

# Conservation genetics of the short-beaked common dolphin (*Delphinus delphis*) in the Mediterranean Sea and in the eastern North Atlantic Ocean

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**Abstract** Mediterranean Sea common dolphins have recently been listed as ‘endangered’ in the IUCN Red list, due to their reported decline since the middle of the 20th century. However, little is known about the number or distribution of populations in this region. We analysed 118 samples from the Black Sea, Mediterranean Sea and eastern North Atlantic at nine microsatellite nuclear loci and for 428 bps of the mtDNA control region. We found small but significant population differentiation across the basin between the eastern and the western Mediterranean populations at both nuclear and mtDNA markers (microsatellite  $F_{ST} = 0.052$ , mtDNA  $F_{ST} = 0.107$ ,  $P$  values  $\leq 0.001$ ). This matched the differential distribution and habitat use patterns exhibited by this species in the eastern and the

western parts of the Mediterranean Sea. The assignment test of a small number of samples from the central Mediterranean could not exclude further population structure in the central area of the basin. No significant genetic differentiation at either marker was observed among the eastern north Atlantic populations, though the Alboran population (inhabiting the Mediterranean waters immediately adjacent the Atlantic ocean) showed significant mtDNA genetic differentiation compared to the Atlantic populations. Directional estimates of gene flow suggested movement of females out of the Mediterranean, which may be relevant to the population decline. Phylogenetic analysis suggested that the observed population structure evolved recently.

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## Introduction

The status of the common dolphin (*Delphinus delphis*) population in the Mediterranean Sea has been a concern for many years. In 2003 the Mediterranean common dolphin ‘subpopulation’ was listed as endangered in the IUCN Red List of Threatened Animals, based on criterion A2, which refers to a “50% decline in abundance over the last three generations”. It is also listed in Appendix II of the Washington Convention (1973), in Appendix II of the Bonn Convention (1983), in Appendix II of the Bern Convention (1986), and in Annex IV of the European Union Habitats Directive (1992). According to ACCOBAMS (Agreement for the Conservation of Cetaceans in the Black Sea, Atlantic, Mediterranean Sea and contiguous waters) this species in these regions should be considered endangered.

The common dolphin shows an extremely wide distribution in all oceans, from warm temperate to tropical waters. In the Mediterranean Sea it shows a non-homogeneous occurrence, and its density seems to vary widely across the basin. In the Alboran Sea, the species is abundant, estimated at 14,736 individuals (95% CI = 6,923–31,366) (Forcada and Hammond 1998). On the other hand, although no abundance estimates exist for the rest of the Mediterranean, the available data indicate that at least a few hundred animals inhabit Greek waters (Ionian and Aegean Seas), and some coastal areas of the Tyrrhenian Sea (Bearzi et al. 2003; Frantzis et al. 2003). Isolated populations are monitored in northern Sardinia, south Tyrrhenian Sea, Malta, and the Ionian Sea (Bearzi et al. 2003). In the Adriatic Sea and Israel, common dolphins are very rare (Bearzi et al. 2004, Kerem, Pers. com.). Literature references, photographic documentation and osteological collections indicate that the common dolphin is disappearing from most of its historical habitat range in the northern region of the basin (Ligurian Sea, Gulf of Lion, Tyrrhenian and Adriatic Sea), where large populations were reported until the middle of the 20th century (Duguy & Cyrus 1973; Casinos and Vericad 1976; Poggi 1986; Cagnolaro 1994; Bearzi et al. 2004). A closely monitored population that used to inhabit the waters surrounding the island of Kalamos (Ionian Sea) has gone through a very rapid decline during the last decade (Bearzi 2003). The causes of this decline are not fully understood and this may be an ongoing trend (Bearzi et al. 2003). In contrast, no trend has been observed in a monitored population in the northern Alboran Sea since 1992 (Cañadas 2006). No information on abundance and trends is available from the southern Mediterranean.

The Mediterranean Sea is an enclosed basin where the intensity of human activities has significantly impacted on the marine environment, especially along the coastal areas. It has been suggested that the most probable factors implicated in the decline of this species in the northern area are global environmental changes, prey depletion, xenobiotic contamination and direct takes and bycatch (for a detailed review of these factors, see Bearzi et al. 2003).

The distribution pattern of the common dolphin differs between the western and the eastern Mediterranean Sea. In the western area (Alboran Sea, Algeria and Balearic Sea), the species is recorded at all depths, but is mainly oceanic, inhabiting primarily waters beyond the continental shelf (>150 m depth) (Viale and Frontier 1994; Forcada and Hammond 1998; Gannier 1995; Cañadas et al. 2002; Cañadas et al. 2005). In this region it frequently forms mixed groups with striped dolphins (Cañadas et al. 2005). Conversely, in the Adriatic Sea and Ionian Sea it has been observed primarily in neritic areas where it often occurs sympatrically with the bottlenose dolphin (Bearzi 2003;

Politi et al. 1992; Frantzis, Pers. com.). In the Aegean Sea, it is also observed mainly in coastal shallow waters over the continental shelf (Frantzis et al. 2003). In the enclosed and deep Gulf of Korianthiakos (Greece) this species is oceanic, and there it has always been recorded in mixed groups with striped and Risso's dolphins (Frantzis and Herzing 2002).

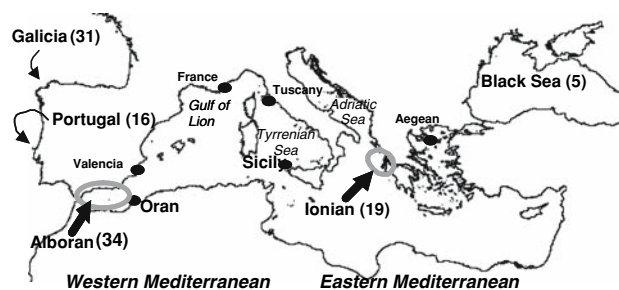
Data for other populations of this species in the North Atlantic (Natoli et al. 2006) would suggest that there should be little differentiation within the Mediterranean system. However, the conservation concerns outlined above mean that an assessment of structure here will be important towards effective management. Therefore, in this study we test the hypothesis that the Mediterranean common dolphin population is homogeneous. We further assess the level of gene flow between Mediterranean and Atlantic populations.

## Materials and methods

### Sample collection and DNA extraction

A total of 118 samples were analysed from stranded animals or biopsy sampling. Samples from the Ionian Sea (except one) were biopsy samples, all other samples analysed were from stranded animals. These samples were from the Black Sea (5), the Ionian Sea (22—mainly from a resident coastal population near Kalamos, Greece), the Alboran Sea (34), Portugal (16) and Galicia (31). Additionally, one sample from the Aegean Sea, one from Sicily, one from the Tyrrhenian Sea (western coast of Italy), two from the eastern coast of France (including one museum specimen from the beginning of the twentieth century), two from Valencia, and three samples from Algeria (eastern Oran) were included in some analyses (Fig. 1).

DNA was extracted from tissue samples preserved in salt saturated 20% DMSO by a standard phenol/chloroform extraction method (Hoelzel 1998). DNA was extracted



**Fig. 1** Map of the Mediterranean and Black Sea. Black dots indicate common dolphin samples from the western, central Mediterranean Sea and Aegean Sea. Grey circles indicate the Ionian and Alboran Sea populations

from bone samples using QIAgen PCR purification columns after grinding 100 mg of bone and digesting it at 37°C for 48 h in 1 ml of digestion buffer (0.01 M TRIS, 0.01 M NaCl, 1% SDS, 2 mg/ml proteinase K, 0.01 PTB). To avoid contamination the extraction and the analysis of the bone specimens were conducted in a different laboratory where no cetacean DNA had ever been manipulated before. An extraction including everything but tissue was undertaken for all analyses as a negative control.

#### Sex determination

Individuals whose gender was unknown were sexed by amplifying portions of the genes ZFX and ZFY as described in Bérubé and Palsbøll (1996).

#### Microsatellite analysis

Samples were genotyped at nine microsatellite loci: KWM1b, KWM2a, KWM2b, KWM9b and KWM12a were derived from *Orcinus orca* (Hoelzel et al. 1998), EV37Mn from *Megaptera novaeangliae* (Valsecchi and Amos 1996), TexVet5, TexVet7 and D08 from *Tursiops truncatus* (Rooney et al. 1999, Shinohara et al. 1997). PCR conditions were as described in Natoli et al. (2006). Amplified DNA was analysed for length variation on 6% polyacrylamide denaturing gels using fluorescent imaging on an automated ABI PRISM 377 DNA sequencer, after incorporation of 1/10 fluorescent labelled primer. An internal standard marker (Genescan-500 ROX, Applied Biosystems) was used to determine the allele sizes.

We analysed the level of relatedness among the individuals using the programme MER (V3) Wang (2002), and thereby identified two re-sampling events ( $R = 1$ ) and one putative mother-offspring pair ( $R = 0.88$ ) in the Ionian population. The duplicates (supported by photo-ID data—data not shown) and the putative offspring were removed from further analyses (leaving  $N = 19$  in the Ionian sample).

The level of genetic diversity was estimated as observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), and allelic richness. Allelic richness controls for variation in sample size by a rarefaction method, and was calculated using the program FSTAT 2.9.3 (Goudet 2001). Evidence for the presence of null alleles or large allele dropout was tested using the programme MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2003). Estimation of  $F_{IS}$  and evaluation of possible deviations from Hardy Weinberg (overall deviation, heterozygote deficiency and heterozygote excess, using Fisher's exact test and the Markov chain method: dememorisation number, number of batches,

iteration per batch set at 1,000, Bonferroni correction applied) were performed using GENEPOP 3.1d (Raymond and Rousset 1995a, 1995b). The level of differentiation among populations was estimated as  $F_{ST}$  (Weir and Cockerham 1984) using the programme ARLEQUIN 2.0 (Schneider et al. 1999).

An asymmetric estimate of the migration rate ( $M = 4N_e m$ ) between pairwise populations, based on microsatellite and mtDNA data, was calculated using MIGRATE 1.7.3 (Beerli 2002). The length of the runs was optimised for both markers (acceptance-rejection > 10%,  $R < 1.2$ ). Initial runs were set estimating  $\theta$  and  $M$  with  $F_{ST}$  and allowing  $M$  to be asymmetric. Reruns were set using the parameter estimated with the first run and lengthening the MCMC chains. In order to verify the result a final run was set using longer chains. For comparison, the migration rate was also calculated according to  $F_{ST} = 1/(4Nm + 1)$ .

Population assignment and possible first generation migrants were assessed using GENECLASS 2.0b (Piry et al. 2004). The Bayesian method described in Rannala and Mountain (1997) was used and the probability that an individual was a resident was computed using a Montecarlo resampling algorithm described in Paetkau et al. (2004) (10,000 repetitions,  $\alpha$  set to 0.01). Considering the observed population differentiation based on the microsatellite results, two putative populations (Ionian samples = 19; Alboran & Atlantic samples = 81) were considered.

GENECLASS 2.0b was also used to assign individuals from unknown populations (Aegean, Tyrrhenian, Valencia and Algeria) to one of two reference populations (the Ionian and Alboran Sea samples in this case). The probability that an individual belonged to each reference population was computed using the Bayesian method (Rannala and Mountain 1997) and applying the Montecarlo resampling algorithm described in Paetkau et al. (2004) (number of simulated individuals set to 10,000;  $\alpha$  set to 0.01).

Sex-biased dispersal was tested using the program FSTAT 2.9.3 (Goudet 2001). Only adult individuals (a total of: 39 females and 55 males) were considered for this analysis.

#### mtDNA analysis

A total of 114 samples were sequenced for 428 bp at the 5' end of the mtDNA control region and compared with four sequences already published (from the Black Sea, Rosel et al. (1994), accession numbers: U02639–U02641). In total 118 sequences were considered for the data analysis.

The mitochondrial DNA control region was amplified with the primers 5'-ACA CCA GTC TTG TAA ACC-3' and 5'-TAC CAA ATG TAT GAA ACC TCA G-3' after Rosel et al. (1994). The PCR reaction conditions are described in Rosel et al. (1994). PCR products were purified with QIAgen

PCR purification columns and sequenced directly using the ABI dye-terminator method. Sequence alignment was performed using ClustalX (Thompson et al. 1997).

Mitochondrial DNA from the French coast bone samples was amplified using two sets of primers designed in order to amplify two overlapping portions of the control region of approximately 200 bps each (Dmtcrf: 5'-TTA GTC TCT CCT TGT AAA T-3' and Dmtcrr: 5'-GGT GAT TAA GCT CGT GAT-3', T annealing: 54°C (Nichols et al. 2007); MTCRf and mtancr: 5'-AAA ATA AAT GAA TGC ACA ATA-3, T annealing: 47°C). The PCR reaction conditions were as follows: 100 µM dNTPs, 2.5 mM MgCl<sub>2</sub>, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 200 nM of each primer, 0.4 µg/(µl BSA, 0.02 U/(µl Taq polymerase. The PCR cycling profile was 5 min at 95°C, 45 cycles of 45 s at 94°C, 1.5 min at T annealing, and 1.5 min at 72°C, followed by 8 min at 72°C. A total of 357 bps were amplified.

The degree of differentiation ( $F_{ST}$ ), the nucleotide diversity ( $\pi$ ), Tajima's  $D$  and Fu's  $F_S$  were estimated using ARLEQUIN 2.0 (Schneider et al. 1999). A median-joining network was generated to infer phylogenetic relationships among the mtDNA haplotypes, using the program NETWORK 2.0 (Bandelt et al. 1999; <http://www.fluxus-engineering.com>). For this analysis sequences were cropped to the shortest sequence length of 357 bps.

## Results

### Genetic variation and population differentiation

Samples were divided into five putative populations based on their geographic origins, though the Black Sea sample was excluded from most comparisons due to its small size. Allelic richness and observed and expected heterozygosities at microsatellite DNA loci were calculated at each locus for each sample (Table 1). The Black Sea sample is included in this table, but likely biased by the small sample size. The Alboran and the Galician populations deviated significantly from the HW equilibrium at one and two loci respectively ( $P < 0.001$ , Bonferroni correction applied). However no evidence for null alleles and/or large allele dropout were detected (using Micro-checker), there was no consistent pattern across populations, and therefore the affected loci were retained in the analyses. The Ionian sample showed lower allelic richness and expected heterozygosity when compared with the Alboran and Atlantic samples.

For mtDNA, 45 different haplotypes (Accession numbers: EU365129-EU365173) were observed based on 42 variable sites (Fig. 2).

Two common haplotypes were shared among all populations. Overall gene and nucleotide diversities were 0.943 and 0.015, respectively (shown by putative population in

Table 2). Fu's  $F_S$  was negative and significant for the Galician population ( $F_S = -11.88$ ,  $P < 0.02$ ), suggesting possible demographic population expansion. However, Tajima's  $D$  test was not significant ( $P = 0.28$  Beta distribution approximation).

Genetic differentiation among pairwise populations was estimated using  $F_{ST}$  (Table 3). Based on the microsatellite data, the Ionian population was significantly differentiated from all other populations, but no significant genetic differentiation was detected between the Alboran and Atlantic (Galicia and Portugal) populations. The mtDNA analysis confirmed the differentiation observed between the Ionian and other putative populations, but also indicated significant differentiation between the Alboran and Atlantic populations.

The spanning network was drawn including the haplotypes of the nine individuals from different areas of the Mediterranean Sea. No clusters reflected geographic origin (Fig. 3).

### Migrants, migration rate and sex biased dispersal

GENECLASS 2.0b identified the Ionian sample as separate from the combined Alboran and Atlantic samples (Fig. 4), and detected three possible migrants ( $P \leq 0.01$ ). One individual belonging to the Ionian population was identified as an immigrant from the Alboran-Atlantic population, whereas two individuals belonging to the Alboran-Atlantic population (one from Alboran and one from Galicia) were putative migrants from the Ionian population (Fig. 4).

Migration rate estimates based on  $F_{ST}$  and MIGRATE were calculated for each pair of contiguous populations with both nuclear and mtDNA markers (Table 4). Values based on the microsatellite markers did not show any clear directional trends, while those based on the mtDNA markers suggested directional migration from the Mediterranean populations towards the neighbouring populations, and within the Mediterranean from east to west.

Sex-biased dispersal was tested among populations. Although  $F_{IS}$  was significantly lower in females than in males (suggesting male dispersal; female  $N = 39$ ,  $F_{IS} = 0.015$ ; male  $n = 56$ ,  $F_{IS} = 0.084$ ,  $P = 0.011$ ), the other parameters analysed (relatedness index, mean assignment test,  $H_o$  and  $H_e$ ) did not show any significant difference between males and females.

### Population assignment of individuals

We attempted to assess the most probable source populations for the nine unassigned samples collected from

**Table 1** Genetic variation at each microsatellite locus for each common dolphin population

		Populations				
		BS <i>N</i> = 5	Ionian <i>N</i> = 19	Alboran <i>N</i> = 34	Portugal <i>N</i> = 16	Galicia <i>N</i> = 31
KWM1b	N. Alleles 4	2 [1.98]	2 [1.54]	4 [1.55]	3 [2.04]	3 [1.82]
	<i>Ho</i>	0.5	0.167	0.147	0.186	0.226
	<i>He</i>	0.53	0.157	0.142	0.375	0.257
KWM2a	N. Alleles 16	4 (1) [3.58]	6 [4.29]	14 (2) [5.59]	10 [5.13]	12 (1) [5.6]
	<i>Ho</i>	0.667	0.842	0.882	0.688	0.839
	<i>He</i>	0.636	0.805	0.887	0.871	0.887
KWM2b	N. Alleles 8	5 [4.56]	6 [3.61]	8 (1) [4.85]	6 [4.61]	7 [4.62]
	<i>Ho</i>	1	0.737	0.882	0.813	0.774
	<i>He</i>	0.788	0.724	0.849	0.829	0.83
KWM9b	N. Alleles 12	4 [3.78]	8 [4.42]	9 [4.87]	7 [4.43]	12 [5.52]
	<i>Ho</i>	0.333*	0.842	0.879	0.875	0.936
	<i>He</i>	0.788	0.79	0.846	0.815	0.886
KWM12a	N. Alleles 10	4 [3.6]	5 [4.01]	9 (1) [4.75]	7 [4.69]	9 (1) [4.68]
	<i>Ho</i>	0.667	0.79	0.765	0.625*	0.871
	<i>He</i>	0.682	0.769	0.823	0.823	0.796
EV37Mn	N. Alleles 24	6 [5.36]	11 [4.97]	15 (1) [5.75]	14 [6.0]	19 (3) [6.1]
	<i>Ho</i>	0.5	0.842	0.941	1	0.968
	<i>He</i>	0.924	0.815	0.899	0.913	0.918
TexVet5	N. Alleles 11	2 [2]	7 [4.68]	10 [4.98]	8 [4.64]	10 [4.93]
	<i>Ho</i>	0.2	0.684	0.656**	0.786	0.5**
	<i>He</i>	0.467	0.827	0.855	0.841	0.854
TexVet7	N. Alleles 7	2 [1.8]	3 [2.35]	5 (1) [3.52]	5 (1) [3.89]	5 (1) [3.7]
	<i>Ho</i>	0.333	0.421	0.879	0.688	0.677
	<i>He</i>	0.439	0.512	0.725	0.788	0.742
D08	N. Alleles 13	3 [2.6]	7 [4.15]	10 [5.05]	10 [5.16]	12 (3) [5.04]
	<i>Ho</i>	0.5	0.632	0.735	0.875	0.645**
	<i>He</i>	0.561	0.781	0.872	0.875	0.845
Average (SD)	N. Alleles	3.6 (1.4)	6.1 (2.7)	9.33 (3.6)	7.8 (3.2)	9.89 (4.7)
		[3.25 (1.24)]	[3.78 (1.13)]	[4.55 (1.29)]	[4.51 (1.09)]	[4.67 (1.27)]
	<i>Ho</i>	0.52 (0.24)	0.66 (0.23)	0.75 (0.24)	0.73 (0.23)	0.72 (0.23)
	<i>He</i>	0.65 (0.16)	0.68 (0.22)	0.77 (0.24)	0.79 (0.16)	0.78 (0.21)

BS = Black Sea. The number of private alleles is given in parenthesis, and allelic richness in squared brackets. \*Indicates significant deviation at  $P < 0.05$ ; \*\*indicate those loci still significant after Bonferroni correction ( $P < 0.001$ )

Algeria, the Tyrrhenian Sea and the Aegean Sea). An assignment test was performed using the Ionian and Alboran Seas as reference populations. A significant assignment was found in five cases (Table 5).

**Discussion**

Both nuclear and mtDNA analyses showed clear differentiation between the Ionian and Alboran populations. The sample from the Ionian Sea is small, and so sampling effects could be important. However, the results based on various different analyses are consistent, all but one Ionian

individuals assigned in the same cluster (Fig. 4), and no kinship was detected among the 19 samples included in the study. Considering the relative proximity of these populations, such marked differentiation was unexpected. The common dolphin is a highly mobile species capable of long distance dispersion, confirmed by the lack of strong population structure observed among North and South Atlantic populations (Natoli et al. 2006). In that context, it is reasonable to conclude that isolation by geographic distance is probably not responsible for the genetic structure observed within the Mediterranean Sea. Moreover, if considered in a broader context, the  $F_{ST}$  values observed between the two Mediterranean populations are quite high. Common

**Fig. 2** Polymorphic sites among 45 haplotypes are shown (left). Haplotype frequencies (right) are given for each haplotype in each population. BS = Black Sea, Ion = Ionian, Tyr = Tyrrhenian Alb = Alboran, Port = Portugal, Gal = Galicia. The total number of haplotypes analysed for each population is reported below the population code

	111111	1111111122	2222333333	3333333334	44	BS	Ion	Tyr	Alb	Port	Gal
	2356113333	3445777934	7779000011	2224557891	11	9	19	5	34	17	30
	9686050234	7356678414	1341568909	0792054361	78						
#BSDD4	-CAAGTCCAT	ATACATTTTT	CTCATCTTCC	TTGAACCTTC	TC	1	4		9	2	2
#BSV1	.....C....	.....A.C.	.....C..	.....C..	C..	3	2	1	2	2	2
#BSV2	.....C....	.....C..	.....C..	C.....-...CT		1					
#BSG11	.....C....	.....C..	.....C..	.....C..	..	1					
#BSDd5	.....C....	.....C..	.....T..	.....C..	..	1					
#DdN423	.....C....	.....G....	.....C..	.....C..	..	1					
#DdN432	.....C....	.....T....	.....C..	.....C..	..	1					
#DELC1	.....C....	.....C..	.....T....	.....T....	..		3				
#DEL2	...ACT... ..T..A.C	..AG..CT..	C.T.....	.....	..		5				
#Dd1.02	.....C....	.....C..	.....C..	C.....CT			4				
#Dd3.02	A....C....	...G...CC	..G...C..	C...G....	..		1	1	1		1
#Bom2	A....C....	...A...C	..G...C..T	..T.....	--			1			
#Bom3	.....C....	...G...C	..G...C..T	.....	--			1			
#ALB1	.....C....	.....C..	.....C..	.....C..	C..				1		
#ALB11	.....C....	.....C..	.....C..T	C.....C..	C..			1	11		
#ALB16	.....C....	.....CC	T.A...C..	C.....C..	C..				2		
#ALB17	A....C....	...A...C	..G...C..T	..T.G....	..				2		
#Alb24	.....CT...	.....C..	.....C..	C.....C..	C..				1	1	1
#Alb25	.....C...C	.....C..	..G...T	C.A.....	C..				1		
#Alb28	.....C....	...G...C	..G...T..	C...G....	C..				1		
#ALB3	.....C...C	..G...C	..G...T	CC.....C..	C..				1		
#ALB4	.....C....	.....C..	.....C..	CC.....C..	C..				1		
#EG10F	.....C....	.....C..	.....C..	.....C..	..				1		
#P8F	.....C...C	.....C..	..G...CC	C.AG.....C..	C..					2	1
#P10F	.....C....	.....CC	.....C..	C.....C..	C..					1	1
#P11F	A....C....	.....C..	.....C..	.....C..	..					1	1
#P14F	..C...C....	.....C..	..C...C..	C.....C..	C..					1	1
#P16F	.....C...C	.....C..	..GG...T	.....C..	C..					1	
#P17F	.....C....	...G...CC	..G...T..	C...G...C..	..					2	
#P19F	.....C....	.....C..	.....C..	C.....A..	C..					1	
#P20F	.....C...C	.....C..	..G...C..T	.....C..	C..					1	4
#P22F	.....C....	G.....C	.....CT..	C.....C..	C..					1	
#P24F	.....C....	.....C..	.....C..	.....C..	..					1	
#G-12	A....C....	...A...C	..G...C..T	..T.G...C..	..						1
#G-34	A....C....	...A...C	..G...C..T	..T.G...C..	..						2
#G10F	.....C..GC	.....C..	..G...T..	.....C..	C..						1
#G16F	.....C...C	.....C..	..G...A...C	CT							1
#G19F	.....C....	...G...CC	..G...T..	C...G....	..						2
#G20F	.....C....	.....C..	..G...C..T	.....C..	..						1
#G23F	.....C....	.....C..	..A...CTT	C.....C..	..						1
#G26F	.....C...C	..G...C	..G...T..	C.A.....C..	C..						1
#G28F	..G.C....	..C...C	T.G...C..	.....C..	C..						1
#G32F	.....C.T.C	..G...C	..G...C..	C.A.....C..	C..						1
#G33F	.....C..G	.....C..	..G..TCCT	.....C..	C..						1
#G35F	A....C....	...G...C	..G...T..	C...G....	..						1
#G5F	.....C....	.....C..	.....C..	C.....C..	..						1
#G9F	-T...C...C	.....C..	..G...C..	.....C..	C..						1

**Table 2** MtDNA genetic diversity values and tests for neutrality for each common dolphin population

	N	Gene Div.	Nucl. Div.	Tajima's D	Fu's Fs
Black Sea	9	0.917	0.009	0.494	-2.0
Ionian Sea	19	0.848	0.016	1.23	3.93
Alboran Sea	34	0.832	0.012	-0.138	-1.29
Portugal	17	0.971	0.014	-0.447	-4.157*
Galacia	30	0.977	0.016	-0.653	-11.880***

\*  $P < 0.05$ , \*\*\*  $P < 0.001$

dolphin populations from different sides of the Atlantic Ocean show similar or even lower genetic differentiation ( $F_{ST}$  values based on microsatellites were between 0.012 and 0.045;  $F_{ST}$  values based on mtDNA were between 0.028 and 0.059 - Natoli et al. 2006).

Differentiation between western and eastern Mediterranean populations has also been observed in other marine species such as the common sole (*Solea vulgaris*; Guarniero et al. 2002), sea bass (*Dicentrarchus labrax*;

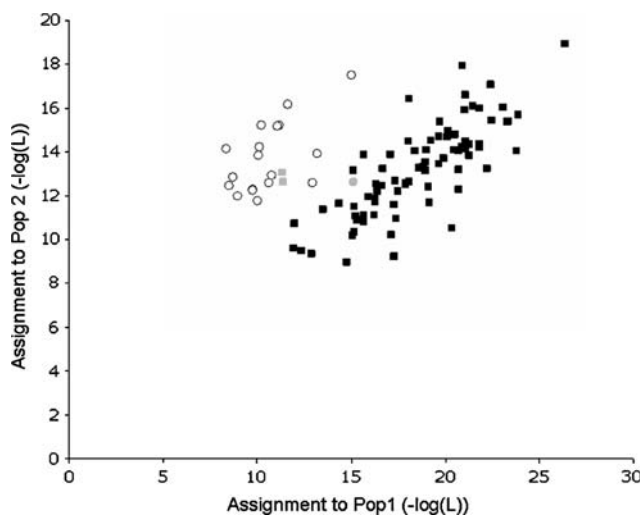
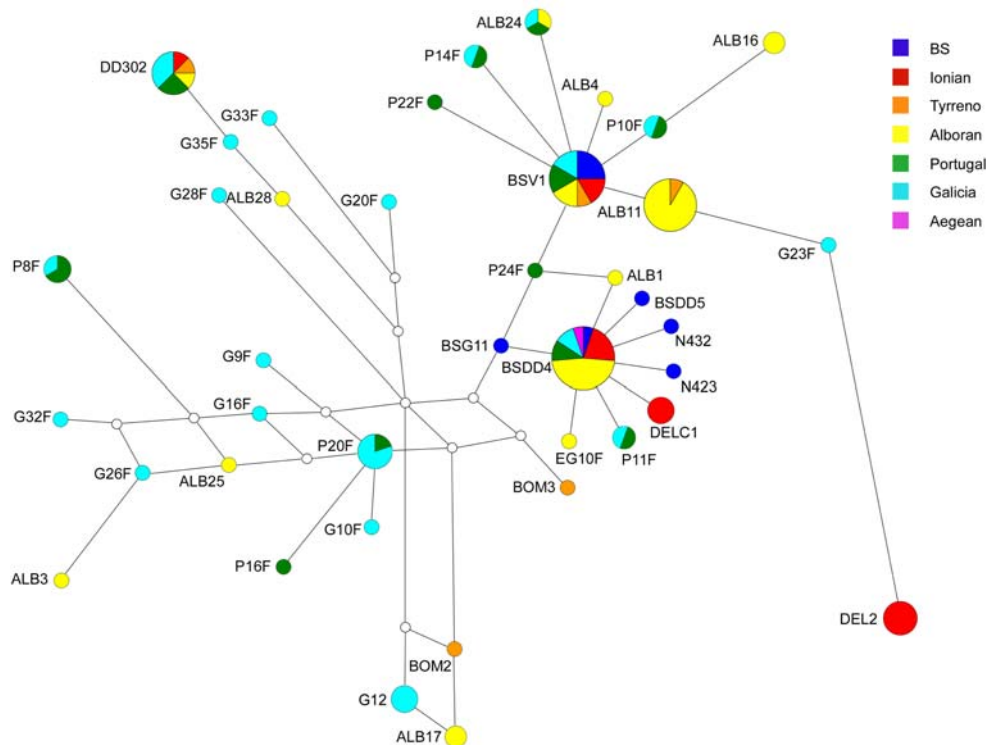
**Table 3** Population differentiation ( $F_{ST}$ ) based on microsatellites (below diagonal) and mtDNA (above diagonal)

Pop	Mitochondrial DNA				
	N	Ionian 19	Alboran 34	Portugal 17	Galicia 30
Ionian	19		0.107***	0.056*	0.064**
Alboran	34	0.052***		0.069**	0.079***
Portugal	16	0.053***	-0.002		-0.009
Galicia	31	0.05***	-0.001	-0.003	

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

Bahri-Sfar et al. 2000), bottlenose dolphin (*Tursiops truncatus*, Natoli et al. 2005) and the striped dolphin (*Stenella coeruleoalba*, Gaspari et al. 2007). The fact that the common dolphin shows different habitat preferences in the western Mediterranean (open water), and eastern Mediterranean (shallow coastal habitat), suggests that the exploitation of different resources may be a

**Fig. 3** Minimum spanning network among common dolphin haplotypes. The size of the circles is proportional to the total number of haplotypes observed. Sectors are proportional to the frequency of each haplotype observed in each population. Populations are identified by scale of greys as reported in the figure. White circles indicate ancestral extinct haplotypes. Haplotype names are the same as in Fig. 2. A colour version is available online



**Fig. 4** Gene class assignments; closed squares denote Alboran and North Atlantic samples, while open circles denote Ionian Sea samples. Squares or circles in grey indicate putative migrants

factor reducing movement between these regions (*c.f.* Natoli et al. 2005).

Comparing the Alboran and North Atlantic populations we found significant differentiation only for the mtDNA locus. Greater differentiation at mtDNA markers can be due to both the 4-fold smaller effective population size represented by the mtDNA marker, and differences in the behaviour of males and females. The possibility of greater male-mediated gene flow is suggested by the lower female

$F_{IS}$  values, but other tests showed no significant difference. Despite the presence of the Strait of Gibraltar, the oceanographic characteristics of the Alboran Sea are similar to those of the eastern North Atlantic Ocean. The Almeria-Oran front situated 350 km within the Mediterranean Sea represents the actual oceanographic shift between Atlantic and Mediterranean waters. Common dolphins in both these areas are generally observed in open waters off the continental shelf (Cañadas et al. 2005; Lopez et al. 2004), and may have adapted to similar habitats, which may facilitate movement of individuals between these two populations. The sample from the Black Sea was very small, but preliminary results suggest isolation from the Mediterranean populations (e.g. see Table 4).

Estimates of gene flow based on a coalescence method using both nuclear and mtDNA markers suggested differences between the sexes with respect to the direction of gene flow. Microsatellite data (reflecting bi-parental inheritance) suggested similar rates in both directions (Table 4). However, mtDNA data (reflecting female movement) suggested a marked directionality of gene flow from eastern to western populations and south to north, indicating directional movements of females towards the eastern North Atlantic populations. A similar result was reported for bottlenose dolphins (*Tursiops truncatus*; Natoli et al. 2005).

The minimum spanning network did not identify any clear correspondence between lineages and geography. However, both the Ionian and Alboran populations were characterised by lower gene diversity compared to the

**Table 4** Estimated migration rate between contiguous populations for both microsatellite and mtDNA markers

Population	Microsatellites (bi-parental)			mtDNA (maternally inherited)				
	Nm	1,2	2,1	95% CI	Nm	1,2	2,1	95%CI
1 BlackSea	2.270	–	9.226	8.31–9.9	7.080	–	36.095	26.16–424.13
2 Ionian		4.184	–	3.68–4.69		5.290	–	3.08–5.36
1 Ionian	4.540	–	30.572	29.16–32.02	4.160	–	1.520	0.18–2.26
2 Alboran		27.174	–	25.84–28.6		13.510	–	0.37–18.25
1 Alboran	inf	–	13.512	12.65–14.41	7.190	–	6.070	3.21–7.41
2 Portugal		16.718	–	15.75–17.70		87.230	–	25.65–105.86
1 Portugal	inf	–	9.58	8.77–10.32	inf	–	0.158	0.14–0.8
2 Galicia		13.46	–	12.58–14.39		93.145	–	70.89–171.85

Nm columns calculated according  $F_{ST} = 1/(4Nm + 1)$ . The other columns represent the asymmetrical estimated migration rate (based on MIGRATE): 1,2 stands for: migration from population 1 to 2; 2,1 stands for: migration from population 2 to 1

**Table 5** Assignment test for individuals from the central Mediterranean

Sample	Prob to belong to		N. Loci
	Ionian	Alboran	
Origin			
Aegean	0	0.0001	9
Tuscany	0	0.0002	9
Sicily	0.207	0.69	8
France*	0.755	0.328	5
Valencia	0	0.0001	9
Valencia	0.0001	0.636	9
Oran	0.131	0.927	5
Oran	0	0.0002	9
Oran	0.943	0.601	5

The probability belong to each of the reference population is reported for each individual. Pop1 = Ionian, Pop2 = Alboran. \*Indicates a pre-decline sample

Atlantic populations, and had a few highly represented haplotypes that were different from one another and not frequent among the other populations analysed. Moreover they showed unique haplotypes, suggesting population discreteness (consistent with the various other analyses). Two of the most common haplotypes among the Mediterranean populations are at the centre of a star-like structure, characteristic of a post-bottleneck expansion, though sampling effects can also generate this pattern. The Atlantic populations showed a diverse pattern with no dominant haplotypes. However Fu’s  $F_s$  neutrality test for this population was significant, suggesting a recent population expansion.

Assignment of nine individual samples from around the Central Mediterranean provided a preliminary indication of the relationship between their genotype and provenance in some cases. Five of these could be assigned to either the Alboran or Ionian population, however, the remaining four were not clearly assigned to either population (see Table 5). We would therefore, recommend a further study

based on a sufficient sample size from the central region to test for possible further population structure within the Mediterranean Sea.

Defining population boundaries is fundamental to the formulation of effective conservation plans. Our results show a clear population boundary between the western and the eastern Mediterranean indicating the presence of discrete population in these two areas, and implying that eastern and western regions of the Mediterranean Sea should be considered independently for further actions towards the conservation of this species. The possible conservation relevance of a signal for directional female gene flow from east to west in the Mediterranean and south to north in the Atlantic, in the context of proposed population declines in the Mediterranean Sea (Bearzi, 2003; Bearzi et al. 2003), needs to be considered further using complementary field and molecular studies.

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