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RESEARCH ARTICLE

New insights on population genetic structure of *Delphinus delphis* from the northeast Atlantic and phylogenetic relationships within the genus inferred from two mitochondrial markers

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Abstract The taxonomic status of common dolphins (*Delphinus* sp.) remains controversial despite the increased number of studies focusing on its populations. Two species are presently recognized, *Delphinus delphis* and *D. capensis*. Apart from a phylogeographic study of the genus *Delphinus*, genetic studies focusing specifically in the northeast (NE) Atlantic remain scarce. Following ecological and morphological evidence for the existence of different common dolphin morphotypes in the Portuguese coast, we examined the population structure of *D. delphis* from the NE Atlantic by comparing DNA sequences from two mitochondrial regions (control region and cytochrome *b* gene). Additionally, we compared the sequences obtained with existing sequences of *D. delphis* from the Azores, Black Sea, Canary Islands, Pacific Ocean, *D. capensis* and also two closely related delphinid species (*Stenella coeruleoalba* and *Tursiops truncatus*). In the analysis of the NE Atlantic populations, we found evidence for the existence of some level of genetic differentiation. In the broader phylogenetic analysis, *D. delphis* and *D. capensis* did not show reciprocal monophyly and we found a group of highly divergent individuals. We discuss the possibility for the existence of two divergent lineages that have evolved independently, a separate subspecies or events of introgressive hybridization. These findings could have important implications on a taxonomic level, although further investigation based on a larger geographical scale and on nuclear loci information will certainly elucidate the origin of these highly divergent individuals.

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Introduction

The common dolphins, genus *Delphinus*, have a worldwide distribution mainly in tropical and temperate waters of the Atlantic, Pacific and Indian oceans. The morphological diversity observed in these dolphins led to the description of more than 20 nominal species (Heyning and Perrin 1994). Currently two taxa are generally accepted: the short-beaked common dolphin, *Delphinus delphis* Linnaeus, 1758, distributed across the Atlantic and the Pacific Oceans and also present in the Mediterranean and Black seas, and the long-beaked common dolphin, *D. capensis* Gray, 1828, which is restricted to near-shore tropical and warm temperate waters of some oceans (Heyning and Perrin 1994; Rosel et al. 1994; Reeves et al. 2002). It was also suggested that some forms of common dolphins from the Indian Ocean should be regarded as a subspecies, *D. capensis tropicalis* (van Bree, 1971) (Jefferson and Van Waerebeek 2002).

The study of two sympatric populations occurring off the California coast supported the separation in two

species based on osteological characters, external morphology, pigmentation patterns and genetic data (Heynning and Perrin, 1994; Rosel et al. 1994). Phylogenetic inference based on the mitochondrial control region revealed reciprocal monophyly of both *D. delphis* and *D. capensis*, with genetic divergence of 1.1% and fixed nucleotide substitutions (Rosel et al. 1994). However, a phylogenetic study of the family Delphinidae, based upon the mitochondrial cytochrome *b* gene, found that the two species were not reciprocally monophyletic (LeDuc et al. 1999). Furthermore, a multilocus nuclear study based on AFLP markers nested *D. capensis* within *D. delphis*, which provides a non-mitochondrial support to the notion that these two species are very recently diverged (Kingston and Rosel, 2004). More recently, a phylogeographic study of the genus *Delphinus* based on control region sequences and microsatellites revealed some differentiation between populations from different oceans and different sides of the same ocean, but little or no differentiation among populations from the same side of an ocean basin (Natoli et al. 2006). The low genetic differentiation observed across a large geographical scale and the high morphological diversity observed in *D. delphis*, suggests that morphotypic variation may be more related with local adaptations than differentiation along phylogenetic lineages (Natoli et al. 2006).

In the NE Atlantic, the short-beaked common dolphin, *D. delphis* is the only species described. It occurs frequently off the south and southwest coasts of Britain and Ireland, in the Irish sea, northeast coast of Scotland (Reid et al. 2003) and off the Atlantic coast of France (Collet 1981), Spain (Lopez et al. 2004) and Portugal (Silva and Sequeira 2003). Although abundant in these areas and despite being the most frequently stranded cetacean species (Lopez et al. 2004; Silva and Sequeira, 2003; Murphy et al. 2006), except from some recent studies on life history (Murphy 2004; Murphy et al. 2005), little is known about migratory patterns, population dynamics or stock structure of short-beaked common dolphins. Recently, the large numbers of dead dolphins arriving on the beaches of western Europe that are evidently a casualty of fisheries by-catch, have raised concern among European authorities.

A morphological study based on cranial characters has suggested that the common dolphin in the Northeast Atlantic should be regarded as a larger form of *D. delphis*, since some morphometric measures (such as tooth counts and rostrum length/greatest zygomatic width ratio) overlapped with those of both short- (*D. delphis*) and long-beaked (*D. capensis*) forms (Murphy et al. 2006). The same study indicated some population differentiation within the NE Atlantic, with female short-beaked common dolphins off Portugal showing segregation from dolphins in other areas, such as in other northerly sampled coasts. These morphometric results were corroborated with ecophysiological evidence that suggested the existence of different feeding ecologies between Portuguese and the French coast (Zhou et al. 2001).

Despite a recent phylogeographic study of the genus *Delphinus* revealing a lack of genetic differentiation among the NE Atlantic short-beaked common dolphin populations (Natoli et al. 2006) based on 369 base pairs (bp) of control region and microsatellites, morphological and ecological evidence prompted us to further investigate the existence of genetic structure in NE Atlantic *D. delphis*. For that we sequenced a larger fragment of the mitochondrial control region and the cytochrome *b* gene and studied populations from Scotland, north Spain and Portuguese coasts. We also performed a broader analysis for both mtDNA regions with sequences existent in GenBank from *D. delphis*, *D. capensis* and we also sequenced two closely related species, *Stenella coeruleoalba* and *Tursiops truncatus*. With this approach we intend to elucidate phylogenetic relationships among these species based on two mitochondrial markers.

Materials and methods

Tissue and tooth samples were collected from stranded common dolphins from four main areas (Fig. 1): Scotland [SCO, $n = 13$ (8 males and 5 females)], north Spain [NSP, $n = 10$ (5 males and 5 females)], west Portuguese coast [WPOR, $n = 35$ (16 males and 19 females)] and south Portuguese coast [SPOR, $n = 10$ (5 males and 5 females)].

Additionally, in order to perform a broader phylogenetic analysis we added sequences from the GenBank to the sequences obtained in this study. For the control region we added 10 sequences of long-beaked common dolphins (U01956, U02656–U02664) and 45 sequences of short-beaked common dolphins, which included the Azores Islands (AY168601–AY168604 and AY422200–AY422203), the Canary Islands (DQ520104–DQ520124), the Black Sea (U02639–U02641) and the Pacific Ocean, (U02642–U02655). We also included sequences of a *Delphinus* sp. from Tierra del Fuego, Argentina, and *S. coeruleoalba* from Portuguese waters, which were sequenced following conditions described in below sections. Sequences had to be truncated in order to have the same size, so the final alignment resulted in 406 bp. For the cytochrome *b*

Fig. 1 Map of the study area. *Acronyms* are according to the text

gene, we added the Argentinean individual previously mentioned, two short-beak common dolphin sequences, one from the Pacific Ocean (AF084085) and one from the Black Sea (AF084084), one *D. tropicalis* sequence (AF084088) and two long-beaked common dolphins from the Pacific Ocean (AF084086 and AF084087). We also included sequences from other delphinid species: four sequences of *S. coeruleoalba* (two from NE Atlantic sequenced in this study, one from the Mediterrenean Sea and one from the Pacific ocean (AF084081 and AF084082) and three sequences of *T. truncatus* from the Iberia Peninsula, which were sequenced as described below.

DNA extraction, PCR amplification and sequencing

All samples (muscle, skin and tooth) were preserved in a solution of saturated NaCl in 20% (v/v) dimethyl sulfoxide in water or in pure ethanol. DNA from muscle and skin was extracted following standard proteinase K digestion and two phenol–chloroform and one chloroform–isoamyl (24:1) extractions followed by ethanol precipitation (Rosel and Block 1996). DNA from tooth samples was extracted using QiaAmp DNA Micro Kit from Qiagen in a separate laboratory facility. Samples were powdered using a mortar with liquid nitrogen and starting with less than 0.5 g tooth powder, the manufacturer's developed protocol for bones was followed. A negative control was used in subsequent analysis to assess the risk of contamination that is due to the susceptibility of this kind of material.

Two mitochondrial gene fragments were PCR amplified. A fragment of 630 bp comprising the proline and threonine transfer RNA genes and the hypervariable region I of the control region was amplified using the primers L15926 (5′ACACCAGTCTTGTAAACC 3′) and H00034 (5′TACCAAATGTATGAAACCT CAG 3′) described in Rosel et al. (1994) and will subsequently be designated as control region. Amplification reactions were performed in 50 µl volumes containing 10–100 ng of extracted DNA, 10 mM Tris–HCl pH 8.4 and 50 mM KCl, 1.5 mM $MgCl_2$, 0.15 mM of dNTPs, 0.3 µM of each primer and 0.02 U/µl Taq polymerase. The thermocycle profile consisted of an initial denaturation step at 94°C for 4 min followed by 35 cycles of 45 s of 94°C, 45 s at 50°C and 1 min at 72°C and a final extension step for 8 min at 72°C. The cytochrome *b* gene was amplified (1,121 bp) using primers on the transfer RNA (tRNA) genes on either side of cytochrome *b*. The L-strand primer was on tRNA glutamine (L14724; 5′ TGACTTGAARAAC CAYCG TTG 3′ and the H-strand primer on tRNA threonine (5′ CCTTTTCCGGTTTACAAGAC 3′) (LeDuc et al. 1999). Amplification reactions were performed in the same way as for the control region. The thermocycle profile for the cytochrome *b* gene consisted of an initial denaturation step at 94°C for 3 min followed by 35 cycles of 45 s at 94°C, 45 s at 48°C and 1 min at 72°C and a final extension step for 5 min at 72°C.

For both mtDNA regions, the PCR products were purified with Qiagen columns following the manufacturer's protocol. Both strands were directly sequenced (BigDye Terminator CycleSequencing; Applied Biosystems) on an ABI 3730 automated sequencer (Applied Biosystems).

To investigate sex-related differences in dispersal, for samples of unknown gender, a PCR-based sex determination was performed using the four primers described by Rosel (2003). Male and female positives were run. PCR products were separated by electrophoresis on 2% agarose gels and gender was determined from the resulting banding pattern.

Data analyses

Population analysis

All sequences obtained for both mitochondrial genes were aligned using the software Sequencher, version 4.2. (Gene Codes Corporation). Nucleotide composition

was examined for variable sites, and the χ^2 homogeneity test of base frequencies was done in PAUP* v. 4.0b10 (Swofford 2003).

Diversity measures included nucleotide diversity (π) and haplotype diversity (Hd) estimated according to Nei (1987) and calculated in DNAsp v. 4.10 (Rozas et al. 2003). To test selective neutrality, Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) were also estimated in DNAsp. Population differentiation for both genes was tested using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) to compute F_{ST} (using haplotype frequencies) and φ_{ST} (using genetic distances) using the appropriate distance model as determined by Modeltest (see below) in the program Arlequin v.2.000 (Schneider et al. 2000). Ten thousand permutations were run to test the significance of variance differences among hierarchical levels and genetic partitioning hypotheses. Sequential Bonferroni corrections for multiple tests were applied using an initial α value of 0.05 (Holme 1979). A Mantel test was conducted with genetic distance matrices based on the models given by Modeltest and geographic distance matrix based on coordinates. A median joining network of all unique haplotypes was constructed using the program Network, v.2.0 (Bandelt et al. 1999).

Phylogenetic analysis

For phylogenetic reconstruction, we used the Neighbour-Joining (NJ) and Maximum-Parsimony (MP) methods, implemented in PAUP* and a Bayesian inference approach implemented using the programme MrBayes 3.1.1b (Huelsenbeck and Ronquist 2001). The partition homogeneity test was generated in PAUP* to test the congruence of the genealogical tree generated with the control region and the cytochrome *b* gene. For the NJ method, a majority-rule consensus tree, rooted with a sequence from *Grampus griseus* (for the control region data set, Accession number AB018584 and for the cytochrome *b* data set, Accession number AF084059) was constructed from 5,000 bootstrap replicates and a 50% criterion for the retention of nodes was applied. The program Modeltest 3.07 (Posada and Crandall 1998) was used to find the best model of evolution that fit the data for NJ analyses. The model suggested for the control region was HKY + *I* (0.6840) + *G* (0.6248) (empirical base frequencies $A = 0.32$, $C = 0.24$, $G = 0.12$, $T = 0.32$) and for the cytochrome *b* gene was TrN + G (0.2642) (empirical base frequencies $A = 0.30$, $C = 0.31$, $G = 0.12$, $T = 0.27$) both selected by the Akaike criterion (Posada and Buckley 2004). In the maximum parsimony analysis, a heuristic search of 100 random additions with tree-bisection-reconnection (TBR) swapping was performed with MULPAR and steepest descent options. For the phylogenetic reconstruction based on a Bayesian approach, the number of generations for the Monte Carlo Markov chains (MCMC) method was set to 100,000 and a tree was saved every ten generations. The burnin value used in the MCMC chains was set to 500. The consensus tree was produced using PAUP* retaining branches with 50% support or greater.

Results

Population analysis

A total of 69 (33 males and 36 females confirmed by genetic analysis) short-beaked common dolphins were sequenced for both mitochondrial regions analysed. For the control region, the 630 bp compared revealed 53 polymorphic sites, from which 2 were insertions/deletions, 40 were transitions, 9 were transversions and 2 sites were both transitions and transversions. Fourty-three haplotypes were defined (GenBank Accession Numbers: DQ378096–DQ378137). Haplotype and nucleotide diversities for the entire data set were $Hd = 0.987 \pm 0.005$ and $\pi = 0.014 \pm 0.001$.

For the cytochrome *b* gene the 1,121 bp analysed revealed 61 polymorphic sites, from which 57 were transitions and 4 sites were transvertions. Twenty-seven haplotypes were defined (GenBank Accesion Numbers: DQ378138–DQ378164). Haplotype and nucleotide diversities were $Hd = 0.921 \pm 0.022$ and $\pi = 0.0056 \pm 0.0009$.

Base frequencies were homogenous across all variable sites for both mtDNA regions and for all *D. delphis* individuals ($\chi^2 = 4.54$, $df = 144$, $P = 1.0$ for the control region and $\chi^2 = 2.01$, $df = 120$, $P = 1.0$ for cytochrome *b* gene). Nucleotide composition showed an A-T bias (for both mtDNA regions) which is characteristic for the mitochondrial genome of cetaceans (Arnason et al. 1993).

The neutrality tests revealed high negative values of Fu's *Fs*, -24.924 ($P < 0.0001$) for the control region and -8.997 ($P < 0.0001$) for the cytochrome *b* gene, which are indicative that the population is in expansion although no significative value for the Tajima's *D* statistic was found, -0.9562 $P > 0.1$) for the control region and -1.8189 ($P > 0.05$) for the cytochrome *b* gene.

The Mantel test failed to reveal a correlation between genetic and geographical distances. No significant genetic differentiation was found when the four sampled areas were analysed. However, when sequences were

analysed separately by sex, we obtained significant F_{ST} values for females and males (0.2743 and 0.0588, respectively) but only for the cytochrome *b* gene.

The median-joining graphs obtained for the control region and the cytochrome *b* gene showed a high degree of complexity and relatedness with no clear geographical structure among haplotypes (Fig. 2). In the control region reticulated graph, a more complex pattern was observed, with many lineages connected to central missing intermediate haplotypes, which suggests that there are unsampled haplotypes or that ancient haplotypes have been replaced by more recent ones. In the cytochrome *b* gene graph, haplotypes that were most common, and shared by more than one geographical region, occupied more internal positions within the network, while haplotypes unique to one geographical region occurred in more external positions. A group of haplotypes (Group X) appeared very well differentiated from the main group in the two graphs, but with higher number of mutational steps separating them in the cytochrome *b* graph.

Phylogenetic analysis

The partition homogeneity test implemented in PAUP* showed the incongruence between the two mitochondrial genes datasets ($P = 0.03$) reason why we analysed them separately. However, phylogenetic trees obtained for the control region resulted in poorly resolved branches with bootstrap support values lower than 50%, and therefore we decided not to present them here as they would not contribute to the understanding of the phylogenetic relationships within and among *Delphinus*. Nevertheless, it is important to note that this dataset of 125 control region sequences, which included sequences from *D. delphis* from NE Atlantic (SCO, NSP, WPOR, SPOR), Azores and Canary Islands, Black Sea, Pacific Ocean, Argentina, *D. capensis* and *S. coeruleoalba*, provided 85 haplotypes. One of these unique haplotypes was shared between Argentina and WPOR and two were shared between the Canary Islands, the Black Sea and the NE Atlantic populations. There were no haplotypes shared by the Azores and other populations.

The phylogenetic trees obtained for cytochrome *b* with the three phylogenetic inference methods used (NJ, MP and Bayesian inference) all resulted in similar topologies (Fig. 3). Within the *D. delphis* clade, it was not possible to detect any geographical structuring. Nonetheless, some interesting results were observed, as the fact that the Black Sea individual joined a haplotype shared by the four NE Atlantic populations studied. The individual from Argentina appeared inside the *D. delphis* clade and it seems to be very closely related with the NE Atlantic populations. Additionally, the *D. delphis* from the Pacific Ocean appeared outside the *D. delphis* clade and it is more distantly related to *D. delphis* from NE Atlantic than from *D. capensis*. Moreover, we observed a highly divergent clade (Clade X) including haplotypes from the Iberia Peninsula, Scottish coast and *D. tropicalis* (from the Indian Ocean). High bootstrap support values for both NJ and MP analysis and high Bayesian posterior probabilities supported this differentiation. These individuals correspond to those that constituted a separate group in the haplotype networks presented in the population analysis section. When genetic divergences between the referred clades were calculated, 1.76% divergence was obtained between Clade X and *D. capensis* clade; 1.59% between Clade X and *D. delphis*

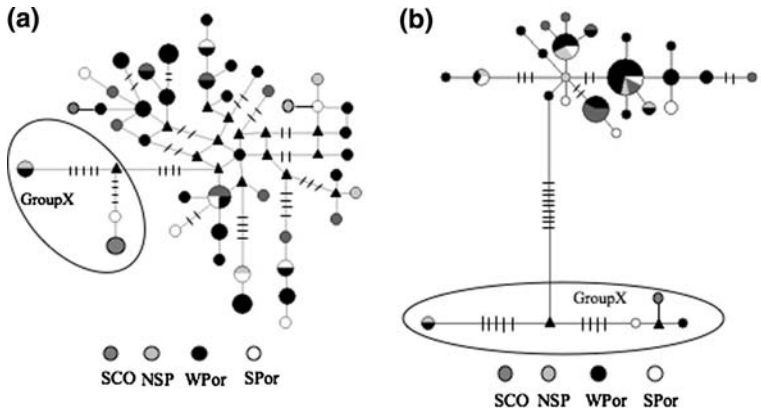

Fig. 2 Median-joining networks of short-beaked common dolphin mtDNA haplotypes for **a** the control region and **b** the cytochrome *b* gene. Circle size is proportional to the number of individuals exhibiting the corresponding haplotype and proportion of each population within each haplotype is shaded according to the legend. Length of lines is proportional to the number of mutational steps separating haplotypes, with *hatch marks* indicating total number of mutations when more than one mutation is present. *Triangles* indicate missing intermediate haplotypes

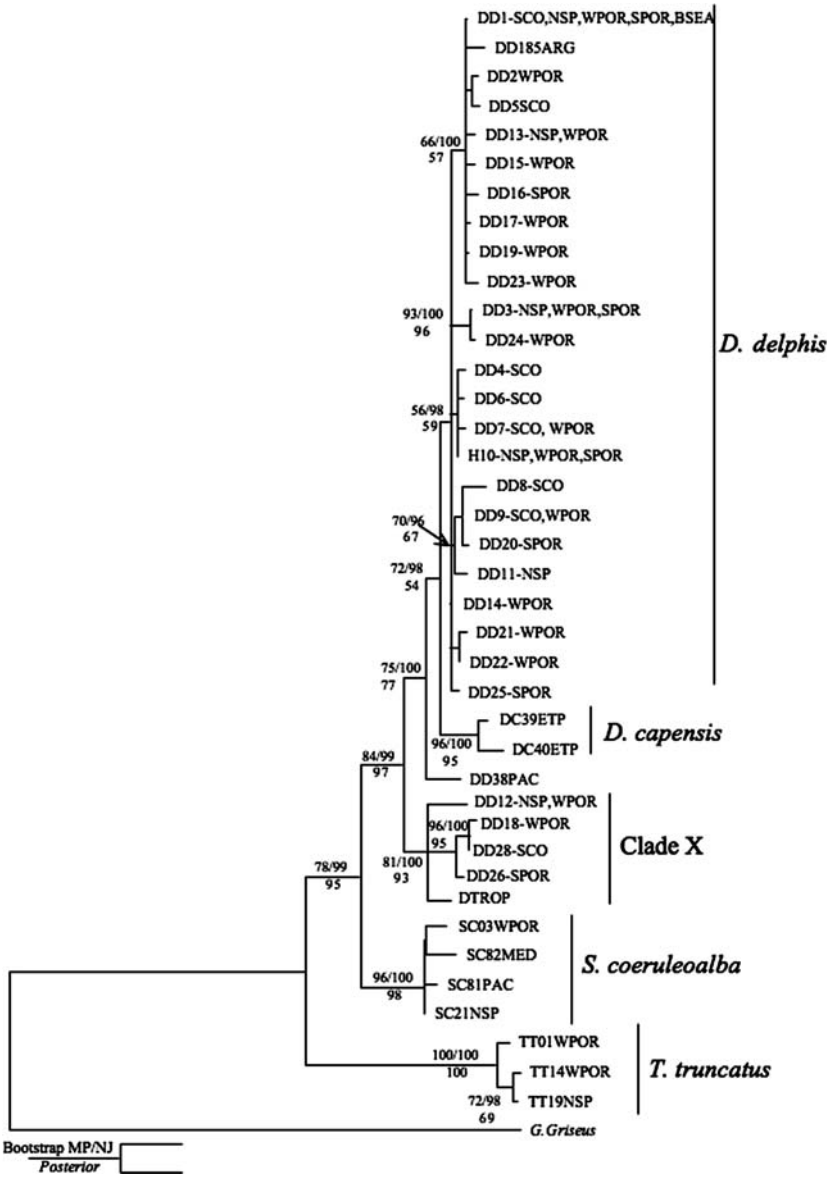

Fig. 3 Phylogenetic relationships recovered based on the NJ, MP and Bayesian methods of phylogenetic inference for cytochrome *b* sequences. *Left*/*right* values above on branches correspond to NJ bootstrap values and Bayesian posterior probabilities, respectively. Values below branches correspond to MP bootstrap support values

clade; and 1.07% divergence between *D. delphis* and *D. capensis* clades. When analysing the sequences of Clade X individuals, 8 fixed nucleotide substitutions (all transitions) were found to separate them from others *D. delphis* and 12 fixed positions (11 transitions and 1 transversion) were found separating them from *D. capensis*. As for *S. coeruleoalba* and *T. truncatus*, they appeared in separated clades, with *S. coeruleoalba* being more closely related to *Delphinus* (as reported by LeDuc et al. 1999).

Discussion and conclusions

In this study we found evidence for the existence of a sex-biased population structure in NE Atlantic common dolphins, which supports previous morphometric evidence based on skull measurements (Murphy 2004). We also found a group of individuals highly differentiated, which appear to be more distant from other NE Atlantic common dolphins than these are from *D. capensis*. Moreover, the two species *D. delphis* and *D. capensis* did not show reciprocal monophyly, as reported by LeDuc et al. (1999).

Population analysis

Overall mitochondrial DNA genetic variability estimates for NE Atlantic were within the range described for other cetaceans (Rosel et al. 1994; Cassens et al. 2005; Escorza-Trevino et al. 2005; Adams and Rosel 2006). Significant genetic differentiation was revealed

when males and females were analysed separately for all populations in cytochrome *b* sequences (F_{ST} = 0.2743 in females and 0.0588 in males). Although F_{ST} estimates for females were twice those of males, which may suggest the existence of female phylopatry, we would need to compare these results with other uniparentally inherited markers such as Y-linked genes to correctly state this. Nonetheless, this is an important indication that should not be disregarded. Even though female site-fidelity and male-mediated dispersal have been described in several cetacean species (Baker et al. 1998; Rosel et al. 1999; Escorza-Trevino and Dizon 2000; Adams and Rosel 2006), it has not yet been described for common dolphins since this species has always been considered to form large, panmictic populations (Reeves et al. 2002). Moreover, both morphometric and ecophysiological differences between Portuguese common dolphins and more northerly sampled animals have been reported (Silva 1999; Zhou et al. 2001), which may support the existence of this fine scale population structure. The fact that φ_{ST} values were not significant may reflect the recent divergence of haplotypes in these populations. Since this statistic is based on both genetic distances and haplotype frequencies, only when there has been sufficient evolutionary time for genetic differences to evolve, can φ_{ST} detect population structure. On the contrary, F_{ST} values are solely based on haplotype frequencies, which implies that when genetic distances are small but haplotype frequencies differ (a recent divergence), only F_{ST} will detect genetic structure (O'Corry-Crowe et al. 1997). More evidence for a recent expansion of the studied populations comes from the star-shaped haplotype networks and from the high negative values obtained for the Fu's *Fs* statistic. These results corroborate what was suggested by Natoli et al. (2006).

Phylogenetic analysis

The phylogenetic analysis performed in this study resulted in some interesting aspects to be discussed, mainly in the tree obtained for the cytochrome *b* gene, since the tree obtained for the control region resulted in a poorly resolved diagram. However, in the control region dataset, we could observe some shared haplotypes between populations from the Canary Islands, the Black Sea and NE Atlantic populations which indicate that some level of gene flow may exist. Also a shared haplotype between the individual from Argentina and a Portuguese common dolphin was found, suggesting that migration from (or to) south Atlantic may exist, as was also reported by Westgate (2005) and Natoli et al. (2006).

In the cytochrome *b* phylogenetic analysis we thus found: firstly, the existence of a highly differentiated group of individuals (Clade X) from the Iberia Peninsula and Scottish coasts, which in turn was very similar to a *D. tropicalis* specimen; secondly, the difference between *D. delphis* from the NE Atlantic and *D. delphis* from the Pacific Ocean, and finally *D. delphis* and *D. capensis* are not monophyletic species. In fact all these aspects are interconnected. The taxonomic controversy surrounding the two species designation (Rosel et al. 1994; LeDuc et al. 1999; Natoli et al. 2006) and the suggestion for the existence of a subspecies (Jefferson and Van Waerebeek 2002) within the genus *Delphinus* makes phylogenetic studies difficult to interpret. Until now, and excluding the phylogenetic study of the family Delphinidae conducted by LeDuc et al. (1999), genetic studies involving common dolphins have used control region, microsatellites and AFLP's. None has focused on a complete study based on full cytochrome *b* sequences in order to clarify *Delphinus* taxonomy. In our opinion, this mitochondrial gene has several advantages when compared to the control region, which seems to be too variable to provide reliable phylogenetic information, mainly when analysing recently separated species whose process of lineage sorting may not yet be completed.

The existence of Clade X individuals is, thus, difficult to explain. We propose several hypotheses: (1) they can be a sample size artefact; (2) two groups that have evolved from independent events; (3) a different subspecies, (4) or the result of introgressive hybridization.

These individuals are all females, one from the Scottish coast (stranded in 2003), one from the northern Spanish coast (stranded in 2004), two from the west Portuguese coast (stranded in 1995 and 1997) and one from the south Portuguese coast (stranded in 2003). Although there is no photographic register available for most of them, misidentification is not a valid hypothesis since it was made by four different institutions/stranding networks, and specimens were fresh when samples were collected.

Common dolphins are amongst the more abundant cetacean species, with large population sizes that account for the high levels of genetic diversity seen in these species. It is reasonable to assume that in our sampling we may have two divergent clades simply because we did not sample the whole genetic diversity of the species. Viricel (2006) has also found two divergent groups in a mass stranding event in the French coast. However, we believe that the cytochrome *b* genetic divergence value separating this group of individuals from *D. delphis* or *D. capensis* being higher

than that separating the two species, and the fact that there was a high genetic similiarity with *D. tropicalis*, provides support for the existence of a separate subspecies. Since morphologically they probably did not differ from typical *D. delphis*, the existence of cryptic species is another possibility. As recently stated by Baker and Bradley (2006), cryptic species are more common in mammals than previously thought, and it was the development of molecular techniques such as DNA sequencing and more recently DNA barcode (Witt et al. 2006) that has allowed its identification in various animal groups.

The existence of highly divergent *D. delphis* groups that may have evolved from independent events, as suggested by Natoli et al. (2006) for *D. capensis*, could also validate the existence of high levels of divergence within the same species, and thus the existence of divergent lineages.

The existence of introgressive hybridization between common dolphin species and other delphinid species is another possibility that cannot be ruled out. Several studies in animals have detected this phenomenon using mtDNA sequences (Sota et al. 2001; Gaubert et al. 2005), although morphometric and nuclear genomic information are always valuable tools for this kind of assessment.

Cetaceans may have the potential to produce viable hybrid offspring more easily than other mammals, since it is unusual for this animal group to display prominent karyological uniformity. Cases of hybridization in captivity support this hypothesis (e.g. Bérubé 2002; Zornetzer and Duffield 2003) but in the wild only a few cases are suspected to exist owing to the difficulties inherent in accurately identifying hybrids (Arnason et al. 1993; Baird et al. 1998). Furthermore, if backcrosses with parental species exist, hybrid morphology can be similar to one of the parental species, making their identification even more difficult (Mallet 2005).

Different patterns of colouration in common dolphins from Portuguese waters and associations with other species have been observed (M. Sequeira, personal communication) in oceanographic surveys but were never thoroughly studied. This existence of anomalously pigmented common dolphins had been previously reported for the NE Atlantic by Perrin et al. (1995). Such morphological variation in a small area has also been reported in New Zealand (Stockin and Visser 2005) and associated with possible introgressive hybridization with other closely related species as *S. coeruleoalba* or *Turiops truncatus* due to observations of mixed groups. Even though individuals from Clade X appeared well differentiated from both species, a more complete molecular study will be necessary to completly rule out the possibility of hybridization. Furthermore, some shared haplotypes between *D. delphis* and *S. coeruleoalba* were obtained in previous studies (unpublished data) both for the control region and cytochrome *b* gene although misidentification in the field could not be ruled out.

According to our results and those obtained by LeDuc et al. (1999), Kingston and Rosel (2004) and Natoli et al. (2006), *D. delphis* and *D. capensis* appear as paraphyletic groups, suggesting the recent separation of the two common dolphin species and an incomplete lineage sorting as the explanation for the phylogenetic pattern observed. The existence of introgression between the two recently separated species could be another plausible explanation for the existence of the individuals of Clade X. Although controversial, there is evidence for the existence of mtDNA recombination in animals (Rokas et al. 2003; Tsaousis et al. 2005) and this possibility has never been studied in the cetacean mitochondrial genome. Furthermore, the morphometric study of Murphy et al. (2006) revealed that short-beaked common dolphins from the NE Atlantic showed intermediate total body length, skull size, rostrum length/zygomatic width ratio and tooth counts between *D. delphis* and *D. capensis*, which is another evidence that supports the possible existence of introgressive hybridization events between these two species, prior to the colonization of this region. Although a recent survey of the nuclear genome of *Delphinus* species using AFLPs concluded that significant nuclear genetic differentiation has arisen between both species despite their morphological similarity (Kingston and Rosel 2004), the majority of the samples used were from the Pacific Ocean. The results obtained in this study suggest that a taxonomic revision in the genus *Delphinus* is due, following a multidisciplinary approach including not only mitochondrial and nuclear genetic information but also morphological characters.

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