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# Mitochondrial DNA control region diversity of the bottlenose dolphin (*Tursiops truncatus*) from the Adriatic Sea

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## Introduction

Bottlenose dolphins - *Tursiops truncatus* (Montagu, 1821) is a species of marine mammals of the order Cetacea - whales which are the most highly aquatically adapted group of mammals. They are cosmopolitan species and they exhibit geographical variation in morphology and genetics. Bottlenose dolphins are the only resident marine mammal species of the Adriatic Sea. This species is considered endangered due to intensive eradication operations in the middle 20th century and is legally protected.

Animal mitochondrial DNA (mtDNA) is circular double - stranded DNA molecule found in mitochondria, organelles that occur in the cytoplasm of most eukaryotic cells. Vertebrate mtDNA contain 37 genes and non - coding sequence, the control region or D - loop. The mutation rate in vertebrate mtDNA is much higher than in nuclear genome, this rate is at its greatest along the control region. Genetic variability is considered prerequisite for the long - term persistence and adaptability of populations.

## The aim of this study

Investigate the level of mitochondrial DNA control region diversity in the bottlenose dolphin population from the Adriatic Sea.

## Materials

In this study 106 muscle samples of bottlenose dolphins found dead and 2 skin samples collected using biopsy darting system were used. All samples were obtained during the period from October 1997 to September 2011 both on Croatian (106 individuals) and Italian (2 individuals) coast of the Adriatic Sea (Figure 1).

## Methods

Total genomic DNA was extracted using Wizard Genomic Purification Kit, Promega. Fragments of the mtDNA control region were amplified with universal MTCrf primer (Hoelzel and Green 1998) and a newly-designed DUPr primer (5' - GGT GAA TAT CAA AGC AGA GG - 3'). 739 bp - long fragments of the mtDNA control region were sequenced in both directions. Sequence analyses were performed using BioEdit (Hall 1999) and Arlequin (Excoffier 2005). Adriatic haplotypes were compared with published haplotypes from north Atlantic, Mediterranean and Black Sea (Natoli et al. 2005, Quérouil et al. 2007, Mirimin et al. 2011), for which our haplotypes were truncated to 439 pb.

## Results

- 11 haplotypes with 26 polymorphic sites were identified. Haplotype DD01 was the most frequent and was shared between Croatian and Italian samples. The second Italian sample was separated into its unique haplotype (table 1).
- The overall nucleotide diversity was  $0.010452 \pm 0.005435$  and gene diversity was  $0.6108 \pm 0.0494$ . Both haplotype diversity and nucleotide diversity of the Adriatic bottlenose dolphin population are lower than those found in the western Mediterranean and Aegean Sea, but are higher than those found in the Black Sea and Israel (Table 2).
- For now, one haplotype, haplotype DD07, is unique to the Adriatic population of bottlenose dolphin (Table 3).



Figure 1. Map of Adriatic sea from where samples of bottlenose dolphins originated

Table 1. Distribution of bottlenose dolphins among haplotypes and frequency of these haplotypes.

Haplotype	Number of individuals	Frequency (%)
DD01	65 (T101)	60,2
DD02	13	12,1
DD03	11	0,2
DD04	7	6,48
DD05	6	5,56
DD06	1	0,926
DD07	1	0,926
DD08	1	0,926
DD09	1	0,926
DD10	1	0,926
DD11	1 (T95)	0,926

Table 3. Matching of Croatian haplotypes with bottlenose dolphin haplotypes from other seas

DD01	AATt2 W Mediterranean Tt27497 E Mediterranean BS1 Black Sea	Natoli et al. 2005
	Ire5 Irish	Mirimin et al. 2011
	TT103, TT099, TT030, TT051, TT047, TT019 Azores TTM002, TTM012 Madeira TT/01/1998, TT/71/1997, TT/43/2004 mainland Portugal	Quérouil et al. 2007
DD03	TUR2 E Mediterranean	Natoli et al. 2005
DD04	Tt2/97 E Mediterranean	Natoli et al. 2005
DD06	TtFil E Mediterranean	Natoli et al. 2005
DD09	AATt25 W Mediterranean	Natoli et al. 2005
DD11	LIDO W Mediterranean	Natoli et al. 2005
	TT084, TT029 Azores TT/61/2002 mainland Portugal	Quérouil et al. 2007
DD02, DD05, DD08 and DD10	CL529, TtG20 E Mediterranean	Natoli et al. 2005
	TT/15/1998 mainland Portugal	Quérouil et al. 2007

Table 3. Gene (H) and nucleotide (p) diversity by population with standard deviations, and number of samples (n).

Population	H	h	n	Reference
Mediterranean	0,935 ± 0,035	0,022 ± 0,012	18	Natoli et al. (2004)
Portuguese	0,857 ± 0,137	0,014 ± 0,008	7	Quérouil et al. (2007)
Aegean	0,65	0,016	11	Viaud - Martinez et al. (2008)
Irish	0,643 ± 0,048	0,011 ± 0,006	82	Mirimin et al. (2011)
Adriatic	0,6108 ± 0,0494	0,010452 ± 0,005435	108	this investigation
Black Sea	0,58	0,003	43	Viaud - Martinez et al. (2008)
Israeli	0,42	0,002	9	Viaud - Martinez et al. (2008)

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