

The Rufford Small Grants Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. 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. 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	Agustin Sanguinetti
Project title	Chloraeinae Orchids from the Pampas and Patagonia: Distribution, Conservation Status and Reproductive Biology
RSG reference	13613-1
Reporting period	September 2013 — February 2015 (Field campaigns started 3 weeks before the grant approval letter was received)
Amount of grant	£5999
Your email address	sangos@gmail.com / sango@bg.fcen.uba.ar
Date of this report	April 2015

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
Presence and Distribution			X	Fieldwork was conducted as planned. One season in Southern Brazil, two seasons in Buenos Aires and Entre Rios provinces and one season in Chubut and Santa Cruz provinces in Patagonia. Remarkably, three pampean species with lack of registries during the last decades were re-found; new occurrence sites were georeferenced.
Reproductive Biology			X	Manual pollination treatments were performed on five pampean and 18 Patagonian species. The reproductive system of the five pampean species studied and their pollinators were uncovered. The same was achieved with almost half of the studied Patagonian species.
Awareness raising and capacity building for local actors		X		An illustrated field guide of the orchids of Lago Puelo National Park was elaborated and given to the park staff. In Los Glaciares National Park rangers were involved in our work on recording orchid diversity and their pollinators. Interviews were given to local and specialised media. We gave presentations on endemic orchid diversity and pollination strategies for 300 high-school students and teachers in Buenos Aires and Tucuman. Unfortunately, we were unable to work together with Buenos Aires park rangers due to delays in obtaining the research permissions.
Conservation strategies		X		All the information gathered regarding distribution, occurrence sites and reproductive strategies constitute novel

				<p>and sound information useful to assess the conservation status of these species. An important part of this information is already available as published articles in international journals. Specifically, we are also carrying some species' conservation assessments according to IUCN criteria with all our and pre-existent data.</p> <p>Asymbiotic germination trials did not rendered positive results. Instead, symbiotic germinations trials are being carried out in partnership with a specialised researcher.</p>
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2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

Research permission to work within Buenos Aires province's protected areas were not emitted on the stipulated time due to bureaucratic problems and delays. This forbade us to work and research inside the province's protected areas and to establish collaborations with their park rangers. The problem was overcome by working outside protected areas in private property along with the owners' consent. The bright side of this situation was that the respective landowners got to know our work and the existence of endemic orchid inside their properties gaining important allies for future discussions on management policies. Besides this inconvenience, work in southern Brazil and Patagonian National Parks was carried out efficiently along with the correspondent research licenses.

Preliminary tests of asymbiotic germination with different culture media on the most compromised species (Pampean Chloraeinae orchids) did not render any positive results. In turn, we contacted a specialist on symbiotic germination, PhD. Sebastian Fracchia from the Micology Lab at CRILAR Institute (<http://www.crilar-conicet.gob.ar/gr07.html>), and we began a partnership to micropropagate these species with aid of symbiotic fungi. Although they seem to be hardy species with very low germination rates, a few protocorms and plantlets developed successfully symbiotically. Future propagative efforts will follow this way together with this new collaboration.

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

During this project we travelled about 12,000 km embracing a latitudinal gradient from -20° S in Brazil to -50° S in Patagonia in which we collected specimens and performed fieldwork in more than 15 different locations. The most important outcomes are detailed below.

I- RESEARCH

Chloraeinae orchids as a whole represent a South American endemic group which biological aspects have been poorly studied in spite of being comprised by about 74 species. We were able to gather new data on presence, distribution and ecological aspects of five pampean and 18 Patagonian species.

a) Presence and distribution

a.i. Pampean species: On our field campaigns to Rio Grande do Sul state (Brazil) and Buenos Aires and Entre Rios province we were able to locate extant populations of all the five non-Andean representatives of genus *Bipinnula*. What is more remarkable is that we could locate the first record of *B. giberti* in Brazil expanding 500 km its range from previous known localities restricted to Uruguay. Also, we found in Argentina populations of two species (*B. biplumata* and *B. polysyca*) that lacked records in herbaria for over a century and four decades, respectively.

As a "by-catch" of this project we could locate in Rio Grande do Sul a minute terrestrial orchid that the only previous known record belonged to the type locality which population was extinguished due to over-collection some decades ago. Another by-catch was the extension of the austral distributional limit of *Habenaria parviflora* which also constitutes a new record for the orchid flora of the Buenos Aires province.

Most of this novel information together with illustrative photographs is now available in the following scientific articles:

Buzatto CR, Sanguinetti A, Romero-González GA, Van den Berg C, Singer RB. 2014. A taxonomic synopsis of Brazilian Chloraeinae (Orchidaceae: Orchidoideae). *Phytotaxa* **158**: 1–22.

Sanguinetti A, Buzatto CR, Singer RB. 2015. The genus *Bipinnula* (Orchidaceae: Chloraeinae) in Argentina. *Nordic Journal of Botany* **33**, in press.

Sanguinetti A. 2015. *Habenaria parviflora* (Orchidaceae), extension of its austral distributional limit and new synonymy. *Nordic Journal of Botany* **33**, in press.

a.ii. Patagonian species: During the field campaign to Lago Puelo National Park in Chubut province we could conclude previous preliminary surveys on the park's orchid diversity. The specimens collected and georeferenced, along with the photographs taken, crystallised in an illustrated field guide we handed over to the park's authorities and rangers. We registered 15 species of 18 cited for the park. This guide is an important tool for the park staff having in account that most of the rangers consulted were aware of the existence of only three to five orchid species (of the most conspicuous ones) within the park. It will be of help to highlight the orchid presence and diversity on future management plans and to increase the visitors' awareness on native flora.

In Los Glaciares National Park in Santa Cruz province we could collect specimens and georeference populations of 10 species from a total of 16 species reported for the region. We

worked there in collaboration and with the assistance of a park ranger (V. Sotelo) who is involved in recording and summarising all the information on orchids from the park.

b) Reproductive Biology

b.i. Pampean species: We were able to perform manual pollination treatments on populations of all the five non-Andean *Bipinnula* species. We could also take photos and videos of their floral visitors and pollinators, hence clearing up the different strategies these plants rely on for their reproduction. All this information is being processed and will be available soon as part of a scientific journal article.

b.ii. Patagonian species: We performed manual pollination treatments on 11 orchid species. Some of them resulted to be autogamous and some dependant on pollinators for their fructification. The pollinator agents of some of the non autogamous species were registered in photos and films.

Our observations permitted to realise that a very significant part of the pollination service on some non autogamous orchids is actually being performed by highly invasive European bumblebees (see Carolina Morales Rufford project #15.01.10). This suggests that Patagonian orchids pollinated by bumblebees are in some way safe from the danger of losing its main threatened native pollinator. This is detailed in the following paper:

Sanguinetti A, Singer RB. 2014. Invasive bees promote high reproductive success in Andean orchids. *Biological Conservation* **175**: 10–20.

II- INCREASED RECOGNITION AND AWARENESS OF NATIVE TERRESTRIAL ORCHID DIVERSITY

Besides gathering important information on orchids we could also register their striking flowers and features and their pollinators in photos and videos. This material is of utter usefulness when comes to outreach and communication. As stated before, a visual guide was produced, printed and handed to Lago Puelo park staff. Also a presentation comprising pictures and videos was produced to explain native orchid diversity and their many strategies for pollination. This presentation was oriented to high-school students and teachers and it was shown in science fairs and gatherings in Buenos Aires city and Tucuman province; it is expected also to be used the current year as an introductory lesson in pollination strategies and orchids in Botany lectures for natural science freshmen at Buenos Aires University.

One of the most important national news agency on science interviewed us on the topic about orchids being pollinated by invasive agents. The interview can be found at: <http://nexciencia.exactas.uba.ar/orquideas-abejorros-agustin-sanguinetti-ecologia-especies-invasoras>

Thanks to this project the biodiversity information system of the National Parks Administration (APN <http://www.sib.gov.ar/>) was enriched with publicly available photographs of 18 orchid species which hadn't previous pictures available.

III- CONSERVATION STRATEGIES

As stated in I.b.ii., our research on some Andean orchids suggest that these plants nowadays do not depend entirely on their unique native pollinator, on the account that this function has been taken over by invasive bees. This novel interaction relieves the orchids from the risk of losing their native pollinator; hence orchid conservation efforts should not address this issue when preparing population management plans.

Micropropagation attempts are being carried on with preliminary positive results. Symbiotic germination tests rendered some successful germination and protocorm development on species with the lesser geographical extent and the most transformed biome (pampean species).

All the information gathered regarding distribution, occurrence sites and reproductive strategies constitute novel and sound information useful to assess the conservation status of these species. An important part of this information is already available as published articles in international journals. Specifically, we are also carrying some species' conservation assessments and categorisation according to IUCN criteria with all our and pre-existent data. We have already submitted an assessment on *Bipinnula polisyka* to the IUCN Red List authority coordinator on Temperate South American Plant Specialist Group. We expect to continue with the rest of the studied species throughout this year.

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

The involvement of local stakeholders and managers (park rangers) is detailed above in point 3.ii

5. Are there any plans to continue this work?

It is our intention to continue researching on native terrestrial orchids and raising awareness on their existence and conservation. Now that we have gained knowledge on basic aspects of pampean Chloraeinae such as distribution and habitat occurrence our goal is to get to know more about their ecological interactions (e.g., mycorrhizas, predators) and how economic activities (such as agriculture and cattle raising) represent a threat for their survival. Besides, we aim to expand our work to Chloraeinae orchids from the north-western part of Argentina which represent a largely unstudied group from this subtribe.

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

We will continue sharing the results of this and forthcoming projects to school students and teachers through presentations and environmental education activities and to assist and training park rangers on learning about orchid diversity and their ecological interactions. An important part of the work done has already been shared through international scientific journals. Some results still need some processing and they will also be published likewise. For local non-English speakers, a Spanish

language version of the published manuscripts will be available as soon as possible as “supplementary material” on a personal ResearchGate page (https://www.researchgate.net/profile/Agustin_Sanguinetti). There is a chance to attend the V Scientific Conference on Andean Orchids in Cali, Colombia on November. There we expect to share our results and experience on Pampean and Patagonic Andean orchids.

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

The project was executed as planned during the period September 2013 — February 2015 during which two spring-summer campaigns could be carried over. We had to start before receiving the acceptance email as a result of an unexpected delay from one of the reviewers proposed.

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	Actual expenses	Difference	Comments
Bus Ticket (2): Buenos Aires-Porto Alegre	230	240	-10	
Bus Ticket (4): Buenos aires-Lago Puelo	540	0	540	See above
Car Rental (95 days)	1545	500	1045	See above
Car Use and Maintenance	0	500	-500	See above
Fuel (500 litres)	484	910	-426	See above
Road Tolls	0	47	-47	See above
Lodging (50 nights)	905	1104	-199	See above
Food (240 meals)	835	988	-153	See above
Insurance for Team members and Volunteers	200	180	20	
First Aid Kit	50	95	-45	We had to buy some other medicines during our campaign in Patagonia.
Outreach Materials (1000 printed brochures)	400	280	120	30 full color reports (70pp) as bounded books for Lago Puelo Park staff and National Parks Administration officers.
Office supplies	100	173	-73	
Camera Flash System	0	0	0	
Macro Lens	310	410	-100	

Digital Camera	0	460	-460	Our preexistent Canon T3i had an obturator malfunction which couldn't be repaired and we had to replace it with a new one. This wasn't contemplated on the proposed budget.
Floral Volatile Trapping Equipment	0	0	0	
Entomological Equipment	0	0	0	
Laboratory supplies	400	310	90	Some lab friends lend us certain reagents and glassware saving us from buying it.
TOTAL	5999	6197	-198	

The main difference on the budget originally proposed is that we were able to borrow a car for fieldwork within Argentina. This provided us more available time, freedom and facilities to move between more localities and to search for more populations to work with. For example, this allowed us also to work in Los Glaciares National Park, further south than we had originally planned. Besides we avoided spending on bus tickets to Lago Puelo and Los Glaciares National Parks; still expenses regarding fuel increased. Some costs on the car use and maintenance had to be included also (e.g., repairing flat tires and punctures, broken wheel joints and shock absorbers, changing oil and filters). As we had more time to do fieldwork -while not depending on a rented car- we had a bigger expenditure in lodging and food.

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

Regarding the work already done we believe it is important to continue and conclude with the IUCN Red List categorisations and conservation assessments for the Pampean species which are the ones that dwell in the most transformed biome and whose areas of occupancy have seriously decreased. Categorising these species would reveal the threats they are exposed to and their extinction risks bringing them into the spotlight for the public opinion and the respective public environmental agencies. Besides, it is important to continue with the outreach with school students and teachers raising aware on the importance on knowing our native biodiversity heritage and its protection.

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

The importance of RSGF funding for this project was acknowledged explicitly in each scientific manuscript we submitted to journals. The logo was used in the field guide and report handed in to the Lago Puelo National Park staff and in all Power Point presentations for school students and teachers. The logo will still be used in forthcoming presentations and reports where results of this project are shown

11. Any other comments?

Without the RSG founding most of the fieldwork done here would have been impossible to accomplish. We believe we have done big improvements on learning about basic aspects of Chloraeinae diversity that will help diagnose their situation and think conservation strategies. We are grateful for giving us the opportunity to boost our work and to scheme future plans on conservation of native terrestrial orchids.