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Progress report: Genetic analysis of population structure and subspecies taxonomy of blue whales

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BACKGROUND

The taxonomy of blue whales remains poorly resolved. Currently, three diagnosable subspecies of blue whales are recognized, with Antarctic (*Balaenoptera musculus intermedia*) and pygmy (*B. m. brevicauda*) blue whales found in the Southern Hemisphere and *B. m. musculus* distributed in the North Atlantic and North Pacific (Rice 1998). In addition, pygmy-type blue whales in the northern Indian Ocean are considered a separate subspecies (*B. m. indica*) on the basis of differences in total length and reproductive seasonality (Perrin *et al.* 2009, SMM Committee on Taxonomy 2014). A fifth un-named subspecies of pygmy-type blue whale has been recognized in the eastern South Pacific based on whaling record data demonstrating that these whales are intermediate in length between the Antarctic whales and the pygmy-type whales in the Indian Ocean (Branch *et al*. 2007; SMM Committee on Taxonomy 2014).

Genetic analyses of blue whale population structure have largely been confined to studies in the Southern Hemisphere (LeDuc *et al*. 2007; Attard *et al.* 2010, 2012, 2015; Sremba *et al.* 2012; Torres-Florez *et al*. 2014). Results of these studies have indicated that pygmy-type blue whales in the eastern South Pacific (ESP) are as different from pygmy-type blue whales in the Indian Ocean (IO) as either is to the Antarctic blue whales (LeDuc *et al*. 2007), supporting previously findings of acoustic and morphological differences between these three groups (McDonald *et al.* 2006, Branch *et al.* 2007). Genetic similarities have also indicated that the blue whales off Chile and those sampled off the coasts of Peru and Ecuador belong to the same population (Torres-Florez *et al.* 2014).

WORK IN PROGRESS

Currently, multiple projects are in progress to integrate genetic data derived from blue whales in the North Pacific with that produced on Southern Hemisphere blue whales. These projects include:

1) *Assessing genetic variation among eastern Pacific blue whales using mtDNA control region sequencing (420 bps) and microsatellite genotypes (n=7 loci*)

This work builds on the analyses described in LeDuc *et al.* 2007, using an expanded sample set from the Southern Hemisphere (n = 78, Antarctic; n = 66, IO; and n= 66, ESP) and adding samples from the eastern Tropical Pacific (ETP, n=46) and the ENP (n=51). Data analysis for this project has been completed, and a manuscript will soon be submitted for publication. Within the Southern Hemisphere, the results support the previous conclusions demonstrating that ESP blue whales are as different from IO blue whales as either is to the Antarctic. However, although significant differences in both mtDNA and nuclear data were detected between the ESP and the ENP, the differences were relatively modest and markedly lower than the degree of differentiation seen in comparisons of the ESP with the Antarctic and Indian Ocean. In addition, while the Antarctic and Indian Ocean strata share no more than two haplotypes with any other stratum, five haplotypes are shared between the ENP and the ESP. Finally, this study examined the genetic similarity of blue whales in the northern (n=21 samples collected near the Costa Rica Dome region) and southern (n=25 samples collected in the waters of Peru and Ecuador) portions of the ETP. As seen in Torres-Florez *et al.* (2014), the results indicated that whales sampled in the ESP are more similar to those in the southern ETP, while whales sampled in the ENP are more similar to those sampled in the northern ETP. Given acoustic evidence indicating that ESP whales are present in all seasons (Buchan *et al*. 2014), it seems likely that the ETP is occupied, at least seasonally and perhaps year-round, by blue whales from both the

Northern and Southern hemispheres.

2) *Mitogenomic analysis of population structure and subspecific taxonomy of blue whales at a global scale*

In recent years, the rapid development of next generation sequencing technologies has made it possible to generate orders of magnitude more data than was previously possible. Here we propose to conduct a comprehensive assessment of blue whale population structure and taxonomy by sequencing the entire mitogenome of blue whales sampled throughout most parts of the species' range. This approach, which will generate >16x more data than traditional mtDNA control region analysis, has been shown to substantially improve taxonomic resolution when utilized in other cetacean species (e.g., killer whales, Morin *et al*. 2010; fin whales, Archer *et al.* 2013). This mitogenomic analysis will focus on examining phylogenetic relationships as well as evaluating the degree and timing of divergence between blue whales from different regions. Approximately 300 samples collected from blue whales in the central and eastern North Pacific, eastern South Pacific, eastern Tropical Pacific, Indian Ocean, and Antarctic waters will be included in this project. These samples were selected to maximize geographic coverage of the species, although it was not possible to identify available samples in some portions of the historic range (most notably, the South Atlantic). Mitogenome library preparation will follow a modified capture array method, as outlined in Hancock-Hanser *et al*. (2013). Three customized capture arrays were designed using a published sequence of the blue whale mitogenome. Libraries are indexed to allow identification of individuals and are then pooled in equimolar amounts and hybridized to the capture arrays for enrichment of the targeted sequences. Library sequencing is conducted on an Illumina Genome Analyzer at the DNA Array Core Facility of the Scripps Research Institute in La Jolla, CA. Samples included on the first two arrays (~200 samples total) have been sequenced, and the data is currently being analyzed. We expect to submit the third library for sequencing in summer 2015, and a report will be submitted to the SC in 2016.

3) *Nuclear analysis of population structure and taxonomy based on genotype by sequencing of ~300 Single Nucleotide polymorphisms (SNPs)*

Subsequent to the design of the capture arrays used in the above approach, a collaborative project (Phil Morin, SWFSC and Life Technologies, the Salk Institute, and the University of Maryland) to sequence the blue whale genome was initiated. While the genome sequence is not yet complete, this work has produced sufficient sequence data to allow a custom SNP panel to be designed that will allow for genotyping-by-sequencing of ~300 to 350 Single Nucleotide Polymorphisms (SNP) loci in ~270 samples. Genotyping will be conducted by Floragenex (www.floragenex.com). The samples included on the mitogenomics array will be used where possible. This protocol relies on PCR-based target selection and high level multiplexing, and requires less DNA to be used than that required by other methods. In most cases, it is expected that a single DNA extraction will provide sufficient DNA for both sequencing approaches, thereby avoiding further tissue sample depletion due to multiple extractions. This analysis of nuclear markers will allow assessment of patterns of reproductive isolation between regions, thereby complementing the results of the mitogenome analysis. The results, if concordant with those of the mitogenome analysis, will provide an additional line of evidence supporting subspecies and/or Distinct Population Segment designation. Genotyping will be completed in summer 2015, and a report will be submitted to the SC in 2016.

COLLABORATORS:

Collaborators and sample contributors in this project include: J. Bannister, Western Australia Museum; J. Calambokidis, Cascadia Research Collective; R. Hucke-Gaete and J. P. Torres-Florez of the Universidad Austral de Chile and the Centro Ballena Azul; K. van Waerebeek, Peruvian Centre for Cetacean Research and the Musée de la Mer de Gorée in Senegal, the Southern Ocean Research Partnership; the International Whaling Commission; and Barb Taylor, Phil Morin, Karen Martien, Eric Archer and Bob Brownell of the SWFSC.

LITERATURE CITED:

Archer F.I., Morin P.A., Hancock-Hanser B.L., Robertson K.M., Leslie M.S., Berube M., Panigada S., Taylor B.L. 2013. Mitogenome phylogenetics of fin whales (*Balaenoptera physalus*): Genetic evidence for revision of subspecies. PLOS One 8:e63396.

Attard C.R.M., Beheregaray L.B., Jenner C., Gill P., Jenner M., Morrice M., Bannister J., LeDuc R., Moeller L. 2010. Genetic diversity and structure of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations. Conservation Genetics 11:2437-2441.

Attard C.R.M., Beheregaray L.B., Jenner K.C.S., Gill P.C., Jenner M.-N., Morrice M.G., Robertson K.M., Moeller L.M. 2012. Hybridization of Southern Hemisphere blue whale subspecies and a sympatric area off Antarctica: impacts of whaling or climate change? Molecular Ecology 21:5715-5727.

Attard C.R.M., Beheregaray L.B., Jenner K.C.S., Gill P.C., Jenner M.-N.M., Morrice M.G., Teske P.R., Möller L.M. 2015. Low genetic diversity in pygmy blue whales is due to climate-induced diversification rather than anthropogenic impacts. Biology Letters, DOI: 10.1098/rsbl.2014.1037.

Branch T.A., Abubaker E.M.N., Mkango S., Butterworth D.S. 2007. Separating southern blue whale subspecies based on length frequencies of sexually mature females. Marine Mammal Science 23:803-833.

Buchan, S. J., Stafford, K. M. and Hucke-Gaete, R. 2014. Seasonal occurrence of southeast Pacific blue whale songs in southern Chile and the eastern tropical Pacific. *Marine Mammal Science* doi: 10.1111/mms.12173.

Hancock-Hanser B.L., Frey A., Leslie M.S., Dutton P.H., Archer F.I., Morin P.A. 2013. Targeted multiplex next-generation sequencing: advances in techniques of mitochondrial and nuclear DNA sequencing for population genetics. Molecular Ecology Resources 13:254-268.

LeDuc R.G., Dizon A.E., Goto M., Pastene L.A., Kato H., Nishiwaki S., LeDuc C., Brownell Jr. R.L. 2007. Patterns of genetic variation in Southern Hemisphere blue whales and the use of assignment test to detect mixing on the feeding grounds. Journal of Cetacean Research and Management 9:73-80.

McDonald M.A., Mesnick S.L., Hildebrand J.A. 2006. Biogeographic characterisation of blue whale song worldwide : using song to identify populations. Journal of Cetacean Research and Management 8:55-65.

Morin P.A., Archer F.I., Foote A.D., Vilstrup J., Allen E.E., Wade P., Durban J., Parsons K., Pitman R., Li L., Bouffard P., Nielsen S.A., Rasmussen M., Willerslev E., Gilbert M.T.P., Harkins T. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Research 20:908-916.

Perrin W.F., Mead J.G., Brownell Jr. R.L. 2009. Review of the evidence used in the description of currently recognized cetacean subspecies. NOAA Technical Memo NOAA-TM-NMFS-SWFSC-450. 35 pp.

Rice D.W. 1998. Marine mammals of the world: systematics and distribution. Society for Marine Mammalogy, Lawrence, KS. 231 pp.

SMM Committee on Taxonomy. 2014. List of marine mammal species and subspecies. Society for Marine Mammalogy, www.marinemammalscience.org, consulted on 15 May 2015.

Sremba A.L., Hancock-Hanser B., Branch T.A., LeDuc R.L., Baker C.S. 2012. Circumpolar Diversity and Geographic Differentiation of mtDNA in the Critically Endangered Antarctic Blue Whale (*Balaenoptera musculus intermedia*). Plos One 7.

Torres-Florez J.P., Hucke-Gaete R., LeDuc R., Lang A., Taylor B., Pimper L.E., Bedriñana-Romano L., Rosenbaum H.C., Figueroa C.C. 2014. Blue whale population structure along the eastern South Pacific Ocean: evidence of more than one population. Molecular Ecology 23:5998-6010.