

SC/66b/SM/16

Brief summary of published data and ongoing research supporting distinct forms of common bottlenose dolphins, *Tursiops truncatus*, in the western North Atlantic

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Abstract

Two distinct morphotypes of common bottlenose dolphins, *Tursiops truncatus*, are present in the western North Atlantic basin and appear to exhibit a parapatric (or possibly sympatric) distribution partitioned into coastal and offshore habitats. Although they can be difficult to distinguish in the field, these two forms differ in cranial morphology, body size, tooth counts, parasite loads, diet preferences, hematological parameters, and in mitochondrial (mtDNA) and nuclear markers. Preliminary results from ongoing genetic analysis of > 700 samples from the western North Atlantic reinforce significant genetic divergence between the two forms at mtDNA, and also show significant genetic differentiation at major histocompatibility complex genes and nuclear microsatellites. Net nucleotide divergence between the two forms in the mtDNA control region is 0.025, on par with other recognized delphinid species. Comparison of mtDNA control region haplotypes from the coastal morphotype to those in GenBank found no matching haplotypes outside the western North Atlantic, while many offshore haplotypes were found elsewhere throughout the world. Overall, the body of genetic evidence suggests the two morphotypes in the western North Atlantic basin deserve at least subspecies, if not species status, with the offshore morphotype remaining *T. truncatus truncatus*. There are two nominal names that may be applicable to the coastal morphotype: *T. erebennus* and *T. subridens*. Ongoing morphological comparisons will further help clarify the taxonomic status.

Introduction

In the western North Atlantic Ocean (wNA), the common bottlenose dolphin, *Tursiops truncatus*, provides an interesting case of morphological differentiation among populations inhabiting similar environments in the absence of obvious barriers to genetic exchange and resulting unresolved taxonomy. These morphotypes differ in some aspects of cranial morphology, body size, tooth counts, parasite loads, diet preferences (Mead & Potter 1995), hematological parameters (Duffield 1987) and in both mitochondrial (Hoelzel et al. 1998, Moura et al. 2013) and nuclear markers (Kingston & Rosel 2004, Kingston et al. 2009), yet they can be difficult to distinguish in the field.

With the advent of remote biopsy techniques (Gorgone et al. 2008), it is now possible to collect skin samples from free-ranging animals in their natural habitat making it feasible to collect biological samples *in situ* allowing a more thorough analysis of the patterns of habitat and ecological differentiation that may exist between the two morphotypes in the wNA. This information can increase our understanding of speciation processes in the open ocean and also has conservation implications. In recent years, genetic data obtained from biopsy and stranding samples have been applied to questions of *Tursiops* distribution, population structure and taxonomy in the wNA (Hoelzel et al. 1998, Kingston & Rosel 2004, Kingston et al. 2009, Rosel

et al. 2009, Moura et al. 2013). More recently, in an ongoing study, we have been collecting genetic data (mitochondrial DNA control region, MHC and Y-chromosome sequences, and microsatellite data) to gain a better understanding of the evolutionary relationships between the two morphotypes and the degree to which they interact on spatial scales. Here we summarize previously published information as well as new preliminary genetic results that examine the degree of genetic divergence between the two morphotypes in the wNA.

Distribution

Between 1978 and 1982 the U.S. Minerals Management Service funded a Cetacean and Turtle Assessment Program (CETAP) to examine the distribution of cetacean and turtle species in U.S. waters between Cape Hatteras, NC and Nova Scotia, Canada and from the shoreline out to 5 nmi seaward of the 1,000 fathom (fm) isobath (Kenney 2000). Most surveys were aerial surveys, although a few vessel platforms-of-opportunity were also used. For *Tursiops*, a pattern emerged of 2 groupings of sightings. Most *Tursiops* sightings were clustered offshore along the 1,000-fm isobath at the continental shelf break from Cape Hatteras to the northeastern end of Georges Bank (i.e., almost completely throughout the surveyed area). A second grouping was seen right along the coastline from Cape Hatteras to southern New Jersey. A fairly obvious distributional hiatus was observed between these sightings (Kenney 2000) over the continental shelf. Vessel surveys south of Cape Hatteras reveal a more even distribution of sightings of *Tursiops* from the coast, across the continental shelf, and along the continental shelf break (Waring et al. 2014).

Morphology, prey preferences and parasite loads

Mead & Potter (1995) examined skull characteristics of coastal and offshore forms in the wNA. They examined skulls from 273 *Tursiops* collected in directed coastal dolphin fisheries that took place in the 1800s along the U.S. east coast and combined those with strandings collected between 1972 and the mid 1990s. To represent the offshore animals, they obtained 15 skulls from animals collected in offshore gillnet fisheries. This analysis did not include a standard principal components-type analysis. Instead, the authors reported on differences between coastal and offshore animals at specific characters, namely that the width of the internal nares differed significantly between the two forms when compared with the zygomatic width and with the condylobasal length. In addition, offshore animals were 15% longer than coastal animals, but the total length distributions of the two forms overlapped to some degree. Mead and Potter (1995) also examined stomach contents and parasite loads and found significant differences between the coastal and offshore forms. Offshore animals (n = 18) fed on pelagic squid and deepwater fishes, notably myctophids, while the coastal animals (n = 117) fed primarily on sciaenid fishes and occasionally coastal squids. Offshore animals were commonly infected with three parasite species absent or rare in the coastal animals. The authors concluded there are significant differences between coastal and offshore populations of *Tursiops* along the U.S. Atlantic coast and suggested there is no interbreeding between them.

Genetics

Some of the earlier molecular work that examined coastal and offshore common bottlenose dolphins examined differences in hemoglobin (Hb) profiles. Duffield et al. (1983) used enzyme electrophoresis to examine Hb profiles in animals sampled from coastal waters in the Atlantic and Pacific as well as offshore animals sampled in the Pacific. The animals sampled in coastal

waters of both oceans shared a Hb profile different from the Pacific offshore animals. In a subsequent study, Duffield (1987) found the Hb profile associated with the Pacific offshore type in several Atlantic samples, suggesting both offshore and coastal forms exist in the Atlantic as well.

Hoelzel (1998), Natoli et al. (2004), and Moura et al. (2013) have all looked at genetic relationships among *Tursiops* worldwide using mitochondrial DNA (mtDNA) sequence data. Samples used to represent the coastal and offshore forms in the wNA that were used in the three studies overlapped. Using 300 bp of control region sequence, Hoelzel (1998) and Natoli et al. (2004) found no shared haplotypes between animals classified as the coastal form and those classified as the offshore form in the wNA (classified based on sampling location, stomach content, parasite load or skull morphology). However, phylogenetic analyses (neighbor joining, parsimony) failed to return reciprocally monophyletic clades of the two forms. Bayesian analysis of complete mitogenome sequences (Moura et al. 2013) did yield reciprocal monophyly between coastal and offshore *T. truncatus* samples from the wNA. In addition, the samples representing the coastal form from the wNA branched off first and were sister to all other *T. truncatus* samples in the data set suggesting a very early split of this group in the evolutionary history of *T. truncatus*.

Rosel et al. (2009) compared mtDNA control region sequences (354 bp) for a larger sample size of coastal animals (n = 451) from the wNA including the Gulf of Mexico to six haplotypes from offshore animals. Bayesian phylogenetic reconstruction did separate the coastal from the offshore haplotypes, but nodal support was relatively low. Haplotypes representing the coastal morphotype have also been found in the Bahamas (Parsons et al. 2006) and are present throughout coastal and estuarine waters of the northern Gulf of Mexico (Vollmer 2011). Expanding to the Caribbean, Caballero et al. (2012) identified 41 *Tursiops* haplotypes, including haplotypes identified as the coastal and offshore forms seen in U.S. waters of the wNA, in 158 control region sequences from the wider Caribbean area.

Analysis of nuclear loci through amplified fragment length polymorphism analysis (AFLP) also revealed separation of the two forms in the wNA. A multi-locus phylogeny inferred from 418 polymorphic genomic markers identified two clades – a coastal clade with high nodal support (posterior probability = 1) separated from an offshore clade (Kingston et al. 2009).

In ongoing genetic analyses, we have sequenced the control region and collected genotypic data for 19 microsatellite loci for 766 dolphins from coastal, continental shelf and slope waters of the western North Atlantic from Florida to central New Jersey. Fixed nucleotide differences were observed in 354 bp of the mtDNA control region, and distinct allele frequency distributions and many private alleles were found at the nuclear microsatellite loci. We have also sequenced Exon 2 of the major histocompatibility complex genes DQB and DRB. Morphotype-specific MHC alleles were seen. Given that MHC alleles are often shared across cetacean taxa, it is surprising that so many private alleles (8 for DQB and 14 for DRB) were found for each of the morphotypes. Interestingly, analysis of Y-chromosome markers from a subset of animals as found no differences.

Overall Summary

The significant biological, ecological and genetic differences between the two morphotypes in the wNA suggest they are on independent evolutionary trajectories and should be managed and conserved independently of one another. Overall, the body of evidence suggests the two morphotypes in the western North Atlantic basin deserve at least subspecies, if not species status, with the offshore morphotype remaining *T. truncatus truncatus*. There are two nominal names that may be applicable to the coastal morphotype: *T. erebennus* and *T. subridens*, and we continue to work to identify which is appropriate. On a broader scale, these unique dolphins with the coastal morphotype (based on skull morphology) and coastal-type mtDNA haplotypes are also found through coastal waters of the Gulf of Mexico from the Florida Keys to the Yucatan Peninsula (Vollmer 2011, Caballero et al. 2012, Vollmer & Rosel 2013), the northern coast of Cuba (Caballero et al. 2012) and in the Bahamas (Parsons et al. 2006), all areas with significant areas of relatively shallow, coastal waters. Caballero et al. (2012) also identified five animals from Puerto Rico and one from the U.S. Virgin Islands that exhibited coastal morphotype mtDNA haplotypes. These are interesting animals as these islands have very narrow continental shelf shelves with little nearshore shallow water.

Morphologically, the offshore form in the wNA is longer and more robust, with a stockier build, and generally shorter rostrum than the coastal form. Mead and Potter (1995) found that the width of the internal nares differs significantly between the two forms when compared with the zygomatic width and the condylobasal length. In a preliminary analysis, Costa and Rosel (IWC 2016 document) performed principal components analysis of 101 skulls and 19 cranial measurements from physically mature specimens of *Tursiops* from the wNA. There was little overlap for principal components (PC) 1 and 2 and significant separation of offshore and coastal form skulls; 95% ellipses for the combined PC1 and PC2 scores did not overlap. This ongoing morphological study further support the taxonomic distinctiveness these morphotypes in the western North Atlantic.

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