

DiSVA, Laboratorio Biologia Evolutiva

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INTRODUCTION

Nowadays, NGS techniques are essential for exploring new genetic and evolutionary features of nonmodel organisms, the study of which involves several difficulties including limited samples availability and lack of baseline information. Risso's dolphin (*Grampus griseus* Cuvier, 1812) is a non-model, highly

MATERIAL AND METHODS

GBS libraries were prepared by digestion with **BamHI/NsiI** enzymes (10 U, 37°C, 1 h), then thermally inactivated (80°C, 20 min). Fragments were ligated with T4 ligases and custom adaptors, purified with SPRI microspheres and PCR amplified

vagile organism of which little is known about population genetic structure in Mediterranean Sea and
Atlantic Ocean; hence the need for a pilot study to find the best Genotype By Sequencing (GBS)
protocol for searching and analysing informative SNPs. A double digest RAD (ddRAD) protocol was
applied to eight samples that represent the diversity of the populations in the study area (Figure 1).
(18 cycles). Indexed libraries (300-750 bp) were sequenced on Illumina
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(FIS) calculated for each individual, whose reference number is represented on the X-axis.

RESULTS

Fixation index FIS

Two samples, from the western part of the Mediterranean Sea

(18 cycles). Indexed libraries (300-750 bp) were sequenced on Illumina NextSeq2000 producing 8 million SE reads (150 bp) per sample. FASTQ files were demultiplexed (bases2fastq), cleaned of artificial adaptors with Trimmomatic software and aligned to the *G. griseus* genome with Burrows-Wheeler Aligner. The variants were called with Freebayes and filtered with Plink 1.9 keeping the chromosomal and biallelic AGCT SNPs genotyped at least 90%. A final set of **15,519** high-quality SNPs was obtained. Heterozygosity and fixation indices were calculated with Plink 1.9. The numeric order of individuals is shown in the table in **Figure 3**.



Figure 1-Samples selected to better represent possible genetic diversity in the study area. The map and point projection were constructed using QGIS 3.34.3 software.

(PD.226) and the French coast of the Atlantic Ocean (FR.11807110), were found to be mixed individuals, suggesting the possibility of gene flow between the two basins. However, given the strong negative individual fixation index (F) (Figure 2) and the low percentage of reads aligned with the reference genome, cross-contamination and possible interspecific hybridization were hypothesized. Bayesian clustering (K=2) revealed clear genetic structure: Mediterranean individuals (green) and Atlantic individuals (red), with individuals 1 and 6 showing partial admixture (Figure 3).

Principal Component Analysis (PCA) in PLINK 1.9 (via RStudio) confirmed these patterns (Figure 4): Mediterranean and Atlantic individuals clustered at opposite extremes, while admixed individuals were positioned centrally.



Figure 3- Clustering analysis K=2 made up by PopCluster. The numeric code of
specimen is shown in the legend in Figure 2. Individuals from the Atlantic are7. FR.12330079
8. FR.12012296shown in red, those from the Mediterranean Sea in green.7. With the second secon



Figure 4- Principal component analysis (PCA) based on geographic origin: individuals from the Mediterranean Sea (green) and Atlantic Ocean (red) show separation along the first principal component, suggesting genetic differentiation between the two populations.

DISCUSSION AND CONCLUSION

This pilot study confirms the presence of two distinct populations of G. griseus in the Mediterranean Sea and Atlantic Ocean, with limited gene flow probably through the Strait of Gibraltar. No T. truncatus-specific variants were detected in samples 1 and 6, excluding hybridization; many no hit reads are ascribable to bacterial contamination due to sampling from necropsies, but did not affect genetic structure analyses because only reads aligned the Risso's dolphin genome were to considered. The study demonstrates the feasibility of using GBS techniques on Risso's dolphins and the efficacy of the ddRAD protocol to identify informative SNPs, with large-scale applications for potential genotyping and study of genomic diversity of

the species.

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