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SHORT COMMUNICATION

Molecular Genetic Identification of Beached Whales in Sri Lanka from Mitochondrial DNA Sequence Data

Ranil P. Nanayakkara[1,4,5], H.M.J.C.B. Herath[1,2,3], Ruvinda K. De Mel[1] and T.G. Tharaka Kusuminda[1*]

[1] BEAR (Biodiversity Education and Research), No: 4, Dr. E.W. Adikaram Mawatha, Pita-Kotte, Sri Lanka.
[2] Postgraduate Institute of Science, P.O. Box: 25, Peradeniya, Sri Lanka.
[3]Department of Biology and Ecology, Faculty of Medicine, South Asian Institute of Technology and Medicine (SAITM), Millenium Drive, New Kandy Road, Malabe, Sri Lanka.
[4] Department of Zoology, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka.
[5] National Aquatic Resources Research and Development Agency, Crow Island, Colombo 15, Sri Lanka.
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ABSTRACT

In the current study we attempt to identify eight baleen whale carcasses that were washed ashore to the Western, Northwestern and Southern coasts of Sri Lanka in 2010, using molecular phylogenetic techniques. Initial physical examination suggested that these carcasses belonged to blue whales (*Balaenoptera musculus*). Analysis of sequences of the mitochondrial control region from baleen whales confirmed that the samples belonged to blue whales (*Balaenoptera musculus*). However, it was impossible to identify the population of blue whales the individuals belonged to, due to the lack of strong population genetic signals in the mitochondrial control region sequences.

Keywords: *Balaenoptera musculus*, Blue Whale, Indian Ocean, phylogenetics

INTRODUCTION

Stranding of marine mammals is a frequent occurrence around the coastal belt of Sri Lanka, yet precise identification is impossible due to the rapid decomposition or due to the lack of local taxonomic expertise in the area. Since all marine mammals are important from the conservation point of view, the precise documenting of their presence would provide valuable information regarding the distribution and migratory nature of different species in the littoral waters around Sri Lanka. Since of late, DNA barcoding or sequencing of mitochondrial genes have been used to indentify species and subspecies of marine mammals, and to which subpopulations these beings belong to.

To date, five species of rorquals (baleen whales) are deemed to occur in the seas around Sri Lanka (Illangakoon, 2002). Amongst the rorquals that inhabit the waters around Sri Lanka, the blue whale is the most abundant and widely distributed and they are found throughout the year in the island's waters; hence the notion of resident populations. These whales are concentrated in areas of high productivity around the island, especially in the southern and eastern coast of the island where constant upwellings are experienced due to the deep sea canyons in these areas. Absolutely nothing is known about the origins and population level relationships of the Sri Lankan or northern Indian Ocean blue whales. As an initial attempt we endeavor to (1) determine the identity of the eight carcasses of beached blue whales that were washed ashore to the Western, Northwestern and Southern coasts of Sri Lanka in 2010 using mitochondrial DNA sequence analysis and (2) determine the population level relationships of the Sri Lankan blue whales using phylogeographic methods.

MATERIALS AND METHODS

Sample Collection and DNA amplification

Tissues samples from the muscles were collected from the eight beached Blue Whale carcasses from Hikkaduwa, Galle, Kahawa, Matara in the Southwestern coast, Dehiwala, Wellawatta,

*Corresponding author's email: t.kusuminda@gmail.com

Negambo in the Western coast and Thalawila in the Northwestern coast of Sri Lanka. A ~580 base pair fragment of mitochondrial control region was used to accurately identify the carcasses of the eight beached whales to the species level. The mitochondrial control region is a highly polymorphic non coding region of the mitochondrial genome with polymorphism concentrated in hypervariable regions. The region was amplified from extracted genomic DNA using primers H16498 (5'-CCTGAAGTAAGAACCAGATG-3') and L15812 (5'-CCTCCCTAAGACTCAAGGAAG-3') (Rosel *et al*., 1994). The PCR amplification cycle consisted of an initial denaturation step for 2 minutes at 94 ºC, followed by 35 cycles of 30 seconds at 94 ºC, 30 seconds at 54 ºC and 40 seconds at 72 ºC and a final extension step for 2 mintues at 72 ºC. The PCR amplification success and sizes of the products were determined by agarose gel electrophoresis with a 1 kb DNA ladder. DNA was successfully amplified only from two specimens (specimens from Hikkaduwa and Talawila) of the eight samples. Purified PCR products were sequenced at the Genetech Pvt. Ltd. Colombo. Sequences of the forward and reverse reads were aligned using the Geneious Pro 5.5 software (Drummond *et al*., 2009) Additional sequences of the mitochondrial control region that were generated in previous studies were downloaded from Genbank (http://www.ncbi.nlm.nih.gov/genbank/). The sequences generated in this study are deposited in the Genbank under the accession numbers HQ456249-HQ456250.

Phylogenetic analyses

To accurately identify the species of the two beached carcasses, phylogenetic reconstruction methods were used. Bayesian and likelihood methods were used to reconstruct phylogenetic trees using the mitochondrial sequences. We used sequences from all extant Baleen Whales (downloaded from the genbank) and used the Sperm whale (*Physator catodon*) as an outgroup since it is well documented that Baleen whales and toothed whales are reciprocally monophyletic groups (Jackson, 2010). jModelTest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) was used to determine the best-fit substitution models for the different partitions and GTR+g was selected as the best-fit substitution scheme. Bayesian analyses were conducted using the software BEAST v1.6.2 (Drummond and Rambaut, 2007) and Maximum likelihood (ML) analyses were performed using RAxML v7.2.6 (Stamatakis, 2006). The Bayesian analysis of the species identification was run for 8 million generations sampling every 1000 generations. The Bayesian analysis of the population genetic identification was run for 12 million generations sampling every 1000 generations using *Balaenoptera physalus* as an outgroup. Convergence was assessed by examining effective sample sizes (ESS values >>100) and likelihood plots through time in TRACER v1.5 (Drummond and Rambaut, 2007), with the first 25% of trees discarded from each run as burn-in. The BEAST maximum credibility trees were summarized in TreeAnnotator v1.6.2 (distributed with BEAST package). Maximum Likelihood analyses were implemented under the GTR+g substitution model performing 200 independent ML searches. Branch support was estimated by 1000 bootstrap searches. DNA polymorphism statistics were calculated using DnaSP 5.0 (Librado and Rozas, 2009) and corrected (HKY) genetic distances between taxa were calculated in Geneious Pro 5.4.

RESULTS

Bayesian (Posterior probability = 0.99) and Maximum likelihood (Bootstrap support = 80) analyses confirmed that the carcasses that were washed ashore in Sri Lanka were of Blue Whales (*B. musculus*) (Figure 1). The genetic divergence between positively identified *B. musculus* and the two samples from Sri Lanka ranged between 1.17-1.91%.

However, population genetic assignment using the mitochondrial-control region sequences failed due to the lack of strong population genetic signals in the mitochondrial coding region sequences used in the current study. As a result, Bayesian and likelihood analysis recovered trees with weekly supported nodes. There was no distinct phylogeographic pattern observed in the blue whale mitochondrial-coding region sequences (Figure 2). However, the sequence analyses indicated that the specimen beached in Thalawila, Sri Lanka was genetically closely related to a blue whale from Mandapan, Tamil Nadu, India. However, this relationship was not strongly supported (bootstrap value<70, Posterior probability<0.7).

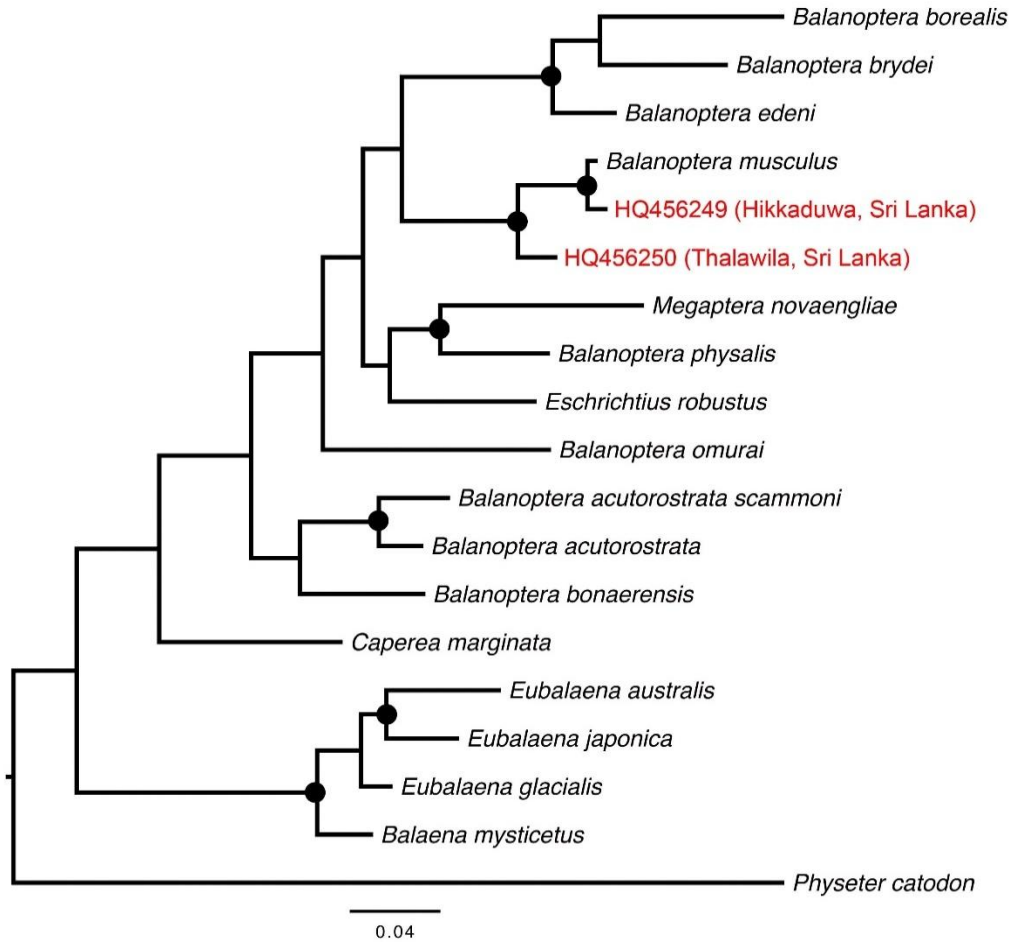

Figure 1. BEAST maximum credibility tree based on the mitochondrial coding region showing the phylogenetic relationships of the two beached whale specimens from Sri Lanka. Nodes with maximum likelihood bootstrap support >70 (above) and Bayesian posterior probability >0.9 (below) are indicated with a dark circle. Scale bar indicates the number of nucleotide substitutions per site.

The sequence of the specimen from Hikkaduwa, Sri Lanka was distantly related to these two sequences and was placed in the clade that consisted of sequences from individuals from the Indian Ocean, Southern Ocean and the Antarctic. Again, these relationships were not strongly supported.

DISCUSSION

Molecular genetics are being increasingly used in identifying unidentifiable species. The practice termed DNA barcoding has now expanded to identifying illegal wildlife products as well as unidentifiable animal parts (Wildlife Forensics). One of the most widely used applications of DNA barcoding is the identification of illegally harvested marine mammal (mainly Whales and Dolphins) meat (Baker *et al*., 2008) and identification of beached cetaceans (Bijukumar *et al*., 2012). In the current study, this method was used to identify two beached Blue Whale carcasses in Sri Lanka. Further, attempts were made to identify the putative population the individual samples belonged to, since it is well documented that populations could be identified from DNA sequences (especially mitochondrial DNA) (Avise, 2000). The genetic analyses suggested that the two samples were from Blue Whales (*Balaenoptera musculus*) indicating that our initial identification of the carcasses based on external morphology was accurate. Currently, three subspecies of the blue whales are recognized in the world, based on external morphology (Rice, 1998). The subspecies found around the Sri Lankan waters is considered to be that of Pygmy Blue Whales (*B. m. brevicauda*) (Alling *et al*., 1991; Anderson, 2005).

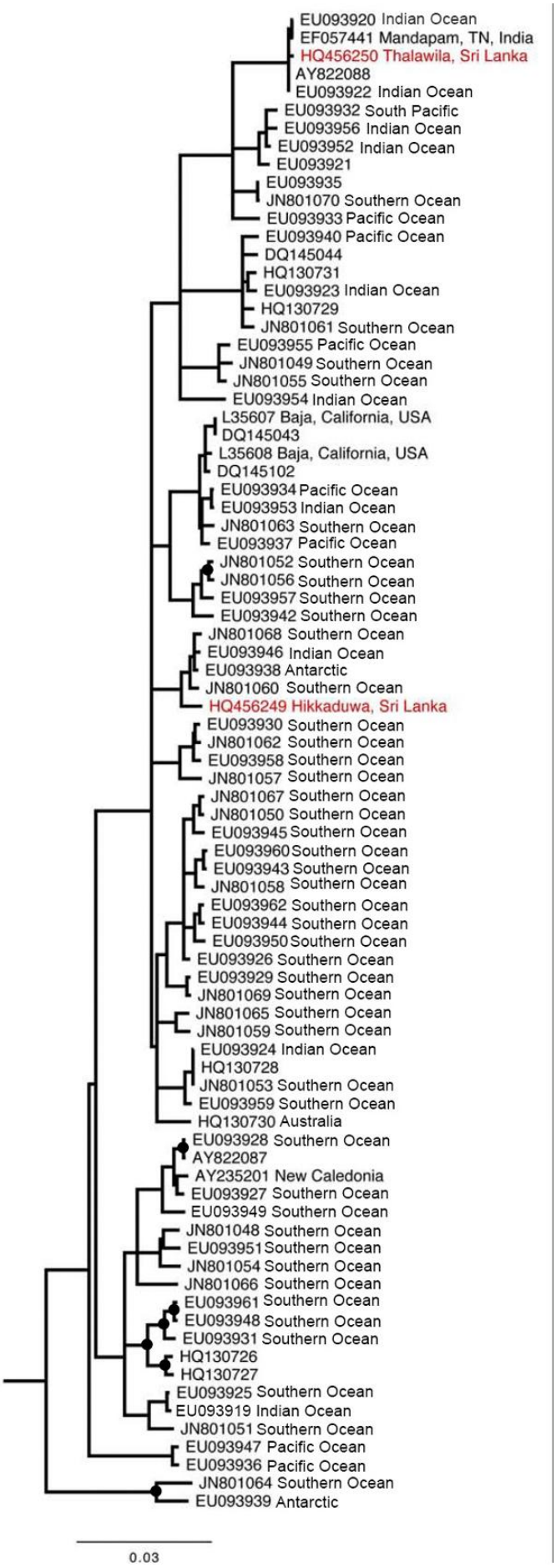

Figure 2. BEAST maximum credibility tree of the mitochondrial coding region showing the relationships of the two beached whale specimens from Sri Lanka with other sampled *B. musculus* specimens from the Pacific, Indian and Southern Oceans. Outgroup *B. physalis* (Fin Whale) is not shown. Scale bar indicates the number of nucleotide substitutions per site.

However, previous studies have not provided molecular biological evidence for the distinction of these three subspecies. Further, earlier studies have also shown that there is no phylogeographic distinction in the mitochondrial control region between Antarctic and 'non-Antarctic' blue whales (Leduc *et al*., 2007; Sremba *et al*., 2012). Similarly, our study also failed to distinguish populations of the blue whales and determine the population to which the two individuals belong, using the mitochondrial control region sequences. It is not known whether the lack of population structure observed in blue whales in this study as well as previous studies (Leduc *et al*., 2007; Sremba *et al*., 2012) is due to contemporary gene flow between the populations or whether it is due to fairly recent isolation of the populations. Although there was no phylogeographic structure among the blue whales, our results support the findings of Leduc *et al*. (2007) that the Indian Ocean blue whale haplotypes are divergent from the Antarctic and Pacific Ocean blue whales. However, microsatellite markers have shown to be more successful in accurately differentiating between *B. musculus* populations (Leduc *et al*., 2007; Sremba *et al*., 2012). Nevertheless, the lack of population structure among the samples does not indicate that there is contemporary gene flow between the Blue whale populations since the haplotypes could be shared even with complete separation of the populations, due to incomplete lineage sorting (Leduc *et al.*, 2007). However, the results of the current study reiterate the need for a reexamination of the blue whale taxonomy (Leduc *et al*., 2007)

Using combined morphological, ecological and genetic methods, establishing the precise lineage of beached blue whales in Sri Lanka is of paramount importance with regard to establishing the ecology of the species / subspecies as well as devising conservation measures. Other molecular methods (*e.g.* microsatellite markers) could provide us with information such as which populations of blue whales migrate through Sri Lankan waters and whether there are hitherto unknown migration patterns. Therefore, studies of this nature would be important in order to understand the animals' ecology as well as its' taxonomy.

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