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	Nemanja Gojković
Project title	Conservation and population status evaluation of the strictly protected Great Capricorn Beetle (*Cerambyx cerdo* L.) on Fruška Gora Mountain (Serbia)
RSG reference	20360-1
Reporting period	August 2016 – August 2018
Amount of grant	£4980
Your email address	nemanja.gojkovic@dbe.uns.ac.rs
Date of this report	21.8.2018.

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
Contribution to the knowledge about the biology of *Cerambyx cerdo*				Based on the sampling sites we surveyed, *C. cerdo* specimens were registered exclusively on sun-exposed *Quercus* trees. Furthermore, the adults were found only on trees where larval galleries were registered, while nearby suitable host trees remained uninfected. It is possible, therefore, that one of the strategies the species developed is to exploit the already infested trees until their eventual decline and then to infest the next suite of hosts. The approximate age of the host trees ranged from 82 to 163 years, with 122 years being an overall average value. Regarding the period of the species' activity, it appears that the traditional May – September period was shortened as we registered adults starting from June, while dead specimens were found in the vicinity of the trees starting from the early August. In terms of the daily activity, adults were registered starting from roughly 7 p.m. All the above inferences should be further confirmed by increasing the scope of the surveyed localities, as well as sampling the same localities for several years.
Non-lethal trapping method				During the first year of sampling *C. cerdo* individuals, the traps which we designed failed to catch any specimens. On the other hand, during the 2nd year of field work activities, we made a few traps based on the design proposed by Redolfi De Zan et al., 2017 (Nature Conservation) and these proved to be successful. These traps were furthermore cheaper to construct and easier to install, therefore we believe that they represent suitable candidates for the establishment of the future monitoring of *C. cerdo* in Serbia.

Non-invasive genotypization				The initial idea of non-invasive genotypisation was unsuccessful since insufficient amount of biological material was retrieved from the faeces of *C. cerdo* individuals. Therefore, our preliminary genetic analyses were based on the DNA extracted from one half of the femur of the middle legs of the sampled individuals. However, as the seasonal peak of the species declined, we collected dead specimens and successfully isolated DNA from their middle leg femurs. Although such an approach was not initially considered, it represents one of the approaches which do no damage to the vulnerable species since the extraction is performed on already dead individuals. These successful DNA extractions were performed both on fresh and dried individuals and therefore could be used to non-invasively genetically assess the populations of insects at the risk of extinction.
Genetic analyses				We tested the performance of universal primers to amplify the 3' end of mitochondrial cytochrome c oxidase subunit I (*3' COI* mtDNA), as well as internally transcribed spacer 2 of ribosomal DNA (*ITS2* rDNA). During the screening, we successfully obtained *3' COI* mtDNA and *ITS2* rDNA sequences for *C. cerdo*, as well as for *C. scopolii*. To our knowledge, *3' COI* mtDNA sequences of *C. scopolii*, as well as *ITS2* rDNA sequences in the case of both species are the first ones ever obtained. Furthermore, the comparison of Serbian *3' COI* mtDNA sequences with those retrieved from studies carried out in Poland (Iwona et al., 2018; Journal of Insect Conservation) and Czech Republic (Drag and Cizek, 2015; Conservation Genetics) showed that Fruška Gora Mountain boasts a considerable *C. cerdo* genetic diversity, where five haplotypes were retrieved from 10 specimens which were assessed. Additionally, four of these haplotypes were private for Serbia, while the most

				common one was shared with the majority of the specimens sampled in Poland. These results are important for future consideration of evolutionarily significant units within *C. cerdo* species, as more stable populations can be used to supplement compatible populations which are more endangered.
Assessment of Fruška Gora Mountain (FGM) habitat conditions				The assessment of FGM living conditions for *C. cerdo* confirmed that many *Quercus* trees belong to the coppice form. Since *C. cerdo* is primarily tied to old, veteran trees, such trees do not represent its primary hosts, though it is too early to exclude the possibility of the beetles exploiting younger trees as well. Several oak trees which were surveyed belonged to a class of suitable hosts, but lacked the exposure to sunlight. Such an issue can be solved by incorporating management strategies such as creating openings within forests to remove shadows cast from trees and shrubs growing around old oaks (Iwona et al., 2018; Journal of Insect Conservation), therefore it is possible to increase the number of suitable host trees of *C. cerdo* on FGM. On the other hand, the invasive relative *C. scopolii* was frequently registered on the cut tree logs. These logs are usually removed from FGM territory and therefore they act as ecological traps for this species. However, other saproxylic beetles are also affected by this practice, meaning that the removal of cut logs should be done cautiously, particularly because preliminary genetic analyses of *C. scopolii* indicate extensive genetic variability and intact evolutionary potential, meaning that this method is not effective enough to suppress this species.
Promotional activities and links				During the course of the project, several promotional activities were carried out. We firstly presented the project at the Faculty of Natural Sciences and Mathematics in Banja Luka (Bosnia and Herzegovina) and then to Ecology students attending the course

				Conservation Biology at the Faculty of Sciences in Novi Sad. We also included several Biology students in the field work (Aleksandar Beatović, Anka Tutulugdžija, Barbara Volarov), but also students of Architecture (Uroš Divac), Economics (Danijela Vukašinović, Darko Obrovački, Stefan Stojšić), Mathematics (Danijel Živanov), Pedagogy (Sanja Branković), Pharmacy and Medicine (Jelena Basta, Vanja Gojković), and Psychology (Ivana Radaković, Sanja Gojković). We made a website (http://cerambyxcerdo.com/) devoted to the project and will keep it updated as the course of investigation further continues. Next, we made brochures summarising the project scope and we will disseminate them among the local people in order to raise the awareness of the issue of saproxylic beetle conservation. During our field work, we encountered many people who got interested in our activities. Since FGM is frequently visited by the local people, the interest they developed in saproxylic beetle protection is very valuable. We also established several links, the most prominent ones being the Institute for Nature Conservation of Vojvodina Province and National Park „Fruška Gora“, both of which helped us with obtaining the sampling permit and the choise of suitable localities for *C. cerdo* surveys. Finally, the results of our project will be presented on the upcoming Rufford conference in Serbia and this opportunity will also be used to meet other local Rufford Small Grant recipients and strenghten the conservation network in Serbia.

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

The greatest difficulty we faced was specimen sampling during the 1st year as we relied on the traps we designed. Having not caught a single individual, we were unable to perform the majority of the project goals. However, upon switching to

mainly manual sample collection and the use of a different trap type, we managed to complete our project tasks during the second year.

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

I) The contribution to the knowledge about *Cerambyx cerdo* biology:

The data concerning site-specific behavioural traits are crucial for implementing adequate local conservation measures. Namely, apart from confirming that *C. cerdo* is primarily tied to sun-exposed, veteran oak trees, we also registered that it only laid eggs on the trees which already have had larval galleries on them. Such behaviour is not necessarily characteristic of other localities (discussed below) and could represent a local adaptation, although it should be more extensively confirmed through future endeavours. Also, registering a slightly shortened activity period from June to early August should be taken into consideration when devising conservation measures since this is a period when the beetles should be least disturbed. Additionally, the installation and removal of ecological traps (as illustrated by the cut logs, but also applicable to the removal of decaying oak trees) could be most harmful in this period as the eggs which beetles laid would be wasted.

II) Minimizing damage done to endangered animals during monitoring:

During the second year of sampling, we managed to catch *C. cerdo* individuals both by hand, but also in non-lethal traps. Such a feat is relevant for monitoring the peak of activity, number of individuals and the range of the species, without causing any damage to the endangered species. Furthermore, we showed that it is possible to successfully amplify and sequence DNA isolated from the dead specimens, therefore demonstrating that genetic monitoring of the species could be established without causing any disturbance to the population size. Additionally, collecting dead individuals allows for the sampling to be extended beyond the period of activity, therefore increasing the number of localities which could be surveyed.

III) The genetic richness of FGM *C. cerdo* population:

The preliminary genetic analyses indicated that FGM population is genetically diverse, meaning that it could be used to supplement more endangered populations through translocations. This feat is particularly important in the context of promoting translocations as a conservation tool for taxa where it is traditionally underrepresented, yet logistically simple. Furthermore, the samples we obtained can be used for future analysis using microsatellite loci, which would enable studying fine-scale structuring of *C. cerdo* populations, identification of evolutionarily significant units and inferences about the population effective size and demography.

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

The local students participated in the field work and collection of specimens, and we also discussed the problems related to the saproxylic beetle protection while in the field. All of them enjoyed spending time in nature and inquired about the other tasks we performed during the project. Additionally, FGM is regularly visited by the local laypeople. Upon seeing us survey the host trees, they would frequently approach us and ask about what we were doing. All the people responded positively to our work and said they were glad that more attention is being given to species and nature conservation in Serbia.

5. Are there any plans to continue this work?

We would like to continue studying *C. cerdo* on FGM, but we would also like to include new sites in our future research. Namely, during the course of the project, we visited localities of Đerdap Gorge, and registered that *C. cerdo* specimens behaved differently than the population on FGM. These beetles attacked both young and veteran oak trees, and exploited many trees simultaneously. Therefore, in order to differentiate between species-specific and site-specific traits, we would like to expand our research to this locality and compare the two populations to see which one is more endangered. We would also like to establish non-invasive monitoring of *C. cerdo* by increasing the number of traps. Finally, using genetic analyses on a bigger sample would allow us to get more precise measurements of genetic diversity and therefore the evolutionary potential of this species.

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

The results of our work will be shared on our website, during the upcoming Rufford conference in Serbia and during lectures with biology and ecology students of the University of Novi Sad. At the end of each calendar year, we also submit our results to National Park "Fruška Gora", the Institute for Nature Conservation of Vojvodina Province and The Ministry of Environmental Protection of the Republic of Serbia.

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 grant was used from August 2016 until August 2018. We initially intended to finish the project by October 2017, but due to the sampling difficulties, the project was completed in August 2018.

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. The local exchange rate is: 1 GBP = 131.32 RSD.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Insect trap material	1200	1250	-50	Apart from the originally planned traps, we made a new set of traps during the second year.
Nikon D3300 camera package	800	800	0	
GPS	150	150	0	
Fuel	850	1350	-500	We used a larger portion of budget for fuel than planned in order to travel to FGM during the second season.
Plastic containers for insect rearing	30	30	0	Instead for rearing the beetles, these containers were used for catching and storing the beetles.
Filter paper	30	0	+30	Since we did not manage to obtain enough beetle faeces to isolate DNA from it, we co-opted this part of the budget for traps and travelling during the second year.
Whatman FTA cards	200	0	+200	Since we did not manage to obtain enough beetle faeces to isolate DNA from it, we co-opted this part of the budget for traps and travelling during the second year.
Laboratory expendables	100	100	0	
NucleoSpin Tissue, DNA isolation kit	300	300	0	
Illustra PureTaq Ready-To-Go PCR Beads	350	350	0	
COI gene primers	30	30	0	
Exonuclease I, FastAP Thermosensitive Alkaline Phosphatase	40	40	0	
Sequencing	350	180	+170	Instead of sequencing two plates, we opted for one.
Brochures	150	110	+40	We managed to arrange a lower price.
Website	400	320	+80	We managed to arrange a lower price.
Total	4980	5010	-30	

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

The most important next steps certainly include increasing the scope of both behavioural and genetic monitoring. Including additional sites in the analysis and studying the degree of differentiation among the populations would allow us to recognise discrete management units and nominate the conservation priorities among them. Furthermore, additional genetic assessment of the species would single out more stable populations which could be used to rescue other populations facing decline. Finally, the incorporation of the analyses of ecological factors of different habitats would give more reliable estimates about the compatibility between different populations for translocations, as well as more robust guidelines for *in situ* species conservation by altering the existing conditions to more favourable ones.

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

We used The Rufford Foundation logo during our presentations, on the website, as well as on the brochures. We also encouraged the students who attended the project presentations to apply for Rufford Small Grant themselves and contribute to the development of conservation biology in our region.

11. Any other comments?

We would like to thank the referees: Dr Borislav Banjac, Dr Smiljana Paraš and Dr Marija Savić Veselinović, for supporting the project from its start. Also, we are grateful to Lazar Kesić, a colleague who helped us with the age estimation of the host trees. Finally, our greatest thanks go to The Rufford Foundation for both supporting the project and allowing us to proceed with the activities when we faced the field work issues. This project represented a great learning opportunity and produced valuable data. Therefore, as researchers at the beginning of their careers, we are truly grateful for being given a chance to conduct a project of our own and to be welcomed to the conservation community.

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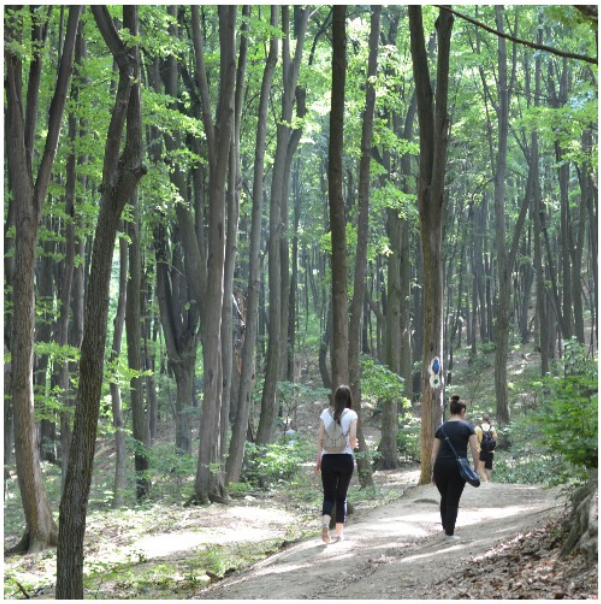

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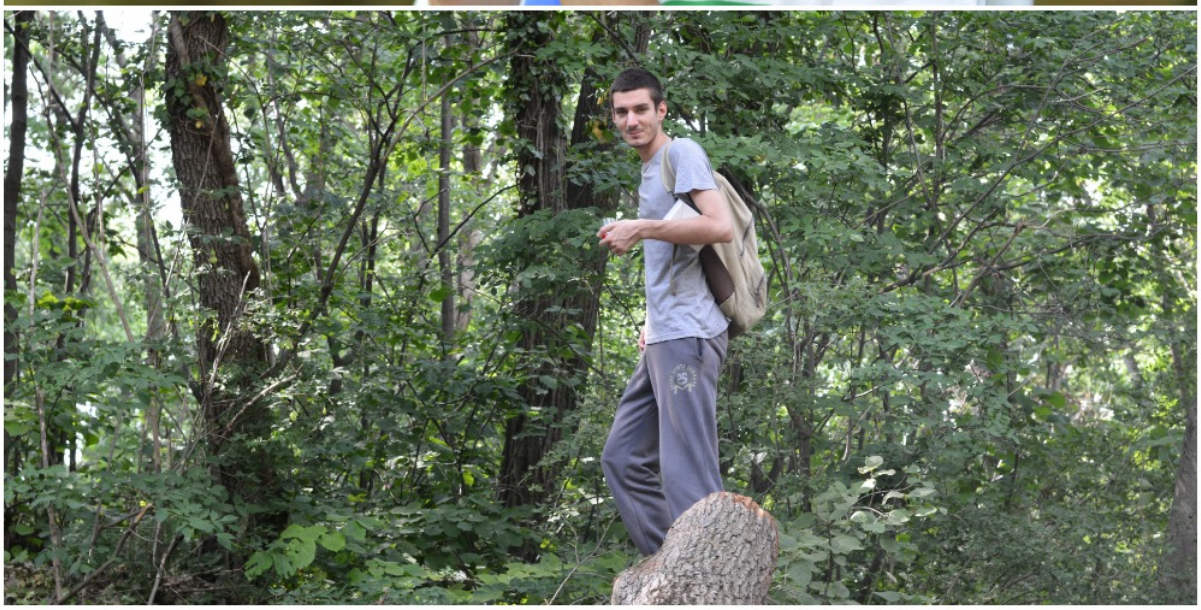

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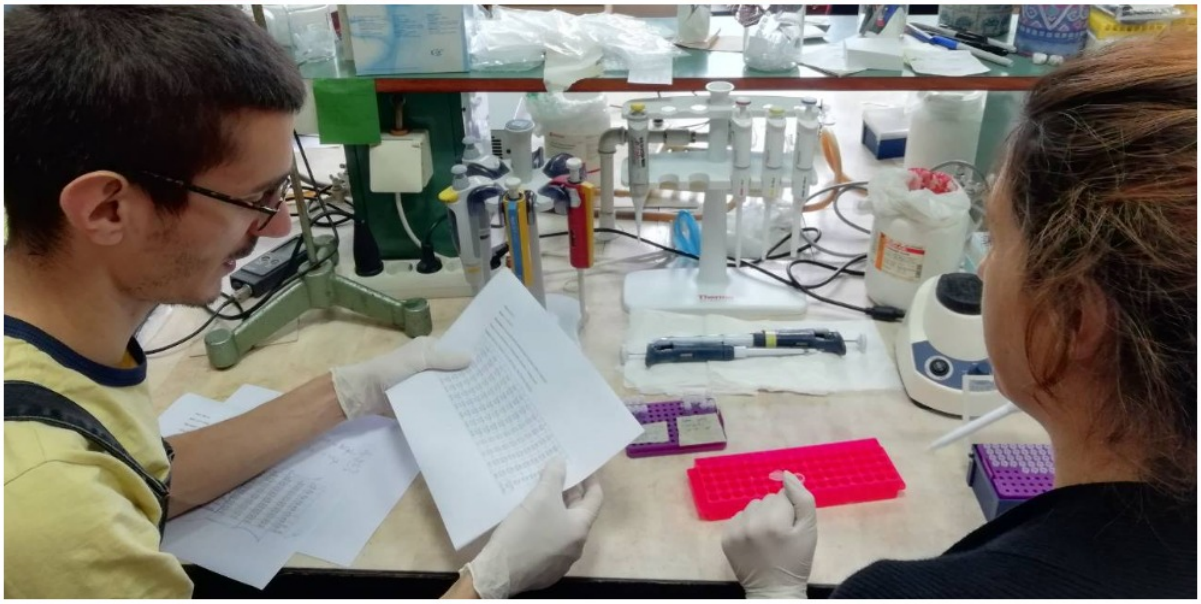


PROJECTS IN COUNTRIES
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Successful reintroduction of an endangered veteran tree specialist: conservation and genetics of the Great Capricorn beetle (Cerambyx cerdo)