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Evidence of introgressive hybridization between *Stenella coeruleoalba* and *Delphinus delphis* in the Greek Seas

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ABSTRACT

Natural interspecific hybridization might be more important for the evolutionary history and speciation of animals than previously thought, considering several demographic and life history traits as well as habitat disturbance as factors that promote it. In this aspect, cetaceans comprise an interesting case in which the occurrence of sympatric species in mixed associations provides excellent opportunities for interspecific sexual interaction and the potential for hybridization. Here, we present evidence of natural hybridization for two cetacean species commonly occurring in the Greek Seas (*Stenella coeruleoalba* and *Delphinus delphis*), which naturally overlap in the Gulf of Corinth by analyzing highly resolving microsatellite DNA markers and mitochondrial DNA sequences in skin samples from 45 individuals of *S. coeruleoalba*, 12 *D. delphis* and three intermediate morphs. Employing several phylogenetic and population genetic approaches, we found 15 individuals that are potential hybrids including the three intermediate morphs, verifying the occurrence of natural hybridization between species of different genera. Their hybrids are fertile and able to reproduce not only with the other hybrids but also with each of the two-parental species. However, current evidence does not allow firm conclusions whether hybridization might constitute a step towards the generation of a new species and/or the swan song of an already existing species (*i.e.*, *D. delphis*). Given that the focal species form mixed pods in several areas of Mediterranean, this study is an excellent opportunity to understand the mechanisms leading to hybridization in the context of gene flow and urges for the evaluation of the genetic status of common dolphins in the Mediterranean.

1. Introduction

In the early years of the modern evolutionary synthesis, natural hybridization had been considered as a rare phenomenon with very little evolutionary significance. Nowadays, this ceased to be the case with the numerous studies of hybridization that have been conducted providing clues on the reproductive behavior, dispersal capabilities and phylogenetic relationships of species (Pyle and Randall, 1994). Even though the evolutionary significance of hybridization is a controversial issue (Schwenk et al., 2008), the study of the causes and consequences of natural hybridization in hybrid zones [areas where genetically distinct groups of individuals meet and mate, resulting in at least some offspring of mixed ancestry (Harrison, 1990)] offers opportunities to evaluate the effects of gene flow, natural selection and recombination in natural populations and provides insights into the phenotypic and genotypic changes during speciation (Mullen et al., 2008).

The frequent occurrence of interspecific hybridization in several groups of animals is indicative of its' key role in animal evolutionary history and speciation mostly by increasing their adaptability to environmental change (Mallet, 2005). Factors considered to promote interspecific hybridization include several demographic and life history traits (population sizes, body sizes, timing of reproduction, behavior, climatic conditions, parental care) as well as habitat disturbance (Crossman et al., 2016; Frantzis and Herzing, 2002; Jahner et al., 2012; May-McNally et al., 2015; Randler, 2006; Rubidge and Taylor, 2005; Scribner et al., 2001; Taylor, 2004; Yau and Taylor, 2013). In a recent review of cetaceans (whales, dolphins, and porpoises) it has been shown that almost 20% of the species hybridize (*i.e.*, Amaral et al.,

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2014; Baird et al., 1998; Glover et al., 2010; Spilliaert et al., 1991) both in the wild and in captivity (for a review see Crossman et al., 2016).

The confusing taxonomy and uncertain phylogenetic relationships observed in some groups of cetaceans have been attributed to incomplete lineage sorting and hybridization (Amaral et al., 2012) as a consequence of the rapid events that characterized their radiation. The relatively recent evolutionary radiation of cetaceans [*i.e.* last 10 million years, (McGowen et al., 2009)] combined with the apparently slow evolutionary rate (Hoelzel et al., 1991; Schlotterer et al., 1991) could justify the constant number of chromosomes ($2n = 44$) and the common karyotic arrangement in most cetaceans (Arnason and Benirschke, 1973; Árnason et al., 1978; Pause et al., 2006). This in turn suggests a lack of major differences in chromosomal rearrangements among species (Amaral et al., 2014) and karyological uniformity (Arnason, 1980) that might indicate a greater potential for cetaceans in respect to other mammals, to hybridize and generate viable and fertile offspring (Amaral et al., 2014). This becomes evident in oceanic cetaceans with a karyotype of 44 chromosomes, where hybridization is known to occur in half of the species (Crossman et al., 2016).

The large diversity of marine habitats in the Greek Seas supports eight commonly occurring and three occasional cetacean species (Frantzis, 2009; Frantzis et al., 2003). In this study we focus on two of the species, the striped dolphin (*Stenella coeruleoalba*) and the short-beaked common dolphin (*Delphinus delphis*). *Stenella coeruleoalba*, which is the most common cetacean in the region (Frantzis, 2009), is typically pelagic, inhabiting the deep waters of the continental shelf and it is observed close to shore only where deep water is found close to the coast (Frantzis, 2009; Gannier, 2005). According to the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Animals, its Mediterranean population is listed as “Vulnerable” (Aguilar and Gaspari, 2012). In the Mediterranean, *D. delphis* occurs in both neritic and pelagic environments, occasionally together with *Tursiops truncatus* (common bottlenose dolphin) and *S. coeruleoalba*, respectively (Bearzi et al., 2003). In the Greek Seas, all known population units of *D. delphis* inhabit shallow (< 200 m) and coastal waters, except the one inhabiting the Gulf of Corinth (GOC) (Frantzis, 2009). The Mediterranean population of this species is listed by IUCN as “Endangered”, since it experienced a 50% decline in abundance over the last three generations (for more details see Bearzi, 2003; Natoli et al., 2008).

Stenella coeruleoalba and *D. delphis* belong to two taxonomically problematic genera of delphinid cetaceans (Natoli et al., 2006), due to the lack of correspondence between their morphological and genetic differentiation. Previous studies on the genetic variation of *S. coeruleoalba* detected significant differentiation among the Mediterranean and North Atlantic and Pacific populations (Bourret et al., 2007; García-Martínez et al., 1999; Gaspari et al., 2007; Valsecchi et al., 2004). Although mtDNA data showed no population subdivision in the Mediterranean Sea (Garcia-Martinez et al., 1995), the use of microsatellite data in two recent studies, revealed population genetic structure within the Mediterranean basin [subdivision in the western Mediterranean population (Bourret et al., 2007) and in inshore and offshore populations in the Tyrrhenian Sea (Gaspari et al., 2007)]. On the other hand, the patterns of genetic differentiation at the population level of *D. delphis* in the Mediterranean showed a marked differentiation between the Ionian and Alboran Seas (Natoli et al., 2008), at a similar or even higher level to that observed between populations of species from different sides of the Atlantic Ocean (Natoli et al., 2006). These patterns were correlated to the different habitat preferences displayed by *D. delphis* in the western (open water) and eastern (shallow coastal habitat) Mediterranean, suggesting the exploitation of different resources as a significant factor reducing movement between these regions (Natoli et al., 2008). Finally, preliminary results indicate the isolation of the Black Sea population of *D. delphis* from the rest of the Mediterranean (Natoli et al., 2008).

The GOC is the only known body of water globally, where three sympatric dolphin species form permanent mixed-species groups: *D. delphis*, the purely pelagic *S. coeruleoalba* and *Grampus griseus* (Risso’s dolphin) (Frantzis and Herzing, 2002) are usually found in the deep waters of the continental slope (Frantzis, 2009). Although the GOC is a semi-enclosed sea, it shows several characteristics of an open sea due to its deep waters and steep slopes along its coasts, the systematic occurrence of wind-driven upwelling currents and the entrance of waters from the Ionian Sea (Frantzis and Herzing, 2002). Sympatric *D. delphis* and *S. coeruleoalba* that form temporal mixed-species groups have been recorded in two more areas of the Mediterranean Sea: the Alboran Sea in western Mediterranean, and the Tyrrhenian Sea in central Mediterranean (García-Martínez et al., 1999). Additionally, recent unpublished observations, indicate that temporal mixed groups of these two species also occur in other Mediterranean areas, such as the Balearic Sea and the Sicilian Strait (Ana Cañadas and Mediterranean common dolphin specialists group, pers. comm. 2017).

The occurrence of sympatric cetaceans in mixed associations provides excellent opportunities for interspecific sexual interaction and the potential for hybridization (Bérubé, 2009). Despite the high number of hybridization events in cetaceans hold in captivity (Bérubé, 2009), wild cetacean hybrids are typically identified based solely on morphology without any prior knowledge of parental interactions. Therefore, hybrid identification in the field is problematic with the number of well-documented incidences being limited (for a review see Bérubé, 2009; Crossman et al., 2016). The collection of genetic data for the verification of the occurrence of alive wild hybrid dolphins is difficult and has associated welfare considerations (Hodgins et al., 2014), rendering the molecular confirmation of hybridization scarce (Amaral et al., 2014; Bérubé and Aguilar, 1998; Willis et al., 2004).

Observations on dolphins in the GOC have reported individuals with unusual pigmentation patterns (intermediate morphs between *S. coeruleoalba* and *D. delphis*) (Frantzis and Herzing, 2002) that either constitute potential hybrids between the two species or represent another incident of the high variability of pigmentation patterns in *S. coeruleoalba* (Acquarone and Notarbartolo di Sciara, 1992). Aiming to test whether natural hybridization is the case and understand the evolutionary mechanisms that may be behind the origin of the intermediate morphs, we used data from highly resolving microsatellite DNA markers and mitochondrial DNA (mtDNA) sequences. Skin samples from several individuals of *S. coeruleoalba* and *D. delphis* from several locations of the Greek Seas with emphasis on the GOC as well as from the intermediate morphs from the GOC were examined and their genetic data were analyzed through several phylogenetic and population genetic approaches. Genetic intermediacy between the two parental forms, nuclear admixture and mitochondrial capture and unique variation would indicate that these morphs constitute the results of recent hybridization.

2. Material and methods

2.1. Samples and DNA extraction

From 1997 to 2013 sixty dolphin skin samples were collected and preserved in ethanol. Twenty-two originated from the GOC and thirty-eight from other areas of the Greek Seas (Fig. 1). In total 45 specimens of *S. coeruleoalba,* 12 specimens of *D. delphis* and 3 intermediate morphs were sampled. The samples from the three intermediate morphs and one *D. delphis* were collected from free-ranging dolphins in the GOC while they were bow-riding by the use of a pole to minimize disturbance. Sampling occurred in accordance with international guidelines and under a research permit from the Greek authorities. All other samples were collected from dead animals stranded along the coasts (Fig. 1 and Table S1). The skin samples were washed three times in 1 mL of 10 mM Tris-HCl (pH 8.0) on a rotary mixer for 24 h per wash to re-hydrate (Austin and Melville, 2006). Total genomic DNA was extracted using the DNA IQ System (Promega, USA).