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SHORT COMMUNICATION

A preliminary genetic study on the harbour porpoise (*Phocoena phocoena*) in the Turkish Seas

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Abstract

Mitochondrial DNA sequences of three individuals of the harbour porpoise (*Phocoena phocoena*) sampled in the Black Sea, Marmara Sea and Aegean Sea, revealed three polymorphic sites, resulting in three different haplotypes. None of the haplotypes obtained in this study clustered with those in the Atlantic populations, consistent with previous findings. The Black Sea and the Aegean haplotypes sequenced in this study had been previously detected from the Ukraine coast of the Black Sea, supporting the idea that harbour porpoises from the Black Sea dispersed into the northern Aegean through the Istanbul and Çanakkale Straits. The third sample from the Marmara Sea had the same unique haplotype as the one found in three individuals from the same sea in an earlier study. This structuring supports the possibility of an isolated population in the Marmara Sea. It should also be noted that this is the first genetic study of the harbour porpoise, in which a sample from the Turkish coast of the Northern Aegean Sea was studied.

Keyword: Harbour porpoise, *Phocoena phocoena,* mitochondrial DNA, D-loop, genetics, phylogeography

Introduction

The harbour porpoise (*Phocoena phocoena*) is a relatively widely but patchily distributed species, found in the Northern Pacific, the Northern Atlantic and the Black Sea (Bjørge and Tolley 2002). The populations in the Northern Atlantic and the Black Sea are considered to have diverged around 5500 years ago (Fontaine *et al.* 2010). In the Turkish waters it is predominantly found in the Black Sea, however its occurrence was first described as "sometimes" in the Marmara Sea and "very rare" in the Mediterranean by Devedjian (1926). There have been records of sightings and strandings in the Turkish Strait System, especially in the Istanbul Strait and the northern part of the Marmara Sea but also in the middle of the Marmara Sea and Çanakkale Strait (Dede *et al.* 2008;

Ozturk *et al.* 2009; Tonay *et al.* 2009; Dede 2010). There are two records of stranded harbour porpoises in the Turkish Aegean Sea coast; one was in Izmir Bay (Güçlüsoy 2008) and the other in Saroz Bay (Tonay *et al.* 2009). Other than these, at least nine records of strandings and sightings of harbour porpoises were reported in the Greek Northern Aegean Sea since 1993 (Frantzis *et al.* 2003; Di Sciara and Birkun 2010).

Morphological and genetic differences of Black Sea harbour porpoises were well studied and resulted in the description of a subspecies, *Phocoena phocoena relicta* (Rosel *et al.* 1995, 2003; Fontaine *et al.* 2007; 2010; Viaud-Martinez *et al.* 2007; Galatius and Gol'din 2011). Genetic signature, and the age and small size of the harbour porpoises found in the Greek coast of the Northern Aegean, suggest that these individuals originated from the Black Sea (Rosel *et al.* 2003; Viaud-Martinez *et al.* 2007). Although the Black Sea and Northern Aegean Sea harbour porpoises have identical mtDNA sequences in the hypervariable control region, suggesting connectivity, it is still possible that they may represent separate subpopulations (Notarbartolo di Sciara and Birkun, 2010). In addition, a private haplotype found in three individuals in the Marmara Sea suggest that harbour porpoises in this water body may be genetically differentiated from both the Black Sea and Northern Aegean Sea populations (Viaud-Martinez *et al.* 2007). In a subsequent study, Birkun (2008) suggested that the harbour porpoise could be containing three or more subpopulations in the Black Sea and neighbouring waters.

In this study three individuals of harbour porpoise were sampled from the Aegean Sea, Black Sea and Marmara Sea and their mitochondrial D-loop was partially sequenced. This is the first genetic study of harbour porpoise that includes a sample from the Turkish coast of the Northern Aegean. Two samples were of the individuals bycaught in Turkish Western Black Sea in 2007 (Tonay 2010) and the Marmara Sea in 2006 (Tonay *et al.* 2007) and the third one was found stranded in Saroz Bay in the Northern Aegean coast of Turkey in 2007 (Tonay *et al.* 2009) (Figure1).

The samples were collected from skin tissues and preserved in 5% dimethyl sulphoxide (DMSO) saturated with sodium chloride at 20 °C. DNA was extracted using Roche High Pure PCR Template Preparation Kit (Indianapolis, USA) following the manufacturer's protocol. The two primers described by Rosel *et al.* (1999), L15824 (5'-CCTCACTCCTC CCTAAGACT-3') and H16265 (5'-GCCCGGTGCGAGAAGAGG-3') were used to amplify a partial 364 base pair-long mitochondrial DNA (mtDNA) fragment of the control region. PCR reactions were performed in a 25 µl volume containing approximately 20 ng genomic DNA, 2.5µl 10X Taq buffer, 2 mM of $MgCl_2$, 0.4 mM of each primer, 0.8 mM deoxyribonucleotide triphosphate and 1 U Taq DNA polymerase (Fermentas). The PCR program was as follows: an initial denaturation step at 94°C for 2 minutes, followed by 35 cycles of 45 seconds at

94°C, 1 minute at 64 °C, 1 minute at 72°C, with a final extension step of 5 minutes at 72°C.

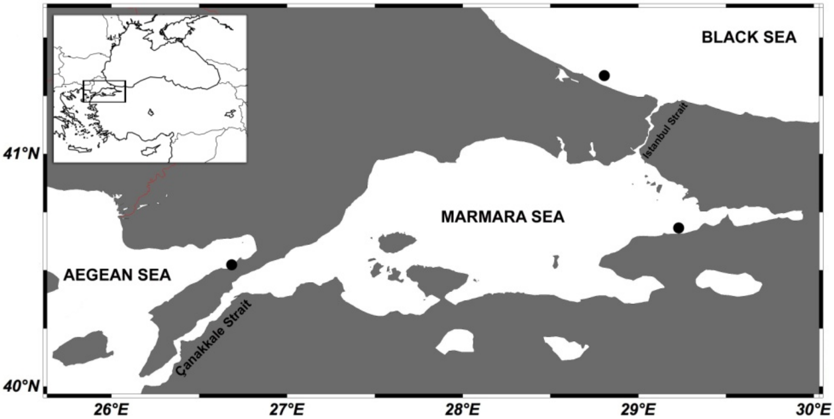

Figure 1. Study area. (Dots show harbour porpoise locations.)

The amplified fragments were purified with Roche High Pure PCR Product Purification Kit (Indianapolis, USA), following the manufacturer's instructions and sequenced in both directions by Macrogen Inc. (South Korea), using the L15824 and H16265 primers. Sequencing data was edited with Sequencher v.3.1 (Gene Codes Corp.). To reveal evolutionary relationships among haplotypes, statistical parsimony network analysis was performed using TCS v.1.13 (Clement *et al.* 2000). Previously reported 31 sequences from the same mitochondrial region (Viaud-Martinez *et al.* 2007) (GenBank accession numbers EF063110, EF063646- EF063675, U09689-U09691) were also included in this analysis. Investigation of 364 bp mtDNA sequences from three harbour porpoises revealed three polymorphic sites in three different haplotypes, differing from each other by two single base substitutions. Each of the haplotypes obtained in our study matched to three different sequences reported by Viaud-Martinez *et al.* (2007) (Figure 2). The haplotype of the Black Sea sample (haplotype XV) had been previously detected on the Ukraine coast of the Black Sea. The haplotype of the Aegean individual was found identical to that of an individual found on the Ukrainian Black Sea coast (Haplotype XVI). This finding supports the idea that harbour porpoises from the Black Sea dispersed into the northern Aegean through the Istanbul and Çanakkale Straits (Rosel *et al.* 2003).

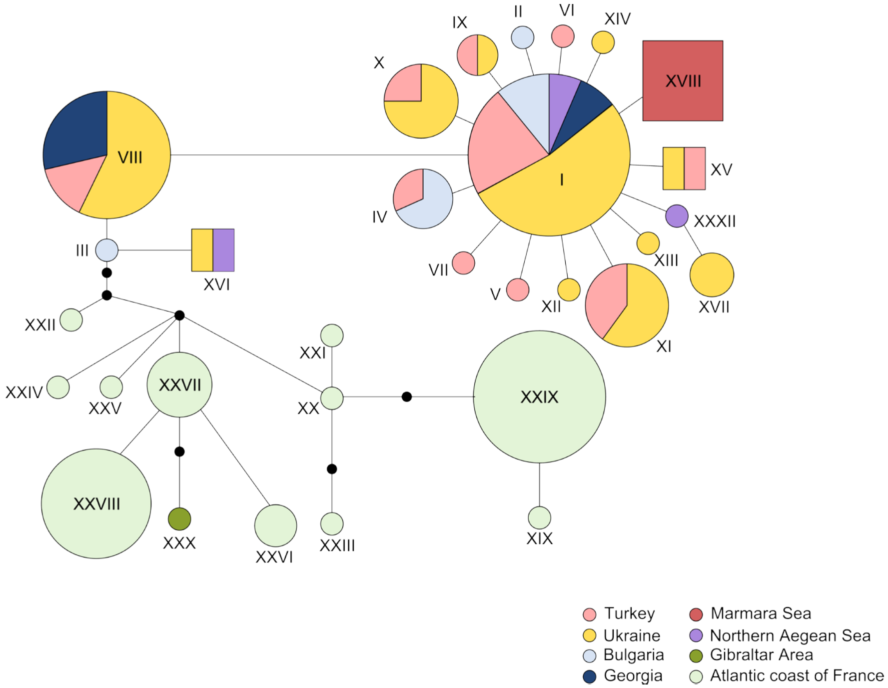

Figure 2. Haplotype network for the mitochondrial control region. The sizes of the circles and squares are proportional to the frequency of each haplotype. Circles represent the haplotypes identified by Viaud-Martinez *et al.* (2007). Squares represent the haplotypes found in our study and by Viaud-Martinez *et al.* (2007). Black circles represent hypothetical haplotypes. Each line between haplotypes represents a single mutational step between haplotypes.

This figure corrected on 5 April 2013.

Viaud-Martinez *et al*. (2007) had investigated three individuals sampled from the Marmara Sea in different months and reported that all of them share a unique haplotype (Haplotype XVIII), which was not observed in other individuals from the Black Sea nor the Aegean Sea. The Marmara Sea sample sequenced in this study had the same haplotype. This pattern supports the possibility of an isolated population in the Marmara Sea, as suggested also by Viaud-Martinez *et al*. (2007).

None of the haplotypes obtained in this study clustered with the Atlantic populations consistent with the idea that Atlantic and Black Sea populations are reproductively isolated and should be considered as subspecies (*Phocoena phocoena phocoena* and *Phocoena phocoena relicta*, respectively) (Rosel *et al*. 1995). It will be necessary to carry out genetic research with more samples to obtain a better understanding of the relationships of the subpopulations of harbour porpoises especially in the Marmara Sea as well as those in the Black Sea.

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Türkiye Denizleri'nde yaşayan muturlar (*Phocoena phocoena*) hakkında genetik bir ön çalışma

Özet

Karadeniz, Marmara Denizi ve Ege Denizi'nden üç tane muturun (*Phocoena phocoena*) mitokondrial DNA analizi, üç farklı haplotipe tekabül eden üç polimorfik bölgenin varlığını gösterdi. Önceki bulgularla örtüşen bir şekilde, bu çalışmada elde edilen haplotiplerin hiçbiri Atlantik'ten olanlarla gruplaşmadı. Karadeniz ve Ege haplotiplerinin daha önceden Ukrayna'nın Karadeniz kıyısında kaydedilmiş olanlarla aynı olması, Karadeniz'deki muturların Ege'ye İstanbul ve Çanakkale Boğaları üzerinden geçtikleri fikrini desteklemektedir. Marmara Denizi'nden olan üçüncü örnekte de, daha önceki bir çalışmada yine aynı denizde üç bireyde tespit edilmiş olan benzersiz haplotip bulunmuştur. Bu yapı Marmara Denizi'nde izole bir popülasyon olabileceği fikrini desteklemektedir. Bu çalışmanın muturlarda Kuzey Ege'nin Türkiye kıyısından bir örneğin genetik analizinin yapıldığı ilk çalışma olması da kayda değerdir.

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