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Identification of a Stranded Whale by Mitochondrial DNA Analysis - www.DNA-Surveillance Program in Action

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Abstract

A whale stranded in 2003 in Colombo, Sri Lanka, was identified based on its morphological characters as Bryde's whale, *Balaenoptera brydei*. Tissue samples from the muscles were collected for sequencing of the mitochondrial control region. The obtained DNA sequence matched that of known common Bryde's whale in a large database. The morphological species identification was thus confirmed. By matching sequenced tissue samples of unidentified whales and dolphins against a reference data base specific identifications are possible.

Introduction

On 2 November 2003, a large whale was washed ashore close to the Colombo commercial harbour in Sri Lanka (longitudes 76.69 and 81.90, latitudes 5.89 and 9.86). Considering its morphological features, such as the shape of the head, shape and position of the dorsal fin, number of ridges on the rostrum, colouration and number of throat plates, the whale was identified as a Bryde's whale. Samples from this whale were taken for

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genetic analysis, as it has been a long-standing necessity in Sri Lanka to establish a method for the identification of whale and dolphin species when only meat samples are available. As the whale species was confirmed as a Bryde's whale, a genetic method that would confirm this would help in establishing a molecular method for identification of whale and dolphin species.

The web-based program DNA surveillance (Ross et al. 2003) has been implemented for the identification of whales, dolphins and porpoises within the order Cetacea. The highly variable mitochondrial DNA control region sequences have been used for this purpose (Baker et al. 2003).

Materials and Methods

Samples collected from the muscle were preserved in 90% alcohol. The DNA extraction was carried out by using a standard phenol/chloroform protocol (Sambrook et al. 1992). The two pairs of primers M13-Dlp1.5-L/Dlp5-H and M13-Dlp1.5-L/Dlp8G-H were used for the amplification of the mitochondrial DNA control region. The primers were generously donated to us by Dr. C.S. Baker of the University of Auckland, New Zealand. The amplification cycle consisted of an initial denaturation step for 2 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 54°C and 40 seconds at 72°C and a final extension step for 2 minutes at 72°C. The sizes of the products obtained from the PCR reaction were determined by running the products with a 1 kb DNA marker on an agarose gel.

Results

The PCR product obtained from the primer pair M13-Dlp1.5-L/Dlp5-H was approximately 550 bp (*Wh 1*) and the product obtained from the primer pair M13-Dlp1.5-L/Dlp8G-H was approximately 800 bp (*Wh 2*) (Fig. 1) (Baker et al. 1993). The PCR product obtained from the primer set M13-Dlp1.5-L/Dlp5-H (*Wh 1*) was purified using the UltraClean PCR clean up kit (Mo Bio Laboratories Inc. USA). This purified sample was then sequenced in a megaBACE 1000 (Amersham Pharmacia Biotech) automated DNA sequencer. When sequenced, *Wh 1* gave a 494 bp sequence (Fig. 2).

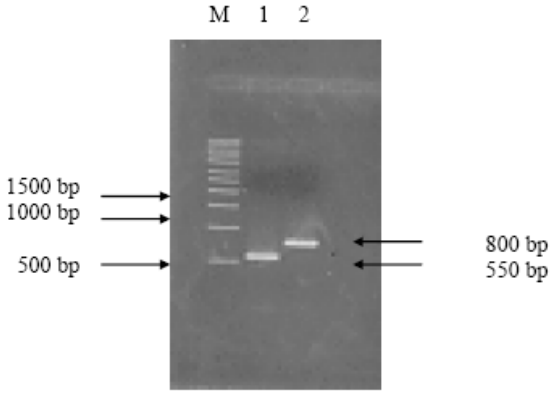

Fig. 1. PCR products obtained from the amplification of the mitochondrial control region for the specimen. (Lane M: 1 kb DNA marker, Lane 1: PCR product obtained with the primer pair M13-Dlp1.5-L/Dlp5-H and Lane 2: PCR product obtained with the primer pair M13-Dlp1.5-L/Dlp8G-H)

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  1  cgagtgaggc gattagtgat atggtctgaa gtagaaccag atgtcttata
 51  aagatcatta aatagctacc ccctacgatt gatgggcccg gtgcgagaag
101  agggatccct gccaagcggg ttgctggttt cacgcggcat ggtggttaag
151  ctcgtgatct aatggagcgg ccataagaat catttgagtg taattgacca
201  ggggatgcat aatgacatgt gctattgtac tattaataaa tattatgtaa
251  tatgtaaaat taataaaatt taatacgagc ttcaactgct cgtggtgaaa
301  ataattgaat gcacagttat acatagcatg tatatataca tccccataag
351  agaagactat tagttaagct atgggaaagt atatacatgt acaaatcaca
401  taataatatg tgacaagaca agtttttttc aatacggaca tagcactgta
451  gccttgtggt tattatcaca tatacttttc agggaatagt ttat
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Fig. 2. Sequence of the PCR product (*Wh 1*) obtained from the amplification of the mitochondrial control region using the primers M13-Dlp1.5-L and Dlp5-H.

The sequence (GenBank accession no. DQ231170) was submitted to the DNA-surveillance website (Ross et al. 2003; Baker et al. 2006) to identify the species to which the specimen belonged. The phylogenetic tree obtained (Fig. 3) showed that the 'user sequence' (*Wh 1*) is closest to the common Bryde's whale.

Further analysis was carried out with the assistance of Dr. C.S. Baker and in this analysis, *Wh1* sequence was aligned with a larger number of reference sequences. This revealed that the specimen is *Balaenoptera brydei* (Bryde's whale). This also revealed that the sample is closest to the mtDNA haplotype of two whales from South Africa.

Fig. 3. Phylogenetic tree showing the affinity of the *wh1* sequence (given as USER SEQUENCE) to the reference sequences of the DNA surveillance database.

Discussion

The use of genetic data to complement morphological identification is being widely used (Tautz et al. 2003). Genetic databases have been useful in such identifications. One such database is the DNA-surveillance web site which has been developed for the identification of cetacean species (Baker et al. 2003).

Determining the patterns of relatedness among species and genera can provide valuable information (Perrin 2004). Mitochondrial DNA analysis has been used to study different populations (Baker et al. 1993) and different species (Dalebout et al. 2002). In addition, when there is confusion as to the existence of more than one species within a given

species name, genetic studies would help in distinguishing the exact number of species present. A large number of species have been analyzed genetically and found to contain more than one species. This has been proven for whale species (Yoshida and Kato 1999) as well as for dolphin species (LeDuc et al. 1999).

Bryde's whale has first been identified as *Balaenoptera edeni*, Anderson 1878 (Cetacean Specialist Group 1996). Later in 1913 a second species had been identified as *B. brydei*, Olsen 1913 (Baker and Madon 2007). In 1950, Junge, a Norwegian scientist had concluded that *B. edeni* and *B. brydei* are one species and that the name *B. edeni* should be retained. However, in a study in 1991, Shiro Wada discovered a distinct Bryde's whale and in 1994 C.S. Baker discovered *B. edeni* in Korean markets (Roach 2003). These findings led to the belief that there was more than one species in the group known as Bryde's whale. Following this, in 2003 a new species of whale, *Balaenopte*ra *omurai* (Wada et al. 2003), commonly known as Omura's whale (Perrin and Brownell 2001), was discovered. In this study, they also concluded that there are 8 species in *Balaenoptera* and that *B. edeni* and *B. brydei* are two distinct species. They named *B. edeni* as Pygmy Bryde's whale (also called Eden's whale) and *B. brydei* as Bryde's whale. This distinction was based on the cranial morphology, the small number of baleen plates and mitochondrial DNA analysis.

Therefore, according to this nomenclature it can be confirmed that our whale specimen is a common Bryde's whale (*Balaenoptera brydei*). This method can now be used in Sri Lanka for the identification of whale meat products in the monitoring of illegal trade and hunting, and in the identification of ambiguous specimens.

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