

SC/68A/SH/08

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INTERNATIONAL
WHALING COMMISSION

Marked phylogeographic differentiation of sei whale based on mitochondrial DNA analyses from Northern and Southern Hemisphere populations

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ABSTRACT

The sei whale, *Balaenoptera borealis*, presents a disjointed geographic distribution, with populations that are separated either by continental landmass, as in the Northern Hemisphere, or by the Intertropical Convergence Zone between hemispheres. Such distribution, together with patterns of seasonal migration, could result in strong phylogeographic structure. Significant genetic divergence between sei whale from the North Atlantic and North Pacific Oceans has been reported, however, limited samples from the Southern Hemisphere precluded a global analysis. The largest whale mass mortality event ever recorded for this species occurred in southern Chile, with at least 340 dead whales. This has become the largest source of samples for the species in the SH. Here we evaluate the population structure between sei whale populations, comparing the North Pacific $n = 27$, the North Atlantic $n = 86$ and the Southern Hemisphere ($n = 91$, including 79 bones from the Chile mass mortality, $n = 16$ reported previously as “Southern hemisphere” and $n = 2$ reported previously for South Atlantic), and the South Atlantic ($n = 2$). At a local level, mitochondrial DNA control region analyses from South Pacific recovered 32 haplotypes, eighteen of which are shared by two or more individuals. High values of haplotype diversity ($h = 0.97$) and nucleotide diversity ($\pi = 0.95\%$) were found. At a global scale, phylogeographic analyses showed a strong genetic differentiation between the Southern Hemisphere and both North Atlantic ($\Phi_{ST} = 0.69$, $p = 0.001$) and North Pacific ($\Phi_{ST} = 0.32$, $p = 0.001$), and a possible migration event from South Pacific to North Atlantic. Together with other recent studies, our results point to a marked phylogeographic differentiation among sei whale in the North Pacific, North Atlantic and Southern Hemisphere, which reflect the existence of three major population units. These results need to be complemented by more samples from other locations within the Southern Hemisphere and the use of nuclear markers.

INTRODUCTION

Species background

The sei whale, *Balaenoptera borealis*, occurs disjoint populations in the North Atlantic, North Pacific and Southern Hemisphere (Rice 1998) with populations that are separated either by continental landmass, as in the Northern Hemisphere, or by the Intertropical Convergence Zone between hemispheres. Population genetic studies of the sei whale are very limited mostly in Southern Hemisphere where no data or analyses of the sei whale population genetic structure within the Southern Ocean have been presented so far (Huijser et al 2018). In the Northern Hemisphere, a pioneer study of Wada and Numachi (1991), analyzed the stock structure of sei whales in the North Pacific based on three polymorphic allozyme loci, reporting no evidence of temporal and spatial genetic heterogeneity, suggesting the existence of a single stock in the area. Later, Kanda et al. (2009) confirmed the results of the previous study demonstrating no evidence of genetic population differences with both microsatellite and mtDNA analysis, indicating that a single stock of sei whales exists in the offshore open water of the North Pacific. A genetic differentiation between North Pacific and North Atlantic populations has been proposed (Baker et al 2004) and recently confirmed (Huijser et al 2018).

In the Southern Hemisphere, sei whales occur mainly between the Subtropical Convergence (40°S) and the Antarctic Convergence (50°S), although some adults have been reported south of the Antarctic Convergence during the austral summer (Gambell, 1974). Whaling exploitation history of this species in the Southern Hemisphere was important. The species was exploited off Brazil, Chile, Peru, South Africa, and South Georgia (Mackintosh 1965, Zerbini et al., 1997; Aguayo-Lobo et al., 1998). An approximate estimation of population decline, based on Japanese catcher and scouting vessels, was from about 64,000 individuals in 1960 to about 11,000 by 1979 (Horwood 1987). The greatest catches exceeded 5,000 per year during 1960–72 with nearly 20,000 in 1964. The IWC (1996) gave a total population estimate for sei whales of about 10,000 south of 30°S. Currently there is not an updated estimation of the total population and the recovery of sei whale stocks after the moratorium on whaling remain mostly unknown (Reeves et al., 2004) while and no recovery of sei whales in Brazilian waters have been detected since that time (Zerbini et al. 1997). In Chilean waters, this whale was the third highest whaling target with at least 1,600 Sei whales caught, with the highest catches off the northern and central coasts of Chile (Aguayo-Lobo et al., 1998).

The recent whale mortality episode (MME) occurred in Golfo de Penas, Chile (ca. 46°30'S Hausermann et al 2017), became the largest source of information for the species in the Southern Hemisphere with over 300 individuals. Therefore, it is a great and unique opportunity to address questions regarding genetic diversity and population structure of the species, and the dynamics of population demography considering historical and contemporary temporal framework. The results should have implications on systematics and conservation of the species comparing genetic information from the Southern and Northern hemisphere.

Here we report (1) a genetic characterization (species identification, genetic diversity and sex identification) of 79 samples taken from MME Gulf of Penas, Southern Chile, and also (2) we evaluate the population structure among sei whale populations, comparing the North Pacific, North Atlantic and Southern Hemisphere

MATERIAL AND METHODS

Southeastern Pacific genetic data

Between 2015 and to date, we have collected a total of 210 samples (160 bone samples, 50 skin samples) of dead whales from the MME at Gulf of Penas. To date we have been able to analyze 79 samples). The skin samples were collected during expeditions undertaken by the Chilean Navy during several weeks after the stranded whales were found. Most bone samples have been collected during private expeditions to the area afterwards (under the Patagonia Project)

DNA from skin samples has been successfully extracted following the salt extraction methods (Aljanabi & Martínez 1997). DNA from bone samples has been extracted using a forensic protocol (Tebbutt et al. 2000), which has proven successful to amplify mtDNA and nuDNA (see below).

A fragment of the mitochondrial DNA control region (Dloop) has been amplified using the primers reported by Dalebout et al. (2005). The taxonomic identity (species level) of sequences have been assessed by comparing with the data bases (1) "Basic Local Alignment Search Tool (BLAST, www.blast.ncbi.nlm.nih.gov) and (2) DNA Surveillance (Ross et al. 2003, <http://www.dna-surveillance.auckland.ac.nz/>). The totality of the samples analyzed to date have been identified as sei whales.

North Pacific, North Atlantic and South Atlantic genetic data

Together with the sequences obtained from dead whales of the Golfo de Penas MME (79 mtDNA control region sequences) we comprise a total of 204 sequences (387pb) for a global phylogeographic comparison.

The data set include sequences from North Pacific $n = 27$ (Baker et al 2015), North Atlantic $n = 86$ (Huijser et al 2018, Baker et al 2015), Southern Hemisphere ($n = 91$, including 79 bones from the Chile mass mortality, $n = 16$ reported previously as "Southern hemisphere" and $n = 2$ reported previously for South Atlantic, Baker et al 2015)

Genetic analysis

Genetic diversity indices segregative sites (k), number of haplotypes (h), haplotypic diversity (H_d), mean number of differences between two random sequences (Π) and nucleotide diversity (π), together with analyses of genetic structure (F_{ST}) and phylogeographic structure (Φ_{ST}) were conducted in Arlequin v3.5.2 (Excoffier and Lischer, 2010) with 1000 permutations and a significance level of 0.05.

A haplotype network was constructed in Hapview, a software that uses phylogenetic trees to construct haplotype genealogies. Previously, a Neighbour-Joining tree was constructed in MEGA v7 (Kumar et al., 2016) to serve as input file for the reconstruction of the haplotype network.

RESULTS AND COMMENTS

At a local level, mitochondrial DNA control region analyses from South Pacific recovered 32 haplotypes, eighteen of which are shared by two or more individuals. High values of haplotype diversity ($h = 0.97$) and nucleotide diversity ($\pi = 0.95\%$) were found. Sex identification analysis show a total of 35 males, 6 females and 13 without sex identification.

From a global phylogeographic overview three haplogroups were recognizable in the resulting haplotype network (Figure 1). A North Atlantic haplogroup composed by seven haplotypes ($h = 0.68$) and ($\pi = 0.27\%$), a North Pacific haplogroup (18 haplotypes, $h = 0.94$ and $\pi = 1.1$) and a Southern hemisphere group with 35 haplotypes ($h = 0.97$) and ($\pi = 1.6\%$). A share haplotype between North Atlantic and Southeastern Pacific hemisphere suggest a possible migration event from South Hemisphere to North Atlantic since the haplotype sampled in the North Atlantic belong genetically to South Hemisphere haplogroup. Two haplotypes sampled in South

Pacific Ocean belong genetically to the North Pacific, although in this case it may just reflect unsorted lineages process and not necessary migration events from north to south.

Phylogeographic structure analyses showed a strong genetic differentiation between the Southern Hemisphere and both North Atlantic ($\Phi_{ST} = 0.69$, $p = 0.001$) and North Pacific ($\Phi_{ST} = 0.32$ $p = 0.001$).

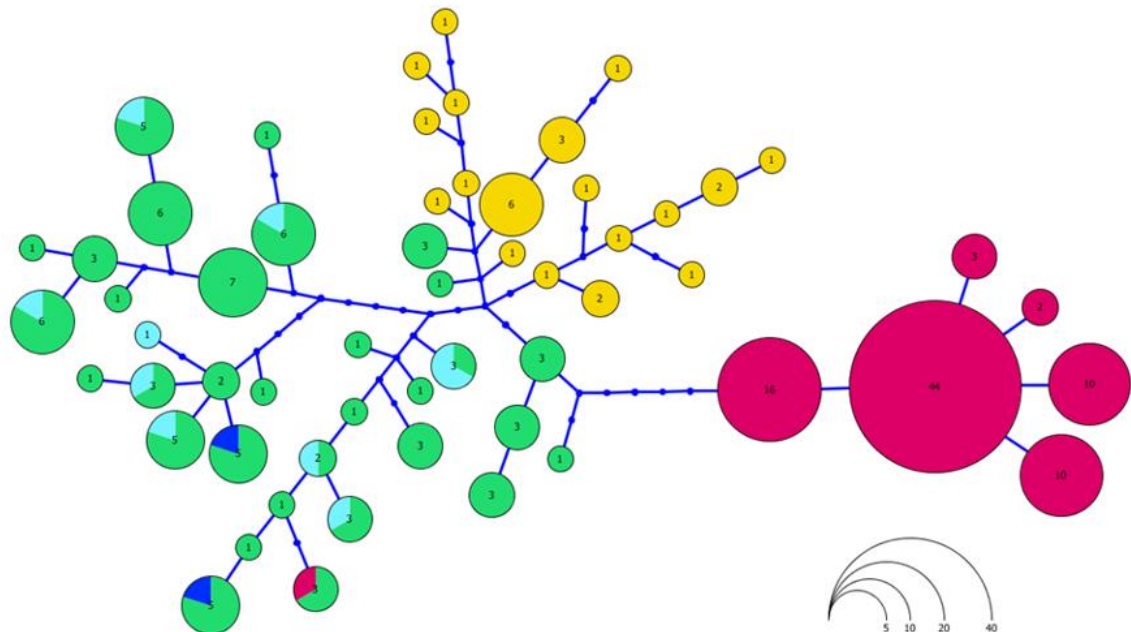


Figure 1: Haplotype network of the sei whale sequences MtDNA (Dloop) from North Atlantic (red) North Pacific (yellow) and Southern Hemisphere (green: Gulf of Penas southern Chile, light blue: sequences from Baker et al 2015 coded as “Southern hemisphere” and blue: sequences from Baker et al 2015 coded as South Atlantic)

CONCLUDING REMARKS

- The totality of the samples collected from the mass mortality event occurred in the Gulf of Penas, Southern Chile (79 whales) has been genetically identified as sei whale, *Balaenoptera borealis*
- The sei whale population from the Gulf of Penas, Southern Chile show high levels of genetic diversity and sex ratio biased to males
- In contrast with North Hemisphere, sei whales do not show evidence of phylogeographic structure in the Southern Ocean
- A possible migration event from South Pacific to North Atlantic is suggested
- Our results point to a marked phylogeographic differentiation between sei whale in the North Pacific, North Atlantic and Southern Hemisphere, which may reflect the existence of three major population units. However, unsorted lineage process and rare dispersal events do not allow to define genetically these populations as different ESU
- These results need to be complemented by more samples from other locations within the Southern Hemisphere and the use of nuclear markers.

ACKNOWLEDGMENTS

We thanks all the colleagues that assisted the collection of samples during Chilean Navy surveys. to Gulf of Penas .We thank Keri Lee Pashuk, Greg Landreth and all the crew from vessel Saoirse for collecting bone samples during their expeditions to Gulf of Penas under Patagonia Project. Biopsy samples were collected under permit from Undersecretariat of Fisheries and Aquaculture, Ministry of Economy of Chile Permits 1502/2013, 1803/2015 and 2982/2015. This study was financially supported by CONICYT Postdoctoral FONDECYT Program 3140513, CONICYT Iniciación 11170182, FONDECYT 1140548, Projects ICM P05-002 and PFB 023 and CONICYT III Fortalecimiento Puente R16A10003.

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