Final Project Evaluation Report

Your Details	
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Project Title	Winter huddling and kinship in the marsupial *Dromiciops gliroides*
Application ID	25578-1
Grant Amount	£4,995
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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
To evaluate genetic relatedness in winter huddling groups			■	We analysed kinship between individuals nesting together during winter by examining the genetic structure of the huddling groups in *Dromiciops gliroides* populations from Chiloé Island using SNPs markers instead of microsatellites as planned. Our results showed individuals observed in the same huddling groups were not more closely related to each other than to other individuals. These results suggest that juveniles tend to form huddling groups with adult individuals independent if they are genetically related or not. Thus, winter huddling may be driven by other factors rather than family bonds such as maintenance of body temperature, energy savings or even reproduction.
To evaluate mating system in *Dromiciops*: multi-paternity, mate preferences and male spatial movements		■		Animals were trapped using custom-made single-doored Tomahawk-like live traps during the beginning (November 2018) and the end of the breeding season (February 2019). A tissue sample from every female and from each of their pouch young were taken when the young was still in the pouch (November to December). Samples will be analysed for kinship in the coming months using SNPs markers. In the meantime, we have analysed a litter of three individuals of a female captured in previous fieldwork. The results showed two pouch young shared the same father whereas one of the young had a different father. These results suggest *Dromiciops* may have a

				multi-paternity mating system. However, we need to increase our sample number to corroborate this finding. Additionally, we collected tissue samples from all the males sharing a nest with a female during winter torpor (August 2018) which will be used to analyse paternity to evaluate if huddling facilitates access to males and identify female mate preferences.
To evaluate the benefits of huddling				We evaluated if thermoregulation benefits are most likely to influence the formation of huddling groups between juvenile and adult individuals during winter by measuring their core temperatures using a thermocouple. We used a thermocouple instead of infrared thermography (IRT) because it gives us a more exact measurement of the core temperature of the animals. Our results are very promising showing juvenile individuals huddling in groups have lower body temperature than those nesting alone suggesting huddling may increase deepness and duration of torpor and affect energy savings. However, more studies need to be done to corroborate our findings such as measuring of energy expenditure and monitoring the temperature inside and outside the nests (data-loggers).
Outreach in the importance of preservation of tree hollows				We performed the two workshops as planned. One with the scientific community of PUCV in Valparaiso where we shared our preliminary results. The second workshop was focused in children instead of adults with the participation of secondary school students from Colegio Bicentenario Domingo Santa María, Puerto Montt. Also, the results generated by this project have been used in educational programmes run by Senda Darwin

				Biological Station (SDBS) and presented in three scientific conferences.

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

Minor difficulties were present in the development of this project. First, the proposed outcomes 'evaluation of mating systems and benefits of huddling' could not be fully accomplished in the 1-year timetable due to low sample number, mainly because we had problems with the renewal of the Australian import permit which was a few months delayed. We have the full permit now and the samples collected in August, November and February fieldwork are ready to be processed. Secondly, we did not use data loggers to measure nest temperature as planned because we did not have enough time to purchase the equipment before the winter fieldwork (August 2018). Despite the complications, we have interesting results of core temperature.

Finally, we could not do the outreaching activities with adults because we had to suspend it due to bad weather and then people were not available (holidays or not enough free time). However, we were able to fully achieve the workshop with local schools and involve them in this project as much as was possible. We also conducted two talks at The Pontifical Catholic University of Valparaiso (PUCV) with pre-graduate students.

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

1. We have increased the knowledge of huddling behaviour and the way in which it may influences the biological fitness of the species (Figure 1). Our results indicated that juveniles huddle with adult individuals independent if they are genetically related and may be using huddling, complementary to torpor, as a strategy to minimise heat loss and thereby lower their energy expenditure, possibly allowing them to reallocate the saved energy to other functions such as growth or reproduction. Thus, natural hollows with enough space to hold a few individuals may be of extreme importance for the survival of this species.

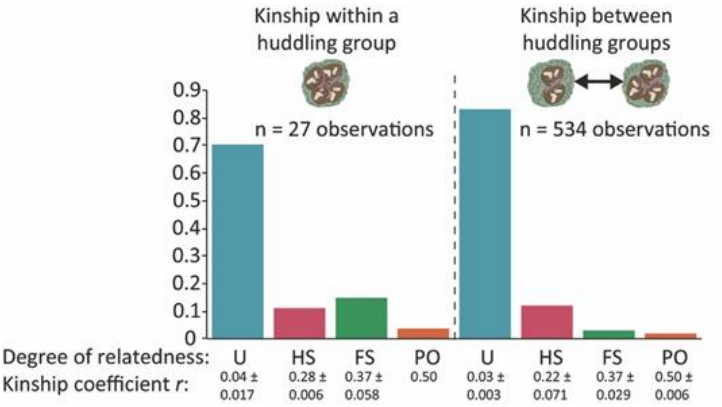

Figure 1-A. Individuals observed in the same huddling groups were not more closely related to each other than to other individuals. U: unrelated, HS: half siblings, FS: full siblings, PO: parent-offspring

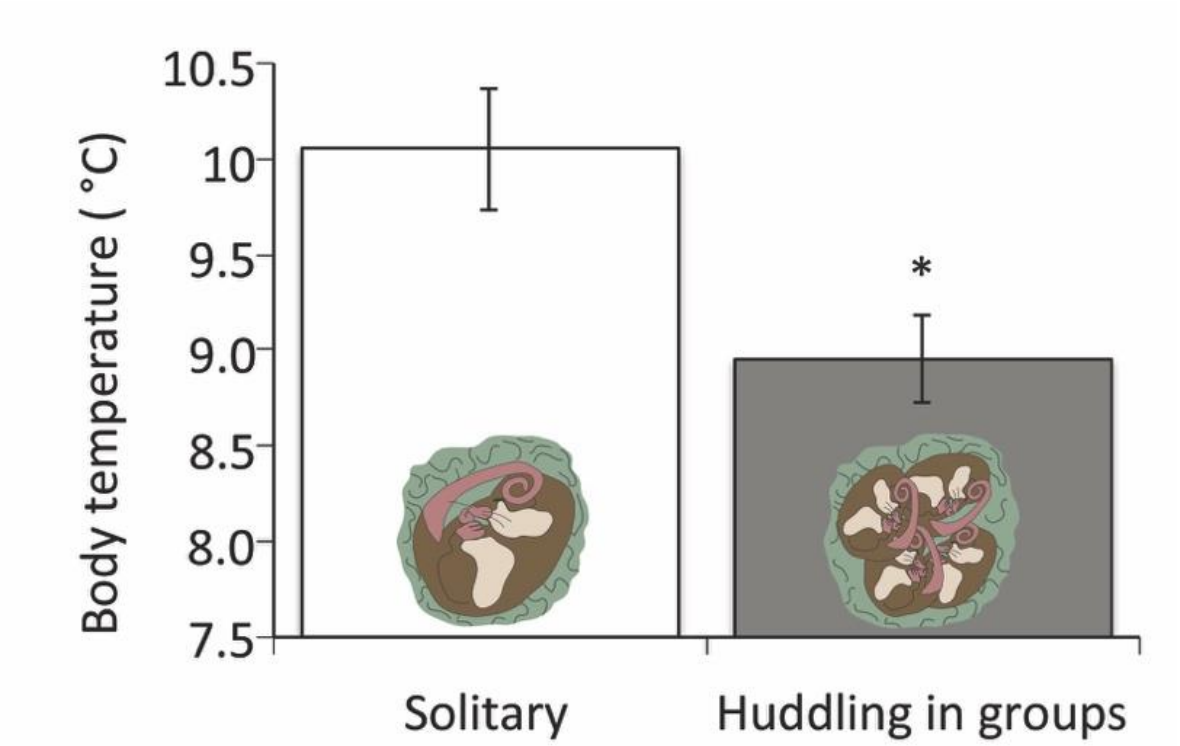

Figure 1-B. Individuals huddling in groups have lower body temperature than those nesting alone suggesting huddling may increase deepness and duration of torpor, affecting energy savings.

2. We have conducted different outreach activities (Figure 2) to increase understanding about *Dromiciops* nesting behaviour and the significance of the use of tree hollows to help in the conservation of these nesting sites. This is of extreme importance because *Dromiciops* rely on tree hollows for shelter and possible breeding, however, land clearing has reduced their availability over the years. Sharing our results with the scientific community allowed us to meet new collaborators and to create a multidisciplinary collaboration group involving top institutions in the field of marsupial research. We also have strengthened our alliance network between Chile and Australia to promote marsupial research in Chile in order to increase the basic knowledge of *Dromiciops gliroides* for future conservation strategies.

3. We have collected enough samples to evaluate if Dromiciops has a multi-paternity mating system. This information is important for the conservation of any species because solid knowledge of reproductive traits is required before developing a successful captivity breeding programme.

Figure 2. Right, poster presentation in the Victorian Biodiversity Conference, Melbourne 2019. Left, workshop with secondary school students from Colegio Bicentenario Domingo Santa María, Puerto Montt, 2018.

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

We performed workshops with local schools where we showed the children the habitat of Monito del Monte, the importance of the conservation of natural hollows and how you can get involve in protecting this species and many others that live in the temperate rainforest of the Chiloé Island. We help them to identify possible nesting sites and the importance of the use of the nest boxes and the impacts of habitat enrichment in this species. The students also had the chance to meet the Monito del Monte, a very elusive species and difficult to see because is almost impossible to find it in the wild and there are no individuals in captivity (Figure 3).

5. Are there any plans to continue this work?

Yes, we had the chance to meet people involved in tourism during our fieldwork. They were very interested in our project so, in terms of outreach activities, our next step will be conducted workshops and training programmes not only for local schools but with people involved in the local tourism industry and governmental agencies. We would like to implement an educational programme with the collaboration of Senda Darwin Biological Station (SDBS) focus on the importance of protecting natural tree hollows as nesting sites, but also how to incorporate the use of nest boxes as a tool for habitat enrichment. At the moment a common practice in the Chiloé Island is to use dead hollow-bearing trees as firewood source because the local community thinks they have no ecological value. So If we continue our work close hand with the local community identifying huddling as a trait that can promote the survival of *Dromiciops* individuals, this may help us to educate the community in regards to the importance of preserving nesting sites and, at the same time, we may use this example as means to show the significance of habitat conservation in a species.

We have the chance to form a new collaboration with Prof. John Rodger, CEO & Executive Director of FAUNA Research Alliance (https://faunaresearchalliance.com/) with whom we are organising the first marsupial workshop/meeting involving marsupial researchers from America and Australia, which will be held in Chile on September 28-October 2, 2020.

In terms of research activities, we will send the samples collected during the 2018-2019 fieldwork for RAD-sequencing analysis to develop more SNPs markers and increase our sample number and our population genetic data.

We would like to evaluate in more detail if the aggregation of individuals in groups during winter is used as a strategy to conserve energy.

Figure 3. Prof. Juan Luis Celis-Diez discussing with the students the importance of the use of the nest boxes for Dromiciops gliroides.

To do so we will measure metabolic rate in the field using two widely used methods, the doubly labelled water (DLW) and heart rate (fH) methods. We will also monitor temperature inside and outside the nest and core temperature (again) during winter torpor.

To finish with the evaluation of the mating system we will analyse kinship in the samples collected in 2018-2019 fieldwork. We also would like to evaluate cooperative breeding by video monitoring (low-light sensitive digital cameras), recording the movements and social behaviour between animals inside the nest boxes.

Finally, we would like to collect samples from different forest fragments in the northern part of the Chiloé Island for sequencing. Then using landscape genetics tools, we would like to evaluate how functional is landscape connectivity and gene flow within and among our different sites. This will give us fundamental information about *Dromiciops* movements within and among primary habitat complexes and which are the most important habitat characteristics that help in the connectivity and dispersal of this species.

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

The results generated by this project have being used in educational programmes (field course 'Ecology and Biodiversity of the South American Temperate Rainforest') organised by the Senda Darwin Biological Station (SDBS). We have also shown our results throughout scientific talks and in poster sessions (Figure 4). We have conducted a seminar with undergraduate students from The Pontifical Catholic University of Valparaiso (PUCV). Also, we have participated in three scientific meetings (the 35th annual meeting from The Australian and New Zealand Society for Comparative Physiology and Biochemistry, Melbourne December 2018, the Victorian Biodiversity Conference, Melbourne February 2019 and Organisms in nature - evolutionary perspective and ecological significance, Berlin September 2019). Also, we are planning to share our results with the general community through local Chilean media, and with the scientific community through scientific publications and reports (i.e. journal articles).

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

The majority of the RSG funds were used to covered fieldwork expenses and outreach activities conducted during the period August 2018-March 2019. The total amount was used over the period of August 2018-June 2019. We have anticipated that the whole project will be finish by September-2020. According to this, the present RSG covered almost a half of the whole schemed project.

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.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Return ticket Melbourne-Santiago	1057	851	-206	
Fieldwork equipment	380	57	-323	Used to purchase walkabout

				radios
Fieldwork food and accommodation	920	900	-20	
Fieldwork transport		825	+825	Included return ticket Santiago-Puerto Montt, bus ticket Puerto Montt-Ancud, car rental and petrol
Consumables fieldwork		935	+935	Included for example flagging, alcohol, eppendorf tubes
Outreach activities		126	+126	Donation to Senda Darwin Biological Station (SDBS) for the use of their facilities for the workshop
Laboratory analysis	2638	2750	+112	
TOTAL	**4995**	**6444**	**+1449**	The additional cost was covered by Prof. Peter Temple-Smith laboratory

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

1. In order to continue our work with the local community regarding raising awareness of the significance of the protection of tree hollows, we need to complete our study to conclude how important is huddling behaviour and the use of nesting sites for the survival of *Dromiciops*. We think it is of extreme importance to establish if huddling may be a unique response to environmental stress and if influences deepness and duration of torpor. So, an important next step is to analyse energy savings in wild populations of *Dromiciops* during winter torpor.

2. Also, we think is important to fully determine the reproductive behaviour in this species in case future captivity breeding programmes are needed. This involves analysing the mating system including multi-paternity, whether huddling facilitate and influence the choice of mate and evaluate if the females choose mates based on relatedness. Also, we think is important to determinate if *Dromiciops gliroides* females are 'cooperative breeders' using huddling as a mechanism beyond thermoregulation and also related to parental care.

3. All this information is important to developing science-based conservation policies and tools for the protection of this species and at the same time educating the public about the importance of healthy ecosystems, which is vital to the protection of the environment.

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 the RF logo in all the conservation talks.

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

Associate Professor Juan Luis Celis Diez. Works in close collaboration with Senda Darwin Biological Station (SDBS). He is one of the lead researchers of the project. Because of his experience working with children, he was involved in conducted the workshop to local schools. He is also responsible of the field course 'Ecology and Biodiversity of the South American Temperate Rainforest'.

Professor Peter Temple-Smith. His is one of the lead researchers of the project and sponsor of the majority of the laboratory analysis. He participated in the November 2018 fieldwork and in the outreach activities. He was responsible of giving two talks, one to undergraduate students from The Pontifical Catholic University of Valparaiso (PUCV) and the other in the scientific meeting 'Organisms in nature - evolutionary perspective and ecological significance', Berlin September 2019.

Professor Marilyn Renfree. Her role in the project was research advisor. She participated in the November 2018 fieldwork.

Professor Eduardo Palma. His role in the project was research advisor. His laboratory was responsible of develop the microsatellites markers that we were planning on using in this study. However, after testing the microsatellites we decide to change the methodology and use SNPs markers.

Dr. Seungmin Ham. His role in the project was laboratory research advisor. He was responsible on helping with the laboratory analysis.

María Ignacia Undurraga. Her role in the project was fieldwork assistance.

Sebastian García. His role in the project was fieldwork assistance.

Rolando Rojas. He works for Senda Darwin Biological Station (SDBS). He was involved in the organization of the workshops to local schools and he was responsible of giving the introductory talk to the students.

12. Any other comments?

We would like to thank RF for their support and funding for this project. We are very excited to continue our work in increasing the knowledge on this amazing and unique species, the *Dromiciops gliroides* and to implement our results on future conservation strategies and tools to help in the protection of this species and their ecosystem.