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GENETIC DIVERSITY OF BOTTLENOSE DOLPHIN (*Tursiops truncatus*) FROM CROATIAN PART OF THE ADRIATIC SEA

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Introduction

The bottlenose dolphin (*Tursiops truncatus*) is the only resident marine mammal species in Croatian part of the Adriatic Sea with the total number of individuals estimated at between 220 to 250 (Gomerčić et al. 2002). This extremely low number and geographical isolation, along with other environmental factors including pollution, coastal development and fisheries interactions, render this population prone to extinction. The bottlenose dolphin is considered endangered and is legally protected species in Republic of Croatia. The maintenance of the genetic variation within populations is a general concern for the conservation of endangered species. Genetic variability is thought to be essential to the long-term persistence and adaptability of populations. With co-dominant inheritance and high degree of polymorphism, microsatellite DNA markers have proved highly informative for population genetic studies (Bruford and Wayne 1993). Mitochondrial DNA (mtDNA) control region is commonly variable on the intraspecific level and is suitable for studies of genetic variability, phylogeography and assignment to management units (Kohn and Wayne 1997).

Materials and methods

We analysed microsatellite loci in 30 individuals found dead from 1994 until 2003 on Croatian coast of the Adriatic Sea (Figure 1) and a 767 bp-long fragment of the mt DNA control region in 22 individuals. DNA was extracted from tissues of carcasses. Multiplex polymerase chain reactions were performed in which 12 polymorphic dinucleotide repeat cetacean microsatellite loci were amplified. The markers used were: EV1, EV14, EV37, EV94 (Valsecchi and Amos 1996), TexVet3, TexVet5, TexVet7 (Rooney et al. 1999), D08, D14, D18, D22 and D28 (Shinohara et al. 1997). PCR products were analyzed on ABI PRISM 310 Genetic Analyzer. Genetic variation analysis was performed using the Genetix 4.05 program (Belkhir i sur. 1996-2004). Fragments of the mtDNA control region were sequenced using universal forward MTCRf primer (Hoelzel and Green 1998) and a newly-designed reverse primer named "DUPr" (5'-GGTGAATATCAAAGCAGAGG-3'). Sequence alignment was performed using ClustalW, implemented in BioEdit software (Hall 1999). MtDNA analysis of the sequences was performed with the ARLEQUIN (Excoffier et al. 2005).

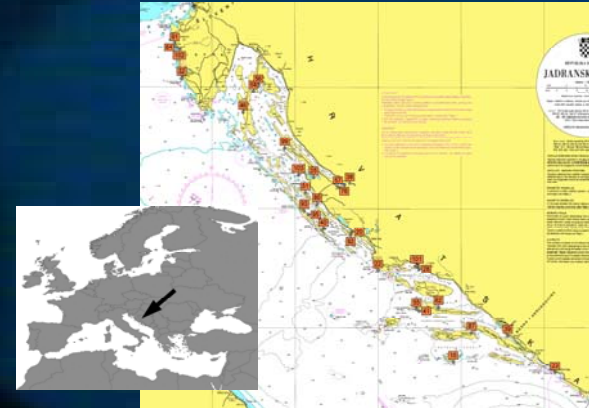


Figure 1. Bottlenose dolphin findings. Individual ID number is placed on the site where the particular individual was found dead

Results and Discussion

All microsatellite loci are polymorphic. We found 86 alleles in total. The mean allelic diversity per microsatellite locus is 7.17, ranging from three to 15 alleles and the mean expected heterozygosity is 0.69, ranging from 0.34 to 0.89 (Table 1). Locus D28 deviate from Hardy-Weinberg equilibrium (Table 1) and should be explored further. The microsatellite results reveal relatively high genetic variability in the population of bottlenose dolphins in Croatia. The analysis of 767 bp-long fragment of the mtDNA identified only four unique haplotypes with 22 polymorphic sites in 22 individuals (Table 2). Haplotype D1 is the most frequent (17 individuals, 77%). Both haplotype diversity (0.403 ± 0.125) and nucleotide diversity (0.008 ± 0.004) of the Croatian bottlenose dolphin population are relatively low. The low haplotype and nucleotide diversities may indicate population bottleneck in the recent past and should be explored further.

These genetic results should prove valuable for future molecular genetic investigations of the Adriatic bottlenose dolphins.

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Table 1. Number of genotyped individuals (N), number of alleles per locus (Na), size range (min-max), observed (Ho) and expected heterozygosities (He) and probability of the data under the assumption of the null hypothesis of Hardy-Weinberg equilibrium (P_{HWE})

locus	N	Na	min	max	Ho	He	P_{HWE}
EV1	27	7	133	145	0.3333	0.4067	0.363
D18	30	9	68	92	0.8000	0.7583	0.803
TV3	23	7	223	237	0.7826	0.8053	0.154
D14	26	6	119	133	0.6538	0.7470	0.408
D08	30	5	103	111	0.6333	0.7728	0.249
TV5	26	6	201	215	0.5000	0.6531	0.163
EV94	30	8	235	257	0.6667	0.7689	0.178
TV7	30	3	154	164	0.3333	0.3378	0.525
EV14	26	6	148	164	0.6923	0.7426	0.109
EV37	30	15	187	245	0.8667	0.8911	0.686
D28	30	7	125	145	0.4667	0.7533	0.002*
D22	30	7	114	132	0.6667	0.6006	0.915
Mean	28.17	7.167			0.6163	0.6865	
(SE)					(0.050)	(0.047)	

Table 2. Polymorphic sites and frequencies of four mtDNA control region haplotypes found in the bottlenose dolphin population from Croatian part of the Adriatic.

haplotype	16	106	157	213	253	272	273	277	286	287	302	311	365	378	399	400	486	509	549	564	612	721	f
D1	-	T	C	C	T	T	A	C	C	T	T	G	T	T	C	C	T	G	T	T	G	C	0.77
D2	A	•	T	T	•	C	C	A	T	C	C	A	C	•	•	T	C	A	C	•	•	T	0.09
D3	A	•	T	T	•	C	C	A	T	C	C	A	C	•	•	T	C	A	•	C	A	T	0.09
D4	A	C	•	T	C	•	•	•	T	C	C	•	•	C	T	A	C	•	•	•	•	•	0.046