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	Andrea Del Pilar Borbón García
Project title	Gut microbiota of Andean Bears: A novel approach for examining health and habitat quality of captive and wild individuals
RSG reference	20909-1
Reporting period	
Amount of grant	£5000
Your email address	ad.borbon174@uniandes.edu.co
Date of this report	

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
Determine the gut microbiota composition of wild and captive Andean bears				We collected 102 samples from wild and captive bears. We found a correlation between habitat degradation and diversity in the gut microbiota. Interestingly, the wild bears showed a higher number of genes related with the degradation of plant sugars instead of animal proteins, which highlights they have a gut microbiota more adapted to plant substrates than meat. The gut microbiota composition showed that permanently captive bears have a much reduced diversity in their gut microbiota and their molecular functions related with nutrients metabolism and detoxification are reduced too. Furthermore, only captive individuals had a large set of pathogens as a core of their gut microbiota. It highlights the usefulness of this tool for the improvement of nutritional and health-related components of *ex-situ* and *in-situ* strategies for Andean bears.
Determine changes in the gut microbiota of captive bears in an enrichment process.				Due to problems in the captive facility for setting up a suitable enclosure for enrichment in wild orphan cubs, we were not able to determine the changes in the gut microbiota during this process. Instead, we evaluated the development of the gut microbiota of those individuals under a standard captivity diet in the same time period. After collecting 24 faecal samples, we determined that cubs are losing the unique diversity of wild bears' gut microbiota, and in

				consequence a functional potential for nutrients metabolism is reduced too. Those microorganisms that have been lost in wild orphan cubs were biomarkers of high nutritional health and degradation of plant polysaccharides. In fact, some symbionts exclusive for wild bears disappear in the orphan cubs after 8 months of sampling, but pathogens and obesity-related microorganisms increases their abundance which also implies that important molecular functions are depleted under a regular diet in captivity and diseases could be highly prevalent. For this reason, further considerations should be taken into account for improving health and preventing diseases in captivity by modifying and enhancing the nutritional content of diets in captivity. Furthermore, it is still important to complete a monitoring for the full period in which the gut microbiota develops in new-born wild cubs maintained in captivity and captive-born cubs, for detect which is the acquisition pathway of pathogens: if they are fully established in adult females and pass through breastfeeding or if they are acquired after contact with humans and enclosure elements. Also, a monitoring of enrichment diets for promoting development of health-related microorganisms should be carried out.
Community outreach				Talks and workshops were conducted successfully with local communities. Alliances with environmental authorities were set up, and attention raising and contributions of community leaders were achieved.
Identification of key microorganisms for health in wild Andean bears				We identified a core of beneficial microorganisms in the gut microbiota of Andean bears that were absent in captive individuals. This core serves

				as a biomarker of good nutritional health that can be tracked in captive bears to detect anomalies and susceptibility for suffering diseases. Also, we expected that future studies on wild Andean bears in degraded habitats also should show the decrease of these biomarkers within their gut microbiota.

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

We faced several problems with the sampling of Andean bear cubs in an enrichment process. Due to low budget in the captive maintenance where they were kept, the wild-caught orphan cubs were not transported to the optimal enclosure and they only received a diet scheme for regular captive individuals (for bears which are maintained in captivity all lifelong) instead of an enrichment feeding. However, since this is a common practice with wild-caught orphan bears, we sampled month-to-month the two individuals and tracked the changes in their gut microbiota, to investigate how it is developed in cubs in captivity and the possible long-term outcomes for their health. Also, because of bureaucratic issues, our sampling in one of the two captive facilities was delayed and processing for subsequent analyses was not possible within the time-scale of the project. Those samples were stored for further steps in the future phases of the project. Consequently, we conducted extra analyses for the other samples and increased the sampling effort for wild individuals. Finally, the transportation hiring was extremely expensive; for this reason we had to shorten the number of workshops, outreach, and audiovisual material production for working with adult community members.

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

a) Scientific communication and community outreach: We successfully achieved results discussion forum with local communities and environmental authority members. Our results confirmed that the gut microbiota of Andean bears is more adapted to an herbivorous diet, which is crucial for conservation of the species to create the awareness that their metabolism is not well adapted to having carnivorous diet and thus actively predating cattle as the community conceives. It is essential for focusing further strategies in the importance of the human habitat use for maintaining the vegetation needed by Andean bears for feeding. One article of science promulgation was presented with the ongoing project information and the very preliminary results at the first stages of the project in the “science section” of a local newspaper (in Spanish) (http://www.eltiempo.com/vida/ciencia/el-cuidado-del-oso-de-anteojos-48030), and in the Universidad de los Andes magazine (in Spanish) (http://uniandes.edu.co/noticias/ambiente-y-sostenibilidad/oso-andino-claves-proyecto-conservacion) to raise attention of the community. After that, we raised interest of a further in-kind contributor which is a leader within the community

of the region for enhancing upcoming steps of community-based work and sustainable strategies for Andean bears conservation, which are under planning now. Also, a manuscript is under construction for sharing with scientific community, and an article for publication in the IBA (International Bear Association for Bear Research and Management) Newsletter Summer issue.

c) We effectively assessed the effect of captivity in the gut microbiota of Andean bears. We determined that permanently captive bears harbour high levels of pathogens which are not observed in wild individuals, but also, they have a decreased nutritional-related metabolism compared with wild individuals. From this, we hypothesised that habitat degradation can lead wild individuals to a gut microbial assemblage towards the captive bears condition. It is an alarming scenario, because key bacterial groups for cellulose, hemicellulose, and other complex polysaccharides degradation could disappear from the gut microbiota of Andean bears, reducing their ability to process food in a wild environment, and increasing the probability for developing and spreading infectious diseases. The high caloric diet the captive bears are fed on paired with a reduced gut microbial diversity could explain the high prevalence of obesity in captivity, and other captivity common diseases could also be related with this microbial condition. Furthermore, the wild Andean bears exhibit a unique gut microbiota, which contains several beneficial microorganisms, only found within this group and absent in captive bears. Also, the results about the gut microbiota development and colonisation in wild-caught orphan cubs under a regular diet scheme in captivity were striking. This colonisation process is crucial in the establishment of beneficial bacteria and the proper immune system development. We observed that wild bear cubs with a regular captive diet (rich in simple sugars and animal protein) are losing key bacterial groups -totally absent in permanently captive bears- specialised in the degradation of complex polysaccharides existing in a wild diet and their gut microbiota is becoming more similar to permanently captive bears. Both results were presented to the co-chair expert team of captive bears of the Bear Specialist Group of the IUCN in an effort to convince them to provide stronger recommendations to captive facilities and the design of effective diets according to the nutritional and metabolic needs of these animals in captivity.

b) Presentation of the microbiome results at the 25th International Conference on Bear Research and Management held in November 12-17th 2017 in Quito, Ecuador. Microbiome results confirmed that feeding in captivity does not provide the enough resource diversity to promote a high functional and taxonomic richness in the gut microbiota, leading to the loss of key microorganisms for the digestive and nutritional proficiency of captive bears and enhancing abundance of pathogens. We highlighted the importance of this scenario for the possible reintroduction of those individuals in the future, which have lost the molecular functions to degrade fibre and polysaccharides that can be found in the diet of wild individuals. Also, as a powerful for monitoring health (e.g. pathogens load, metabolic and food processing potential). These results were discussed with the co-chair of the captive bear's expert team for the Bear Specialist Group (BSG) of the IBA, allowing us to expand the study to captive facilities in Europe for studying the efficiency of several

diet schemes, and in the middle future being able to provide an optimal diet scheme for captive bears.

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

The water resource from paramo depends on long-term maintenance of Andean bear populations, but the main human-bear conflict arises due to habitat fragmentation by human settlements and illegal retaliatory hunting of bears because of cattle consumption. In consequence, local communities can be benefited from the coexistence with these Andean bear populations. Activities with local communities and environmental authorities were conducted -including talks and field trips- in which we discussed our results to create awareness about the adaptations of the gut microbiota of Andean bears for having a mostly plant-based diet and demonstrate that predation of cattle primarily occurs under resources reduction and habitat fragmentation under ranching and farming conditions. Also, that given Andean bears gut microbiota is not completely specialised for herbivory, these bears depend on large quantities of plants per day, which highlights the negative outcomes of habitat reduction. The participants of discussions also included specialists and academic representatives. Also, the development of audiovisual material for children was a crucial way to engage the younger members of communities, now a storybook is under editing and printing process.

5. Are there any plans to continue this work?

Yes. Our long-term goal is to convince captive facilities to design adequate diet schemes for Andean bears, especially depending on the future outcome for individuals: permanent captivity or reintroduction. It is crucial because that determines the nutritional health of bears. Furthermore, diet schemes are very variable between facilities. For this reason, it is important to conduct further comparisons among captive facilities to determine its effect on Andean bears' health, gut microbiota composition and effectiveness. To date, we have established a network of new captive facilities harbouring Andean bears for future screening and also we have designed a proposal of a pilot diet for monitoring gut microbial changes in captivity. It is one of our next steps which will allow us to conclude our project goals and broaden the scope of the project. Furthermore, we are convinced this tool could be cost-effective for diagnostic of health in wild populations. This methodology can also provide additional and a high-resolution evidence of the effect of habitat degradation on Andean bears and strengthen current evidence for management plans. For this, we wanted to expand our sampling areas covering degraded regions were foods are scarce for bears. We are laying out a proposal for the Andean Bear Specialist group for further contribution with sampling along the Andean bear's distribution in Colombia.

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

Firstly, we plan to focus our sharing strategies on community at large, and to create participatory channels of Andean bear research. For this reason, some publications

were released in local newspaper and magazines highlighting the ecological importance of Andean bears, the work we are advancing on, and our preliminary results so far. Furthermore, we are organising all the information to create an uploaded webpage of the project, with a "how to get involved" tab for students, researchers, and community members interested in to participate on the project.

After printing the finished storybook for local children, we'll contact educators on the region to find a way to include this book on school practices, as a supplementary material for children. So far, this last step is some premature but is ongoing. We are preparing manuscripts of the research to be published in peer-reviewed journals. Also, we are preparing a divulgation manuscript to be published in the BA Newsletter Summer issue.

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

The Rufford Small Grant was used over the period of February and January 2018. Initially was planned one year and a half of project length. Due to higher expenses in fieldwork, we had to shorten the period planned for working with local communities.

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
Transportation Bogota-Guasca and Bogota-Mesitas (twenty-four fieldtrips for two people)	450	967	517	This value describes the payment for transportation for all the fieldtrips. Differences in the values were due to increased rates for hiring transportation (SUV with fuel and tolls). **RSG spent = £900**
a) Liquid nitrogen tank	3737	400	27	This value describes the cost of equipment and supplies for sample collection. The difference in the values were due to increased price and importing tax fees.
b) Supplies and materials for sampling	100	105	5,3	**RSG spent = £691,13**
c) GPS	174	186	12	

Meals	405	326	-79	This value describes the food expenses during all fieldtrips. The difference arises because we saved money preparing meals in the field. **RSG spent = £325,71**
DNA extraction	0	375	375	This value describes the expenses for DNA extraction kit and reagents. The differences were due the DNA extraction kit was contaminated, so we had tu buy new kit and reagents. **RSG spent = £0**
Primers manufacturing and importation	0	83	83	This value describes the expenses for manufacturing and importing sequencing and barcoded primers. The differences were due to we performed a previous amplification for improving further results. So, we had to order new primers. **RSG spent = £0**
Amplicon Library Preparation	989	1215	226	This value describes the expenses for reagents and procedures for preparation of amplicon libraries. The differences were due to additional fees for needed reagents that were not considered for the initial budget and changes in the change rate of USD to COP (applied for importing some reagents) **RSG spent = £0**
Amplicon libraries sequencing	987	1105	118	This value describes the expenses for amplicon libraries sequencing using Illumina MiSeq PE 250bp. The differences were due to an increase in the cost for sequencing compared with the time when the quotation was received and the change rate of USD to COP when the libraries were sequenced. **RSG spent = £0**
Metagenomic libraries. Illumina gDNA Library Preparation (with PCR)	1425	1524	99	This value describes the expenses for metagenomic libraries preparation and sequencing. The differences were due to an increase in the cost for sequencing compared with the time when the quotation was received and the change rate of USD to COP when the libraries were sequenced. **RSG spent = £0**

Metagenomic libraries. Illumina HiSeq PE 125bp sequencing lane	1975	2121	146	This value describes the expenses for sequencing of metagenomic libraries. The differences were due to an increase in the cost for sequencing compared with the time when the quotation was received and the change rate of USD to COP when the libraries were sequenced. **RSG spent = £1025**
Divulgation material preparation for local communities	1000	610	-390	This value describes the expenses for divulgation material for working with communities. The differences are due to increased expenses related to the illustration and editing of a storybook for children (£500 budgeted), which is under production now. However, we had to omit the divulgation material for adults (£500 budgeted), since the transportation costs reduced considerably the budget for this item. **RSG spent = £610**
Workshops organization	1800	1460	-340	This value describes the expenses for workshops organization. Differences in the value were because we could save some money with equipment rental, and we had to shorten them because an increased cost for transportation. **RSG spent = £1460,2**
Total	**9678**	**10476**	**798**	**RSG spent = £5012.04**

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

Currently, the next most important step is to design and implement an effective diet scheme for captive bears. For this, we consider that comparing captive facilities which provide a diet with high fibre content would be useful to determine its effects on the crucial beneficial microorganisms in the gut of Andean bears, and a further enrichment of those microorganisms via food. The other important step, is to set up a complete sampling design which allow us to examine the health of Andean bears in areas with higher human impact and fragmentation, with food scarcity. In this way, we can detect the degradation effects of the gut microbiome of Andean bears and find an effective way to recover them in wild individuals.

We found several clinical pathogens in captive individuals, so we need to establish a method for rapid identification of this pathogens in both captive and wild individuals, because its presence can have a negative impact on the animals' health and can be related with the high prevalence of some diseases in captivity.

Our results demonstrated the high vulnerability of Andean bears to habitat degradation: they strongly depend on highly fibrous diet to keep beneficial symbionts in the gastrointestinal tract, nutritional health, and an adequate immune system development. Based on this, it is necessary to provide and support local authorities with this extra evidence of the plausible negative impacts on bears' health as a result of habitat degradation, to promote stronger regulations of land use and protection of Andean bear habitats.

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?

We used The Rufford Foundation logo in the material produced under this project. During the course of this work, the Rufford Foundation has been mentioned in the presentations during the 25th International Conference on Bear Research and Management held in Quito, Ecuador in November 2017, and during the 3rd International Forum on Computational Biology held in Bogota in December 2017.

The Rufford Foundation logo will also be used in the webpage of the project to broaden the scope of publicity for The Rufford Foundation. Also, the RSGF will be mentioned in the manuscripts we are preparing for peer-reviewed journal and IBA Newsletter Summer issue.

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

Andrea Borbon was leading the project and expanding the future alliances with local authorities, regional, and international experts. She performed the sampling of wild animals and some captive bears, the laboratory work for analyzing the gut microbiota composition along samples, and the starting work with communities and preliminar design of the storybook for children.

Orlando Feliciano performed the sampling of captive bears from Fundacion Bioandina, both the permanently captive and the wild-caught orphan cubs. He provided important veterinary information and considerations about the nutritional care of Andean bears. His strong link with the community was crucial to create communication channels and organize the workshops.

María José Pitta is a professional on food security and community-based work. She kindly accepted joining the team after the set-up of the project, and her participation in the project allowed us to implement social sciences methods for getting a closer relationship with the community, spreading our discoveries and engaging them. Finally, María José helped substantially to lay out strategies for upcoming promotion of coexistence and sustainable development in the region. Also, she played a crucial role designing effective educational tools for children through scientific communication which are still under construction.

12. Any other comments?

Most of the work achieved in this project and the most important outcomes of this project related with the conservation of Andean bears was only achieved by The Rufford Small Grant funding. For this reason, we want to thank the strong and invaluable support of the board committee. You have broadened the scope of this project and had let us the opportunity to connect biological research with conservation practices. For us, your financial support was essential for empowering our role as young Latin American women doing scientific research coupled with conservation. This project was conceived with a strong Microbiology basis and since then, little attention received and a non-significant importance was attributed to it related to its conservation implications. You allowed us to prove that microbiology can make important contributions for conservation. You made possible to us to pursuit our career goals and make efforts to contribute for the conservation of endangered species possible.

Claw-marks of Andean bears in the trees seen during fieldtrips with the community. © Andrea Borbon

Workshops with local communities. The participatory tools were applied, for community to identify the main characteristics of their territory, in terms of human and natural resources. © Andrea Borbon

Facilitators working with communities to lay out proposals for coexistence with Andean bears. © Maria Jose Pitta

Essential guidelines to ensure participation and correctly show up ideas. ©Andrea Borbon


Fresh fecal samples of an Andean bear, the small size suggested a possible juvenile origin. ©Andrea Borbon


Left: Kid working on ideas for preserving the biodiversity that surrounds his community. © Maria Jose Pitta. Right: Collecting fresh fecal samples of Andean bears. ©David Garcia

Obtaining samples from fresh faeces of Andean bears. ©David Garcia

Consumed Puya sp. by Andean bears, the foliar base is extracted and eaten. Feces are commonly found close to consumed plants. © Andrea Borbon

Field trips achieved successfully with some community members and environmental authorities, checking the used paths by Andean bears. © David Garcia

Left: Working with environmental authorities for finding and collecting Andean bears' fecal samples and train them with the complete process of samples preservation. © David Garcia. Right: Training community member with the collecting process, which includes a detailed register of geographic location and metadata from each sample: color, texture, moisture, freshness, etc. © Alfonso Espinosa

Fieldtrips with experts from academia and community leaders to the Andean bears' habitat, exposing them the bears' mostly used trees for resting and looking for claw marks, hair and stool. © Alfonso Espinosa

Nine-month-old-cubs (left: female and right: male) under a standard diet for captive bears in Fundacion Bioandina, Mesitas – Cundinamarca. © Andrea Borbon


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