and Ardestan Free Area.

Eva Bärmann, PhD., who is an expert on gazelle phylogeny, was supervisor for sequencing and data analysis which was done in the Zoological Research Museum Alexander Koenig in Bonn, Germany.

Hannes Lerp, PhD., who is an expert on gazelle biogeography and taxonomy using molecular methods, was advisor for population genetics.

Hamid Reza Rezaei, PhD., was advisor for population genetics and ecology of Chinkara in Iran and brought his experiences of studying bovid taxonomy using molecular methods. Dr. Rezaei helped our fieldwork in some areas and islands in Persian Gulf.

Ali Ostowar, Master's student at IUT, helped the project on the field trips in more than 60 days and organized meetings with environmental groups (Yarane Baran at Isfahan University of Technology), students, experts, wildlife photographers, and game wardens.

Volunteers helped me in the fieldwork, collecting samples, and present points: **Farshad Behzadi** (Shiraz Province); **Davoud Pakniat** (Kavir NP, Touran NP and PA); **Mohsen Ahmadi** (analysing present points); **Shima Malakouti Khah** (Kavir NP and Abas Abad WR fieldwork); **Ali Khani** (Khorsan Province); **Mojdeh Ram** (Museum at Hormozgan DOE); **Masoumeh Mirzakhah** (fieldwork at southern Iran); **Maryam Naseri** (fieldwork at southern and northeastern Iran); **Azita Rezwani** (Kavir NP and Abas Abad WR fieldwork); **Ahmad Fadakar, Mohammad Fadakar,** and **Sajjad Fadakar** (Touran NP and PA fieldwork); **Masoud Azizi** (Kavir NP and Yakhab Free Area fieldwork); and **Amir Vafa Khosravi** (Kerman Province).

Local People helped in fieldworks: **Abdolnaser Rahmatzehi** (Mokke Sorkh FA Semi Breeding center), **Hamid Abasabadi** (collecting samples in Bandare Jask Free Area), **Mosa Jalali** (Sohran Village in Jask free area), and **Hasan Ragh** (Barkohi Village in Bandar Lengeh free area).

More than 40 game guards in whole protected areas network during fieldworks.