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Population genetic structure of common bottlenose dolphins (Tursiops truncatus) *in the Adriatic Sea and contiguous regions: implications for international conservation*

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ABSTRACT

1. Habitat diversity plays a significant role in shaping the genetic structure of cetacean populations. However, the processes involved in defining the genetic differentiation of these highly mobile marine mammals are still largely unknown.

2. Levels of genetic differentiation and dispersal patterns of common bottlenose dolphins (*Tursiops truncatus*) were assessed in the north-eastern Mediterranean Sea, with a focus on the Adriatic Sea. This is a region characterized by diverse marine ecosystems and high levels of human-induced habitat degradation.

3. Although this species seems almost uniformly distributed throughout the Adriatic Basin, genetic evidence rejected the hypothesis of a single stock. Pairwise estimates of genetic differentiation at 12 microsatellite loci, and mitochondrial DNA (entire control region, 920bp), revealed diverse levels of genetic differentiation among five putative populations from the Tyrrhenian Sea to the Aegean Sea.

4. A fine-scale genetic structure was recorded within the Adriatic Sea, where females appear to be the principal gene flow mediators. The assessment of recent migration rates indicates a relatively high level of gene flow from the North Adriatic towards adjacent areas.

5. Indication of a fine-scale population structure across the Adriatic Sea is a factor to be carefully considered in the emerging marine management scenario set by the implementation of the EU Marine Strategy Framework Directive (2008/56/CE), particularly when it comes to assessing and managing direct mortality caused by human activities (e.g. fisheries or maritime traffic). A good knowledge of population structure at the basin level is also fundamental for the identification of potential Adriatic Special Areas of Conservation for the bottlenose dolphin under the Habitats Directive (Council Directive 92/43/EEC).

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KEY WORDS: cetacean conservation; *Tursiops truncatus*; Mediterranean Sea; Adriatic Sea; population structure; gene flow

INTRODUCTION

Several cetacean species have greater population structure than would be expected over relatively small geographic scales. This is predominantly due to their demographic history, habitat association and foraging behaviour (Hoelzel *et al*., 2002). Furthermore, dispersal may be limited by oceanographic processes such as salinity and temperature gradients (Fullard *et al*., 2000; Jørgensen *et al*., 2005; Natoli *et al*., 2005). The common bottlenose dolphin (*Tursiops truncatus*) shows strong genetic structure among populations across its worldwide range (Hoelzel *et al*., 1998; Natoli *et al*., 2004). This pattern is not always associated to geographical distance and appears to be highly dependent on the type of environment that the constituent individuals inhabit. The identification of genetic discontinuities is critical when evaluating processes affecting the distribution of genetic variation both within and among populations. This is particularly important for marine species, such as *Tursiops truncatus*, that are often discretely distributed, but can nevertheless be genetically structured.

One previous population genetic study on common bottlenose dolphins was conducted on a broad geographic scale from the eastern North Atlantic to the Black Sea. It reported a general pattern of genetic divergence between the east and west Mediterranean basins (Natoli *et al*., 2005). Common bottlenose dolphins have been studied in only relatively limited parts of the Mediterranean, therefore wide areas remain largely unexplored, particularly in the eastern basin (see Bearzi *et al*., 2009 for a review). Moreover, the distinction between inshore and offshore populations has yet to be defined, especially in the Adriatic Sea, which is characterized by very diverse marine coastal habitats within a relatively small geographic area (Cushman-Roisin *et al*., 2010).

The common bottlenose dolphin, living mainly in coastal areas, faces numerous human-induced threats and has therefore attracted the attention of both national and international conservation authorities (Bearzi *et al*., 2009). The European Habitats Directive (Council Directive 92/43/EEC) lists *Tursiops truncatus* in Annex II and requires European Union (EU) member states to establish Special Areas of Conservation where populations are resident. This species is also listed in Annex IV, which demands national authorities to monitor the status of extant populations and human-induced mortality. Moreover, the Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC) requires all EU member states to assess and monitor the status of the marine environment in relation to the main pressures caused by human activities in order to achieve a 'Good Environmental Status' by 2020. Core aspects of this framework are the development of monitoring and management programmes at the regional level. This includes the assessment of the status of cetacean species and analysis of population genetic structure (MSFD indicator 1.3.2) that may help clarify migratory routes and identify distinct units for conservation (European Commission, 2011). This is of particular importance in the Adriatic Sea, a semi-enclosed basin that represents one of the four Mediterranean MSFD sub-regions and is affected, particularly on the north-west coast, by human encroachment and water pollution.

This study evaluated whether common bottlenose dolphins living in the Adriatic Sea represent a genetically distinct unit and/or show patterns of population genetic structure. Dispersal routes were also assessed across the Adriatic, Ionian and Aegean Seas, and the non-contiguous area of the Tyrrhenian Sea.

MATERIALS AND METHODS

Study area

This study was conducted in the Adriatic, Ionian, Aegean and Tyrrhenian Seas (Figure 1). The Adriatic Sea shows clear differences in coastal and submarine topography along its longitudinal and transverse axes, and it is divided into three sub-basins (Artegiani *et al*., 1997; Cushman-Roisin *et al*., 2010).

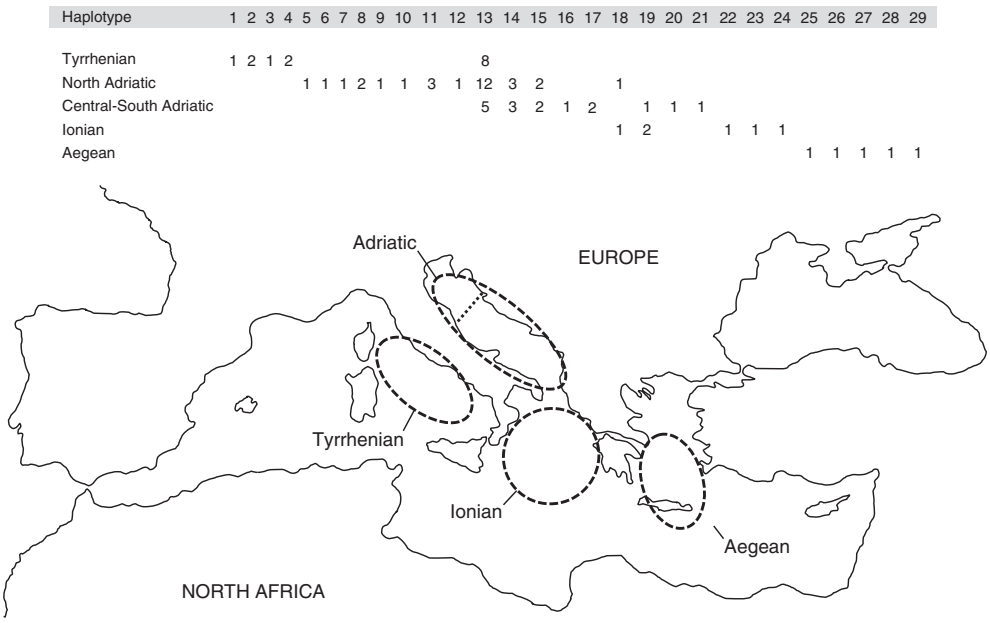

Figure 1. Map of the study area. Samples were obtained from stranded and free-ranging bottlenose dolphins in the Tyrrhenian, Adriatic, Ionian and Aegean Seas (dashed circles). Dotted line in the Adriatic Sea shows approximate limit between north and central-south basins. Number of haplotypes is reported for each sampling area.

The northern section is shallow (average depth of 35 m) and extends south-eastwards to the 100 m bathymetric contour. The central Adriatic is a transition sub-basin with an average depth of 140 m and extends as far as the 170 m deep Pelagosa sill. The southern sub-basin is characterized by a wide depression, more than 1200 m deep and is divided from the Ionian Sea by the Otranto sill. The west coast of the Adriatic Sea is fairly linear, sandy with gentle slopes, while the east coast is irregular, has many islands and a rocky, steeply sloping bathymetry. Despite the north-west–south-east topographic division, Artegiani *et al.* (1997) and Cushman-Roisin *et al.* (2010) considered the North Adriatic sub-basin dynamically independent from the Central and South Adriatic sections. Therefore, samples were collected from the central and southern sections of the basin and pooled as the Central-South Adriatic sampling area.

Sample collection and genetic analysis

Tissue samples from 89 adult common bottlenose dolphins were collected between 1992 and 2009 from stranded animals ($N = 69$) and free-ranging specimens ($N = 20$) in the Adriatic Sea (43 stranded and 20 free-ranging), and in the Ionian ($N = 6$ stranded), Aegean ($N = 6$ stranded) and Tyrrhenian ($N = 14$ stranded) Seas. Biopsy samples from free-ranging animals were collected from an average of one individual per social group encountered during surveys. Samples from stranded dolphins were collected opportunistically from isolated individuals at different times of the year.

DNA was extracted with phenol/chloroform and ethanol precipitation from tissue samples preserved in salt-saturated 20% DMSO. Samples were genotyped at 12 microsatellite loci, including EV37Mn and EV14Pm (Valsecchi and Amos, 1996), TtruGT6 (Caldwell *et al.*, 2002), D08 (Shinohara *et al.*, 1997) and Ttr04, Ttr11, Ttr19, Ttr34, Ttr58, Ttr63, TtrRH1 and TtrRC12 (Rosel *et al.*, 2005). To ensure accuracy in genotyping and to standardize allele sizing for each locus, about 30% of the samples were re-amplified as controls. Microsatellite genotypes were screened for duplicate sampling using the Excel Microsatellite Toolkit 3.1.1 (Park, 2001) and tested for scoring errors due to allelic dropout, null alleles and stuttering with Micro-Checker 2.2.3 (Van Oosterhout *et al.*, 2004).

The mitochondrial DNA (mtDNA) entire control region was amplified and sequenced using the light-strand primer TURCRL5483 (5′ - GGTCTTGTAAACCGGAAAAGG - 3′) and the heavy-strand primer TURCRH6379 (5′ - GCAGACTTACACATGCAAGCA - 3′) designed specifically on the threonine tRNA and 12S rRNA genes, respectively. Primer numbers refer to the 3′ base of the published *T. truncatus* mitochondrial genome sequence (Xiong *et al.*, 2009). Microsatellite alleles and cycle sequencing reactions were resolved on an Applied Biosystems 3100 genetic analyser. Raw sequence chromatographs from both strands were edited and aligned using CodonCode Aligner 3.0.1 (CodonCode Corporation). The consensus sequence consisted of 920 nucleotides corresponding to the entire common bottlenose dolphin mtDNA control region sequence.

Analysis of genetic variation and population structure

Allele diversity, observed heterozygosity, and unbiased gene diversity were assessed using GenAlEx 6.5 (Peakall and Smouse, 2012), and tested for departure from Hardy–Weinberg equilibrium (HWE) using the Markov chain randomization implemented in Genepop 4.1 (Rousset, 2008). Allelic richness was calculated to account for variation in sample size using the rarefaction method implemented in Fstat 2.9.3.2 (Goudet, 1995). Haplotype and nucleotide diversity for mtDNA sequences and population comparisons based on *F*-statistics for mtDNA and microsatellite loci were calculated using Arlequin 3.5 (Excoffier and Lischer, 2010). Genetic differentiation among populations was also evaluated using principal component analysis (PCA) on multilocus genotypes implemented in GenAlEx 6.5. This analysis transforms a number of correlated variables (the alleles) into a smaller number of uncorrelated variables (the principal components) to best represent the original relationships among sampling sites and alleles. A Mantel test for matrix correspondence and a spatial autocorrelation analysis were also performed using GenAlEx 6.5 to compare patterns of genetic variability as a function of geographic distances in the Adriatic Sea. Pairwise genetic distance matrix was calculated following Peakall *et al.* (1995) and Smouse and Peakall (1999). Pairwise geographic distances were calculated based on geographic coordinates of individual sampling locations. The spatial autocorrelation coefficient of genetic distance (r) was calculated for 10 geographic distance classes of 20 km and 95% confidence intervals were generated based on 1000 bootstrap replicates. Significance of Mantel test was based on 1000 permutations. The Bayesian clustering method of Structure 2.3.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003) was implemented to estimate patterns of genetic structure. Markov Chain Monte Carlo (MCMC) runs were conducted without prior population information to assess the most appropriate number (K) of populations needed for interpreting the observed genotypes. The analysis was conducted for K values ranging from one to seven (number of sampling areas plus two) using a burn-in period of 100 000 iterations followed by runs of 10^6 steps. Given the contiguous geographic range and the probability of gene flow, the admixture and the correlated frequency models were chosen. The LOCPRIOR option (Hubisz *et al.*, 2009) was used to include sampling locations information and assist clustering at lower levels of divergence or with fewer data, as was the case with the Ionian and Aegean sample set. The mean likelihood $L(K)$ was calculated over 10 runs for each K. The mean difference was assessed between successive likelihood values of K, $L'(K)$ and the absolute value of the difference between successive values of $L'(K)$, $|L''(K)|$. The modal value of ΔK (the mean of $|L''(K)|$ averaged over 10 runs divided by the standard deviation of L(K)) was estimated using Structure Harvester (Earl and vonHoldt, 2012) and it was taken as the most likely number of populations K as described in Evanno *et al.* (2005). The K value was then used as prior information to estimate the probability that an individual belongs to a given population. The program BayesAss (Wilson and Rannala, 2003) was used to estimate bi-directional recent migration rates among populations. The program implements a Bayesian procedure using MCMC procedure allowing for deviations from Hardy–Weinberg equilibrium. MCMC runs of 10×10^6 iterations were conducted and sampled every 1000 iterations

to infer posterior probability distribution using default Delta values. The Bayesian coalescent approach implemented in Migrate 3.4.2 was also applied to estimate average mutation-scaled effective migration rates (M) between sampling areas assuming migration-drift equilibrium (Beerli, 2006). An infinite allele microsatellite mutation model (IAM) with constant mutation rate, an F_{ST} based measure as starting parameter and an uniform prior distribution were used. The Bayesian run consisted of one long chain with 5×10^6 parameter values sampled every 100 iterations and a burn-in of 10 000 genealogies discarded at the beginning of each chain.

RESULTS

Measures of genetic diversity

Common bottlenose dolphins showed a relatively high degree of genetic diversity. Mean number of alleles per sampling area varied from 4.9 to 9 and allelic richness was similar among areas, ranging from 4.1 to 4.3. Expected and observed heterozygosities were 0.764 ± 0.005SE and 0.692 ± 0.023SE, respectively (Table 1). There was no evidence of scoring errors due to stuttering, large allele dropout or null alleles. However, significant departure from Hardy–Weinberg equilibrium was recorded in the North Adriatic and Tyrrhenian seas after applying sequential Bonferroni correction. Deviation from equilibrium was detected at four loci for the Adriatic and Tyrrhenian populations, suggesting possible population substructure. Population comparison analyses were run removing those loci that showed heterozygosity deficiency for one or more populations (Supplementary material, Table S1). However, patterns of population divergence did not change significantly and therefore the original set of 12 loci was maintained. Sequencing of the mitochondrial DNA control region revealed a total of 29 haplotypes with five to 12 haplotypes per sample area (Table 1). Mean number of pairwise differences was 9.8 ± 0.9SE with the lowest value (6.7 ± 1.5SE) recorded in the Tyrrhenian Sea and the highest (11.8 ± 2.8SE) reported for the Aegean Sea. Haplotypic diversity was generally high, ranging from 0.67 in the Tyrrhenian to 1 in the Aegean Sea. Twenty-four unique haplotypes were recorded. The Aegean Sea was represented by five samples and each revealed a unique haplotype. The Ionian and Tyrrhenian Sea had 60% and 80% unique haplotypes, respectively. The North and Central-South Adriatic shared three haplotypes, and the most common sequence was shared by 25 dolphins in the Tyrrhenian and Adriatic Sea (Figure 1).

Population structure

Genetic divergence among common bottlenose dolphins from the five sampling areas of the Mediterranean Sea at microsatellite loci and mtDNA is reported in Table 2. Microsatellite data detected significant differentiation among all putative populations except for the Aegean Sea, which showed a low level of genetic divergence from the Ionian and Adriatic Sea. A low but significant F_{ST} was also estimated between the North and Sentral-South Adriatic areas. This pattern of genetic structuring was in part corroborated by

Table 1. Microsatellite loci and mitochondrial DNA diversity measures for bottlenose dolphins from five sampling regions across the Mediterranean basin. N, sample size; A, allele diversity; A_R, allelic richness; H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; k, number of haplotypes; S, number of segregating sites; h, haplotype diversity; π, nucleotide diversity. Standard error values in parenthesis

	Microsatellites					Mitochondrial DNA				
Sampling site	N	A	A_R	H_E	H_O	N	k	S	h	π ($\times 10^{-2}$)
North Adriatic	39	9.0 (1.0)	4.3 (0.2)	0.77 (0.02)	0.71 (0.03)**	29	12	43	0.82 (0.02)	1.07 (0.09)
Central-South Adriatic	24	8.1 (0.9)	4.4 (0.2)	0.78 (0.02)	0.77 (0.03)*	16	8	23	0.87 (0.02)	0.95 (0.15)
Ionian	6	4.9 (0.4)	4.2 (0.3)	0.76 (0.03)	0.68 (0.05)	6	5	26	0.93 (0.05)	1.26 (0.32)
Aegean	6	4.9 (0.5)	4.3 (0.3)	0.76 (0.04)	0.63 (0.04)	5	5	24	1.00 (0.06)	1.28 (0.37)
Tyrrhenian	14	6.1 (0.5)	4.1 (0.2)	0.75 (0.03)	0.67 (0.05)**	14	5	20	0.67 (0.06)	0.73 (0.11)

*and **denote significant heterozigosity deficiency ($P < 0.05$ and $P < 0.01$, respectively) before Bonferroni correction.

Table 2. Genetic differentiation based on *F*-statistics for the bottlenose dolphin. F_{ST} values are reported on the left triangulation matrix for microsatellite loci (below diagonal) and mtDNA sequences (above diagonal)

Sampling site	North Adriatic	Central-South Adriatic	Ionian	Aegean	Tyrrhenian
North Adriatic	—	– 0.003	0.131(*)	0.112(*)	0.021
Central-South Adriatic	0.009(*)	—	0.080(*)	0.073	0.057
Ionian	0.026(*)	0.025(*)	—	0.034	0.221(**)
Aegean	–0.010	0.011	0.015	—	0.200(*)
Tyrrhenian	0.051(**)	0.033(**)	0.042(*)	0.043(*)	—

Significance of fixation indices at 5% (*) and 1% (**) levels was tested by 10 000 permutations.

principal component analysis (PCA) (Figure 2). The first three components of PCA explained 88% of the total inertia, with components 1 and 2 explaining 44% and 25% of the variation, respectively. The Mantel test found a weak correlation between geographic and genetic distance in the Adriatic Sea ($Rxy = 0.009$, $P = 0.048$). Spatial autocorrelation analysis revealed no significant correlation. Analysis of mtDNA sequences partially supported these results. Significant differentiation was, in fact, recorded between the North Adriatic and the Aegean sampling locations and no significant divergence was recorded between the Tyrrhenian and the Adriatic Sea. Genetic comparison between West and East Adriatic animals was highly significant at nuclear DNA loci ($P < 0.001$) but not significant for mitochondrial DNA sequence comparison.

Mean values of the log likelihood of the data estimated using the Bayesian clustering approach did not provide a *K* value with a significantly high posterior probability. Conversely, using the statistic ΔK based on the rate of change in the log probability of the data between successive *K* values, a modal value of $\Delta K = 21.1$ was found for $K = 5$. This value was used as prior population information for calculating the posterior probability of individual assignment. However, different proportions of dolphin multilocus genotypes were assigned to different clusters and no strong pattern of population structure could be detected. High rates of gene flow were observed from the North Adriatic to the other regions (Table 3). Migration rates among the Central-South Adriatic, Ionian, Aegean and Tyrrhenian seas appeared to be negligible. Comparison between the West and East Adriatic coastal areas revealed a pattern of gene flow from west to east. For the West Adriatic, the proportions of resident individuals and migrants from the East Adriatic were 0.970 and 0.029, respectively. For the East Adriatic, the proportions of residents and migrants from the West Adriatic were 0.680 and 0.320, respectively. The coalescent approach recovered relatively high migration rates from the Adriatic to the Ionian Sea and between the Ionian and the Aegean Sea (range of posterior distribution values of *M*: 93.1–133.6). Minor rates of gene flow were instead recorded among other possible migration routes (*M* values from 2.9 to 55.1).

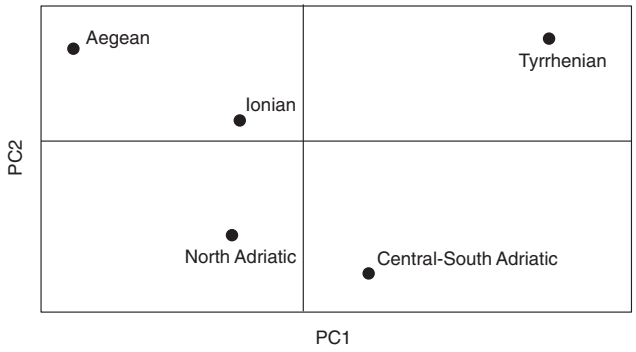

Figure 2. Principal component analysis of common bottlenose dolphin multilocus genotypes from the five study areas (North Adriatic, Central-South Adriatic, Ionian, Aegean, and Tyrrhenian Seas).

DISCUSSION

Results from this study revealed different degrees of genetic differentiation across the study area. An overall pattern of population structuring was detected by principal component analysis and, to a minor extent, by a Bayesian clustering approach. Pairwise comparisons based on *F*-statistics showed

Table 3. Means and 95% confidence intervals of the posterior probabilities for migration rates between bottlenose dolphin populations. Sampling sites listed in the rows represent populations of origin of migrants. Recipient populations are listed in the columns

	Recipient population				
Population of origin	North Adriatic	Central-South Adriatic	Ionian	Aegean	Tyrrhenian
North Adriatic	0.981 (0.951, 0.997)	0.298 (0.249, 0.327)	0.219 (0.103, 0.307)	0.189 (0.073, 0.298)	0.273 (0.200, 0.324)
Central-South Adriatic	0.004 (0.000, 0.025)	0.679 (0.667, 0.711)	0.023 (0.000, 0.097)	0.026 (0.000, 0.107)	0.015 (0.000, 0.065)
Ionian	0.005 (0.000, 0.022)	0.007 (0.000, 0.034)	0.711 (0.667, 0.810)	0.026 (0.000, 0.110)	0.012 (0.000, 0.052)
Aegean	0.004 (0.000, 0.022)	0.006 (0.000, 0.032)	0.023 (0.000, 0.101)	0.728 (0.669, 0.858)	0.012 (0.000, 0.053)
Tyrrhenian	0.004 (0.000, 0.020)	0.007 (0.000, 0.037)	0.023 (0.000, 0.102)	0.029 (0.000, 0.113)	0.688 (0.667, 0.735)

significant differences in nuclear DNA between all areas except between the Aegean Sea and the other east Mediterranean sampling sites. Mitochondrial DNA data revealed a strong divergence between the geographically distant sampling sites of the North Tyrrhenian Sea and Aegean Sea, but did not show a clear separation between the Tyrrhenian Sea and the Adriatic Sea. These results are in general agreement with the previous study conducted by Natoli *et al.* (2005) in which an overall pattern of genetic differentiation was found between the west and east Mediterranean basins, and mtDNA sequence divergence was weaker than nuclear DNA data. Lack of genetic differentiation among the Aegean, Ionian and Central-South Adriatic in both mitochondrial and nuclear markers mirrored the general south-eastwardly pattern of gene flow detected by the Bayesian inference methods.

Further subdivision was found within the Adriatic Sea, where common bottlenose dolphins revealed a fine-scale genetic structure at nuclear DNA markers, showing a differentiation between north and central-south sub-basins, as well as between the west and east coasts. This subdivision seems to reflect the physiographic differences found along both latitudinal and longitudinal axes of the Basin. The Adriatic Sea presents very diverse oceanographic features between the sandy and fairly linear west coast and the karst rocky and rugged east coast. Important environmental differences are also present between the North and Central-South Adriatic sub-basins, which are characterized by different depth gradients and water mass circulations (Artegiani *et al.*, 1997). Differences in marine habitats and resources could be among the mechanisms by which this differentiation has evolved and is maintained. Different degrees of resource specialization, with respect to prey and specific habitats, have been documented in bottlenose dolphins by Barros and Wells (1998), Connor (2000), and Sargeant *et al.* (2005). Combined results between genetics, photo-identification and stable isotope analyses suggest, as a preliminary hypothesis, that factors such as local site fidelity and/or physiographic features, rather than prey specialization, are the mechanisms maintaining the observed population structure. In fact, stable isotopes analyses conducted in the central Adriatic shows that common bottlenose dolphins readily shift prey, probably dependent on which prey is available (Holcer, 2012).

The genetic structure of bottlenose dolphin populations at a relatively small geographic scale was also suggested by Krützen *et al.* (2004) at Shark Bay, Australia, by Sellas *et al.* (2005) in the western North Atlantic, and recently by Ansmann *et al.* (2012) for inshore bottlenose dolphins (*Tursiops aduncus*) in Moreton Bay, Australia. These studies and our data imply a broader, worldwide pattern of small-scale differentiation for this genus. Photo-identification data have suggested that common bottlenose dolphins of the Adriatic Sea are structured in putative local populations (Fortuna, 2006; Genov *et al.*, 2008, 2009; Holcer, 2012; Pleslić *et al.*, in press), corroborating the results of this study. For instance, in the North Adriatic, no matches were found between individuals from the Gulf of Trieste (Italy, Slovenia and Croatia) and the

Kvarnerić archipelago, Croatia, only 120–150 km apart (Genov *et al*., 2008, 2009). Similarly, long-term studies based on photo-identification show an even finer-scale population structure in this region, reporting very little exchange among local populations along the Croatian coast (Holcer, 2012). Conversely, some individuals were found in both catalogues of the Kvarnerić and Kornati archipelagos (North Adriatic), approximately 80–100 km apart (Fortuna, 2006; Kammigan *et al*., 2008). Data from the Vis and Lastovo archipelagos (Central-South Adriatic), 80–150 km south of Kornati archipelago, (Holcer, 2012) did not show any match with other sites in the region.

The use of stranded samples may lead to an underestimate of genetic differentiation, particularly when population structure is assessed over a relatively small geographic scale (Bilgmann *et al*., 2011). This may render difficult the identification of management units for conservation. Although the majority of tissue samples collected for this study were obtained from stranded specimens, the results did show evidence of population structure in the Adriatic Sea, which was also strengthened by a strong match with photo-identification and stable isotopes data.

When considering the discrepancies between nuclear and mitochondrial DNA the data may be influenced by sex-biased gene flow. Differential dispersal of males and females can have a major influence on the distribution of maternally and bi-parentally inherited genes in bottlenose dolphin populations (Hoelzel *et al*., 1998). Diverse dispersal behaviours may be adopted in different regions. In some populations, male bottlenose dolphins disperse more often and further than females (Krützen *et al*., 2004; Möller and Beheregaray, 2004), while in other populations there are no significant differences in dispersal between the sexes (Natoli *et al*., 2005). Unlike the nuclear DNA data, the mitochondrial DNA sequences comparisons recovered no differences among sampling sites in the Adriatic Sea, suggesting that female bottlenose dolphins may have an important role in mediating gene flow across the basin.

The absence of a clear assignment of individual multilocus genotypes to distinct genetic clusters, despite the Bayesian clustering approach distinguishing five partitions, may be due to limitations of the software in detecting genetic differentiation when F_{ST} values are low (Latch *et al*., 2006). The existence of both resident and visiting individuals, as indicated by photo-identification studies (Genov *et al*., 2008; Pleslić *et al*., in press) may be an additional or concomitant cause of why consistent assignment to distinct clusters could not be detected. In particular, a significant proportion of either migrants or individuals of mixed ancestry may result in a degree of gene flow that potentially obscures population structure.

Analysis of recent migration rates recorded a significant rate of gene flow from the North Adriatic towards the other study areas, and negligible movements of individual dolphins between all other sampled areas. This study supports the findings that there are differences in movement patterns of resident and visiting animals according to the data derived from different projects in the North Adriatic (Fortuna, 2006; Genov *et al*., 2008, 2009; Kammigan *et al*., 2008; Holcer, 2012; Pleslić *et al*., in press). The Kvarnerić bottlenose dolphin population showed up to 30% rate of non-random temporary emigration (Fortuna, 2006), while in the Vis and Lastovo archipelagos Holcer (2012) described areas with locally defined groups mixing with transient individuals. Similarly, 48% of about 100 identified individuals in the Gulf of Trieste were recorded only once and therefore considered transient or visiting dolphins (Genov *et al*., 2008).

Conservation implications

The Adriatic Sea common bottlenose dolphins inhabit an environment greatly affected by human activities, including intensive fishery, gas and oil exploitation, maritime traffic, tourism and chemical pollutants. These pressures, particularly fishery bycatch, may have a strong, adverse impact on population viability and need to be carefully assessed and managed at scales that are consistent with the population structure of bottlenose dolphins (Fortuna *et al*., 2010). Indeed, scientific assessment of population structure, distribution and status, identification of threats affecting a specie's viability and implementation of conservation and

management measures should not prescind from an international and coordinated plan of actions. The Mediterranean 'subpopulation' (sensu IUCN) of *Tursiops truncatus* is listed as Vulnerable (A2cde) in the IUCN Red List (http://www.iucnredlist.org/details/16369383/0). The IUCN assessment noted that 'the listing of Mediterranean common bottlenose dolphins as a single subpopulation should not be interpreted to mean there is no further subpopulation structure within the region'. This study provides evidence of fine-scale population structure and dispersal patterns in the Adriatic Sea and the eastern Mediterranean basin and supports the necessity of identifying separate areas for conservation. Genetic divergence between the North and Central-South Adriatic dolphin populations strengthens the case for conservation actions targeting different sites. Population management actions should also consider how the impact of human activities differs across geographically distinct areas. The results, describing possible south-eastward migration routes from the North Adriatic, advocate the development of an international network of marine protected areas and connecting corridors (Bearzi *et al.*, 2011). The Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona 1995) considers the development of protected areas for cetacean species in the Adriatic basin. Croatia joined the EU in 2013, and although there is no control over marine regions currently under the jurisdiction of Bosnia-Herzegovina, Montenegro and Albania, the application of these countries to become EU members would require a certain level of cooperation. In this instance, the development of the EU Natura 2000 network of the Habitats Directive (Directive 92/43/EEC) and the extension of the Pan European Ecological Network of the Convention on the conservation of European wildlife and natural habitats (Bern, 1979) to non-EU States, provide the instruments to coordinate international conservation strategies within the Adriatic Sea (see Mackelworth *et al.*, 2011, 2013 for a review; Genov *et al.*, 2012). In addition, the implementation of the Marine Strategy Framework Directive (MSFD) (Directive 2008/56/CE) and its monitoring activities (Article 11) calls for the cooperation between EU and third party States in regions of shared marine waters (Article 13). This is of particular importance as the Adriatic Sea is one of only four defined sub-regions of the Mediterranean basin and it is considered to be the future pilot area for the development of marine spatial planning which includes the definition of MPAs. While there are clear arguments for the development of spatial conservation measures for the Adriatic Sea, the paucity of data available requires that further genetic research is undertaken to help define these regions in the near future, especially to help in the effective evaluation of fishery-induced mortality at the level of 'units-to-conserve' (Taylor *et al.*, 2010). The MSFD requires member states to work together to establish programmes for monitoring the 'Good Environmental Status' of shared waters by July 2014. This would be an excellent timeframe for the development of further genetic research in the Adriatic region.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table S1. Observed and expected heterozygosity per locus per putative populations (four loci were excluded).