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	Italo Fernández Cisternas
Project title	"Exploring molecular tools for conservation of a heavily fished species in Chile: the case of the darwini's Sheephead (*Semicossyphus darwini*)"
RSG reference	21864-1
Reporting period	May 2018
Amount of grant	5,000
Your email address	itfernandez@uc.cl
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
Obtain samples along the entire coast of Chile (complete geographic distribution of Chile)			■	We identified two distinct populations within the frame of our study. In order to deepen our understanding of the evolutionary mechanism behind this separation it is necessary to obtain samples to expand the geographic range of our study such as the southern distribution which were part of our southern population, belonging to latitudes 32º - 33º S.
Study the gene flow between *Semicossyphus darwini* and *Semicossyphus pulcher* (Possible anti-tropical connectivity between hemispheres)			■	With this grant we were able to confirm that the fish species in California and Chile are similar in terms of genetic makeup which was surprising considering that both species are separated but face similar threats (i.e., fishing). However, it is necessary obtain samples from Ecuador, Perú and Galapagos island to look for hybrids of *S. darwini.* (More information in results)
Study the genetic connectivity of the *S. darwini* along of the coast of Chile			■	We were able to achieve this goal using the 54 samples from *S. darwini.* The studies covering 1307 km of coast approximately, resulting in a continue connectivity along the coast (More information in results).
Study the genetic population structure of the *S. darwini* along of the coast of Chile		■		It is necessary obtain more samples of the southern population of *S. darwini* (29º- 33º S) (More information in results)

Study the genetic diversity of the population of *S. darwini* for known the health of the stock			■	We were able to achieve this goal using the 54 samples and analysing the proportion of polymorphic loci founded. (More information in results).
Estimate the effective population size (Ne) of the *S. darwini* in Chile		■		We found that that the effective population size (Ne) is not bigger than 400,000 which is very low considering the impact of fishing. In that sense, fishing may surpass the effect of climatic events on populations and soon they will be facing a bottleneck. We aim to continue this work in a second grant to effectively know the mechanism behind (such age at maturity, reproductive output, recruitment)
Generate data to implement a sustainable management plan for the spearfishing of *S. darwini*		■		We succeeded in understanding with molecular data the population size of this heavily fish species, the genetic makeup of two different populations of this species, and the overlap with its sister species (*S. pulcher*) from California. Moreover, we identified two possible genetic stock in the coast of Chile. With this molecular information, we aim to complimented with fishery information in a subsequent second small grant (which I aim to apply) in order to underpin the evolutionary and ecologically consequences of fishing

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

There were difficulties in obtaining samples in some representative areas, especially in populations further south of the distribution of *S. darwini*. Also, we had difficulties with the low quality of DNA in several samples obtained, reducing the number of replicates to separate populations or genetic stock.

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

At the moment, we have obtained very good results from the sequencing of the *S. darwini* genome that will contribute in proposing long-term management plans for the species which at the moment are inexistent. Importantly that we generated a good link with the fisherman who contributed to samples and natural history information. Most of them are willing to change the ways of fishing to reduce the impact of the fishery. Fisherman and divers contributed to more than 80% of the samples and location. We will simplify the results herein and provide a series of small outreach material to compliment findings of this species. Moreover, we presented results of this study in a national Marine Science Conference, Valdivia. I will summarize the main results herein:

1) History of live of *S. darwini*: phylogeny in sister species

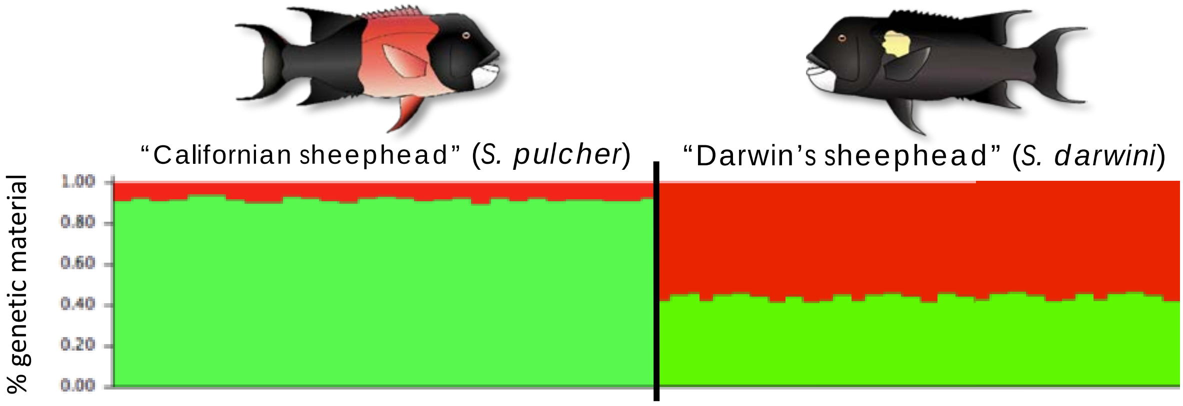

Figure 1. Bayesian test of population assignment based in 10809 genomic loci finded. Highest likelihood was found when data were partitioned in two clusters (K = 2) represented in green and red. Vertical lines represents one individual and its assignment likelihood to belong to one of the cluster (% of genetic material) is shown by the color. The vertical line represents the separation between Californian sheephead (left) and darwin's sheephead (right) The graphic was make with STRUCTURE© software.

The results of population genetic using all the loci obtained in both species, generate with the software "Structure" (**Figure 1**), show that about a 30% of the genetic material is shared between the two population, or in this case, species. With a Fst (The fixation index; which is a measure of population differentiation due to genetic structure) of 0.282 (P= <0.001), generated in software "Arlequin©", this result indicates that there is a gene flow between both species, suggesting finally that they could be individuals of the same species or *S. darwini* as a subspecies.

In conservation, for a correct implementation of a management plan, it is necessary to correctly identify the conservation units, this means we need to know if we protect the environment, a population of threatened species, a subspecies, and finally genes. Understanding the phylogeny of an animal and history life, it is possible to understand how local adaptations influence the evolutionary divergence of a stock or genetic stock. Also, how much of the genetic pool of a sub-species would be influencing the pool of another sub-species (gene flow). The conservation of multiple populations, not just one or two, is critical for insuring the long-term persistence of species. Finally, an existing management plan for one sub-specie (in this case *S. darwini* in California) is very useful for implementing a new management plan in another sub-species.

2) Gene flow in *Semicossyphus darwini* in Chile

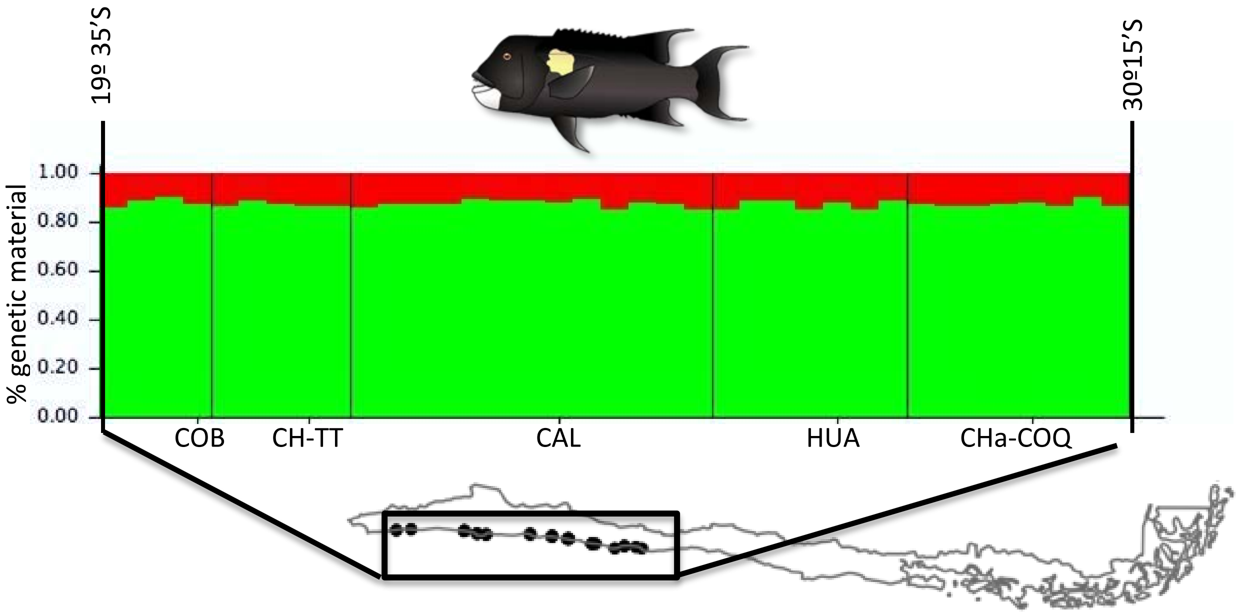

Figure 2. Bayesian test of population assignment based in 10320 genomic loci found. Highest likelihood was found when data were partitioned in two clusters (K = 2) represented in green and red. Vertical lines represents one individual and its assignment likelihood to belong to one of the cluster (% of genetic material) is shown by the color. The vertical lines represent the separation between populations of *S. darwini* along the coast of Chile (COB= Cobija, Ch-TT= Chañaral and Taltal, CAL= Caldera, HUA= Huasco, Cha-COQ= Chañaral de aceituno and Coquimbo). The entire zone is represented with a map of Chile and the black square is the sample zone, ubicated between 19º35' S and 30º15' S. The graphic was make with STRUCTURE© software.

The results of population genetic using all the locis obtained in the samples of *S. darwini*, generate with STRUCTURE© (**Figure 2**), it does not show a genetic structure that separates the five populations sampled within the distribution range of *S. darwini*. There is a permanent gene flow in the population of *S. darwini* along the entire coast.

When we were working with the outlier loci (loci selected by the environment) and separating the total samples obtained from *S. darwini* in two populations predefined by the biogeographic break found in the 30º S (North zone and center-north zone), we can observe an important population structure between these two zones (**Figure 3**). With a Fst of 0.327 (P = <0.001), these results suggest that different selective pressures would be acting in these two biogeographic zones, selecting different loci possibly associated with genes.

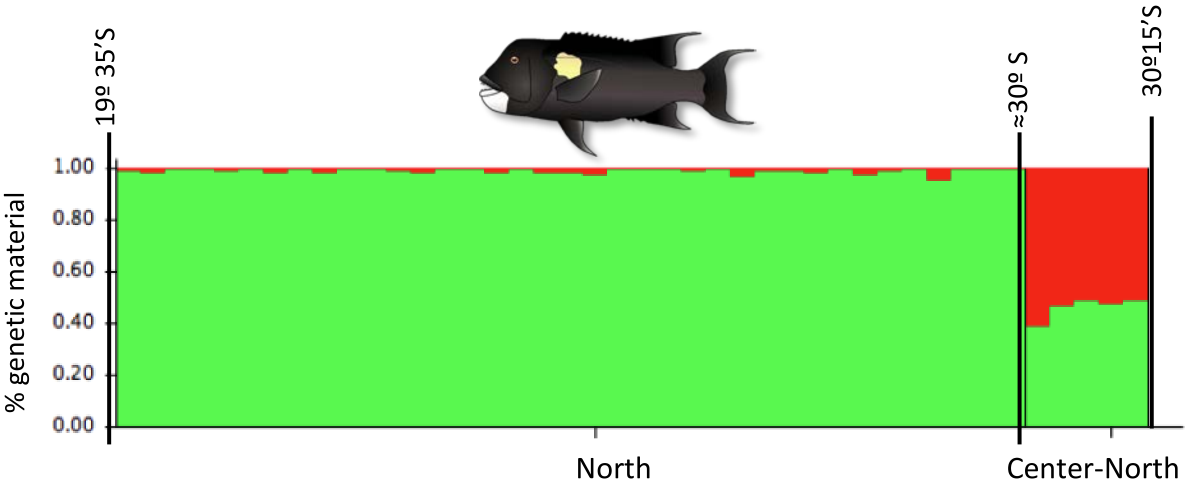

Figure 3. Bayesian test of population assignment based in 452 genomic outlier loci finded. Highest likelihood was found when data were partitioned in two clusters (K = 2) represented in green and red. Vertical lines represents one individual and its assignment likelihood to belong to one of the cluster (% of genetic material) is shown by the color. The vertical line represents the separation between northern and center-north populations of *Semicossyphus darwini* separated on the biogeographical break (30º S) along the coast of Chile, ubicated between 19º35' S and 30º15' S. The graphic was make with STRUCTURE© software.

3) Genetic diversity of *Semicossyphus darwini* in Chile

We explored the genetic diversity of *S. darwini* samples with the polymorphyc percentage in the loci found. First, a quality filter was performed on the samples and subsequently a gene population analysis in the Arlequin© software. We compared the diversity of *S. darwini* in Chile with respect to *S. pulcher* in California (**Figure 4**). Both species having a low polymorphic loci value, with *S. darwini* having a lowest genetic diversity of both species. When the population of *S. darwini* in the north and central-north zones was subsequently explored, the diversity found was lower in the center-north of the distribution (**Figure 5**).

A low genetic diversity in a species with a wide range of distribution in Chile could be the product of the strong fishing pressure that exists on *S. darwini*, due to the non-existent fishing management that this species has in the country. Finally, due to the sequential hermaphroditic character of the species, the proportion of males would decrease year by year, drastically reducing the effective size of the population, eventually causing the collapse of the population of *S. darwini* in Chile.

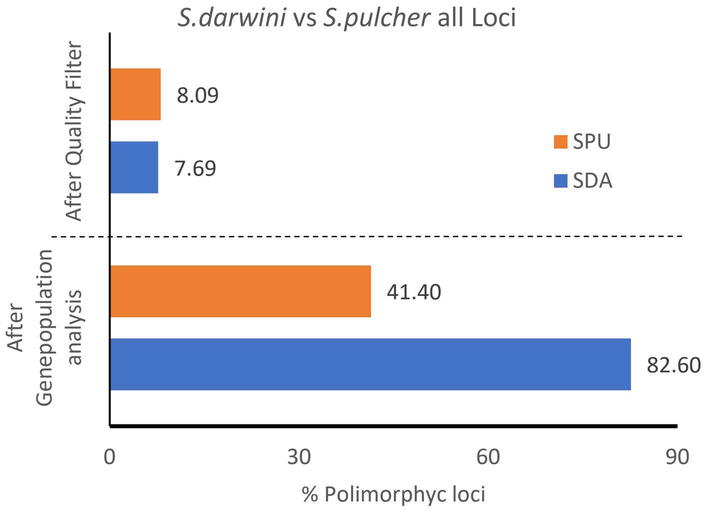

Figure 4. (%) Percentage of polymorphic of all loci obtained for *S. darwini* (blue) and *S. pulcher* (red). The dotted line separate between the result after quality filter (up) and after the gene population analysis (down).

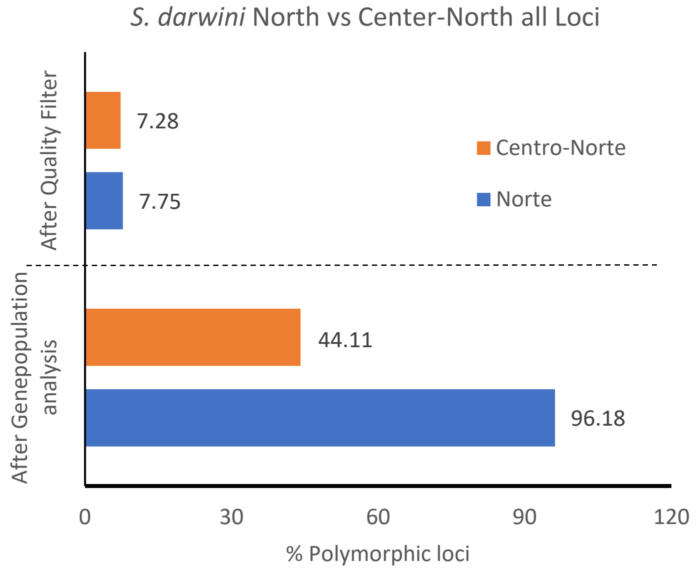

Figure 5. (%) Percentage of polymorphic of all loci obtained for *S. darwini* of the north population (blue) and center-north (red). The dotted line separate between the result after quality filter (up) and after the gene population analysis (down).

Molecular tools allow us to generate long term management information, special for conservation of a species and populations, avoiding their extinction. With this information, we can define possible "genetic stock" (recognise a stock with a genetic structure different from another stock), found it within the range where a species is exploited.

Our results indicate that there is a gene flow along the entire coast, and that we can treat *S. darwini* as a great and unique genetic stock. However, focusing on the outlier loci, these indicate that we could find two genetic stocks, one in the north and other in the center-north. Changes in genetic structure can be associated with changes in population fitness, due to the process of adaptation to environmental or anthropological factors *(e.g.* overfishing), independent of the local immigration to other stock (gene flow), and are therefore relevant to fishery management. Knowing well the stock present in the area is of vital importance, undetected stocks may become eliminated, with a corresponding reduction in inter-population genetic variance. It is necessary to apply a management approach to conserve locally adapted stocks. A stock where the population is being reduced by overfishing, like the stock founded in the center north, could be causing a significant loss of genetic diversity for the species in Chile.

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

The community involvement in this project correspond mostly to the sport and artisanal fishermen (spearfishing) that capture the species. Spear fishermen are fully aware of the reduction of the population of *S, darwini* in certain areas of the country (especially in the centre-north of the country), we know that for speaking directly with them or through the comments made on social networks. Adding more fishery information (age at maturity, size at reproduction, and reproductive output between sexes) alongside with the information of traditional ecological knowledge will be crucial.

On our social media as outreach for this study, the recreational spear fishermen community and the people in general has been quite interested in our work and benefits from the information we provided for the species, giving more awareness of the urgency of conserving a species like the *S. darwini* and the species on temperate reefs **(Figure 6).**

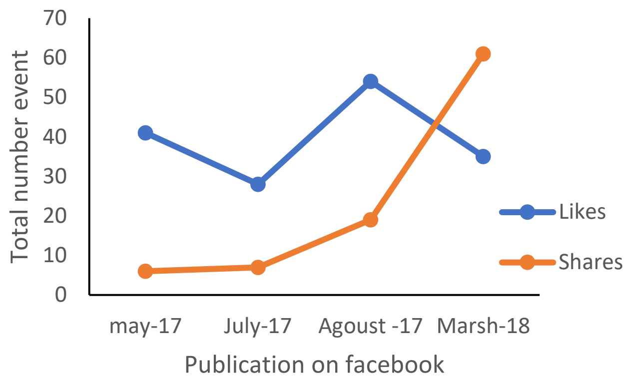

Figure 6. Graphic representation of participation of the Facebook© community. Blue dots represent total "likes" and red dots represents total "Share" in the publications of Subelab account trough the project. The first publication was the promotional video of the project (https://vimeo.com/218036349). The last publication was the first preliminary results of the project.

5. Are there any plans to continue this work?

With our results, we found a possible two genetic stock along the coast of Chile. However, it is important notice that the diversity of *S. darwini* also turned out to be relatively lower in genetic diversity compared with the sister species, *S. pulcher*. This could be a consequence of the overexploitation along Chilean coast, especially in the southern distribution. We will proposed to close the fishery in the southern end of the population due to a differentiation of the genetic makeup.

The main objective of this project was to generate sufficient information to implement an efficient extractive management plan. For this, we need to continue obtaining samples for new genetic analyzes, and complete the range of distribution of the species in Chile, and possibly more to the north of the country (Perú, Ecuador and Galápagos islands). Furthermore, it is important to complement this information with fishery and ecological data of the species for implement a short-term management (harvesting stock), that allow us to protect the fishery of this species, avoiding the overexploiting of the population. It is necessary to describe these two genetic stocks. We aim to create a multidisciplinary work for this project. This is why we intend to continue this project by applying for a Second Small Grant, which will allow us to complete and create new study objectives for the conservation of an overexploited species in Chile, the darwini's sheephead.

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

We have disseminated our preliminary results in small presentations, such as the one recently held at the XXXVIII Congress of Marine Sciences 2018, held in the city of Valdivia, Chile in which more than 500 participants. In addition, through the social media of the laboratory, the project has been successfully shared during this year of work. Also, we will prepare the outcomes of the study as an outreach format such as in digital magazine (http://chileesmar.cl/categoria/rema/). This information will be crucial to many spear fishermen. We also shared our results with government agencies such as the Undersecretary of Fishing and the Institute of fishing (IFOP).

We also aim to use international platforms to outreach our results such as the Seymour Discovery Centre, at Santa Cruz, California to a very large public *(about 300,000 visitors every year). Lastly, but importantly we are currently working on two manuscripts (one for the comparisons of genetic makeup between S. darwini and S. pulcher and another for the genetic structure of S. darwini in Chile) that will be submitted to scientific journal (please see our publication record in the Lab I work (www.subelab.cl) and Bernardi´s lab (http://bernardi.eeb.ucsc.edu).

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 Foundation grant was used gradually throughout the period contemplated for the project (duration of approximately 1 year), the timescale of the Project can be divide in three periods:

1) **May 2017– January 2018:** Outreach of the project in social media and collection of samples from fish tissue in coves and sports fishermen.
2) **February – March 2018:** Extraction and sequencing of DNA of samples on the molecular ecology and evolution of fish's laboratory, of the University of Santa Cruz, CA.
3) **April – May 2018:** Analysis of results from the sequencing obtained in the laboratory.

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**
Air fare	289	687	398	
Per diem and travel	645	615	30	
Lab Equipment	280	1000	720	
Laboratory analysis	2005	2000	1005	
Petrol-car rental	801	0	801	Item not used
Team salaries	980	980	0	
Total	5000	5282		

*All the budgeted amount were calculate in pounds

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

It is necessary to continue analysing the actual sequences, use new molecular tools and, of course, obtain more tissue samples from the areas with less presence of *S. darwini* in Chile (29º-33º S), and from new study sites. Galapagos Islands corresponds to the northern limit of the distribution of *S. darwini* and a good site to broaden the understanding of the connectivity between the two species.

The most important step, however, is to generate more information related to the ecology and life attributes (fishery studies) of the species, allowing the creation of a complete fishery and management plans for *S. darwini* in Chile. Fisheries and ecological studies are basic and completely necessary to regulate the extraction of a species. For this, we need to perform studies of age and growth, habitat for recruitment, length at sexual maturity and reproduction seasons. It is necessary create a complete short-term management (estimation of production models, catch forecasting and effort regulation), and long-term management objectives (long-term sustainability and genetic conservation), to protect a specie with an important fishery in Chile.

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?

The Rufford Foundation was constantly publicised when we were talking about the project to different scientific entities, in Chile and internationally (California, USA). In addition, the project developed thanks to the Rufford Foundation grant is presented on the official website of my laboratory (please visit: http://www.subelab.cl) and on social media (Facebook: /Subelab, Instagram: @Subelab), naming the participation of the foundation.

Finally, the logo of the Rufford Foundation was used in the video of the project (https://vimeo.com/218036349) and in the presentation of preliminary results at the XXXVIII Congress of Marine Sciences 2018, held in the city of Valdivia, Chile.

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

The team of this project were conformed to members of the Subelab of The Pontificia Universidad Católica de Chile and members of the Molecular Ecology and Evolution of Fishes (Bernardi's Lab) of the University of Santa Cruz, California:

Italo Fernández (Subelab): Subelab laboratory researcher who is currently leading the project, was in charge of gathering and obtaining samples of *S. darwini* tissue. He performs molecular laboratory work at the University of Santa Cruz, California and then analyze the samples.

Alejandro Pérez Matus (Subelab): Principal researcher of the Subelab laboratory, assistant professor at Pontificia Universidad Catolica de Chile project guide, participated in the collection of samples, data analysis and contact with other researchers.

Eric Garcia (Bernardi's Lab): Provide samples of *S. pulcher* and supported throughout the molecular work done at University of Santa Cruz, California. In addition, he attended all the work of analyzing the sequences obtained.

Giacomo Bernardi (Bernardi's Lab). Principal researcher of the Molecular Ecology and Evolution of Fishes lab. He gives the tools and space to perform molecular work in the laboratory at the University of Santa Cruz, California. He guided and led the molecular work, performed the data analysis and actively supported the work that it is still in process.