

## 2016 SMALL GRANT IN AID OF RESEARCH – ANNUAL SUMMARY REPORT

### RANGE-WIDE PHYLOGEOGRAPHY OF THE SOUTH AMERICAN FUR SEAL: EVOLUTIONARY HISTORY OF FUR SEALS IN SOUTH AMERICA USING HIGH-THROUGHPUT SEQUENCING

Fernando Lopes (nandulopes@gmail.com; fernando.ricardo@acad.pucrs.br)

Laboratory of Genomics and Molecular Biology  
Pontifícia Universidade Católica do Rio Grande do Sul

#### SUMMARY REPORT

A total of 10 samples were collected from South American fur seals (*Arctocephalus australis*) stranded along the coast of the State of Rio Grande do Sul, southern Brazil. The samples were cryo-preserved (-20 °C) in 70% ethanol at the Laboratory of Genomics and Molecular Biology - Pontifícia Universidade Católica do Rio Grande do Sul, where genomic DNA was extracted following a standard QIAmp DNeasy Blood and Tissue Kit (Qiagen). The integrity and concentration of the DNA were evaluated in agarose gel electrophoresis 1% and NanoDrop Lite equipment. The DNA libraries were prepared (for these 10 samples and other samples evaluated in the project – plus 42 samples collected along the South America continent for *Arctocephalus australis*; 10 *Arctocephalus galapagoensis*; 9 *Arctocephalus forsteri*) using a Double Digest Restriction-Site Associated DNA sequencing (ddRADSeq) protocol published by Kess et al. (2016). In order to achieve a coverage of 50x and the optimal size of the loci to be sequenced, we simulated (*in silico*) the best pair of enzymes, the number and size of the fragments to be cleaved. The simulation was based on Da Costa and Sorenson (2014) pipeline and *Arctocephalus gazella* reference genome. The SphI and EcoRI enzymes were chosen resulting ~31.700 loci that ranged from 355 to 555 base pairs. After prepared, the DNA libraries were sent for sequencing in an Illumina HiSeq2500 platform at The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada. The reads were demultiplexed and trimmed in order to remove the remained adaptors. A total of 112.000.000 of reads were generated. The reads are unevenly distributed among the individuals. Besides that, 77% of the reads were correctly assigned to its samples and the remaining 23% were discarded and not used in the following analysis. We repeated the library preparation for those samples with a low number of reads applying some modifications in the original protocol (for instance, the number of PCR cycles and the DNA input). The trimmed sequences were mapped against the *A. gazella* reference genome. As perspective, we will follow the pipelines for population genomics and

phylogenomics analysis in order to solve the major taxonomic and phylogeographical questions reported in the PhD and grant purposes. In March 2019 I intend to defend my PhD thesis. There, I will present and debate the results generated along the project development and, after that, submit the respective articles to specialised journals. I'm thankful for my collaborators that have made possible the execution of this project. I would like to thank the Society for Marine Mammals that provided funds for extend a wide-range South American fur seals analysis. In a time of economic crisis, where science, technology and education are suffering drastic budget reductions by the governmental Brazilian agencies (the investments in these areas were frozen by 20 years), grants like the SMM Small Grants-in-Aid of Research are very stimulant, keeping alive our seek for make science and for help the conservation of marine mammals.