

Untangling the complex speciation process of the Peruvian-Chilean fur seals hybrid group and the role of Isla Foca in its population dynamics

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SUMMARY

The hybrid speciation is an intriguing phenomenon in Evolutionary Biology being extremely rare in vertebrates. We previously detected the hybrid origin of the Peruvian-Chilean fur seals, their ancient history and backcrossing occurring between Galapagos and Peruvian-Chilean fur seals in Isla Foca, Peru, distant 1,000 km from their main distributions. We detected that the speciation events were more complex than expected, meaning that further investigation is needed to provide a correct baseline for in-situ management and conservation. Studying whole genomes, we aim to investigate historical spatio-temporal interactions and the role that Isla Foca plays on the group population dynamics.

Project Description

What are the central research questions?

Hybridization occurs due to incomplete reproductive isolation when two populations or distinct species mate. The evolutionary consequences of this phenomenon can vary depending on the viability and fertility of the offspring. The effects of interspecific hybridization can extend further than the contact zone, causing the influx of foreign alleles from either or both parental species into the genome of other species, resulting in genomic introgression (Downing and Secor 1997). One of the most extreme cases of hybridization is the hybrid speciation, that is extremely rare in mammals. The homoploid hybrid speciation is less frequent in nature because the genes and alleles are frequently overcome by gene flow from the parental species, difficulting its stabilization as a new lineage (Schumer et al. 2018). Despite this, new genetic recombinations, that emerge from parental alleles in the introgressed groups can lead to new phenotypes, which may be well-adapted to the environment, improving the fitness and giving an evolutionary advantage to the hybrids (Abbott et al. 2013). Besides that, hybrid speciation is hard to distinguish from current interspecific gene flow, needing the interpretation of combined analyses to be detected.

The systematics and phylogeny of Otariidae have been extensively studied for over two centuries since the first species descriptions by European explorers (e.g., Sclater 1897; Berta et al. 2018). Yet, several relationships within the family remain unclear. Similarly, the species delimitation of the clade that encompasses the Galapagos (*A. galapagoensis*), New Zealand (*A. forsteri*), South American and Peruvian-Chilean fur seals (currently recognized as *A. australis* subspecies) are also debated (e.g. Yonezawa et al. 2009; Berta and Churchill 2012).

Seeking for solving this problem and based on six whole-genomes and reduced representation libraries (ddRAD-seq) of 47 individuals, representing South American/Peruvian-Chilean fur seals (Evolutionary Significant Units of *A. australis* - Oliveira et al. 2008), New Zealand and Galapagos fur seals, we found well-supported species tree and well-delimited taxa encompassing the clades above mentioned. The admixture analysis, however, suggested three as the most appropriate number of genomic clusters for these populations. This indicated all Peruvian-Chilean fur seals with shared genomic components with Galapagos and South American fur seals with ~50% of each parental species. Maximum-Likelihoods

windows-based phylogenies also reflected this grouping proportions. Posterior phylogenetic analyses with admixture dating and ABC supported that this admixture reflects the hybrid origin of the Peruvian-Chilean fur seals and the ancient evolutionary history of this taxon with 100% of support. These analyses combined with previous morphological studies and Admixture of K=4 population clusters also supported Peruvian-Chilean fur seals having their own genomic component. Thus, justifying an extant and well-delimited biological entity.

Until now, phylogenetic relationships (Wynen et al. 2001), biogeographical histories (Churchill et al. 2014) and even taxonomic reclassification (Berta and Churchill 2012) within this group were inferred with few mtDNA sequences. Among these studies, those that applied more than one individual per species supported the paraphyly for the crown group (Yonezawa et al. 2009). In our study, we detected the same pattern for the mtDNA genome. However, we analyzed, for the first time, many individuals of the Peruvian-Chilean fur seals in a dataset with South American, Galapagos and New Zealand fur seals. For the Peruvian-Chilean group, we detected two paraphyletic and very distant lineages: the first one grouping with South American fur seals and the second one grouping with the New Zealand fur seals, the basal taxa of this clade. This contributes to the taxon ancient evolutionary history and elucidates that the clade is a potential system to study gene-tree vs. species tree dynamics, given the genealogical discordance within genomes originated from events of introgression or Incomplete Lineage Sorting.

We also detected two pure individuals of Galapagos fur seals in Isla Foca, a remote rookery located ~1,000 km from the main distributions of the Peruvian-Chilean and Galapagos fur seals, and one backcrossed individual with genomic components of Peruvian-Chilean and Galapagos fur seals. Here is important to highlight that the Galapagos fur seals are reported as endemic to the Galapagos archipelago so far, but it seems to be extending its area of distribution. Considering the mentioned above and that the Eastern Equatorial Pacific Ocean is frequently facing climatic anomalies (El Niño and La Niña), that affects the food chain and primary productivity in the Peruvian coast and Galapagos Archipelago, the influence of climate change and oceanic anomalies on the population distributions and the role that Isla Foca plays on the Peruvian-Chilean and Galapagos fur seals extant populations need further investigation.

What are the main aims of this research project?

This project has three main goals: to investigate the spatio-temporal interaction during speciation (origin and dispersal) of the Peruvian-Chilean fur seals, given the mtDNA paraphyletic lineages; the role of climatic anomalies and climate change on the population dynamics of the Peruvian-Chilean and Galapagos fur seals; and the role that Isla Foca plays on the population dynamics of these taxa.

Which theories and methods will be employed?

For this, our research group will sequence 25 genomes (coverage ~25X) from individuals already sampled: Peruvian-Chilean fur seals, Punta San Juan - Peru (n = 5), Galapagos fur seals, Galapagos Islands (n = 2), New Zealand fur seals, Otago Harbour, New Zealand (n = 1) and South American fur seals (n = 4, one from each locality: Brazil, Argentina, Falklands and Chile). The fur seals sampled in Isla Foca (pure Galapagos fur seals and backcrossed with Peruvian-Chilean fur seals - n = 3) will be also sequenced. We will collect in a jointed field trip with Peruvian collaborators additional samples from Isla Foca. The total amount of samples will depend on the success of the field campaign, but we estimate a maximum of 10 genomes. The sequenced genomes will be assembled to the six high coverage genomes

previously sequenced (~25X) and that will be also used as a pseudo reference to ensure the mapping quality. For genomes sequencing, there is budget available from Brazilian funding (Brazilian funding PRONEX 12/2014 and FAPERGS PqG 05/2019 to Sandro Bonatto and Larissa Oliveira, my supervisors). However, no budget is available for field trips, supplies travel to Isla Foca and equipment and this is the reason for this proposal.

Sequenced reads will be trimmed for vestigial adapters, mapped against reference and locally realigned using the bam_pipeline implemented on PALEOMIX 1.2.13.2, following the parameters: reads with length-size <100 bp and Phred-score < 30 will be filtered out; the remaining paired-end reads will be mapped with BWA 0.7.17 and -mem algorithm; paired-end reads with mapping quality Phred-score 3) and significantly negative ($Z < -3$) values reject the null hypothesis.

Different scenarios of speciation will be tested with Approximate Bayesian Computation implemented on ABCtoolbox and fastsimcoal2 to test the most likely demographic and evolutionary scenarios. Demographic reconstructions through time will be also recovered with MSMC2 and the Isolation-Migration model, that allows inferring events of ancient introgression (Wang et al. 2019).

Y-chromosome will be recovered to test the role of natural selection on the process of speciation by looking on the X chromosome vs. the autosome. A similar approach of Juric et al. (2016)

What is the innovative potential that merits funding? How will the project contribute to your field of research?

Our study was the first to apply genomics to understand the evolution of Otariidae and the clade comprising Peruvian-Chilean, South American, Galapagos and New Zealand fur seals, shedding light on two centuries of scientific debate. The whole mitochondrial DNA analysis indicates that the process of diversification of the Peruvian-Chilean hybrid taxa had a complex history by showing two very divergent and paraphyletic lineages. We also found a secondary contact of the hybrid Peruvian-Chilean with Galapagos fur seals in Isla Foca and Punta San Juan, Peru, indicating that the Galapagos fur seals are generating offspring beyond the Galapagos Islands and potentially extending its distribution towards the South American continental coast. Our findings, so far, also highlight that new studies are needed to understand the role of Isla Foca and Peruvian coast in the population dynamics, distribution, and conservation of the threatened Peruvian-Chilean and Galapagos fur seals. Understanding the population dynamics and distribution of these taxa can help us to assess the impact of El Niño and climate changes in the backcrossing events, species range distributions and the consequences for their respective populations.

How and in what form will the results of this research project be disseminated?

The results of this research will be published in up two manuscripts of high impact. Journals as Genome Biology, Molecular Biology and Evolution, Current Biology and PNAS are good candidates for future submissions. During the period of research, the results will be presented in International meetings like the World Marine Mammal Science Conference of the Society for Marine Mammalogy, the International Meeting of the Society Latin American of Specialists in Aquatic Mammals and the International Meeting for the Study of Marine Mammals of the Mexican Society of Marine Mastozoology.

What is the added value of your collaboration with and secondment to an international academic institution /your cross-sectoral collaboration with a non-academic institution for your research project, and how will it contribute to the successful completion of your project?

The project will generate fundamental knowledge about the marine biodiversity, providing a baseline for in-situ management and conservation of threatened fur seals species in face of climate change and oceanic anomalies. This information will be also important for political and economic initiatives since these fur seals species are essential for the maintenance of the ecological equilibrium in key areas for the use of natural resources in South-eastern and Equatorial Pacific coast (e.g., Guano exploration in the Peruvian coast and Ecotourism in the Galapagos islands). Throughout the collaboration with nine institutions in the whole America, New Zealand and Europe, this project also aims to promote interdisciplinary marine mammal knowledge interchange.

Project Timeline

Field Sampling - Jan-Feb/2021

DNA extraction and evaluation – Mar/2021

Libraries preparation and DNA sequencing – Mar/2021

Data treatment – May/2021

Data Analyses – Jun-Sep/2021

Manuscript writing – Jun-Jan/2022

Literature review – Jan/2021 – Jan/2022

Project Budget

(estimated in June 2020)

Flight ticket Porto Alegre (Brazil) - Lima (Peru) - Porto Alegre US\$ 500

Accommodation US\$ 70

Field trip Meals US\$ 50

Fuel Lima - Isla Foca - Lima US\$ 100

Latex gloves US\$ 10

External Hard Drives 5 Tb (Big Data storage): \$ 250

Basic reagents Total US\$ 1,020

Details of the basic reagents cost (included in the US\$ 1,020)

Allprotect Tissue Reagent - for DNA stabilization US\$ 720

DNA extraction kit US\$ 250

Eppendorf tubes: \$ 30

Biopsy punches (3 units) US\$ 20

Total requested: US\$ 2,000

LEGAL PERMITS

Brazil – N° 105/98 – IBAMA; Peru 022-2004-INRENA-IFFS-DCB, Peru 119-2006-INRENA-IFFS-DCB;

Falkland Islands CITES 14US54398B9; Argentina – Secretaria de Turismo, Áreas Protegidas e Parques

Naturales: N°008/05, N° 178/07, N° 012/08, N° 003/09, N° 196/09, N° 001/10, N° 011/11, N° 082/12, N° 304/13 e N° 032/14; Uruguay N°001205

New permits will be requested to CITES and Peruvian authorities for the project execution.

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SHORT CV

Fernando Lopes, PhD (defense in Aug/2019). from Porto Alegre, Rio Grande do Sul – Brazil
Current occupation: Researcher fellow at Laboratory of Genomics and Molecular Biology of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). Av. Ipiranga, 6681 - Partenon - Porto Alegre, RS, Brazil.

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Primary research interest: Conservation genetics and genomics of South American fur seals and sea lions. Currently studying phylogenetic relationships, gene flow, demographic oscillations, hybridization and hybrid speciation of fur seals and sea lions.

Member (as student) of “Sociedad Latinoamericana de Especialistas en Mamíferos Acuáticos” since 2013
Member of Society for Marine Mammalogy (SMM), founder and administrator of Latin American Student Chapter SMM, since 2016