

UC San Diego

Capstone Papers

Title

Phylogenetic and Phylogeographic Analysis of Beaked Whales in the Northern Bahamas:
Implications for Conservation

Permalink

<https://escholarship.org/uc/item/52m5j959>

Author

Lee, Nicole M

Publication Date

2012-04-01

Phylogenetic and Phylogeographic Analysis of Beaked Whales in the Northern Bahamas: Implications for Conservation

Nicole M. Lee
MAS Marine Biodiversity and Conservation
Scripps Institution of Oceanography
2012

Committee Members

Phillip Morin, Ph.D, NOAA Southwest Fisheries Science Center Marine Mammal Genetics Group
(Chair)

Greg Rouse, Ph.D, Scripps Institute of Oceanography, Professor, Marine Biology

Phaedra Doukakis, Ph.D, Scripps Institute of Oceanography, NOAA

David Woodruff, Ph.D, Professor, Ecology, Behavior, and Evolution, UCSD

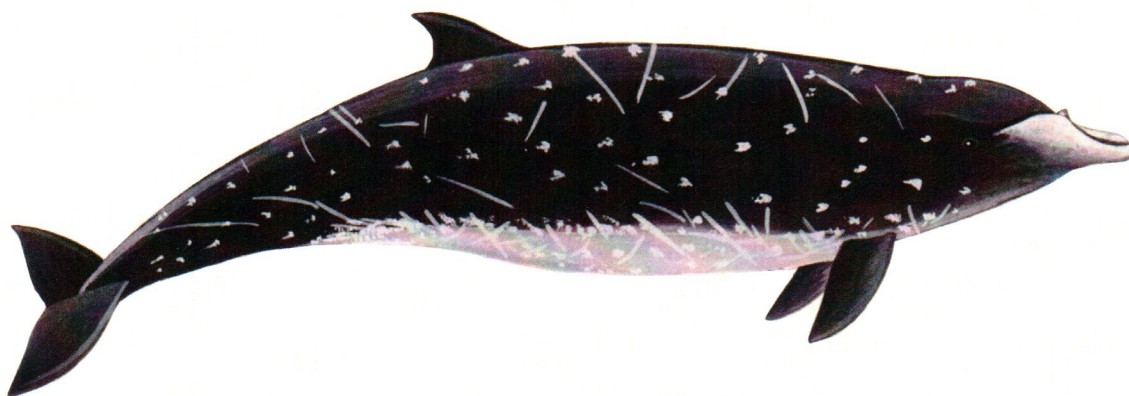


SCRIPPS INSTITUTE OF
OCEANOGRAPHY
GLOBAL DISCOVERIES FOR TOMORROW'S WORLD





Ziphius cavirostris

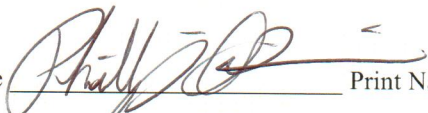


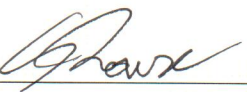
Mesoplodon densirostris

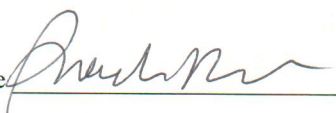
Acknowledgements

I wish to begin by acknowledging and thanking those who helped make this project possible. First, thank you to Greg Rouse for introducing me to the field of molecular biology and phylogenetics, and for the countless hours he helped me. Without Greg's influence and guidance, this study would not have occurred. I also want to thank Phil Morin for allowing me to have access to such incredible data he sequenced and for his patience while working with me on this project. I'd like to thank John Durban and the Bahamas Marine Mammal Research Organization for the many hours of arduous fieldwork necessary in obtaining the samples that were used in this study. I want to also express gratitude to Eric Archer for the many problems he helped me overcome and the kindness he showed while doing so. I would also like to thank Summer Martin and Matt Leslie for their support and guidance. I also wish to acknowledge Brittany Hancock-Hanser and the Marine Mammal Genetics lab at NOAA SWFSC for their work in processing the samples. Also, thank you to Phaedra Doukakis-Leslie and David Woodruff for serving on my committee and helping me achieve my goals, as well as Jane Weinzierl and the MAS CMBC family. I wish to acknowledge my partner, Trey Doig, for his love and support through this year and for all the technical and artistic support he provided that made this project possible. Finally, I wish to thank my grandmother, Jean Hersey-Spritzer, for instilling in me a love of wildlife and the ocean. You believed and supported me no matter what crazy adventure I was on. For this, I am forever grateful.

Capstone Advisory Committee

Signature  Print Name Phillip A. Morin Date 6/14/12
Affiliation SWFSC/SIO Email Phillip.Morin@NOAA.GOV Phone 858-546-7165

Signature  Print Name G. Rouse Date 6-14-2012
Affiliation SIO Email grouse@ucsd.edu Phone 858 534 7773

Signature  Print Name P. Dauke Date 6/14/12
Affiliation SIO/SW FSC NOAA Email pdauke@ucsd.edu Phone 45022

Abstract

Little is known about Ziphiidae, elusive deep-diving beaked whales that spend little time at the surface and occur exclusively in deep waters off the continental shelf. Mass strandings of Blainville's (*Mesoplodon densirostris*) and Cuvier's (*Ziphius cavirostris*) beaked whales have been correlated with sonar exercises conducted by the United States Navy at their sonar-testing facilities in the northern Bahamas (Balcomb, 2001). It is not known how naval sonar may be affecting local populations of *M. densirostris* and *Z. cavirostris* that inhabit the waters surrounding the testing site. Current regulations and conservation measures established to protect the whales are limited due to an overall lack of understanding in the distribution, abundance, and population structure of beaked whales in the region and globally. To better understand the population structure of these animals, full mitochondrial genomes of both species were generated from 41 stranded and live animals across global ocean basins to analyze the mitogenomic diversity and phylogeographic patterns both around the Bahamas and globally. Within *M. densirostris*, haplotypes were found to be divided into two distinct geographic clades representing possible Atlantic and Pacific sub species or species. In *Z. cavirostris*, 3 major clades were discovered, however, in two clades both Atlantic and Pacific animals were found, indicating that inter-ocean migration events have occurred relatively recently, or that there is ongoing gene flow for this species between ocean basins. Further sampling of both species and additional data from nuclear DNA will be required to fully resolve this and to better understand and mitigate the effects of naval activities on beaked whale populations within the northern Bahamas and elsewhere.

Section I.

General Introduction

Family Ziphiidae

Beaked whales are among the least understood of all marine mammals. The family consists of 21 rarely seen oceanic species who spend the majority of their time foraging for cephalopods and benthic fish (Clarke 1986, Heyning 1989) at depths of up to 2,000 meters (Tyack et al., 2006). They are typically found in small elusive groups in waters beyond the continental shelf (Cox *et al.*, 2006) and are not active at the surface, making them difficult to detect and study (Barlow, 1999). Beaked whales show a habitat preference for topographically diverse environments such as deep-water canyons, seamounts, and continental shelf edges (Whitehead et al. 1997, Waring et al. 2001, D'Amico et al. 2003). As encounters in the wild have been limited, most of what is known about these animals has come from studies on individuals who have mass stranded, oftentimes coinciding with anthropogenic noise events (Simmonds and Lopez-Jurado 1991; Frantzis 1998; Jepson et al. 2003).

Beaked whales and Anthropogenic noise

As far back as 1874, mass stranding events involving beaked whales have occurred. Of 136 beaked whale mass stranding events reported from 1874 to 2004, 126 occurred between 1950 and 2004, after the introduction and implementation of modern, high-power mid-frequency active sonar (MFAS) (D'Amico et al, 2009). The correlation

between strandings of beaked whales and offshore naval maneuvers was first noted in 1991 (Simmonds and Lopez-Jurado, 1991). Since that time, there have been five mass stranding events of beaked whales following naval sonar exercises, including a mass stranding in the Bahamas in March 2000 (Cox et al. 2006). While the mechanistic cause of the strandings is still being investigated, researchers hypothesize that it may be due to a variety of behavioral responses that alter normal dive patterns, contributing to gas bubble formation, hypoxia, cardiac arrhythmia, hypertensive hemorrhage or other forms of trauma (Cox et al., 2006). It is also thought that beaked whales may experience direct tissue damage from the sound exposure itself through gas bubble and growth formation in auditory pathways (Fernandez et al. 2005, Cox et al., 2006).

Necropsies on stranded individuals have supported these hypotheses by revealing hemorrhaging around the brain, the inner ears, and in the acoustic fats (i.e., fats that are located in the head and are involved in sound transmission) (Cox et al., 2006). Gas bubble-associated lesions and fat embolism in the vessels of vital organs have also been found in stranded beaked whales (Fernandez et al. 2005). Scientists believe that normal beaked whale diving patterns may already lead to an accumulation of excess nitrogen in the tissues, making them especially vulnerable to diving related pathologies, such as decompression sickness, if normal patterns are disrupted by intense anthropogenic noise (Cox et. al, 2006). Most recently, a study conducted using two live beaked whales, one *M. densirostris* and one *Z. cavirostris* assessed behavioral changes in dive patterns. The animals were outfitted with time, depth, and auditory recording devices and exposed to controlled levels of low and mid-frequency active sonar. The behavioral responses included ceasing echolocation-based foraging and ascending unusually slowly to the

surface (Tyack et al., 2011). Interestingly, researchers found that playing recordings of orcas, their main predator, caused the beaked whales to alter their behavior in similar ways, leading to the notion that beaked whales display avoidance behaviors when exposed to sonar (Tyack et al., 2011).

Section II.

Beaked Whales in the Northern Bahamas

Beaked whales in the Grand Bahama Canyon

While it has been difficult to measure effects of multi-national, multi-ship sonar exercises on global beaked whale populations, substantial effort is now being focused on local populations surrounding the Grand Bahama Canyon in the northern Bahamas where the United States Navy's sonar testing facility, the Atlantic Underwater Test and Evaluation Center (AUTECE), is located. This canyon (Figure 1) is a deep ocean basin approximately 100 nautical miles (190 km) long by 15 nautical miles (28 km) wide with depths of up to 1,800 meters (NAVSEA, 2012). The canyon is frequented by three species of beaked whales including *M. densirostris*, *Z. cavirostris* and *M. europaeus* (Balcomb and Claridge, 2001). It is thought that these whales frequent the basin due to its oceanographic and topographical features that allow for optimal sound propagation for echolocation based foraging, navigation, and communication (Balcomb and Claridge, 2001). Similarly, humans have learned to exploit the canyon for many of the same reasons in order to test sonar weaponry for anti-submarine warfare.

AUTEC

The United States Navy currently tests its mid-frequency active sonar for anti-submarine warfare in the Northern Bahamas at the AUTEC (Figure 1). The range is under the jurisdiction of the US Navy; however, the sea floor, water column and any living organisms within the testing range are not (NAVSEA, 2012). Currently, the US Navy leases the range from the Bahamian government for approximately \$11,050,000 US Dollars annually (The Government of the Bahamas 2011). Because of this, any exercises conducted in the range are under the authority of the Bahamian government. In 2005, the Bahamian government enacted The Marine Mammal Protection Act, which is currently regulated by the Minister of the Department of Marine Resources (The Government of the Bahamas, 2012). This act, much like the Marine Mammal Protection Act of the United States, states that no person “shall take, harass, hunt, kill or attempt to take, harass, hunt, kill any marine mammal within the Bahamas” without a license or permit granted by the government (The Government of the Bahamas, 2012). Interestingly, the United States Navy does not need to complete an environmental impact statement (EIS) since they are in the territorial waters of another country. With an exemption from filing an EIS, the US Navy is not legally held responsible for any whales or other marine mammals that may be negatively impacted by their sonar exercises. However, in light of environmental groups and scientists speaking out against the legislative loopholes, the US Navy has acknowledged that their sonar exercises likely have detrimental environmental impacts on marine mammals and now help fund and work with academic and governmental entities to research and monitor the effects on the animals within the

Bahamas region, including this research endeavor.

Mass Stranding Event- March 15-16, 2000

On March 15, 2000, a mass stranding event occurred coinciding with a naval sonar exercise in the northern Bahamas at the US Navy's AUTEC range. During this exercise, 4 vessels attempting to locate a submarine within the Grand Bahama canyon used mid-frequency active sonar. A total of 16 animals, including 9 *Z. cavirostris*, 3 *M. densirostris*, and 3 unidentifiable beaked whales, stranded and died (Balcomb and Claridge, 2001). Eight other animals also stranded, but were pushed back to sea and never seen again (Balcomb and Claridge, 2001). Necropsies on five of the beaked whales revealed hemorrhaging in the brain, inner ears, and acoustic fats, which are used for sound transmission. Gas and fat embolism was also reported and thought to possibly be due to an excess buildup of nitrogen in blood from an alternation in dive patterns that could have led to decompression sickness-like symptoms similar to what human divers would experience as "the bends" (Tyack et al., 2011). Since this mass stranding event, researchers from the Bahamas Marine Mammal Research Organization (BMMRO), NOAA Southwest Fisheries Science Center and the US Office of Naval Affairs have focused on gaining a better understanding of the distribution, abundance, and population structure of the species of beaked whales that are commonly found around the navy testing site.

Conservation Status of beaked whales in the Bahamas

While little is known about these cryptic beaked whales that frequent the northern Bahamas, the International Union for Conservation of Nature's (IUCN) Red List of

Threatened Species gives us some insight as to the current conservation status of these animals. Currently, the IUCN lists *M. densirostris* as data deficient (DD), meaning appropriate data on abundance, distribution, and population structure is lacking (IUCN, 2008). *Z. cavirostris* is listed as a species of least concern (LC), with their range being among the most widespread of all beaked whales, and an abundance of at least 100,000 individuals (IUCN, 2008).

Although the global population status of these whales isn't well known due to a lack of data, photo-identification studies of local populations in the Northern Bahamas have indicated that localized, genetically distinct populations of some species may exist (Durban, per. comm.). This challenges the current classification of the three species, as each are individually recognized as single, cosmopolitan species (Taylor, 2004). Photo-identification has also revealed that certain individuals and groups of *M. densirostris* exhibit high site fidelity; meaning they may have an isolated geographic range (Durban, pers. com). These findings are an indication that while these species of beaked whales may be found in temperate to tropical waters worldwide, there may be certain genetically isolated sub-populations existing. And, if negatively impacted over time, these populations could decline and lose the genetic variability needed to sustain their population in the future.

Because the population structure and status of the beaked whales found in the northern Bahamas is not known, appropriate conservation actions and effective management cannot be implemented. If populations were found to be genetically, morphologically, and socially different from their global counterparts, conservation measures could be enacted to protect them. An example of a similar scenario can be seen

with the killer whale or orca, *Orcinus orca*, which was once thought to comprise a single, cosmopolitan species with a diverse diet, has recently been discovered to be at least three genetically distinct species along with many other sub-species representatives (Morin et. al, 2010). Furthermore, the geographically and genetically distinct Southern Resident orca population found in the Pacific Northwest United States has now been identified as a distinct population segment (DPS) under the Endangered Species Act based on diet, social structure, and genetic and morphological differentiation (<http://www.nwr.noaa.gov/Marine-Mammals/Whales-Dolphins-Porpoise/Killer-Whales/ESA-Status/Index.cfm>). As much is not know about morphology, diet, and social structure in beaked whales, an investigation of molecular data as was done with orcas was imperative both to further our understanding of these animals and to influence conservation measures.

Section III.

Investigating Population Structure Using Genetics

A variety of research methods are currently being used to study the beaked whales that inhabit the northern Bahamas. These include photo-identification, tagging and tracking, post mortem necropsies of stranded individuals, acoustics, and most recently, genetics. For this project, a mitogenomic phylogenetic structure analysis on the two most commonly observed species, *Z. cavirostris* and *M. densirostris* was conducted. Samples of skin biopsies, sloughed skin, feces, and bone were collected in order to better understand the population structure, habitat use and movement patterns of the beaked whales that

may be affected by Navy sonar in the AUTECH range. These samples were obtained as part of the ongoing study of the *Distribution, Abundance and Population Structuring of Beaked Whales in the Great Bahamas Canyon*, led by Claridge et al. To investigate gene flow and population sub structuring around the Bahamas and globally, full mitochondrial genomes for 41 individuals (22 *Z. cavirostris*, 19 *M. densirostris*) were sequenced and assembled for phylogenetic and phylogeographic analysis.

Materials and Methods

Samples

Samples were collected from stranded animals and by remote biopsy of free-swimming whales (e.g., Hooker *et al.*, 2001) and stored frozen in the Southwest Fisheries Science Center's marine mammal and turtle tissue collection, with or without preservative (e.g., salt-saturated 20% DMSO solution or ethanol). DNA was extracted using silica-based filter purification (Qiaxtractor® DX reagents, Qiagen, Valencia, CA, USA) following manufacturers' instructions, performed on a JANUS® automated workstation (Perkin-Elmer, Waltham, MA, USA). Samples were selected from the SWFSC collection to include multiple samples from *M. densirostris*, and *Z. cavirostris* around the Bahamas, and additional samples were selected to maximize representation of the geographic distributions of these species (Table 1).

Mitogenome assembly

Because a reference mitogenome for either species did not exist, reads from four

individual of each *Z. cavirostris* and *M. densirostris* were pooled and assembled to *H. ampullatus* reference sequence using CLC Genomics Workbench v. 4.0 (CLC Genomics, Muehlthal, Germany), using relaxed criteria (Similarity = 0.8, Length fraction = 0.8, Insertion cost = 3, Deletion cost = 3, Mismatch cost = 3). The assembly was visually checked for clusters of differences, indels, and stop codons or frame shifts in coding regions, and manually edited.

The consensus sequence for each species was then used as the reference sequence for assembly of mitogenomes for individuals of each respective species using a custom pipeline written by Eric Archer in R (R Development Core Team, 2006).

The mpileup module in SAMTOOLS (Li *et al.*, 2009) was used to convert the resulting BAM-format alignment file into a “pileup” text format that lists the base composition across reads at each site in the reference sequence. This text file was then parsed by custom R code to create the consensus bases for each individual sequence, using the following rules: If a given site had fewer than three reads covering it, an “N” was placed for the consensus base. If the coverage was between three and five, and all reads contained the same nucleotide, then that nucleotide was used as the consensus base, otherwise, the consensus received an “N” for that site. If coverage was greater than five, then the nucleotide that occurred in 70% or more of the reads was used as the consensus base. If no nucleotide frequency exceeded 70%, then an “N” was inserted (Morin *et al.*, 2012).

Phylogenetic and phylogeographic analysis

To assess phylogenetic relationships among the species and ocean basins, the full

mitochondrial genomes of 41 individuals, and all odontocetes (Table 1) from Dornberg et al. (2012), excluding dolphins whose families were already represented, were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. The trees were rooted using the sperm whale (*Physeter macrocephalus*) and pygmy sperm whale (*Kogia breviceps*), based on results from previous analyses (e.g. Dornberg et al., 2012). Datasets were assembled using Mesquite (Maddison and Maddison, 2011) and aligned using Muscle (Edgar, 2004).

Maximum Parsimony and Maximum Likelihood analyses

Maximum parsimony analyses were carried out using PAUP* 4.0a122 (Swofford, 2002) with all characters unordered and equally weighted. Heuristic searches were conducted using random stepwise addition for 1000 replicates with the tree bisection reconnection (TBR) branch-swapping algorithm with maximum zero-length branches collapsed. Resulting trees were summarized via strict consensus. Clade support was assessed using jackknifing (Farris et al., 1996) on 1000 replicates with 10 random additions per iteration. Uncorrected and corrected (using GTR model) pairwise distances were calculated for the whole mt genomes within the *M. densirostris* and *Z. cavirostris* species sets using PAUP*.

Maximum likelihood results were inferred using RAxML 7.2.3 (Stamatakis, 2006; Stamatakis et al., 2008). The model used was GTR+G with the mitochondrial genomes treated as a single partition and 1000 bootstrap iterations of the data were performed to assess clade support.

Connectivity

The available sample sizes were not large enough to assess if the genetic distances between and within populations were significantly different using an approach such as AMOVA. However, the nucleotide diversity was examined in a phylogeographic sense. Computational difficulties precluded using the whole mitogenomes for this purpose so only the Cytochrome Oxidase subunit I (COI) gene in each the new mt genomes was used. The COI sequences were identified and annotated by importing GenBank sequence annotations for the *H. ampullatus* mitogenome into the newly assembled mitogenomes, and aligning the new data set using Geneious (Drummond et al 2011). COI was then extracted and imported into Network 4.6.1.0 (<http://www.fluxus-engineering.com/sharepub.htm#a1>) where median-joining (Bandelt et al. 1999) haplotype networks for *Z. cavirostris* and *M. densirostris* were generated.

Bayesian analyses using Beast

Two different analyses were run using Beast v1.6.2 (Drummond and Rambaut, 2007) to co-estimate divergence times and tree topologies under a Bayesian Inference (BI) framework, though only the second result was found to be sufficiently accurate.

For the 1st analysis, used for a preliminary report to the International Whaling Commission (IWC) Scientific Committee (Morin *et al.*, 2012), we used a subset of the available Odontocete whole mitochondrial genomes along with the 48 mitogenomes generated from the beaked whale data in this study (19 *M. densirostris*, 22 *Z. cavirostris*, and 7 individuals of *Mesoplodon europaeus*). The other odontocetes used were *Monodon*

monoceros (Narwhal), *Phocoena phocoena* (Harbor porpoise), *Lipotes vexillifer* (Baiji river dolphin), *Hyperoodon ampullatus* (Northern Bottlenose whale), and *Orcinus orca* (Killer whale).

Settings for the 1st analysis were as follows: Relaxed log-normal molecular clocks and BIC selected substitution models for each of the three partitions (1. 12S and 16S rRNA, 2. Protein coding genes, and 3 D-loop control region), as this method has proved appropriate for complete mitogenomes in cetaceans and other vertebrates (Ho and Lanfear, 2010); and a single tree prior assuming a Yule speciation process, as this model tends to be a good fit for phylogenetic studies including different species (Drummond and Rambaut, 2007). Four prior calibrations from previous molecular-based dates (McGowen *et al.*, 2009) were used for the time (in millions of years, MYA) to the most recent common ancestor (TMRCA) for the following taxonomic groups; Delphinida and Ziphiidae (34.08 MYA), Delphinida (29.08 MYA), Delphinoidea (22.27 MYA), and Phocoenida and Monodontidae (17.68 MYA). Lognormal calibrations were used and a MCMC chain length of 500,000,000, sampling every 5000 generations, and a second identical run to assure convergence of independent analyses. Convergence of parameter estimates was determined in *Tracer* (Rambaut and Drummond, 2003), verifying that effective sample sizes were at least 500 for all parameters.

For the second analysis, all published whole mitochondrial genomes for Odontoceti were used (Table 1). This included a much greater diversity of odontocetes than used in Analysis 1 and allowed for several fossil-based prior calibrations to be used. Different prior date calibrations were used compared to the 1st analysis. This was based on results in Dornberg *et al.* (2012) that showed the rate of mitochondrial genome evolution

differing between Odontoceti and Mysticeti, with the rate of evolution for Odontoceti being much faster than in Mysticeti. This means that the four molecular-based dates calibration priors used in Analysis 1, which were based on the McGowen *et al.* (2009) analysis that was across all Cetacea, were inappropriate and were much older than the actual TMRCA for these nodes. For instance the TMRCA for Phocoenida and Monodontidae was set at 17.68 MYA, though the fossil calibration for this group is in fact closer to 10 MYA (McGowen *et al.*, 2009; Dornberg et al. 2012). Also problematic was the date for the TMRCA for Delphinida and Ziphiidae which was set at 34.08 MYA, which is much older than the accepted crown group fossil for Odontoceti as a whole (Dornberg et al. 2011, 2012), which dates to only 23.7 MYA. Also generally problematic is the use of prior calibration points based on previous molecular clock studies, rather than using the actual priors these studies were based on, which were fossils in the first place (McGowen *et al.* 2009). For these reason actual fossil calibration priors were used in Analysis 2.

The three fossil-based calibration priors used for Analysis 2 were obtained from Dornberg et al. (2012). The fossil ages were used to set the minimum age for the node (as the offset). Lognormal distributions were then used with a mean of 1 and the standard deviation (SD) set to give older age distributions for the nodes in question that reflected Marshall's (2008) method of absolute age bracketing (FAc95), as calculated in Dornberg et al (2012). The TMRCA for crown Odontoceti was set at 23.7 MYA, the TMRCA for *Monodon* and *Phocoena* was set at 10 MYA and the TMRCA for *Inia* and *Pontoporia* was set at 11.2 MYA. Though a date of 12 MYA for crown Ziphiidae is available based on fossils (Dornberg et al. 2012) this date was left as free to vary in the Beast analyses to

assess the appropriateness of the other calibrations. For each of the analyses, the genomes were treated as a single partition, though partitioned analysis is warranted but was not done owing to timing issues. A GTR+ gamma model was used, with a single random tree prior assuming a Yule speciation process. Four independent MCMC runs of fifty million generations were completed and appropriate burn-in and convergence assessed using Tracer. The last 5,000 trees for each run were combined using Log combiner (Drummond and Rambaut, 2007) and a majority rule consensus tree generated using Tree annotator (Drummond and Rambaut, 2007).

Section V.

Results

Complete mitogenome sequences from 19 *M. densirostris*, and 22 *Z. cavirostris*, with sequence lengths between 16330 and 16352 bp were analyzed. Because low resolution and variability can be seen in some mtDNA genes in beaked whales, full mitochondrial genomes were used for phylogenetic and phylogeographic analysis. However, Cytochrome oxidase subunit I (COI) was found to be variable and was used for haplotype networking (Figure 5-6). Based on COI, *M. densirostris* was found to have 12 unique haplotypes, and *Z. cavirostris* to have 16 unique haplotypes.

MP and ML analyses

Of the total of 16780 characters, 9463 characters were constant, 1943 were variable, but parsimony-uninformative, while there were 5374 parsimony-informative sites. For the MP search 88 shortest trees were found with a length of 22186 steps. The

ML analysis gave a tree with a maximum likelihood score of (ln) -112988.648. The two analyses gave similar results and support values and the overall odontocete tree topology largely matched that of Dornberg et al. (2012). Only the ML tree topology is shown here (Figure 3). Both analyses showed the included Ziphiidae terminals (*Berardius beardii*, *Hyperodon ampullatus* and the *Ziphius* and *Mesoplodon* specimens) forming a clade. The two species of *Mesoplodon* were reciprocally monophyletic and formed a clade that was sister group to *H. ampullatus*. This clade was sister group to *Z. cavirostris* with *B. bairdii* then sister to all the other ziphiids. In both the MP and ML analyses Ziphiidae was sister group to Delphinoidea rather than to *Platanista minor* (the Indus River Dolphin), as was shown in Dornberg et al (2012). Otherwise results were essentially similar to those shown in Dornberg et al (2012), though *Orca orcinus* and the *Ziphius* and *Mesoplodon* sequences had been added here.

Within *M. densirostris* there were two major clades, each with high bootstrap and jackknife support (Figure 6). One comprised the Atlantic (Bahamas and Florida) specimens and the other the Pacific (Hawaii, New Zealand and California) specimens. Within each of these clades there were very short branch lengths but some sub-clades still showed good support. In contrast to *M. densirostris* there was no major geographic structuring among the specimens of *Z. cavirostris*. The samples from the Bahamas were instead spread across three distinct clades, with two of these mixed with samples from the Pacific (Figure 5). The specimens of *M. europaeus* were all Atlantic and these formed a single well-supported clade with no marked sub structuring (Fig 1).

The minimum uncorrected pairwise distance based on the whole mitochondrial genomes between the Pacific and Atlantic clades of *M. densirostris* were 1.597%

(Bahamas2 vs. Hawaii1) and with GTR model this was 1.827%. The maximum uncorrected distance within each basin was 1.022% (Bahamas 2 and 4 vs. Bahamas 7) for the Atlantic clade and 0.262% for the Pacific clade (Hawaii Maui vs. SBCA). For *Z. cavirostris* the greatest uncorrected distance found was 1.481% between the Florida2 and the Bahamas4 specimens (GTR distance= 1.505%). The maximum pairwise uncorrected distance found between the samples from the Bahamas 4 and 5 was 1.445% (GTR distance= 1.467%).

The COI dataset showed essentially similar distances with respect to those outlined above and this dataset was used to generate the haplotype networks. The median-joining haplotype network for *M. densirostris* (Figure 7) clearly shows the genetic break between the Atlantic and Pacific with 17 changes in COI. There is then little haplotype variability within each of the ocean basins. For *Z. cavirostris* (Figure 8) there was a break of similar magnitude (14 changes) to that seen between Pacific and Atlantic *M. densirostris* but there is clearly no geographic correlation with this. There was also a Bahamas haplotype isolated from the rest by 15 changes in COI alone.

Divergence using BEAST

With respect to the ages of interest for this report the analysis in the IWC report gave a TMRCA for Ziphiidae of 20 MYA, for *Z. cavirostris* of 2.5 MYA, for *M. densirostris* of 2.13 MYA and for *M. europaeus* of 0.88 MYA.

In Analysis 2 (Figure 4), which used a broader sampling of mt genomes for Odontoceti and fossil based prior calibrations, the results showed a younger age for Ziphiidae and also for the *Mesoplodon* and *Ziphius* species. In analysis 2, the prior for

crown Odontoceti was set at 23.7 MYA and the posterior was recovered at 27 MYA. For *Monodon* and *Phocoena* the prior was set at 10 MYA and the posterior was 11 MYA. For *Inia* and *Pontoporia* the prior was set at 11.2 MYA and the posterior was 13MYA. Although a prior date of 12 MYA for crown Ziphiidae was available based on fossils (Dornberg et al. 2012), this date was left as free to vary in the analysis to assess the appropriateness of the other calibrations. The date recovered for the TMRCA for Ziphiidae was 11.75 MYA. Analysis 2 showed the TMRCA for *Z. cavirostris* at 1.68MYA, for *M. densirostris* at 1.43MYA and for *M. europaeus* of 0.54MYA. The Pacific and Atlantic clades of *M. densirostris* showed TMRCA of 0.37 and 0.68 MYA respectively. For *Z. cavirostris* the main clade that comprised most of the samples, including several from the Bahamas samples, showed a TMRCA of 1.28 MYA and was sister to a clade that comprised the remaining two Bahamian specimens (Figure 4).

Section VI.

Discussion and Conclusions

Through phylogenetic and phylogeographic analyses, it was found that for both species studied, intra-specific diversity is high. Within *M. densirostris*, haplotypes were found to have divided into two distinct Atlantic and Pacific clades approximately 1.4 mya. There was found to be little haplotype variability for *M. densirostris* in the Bahamas. If low diversity in this species is due to low population size, *M. densirostris* could be of particular importance for conservation reasons. Due to gene flow between ocean basins being unlikely, the population seen in the northern Bahamas may represent

part of an Atlantic sub-species or species whose population could suffer if negatively impacted over time. Taxonomic revision would be dependent on determination that the break of 1.597% (GTR=1.827%), while also correlated with a geographic break, is large enough to warrant subspecies or species status. Further genetic evidence from other parts of the Atlantic is needed to further elucidate the population status of the Bahamas. Because isolated gene flow is occurring in the *M. densirostris* population found around the AUTECH testing range, an argument could be made for listing as a discrete population segment (DPS) under the Endangered Species Act. Sub-species of marine mammals have been erected and granted conservation measures from far less molecular data, such as the Southern Resident orcas who were listed as a DPS based majorly on morphological data (<http://www.nwr.noaa.gov/Marine-Mammals/Whales-Dolphins-Porpoise/Killer-Whales/ESA-Status/Index.cfm>). If granted DPS status under the Endangered Species Act, conservation measures could be taken to protect these whales against anthropogenic noise and other threats. However, this is further complicated by the fact that the US ESA is a US law, and these whales are in Bahamian territory.

In contrast to *M. densirostris*, the more widely distributed *Z. cavirostris* was found to have limited clustering of haplotypes by ocean basin, with a TMRCA of 1.68 mya. Three major clades were discovered, however, and in two clades both Atlantic and Pacific animals were found, indicating that inter-ocean migration events have occurred evolutionarily, or ongoing gene flow is occurring between ocean basins. There was found to be very high diversity in haplotypes, which could indicate a large population and could give insight to population abundance calculations. The large age differences between some of the Bahamian specimens of *Z. cavirostris* (Figure 4) further supports the results

shown in the haplotype network indicating that there is relatively high genetic diversity of this species in the Bahamas. There is a similar sized break in *Z. cavirostris* with a distance of 1.481% (GTR= 1.505%) but unlike *M. densirostris*, there is no geographic sense to this. Further sampling will be needed before any inferences can be made regarding contemporary gene flow in this species.

Although this study represents the largest number of ziphiid mitogenomes to date, it is limited by the number and geographic diversity of samples sequenced. Further sampling of both species and additional data from nuclear DNA will be required to fully resolve this and to better understand and mitigate the effects of naval activities on beaked whale populations within the northern Bahamas and elsewhere.

Table and Figures

Table I

Sample ID	Haplotype name in analysis	Species	Geographic Location
NC5273.1+AJ554056.1	Hyperoodon_ampullatus	<i>Hyperoodon ampullatus</i>	-
gi 270301949 gb GU187185.1	Orcinus_orca	<i>Orcinus orca</i>	-
gi 38602571 emb AJ554062.1	Monodon_monoceros	<i>Monodon monoceros</i>	-
gi 38602585 emb AJ554063.1	Phocoena_phocoena	<i>Phocoena phocoena</i>	-
gi 55274416 gb AY789529.1	Lipotes_vexillifer	<i>Lipotes vexillifer</i>	-
NC_005275.1+GI:38707626	Platanista_minor	<i>Platanista minor</i>	-
NC_005274.1+GI:38707598	Berardius_beardii	<i>Berardius beardii</i>	-
NC_002503.2+GI:34582601	Physeter_macrocephalus	<i>Physeter macrocephalus</i>	-
NC_005272.1+GI:38707548	Kogia_breviceps	<i>Kogia breviceps</i>	-
NC_012062.1+GI:223471661	Grampus_griseus	<i>Grampus griseus</i>	-
NC_005278.1+GI:38707534	Lagenorhynchus_albir	<i>Lagenorhynchus albir</i>	-
NC_005276.1+GI:38707450	Inia_geoffrensis	<i>Inia geoffrensis</i>	-
NC_012059.1+GI:223471647	Tursiops_truncatus	<i>Tursiops truncatus</i>	-
NC_005277.1+GI:38707584	Pontoporia_blainvilli	<i>Pontoporia blainvilli</i>	-
z0004010	MdenSBCA	<i>Mesoplodon densirostris</i>	Santa Barbara, CA, USA
z0004472	ZcavFLUSA1	<i>Ziphius cavirostris</i>	FL, USA

z0004967	ZcavHIUSA1	<i>Ziphius cavirostris</i>	HI, USA
z0005565	ZcavPhillippines	<i>Ziphius cavirostris</i>	Philippines
z0007445	ZcavFLUSA2	<i>Ziphius cavirostris</i>	FL, USA
z0008681	MdenFIUSA	<i>Mesoplodon densirostris</i>	FL, USA
z0009110	MdenStrndNZ	<i>Mesoplodon densirostris</i>	New Zealand (Stranding)
z0009122	ZcavNZ	<i>Ziphius cavirostris</i>	New Zealand
z0009561	ZcavTaiwan	<i>Ziphius cavirostris</i>	Taiwan
z0014950	ZcavPR	<i>Ziphius cavirostris</i>	Puerto Rico
z0024816	ZcavAKUSA1	<i>Ziphius cavirostris</i>	AK, USA
z0026279	ZcavWestCoastUSA	<i>Ziphius cavirostris</i>	West Coast, USA
z0030065	ZcavHIUSA2	<i>Ziphius cavirostris</i>	HI, USA
z0033737	MdenHI1	<i>Mesoplodon densirostris</i>	HI, USA
z0050723	MdenHINorth	<i>Mesoplodon densirostris</i>	North HI, USA
z0061950	ZcavCAUSA1	<i>Ziphius cavirostris</i>	CA, USA
z0068606	ZcavBajaMX	<i>Ziphius cavirostris</i>	Baja California, Mexico
z0072279	ZcavETPMX	<i>Ziphius cavirostris</i>	ETP, Mexico
z0074425	MdenBahamas7	<i>Mesoplodon densirostris</i>	Bahamas
z0074424	MdenBahamas7a	<i>Mesoplodon densirostris</i>	Bahamas
z0079632	ZcavAKUSA2	<i>Ziphius cavirostris</i>	AK, USA

z0079824	MdenBahamas1	<i>Mesoplodon densirostris</i>	Bahamas
z0079835	MdenBahamas2	<i>Mesoplodon densirostris</i>	Bahamas
z0079837	MdenBahamas3	<i>Mesoplodon densirostris</i>	Bahamas
z0079838	MdenBahamas4	<i>Mesoplodon densirostris</i>	Bahamas
z0079840	MdenBahamas5	<i>Mesoplodon densirostris</i>	Bahamas
z0079839	MdenBahamas5a	<i>Mesoplodon densirostris</i>	Bahamas
z0079887	ZcavBahamas1	<i>Ziphius cavirostris</i>	Bahamas
z0079893	ZcavBahamas2	<i>Ziphius cavirostris</i>	Bahamas
z0079898	ZcavBahamas3	<i>Ziphius cavirostris</i>	Bahamas
z0074262	ZcavBahamas3a	<i>Ziphius cavirostris</i>	Bahamas
z0079900	ZcavBahamas4	<i>Ziphius cavirostris</i>	Bahamas
z0087481	ZcavCAUSA2	<i>Ziphius cavirostris</i>	CA, USA
z0094563	MdenBahamas6	<i>Mesoplodon densirostris</i>	Bahamas
z0074263	MdenBahamas6a	<i>Mesoplodon densirostris</i>	Bahamas
z0074264	MdenBahamas6b	<i>Mesoplodon densirostris</i>	Bahamas
z0094591	ZcavBahamas5	<i>Ziphius cavirostris</i>	Bahamas
z0094595	ZcavBahamas6	<i>Ziphius cavirostris</i>	Bahamas
z0094785	MdenHIMAUI	<i>Mesoplodon densirostris</i>	Maui, HI, USA
z0098730	MdenHI2	<i>Mesoplodon densirostris</i>	HI, USA
z0033736	MdenHI2b	<i>Mesoplodon densirostris</i>	HI, USA

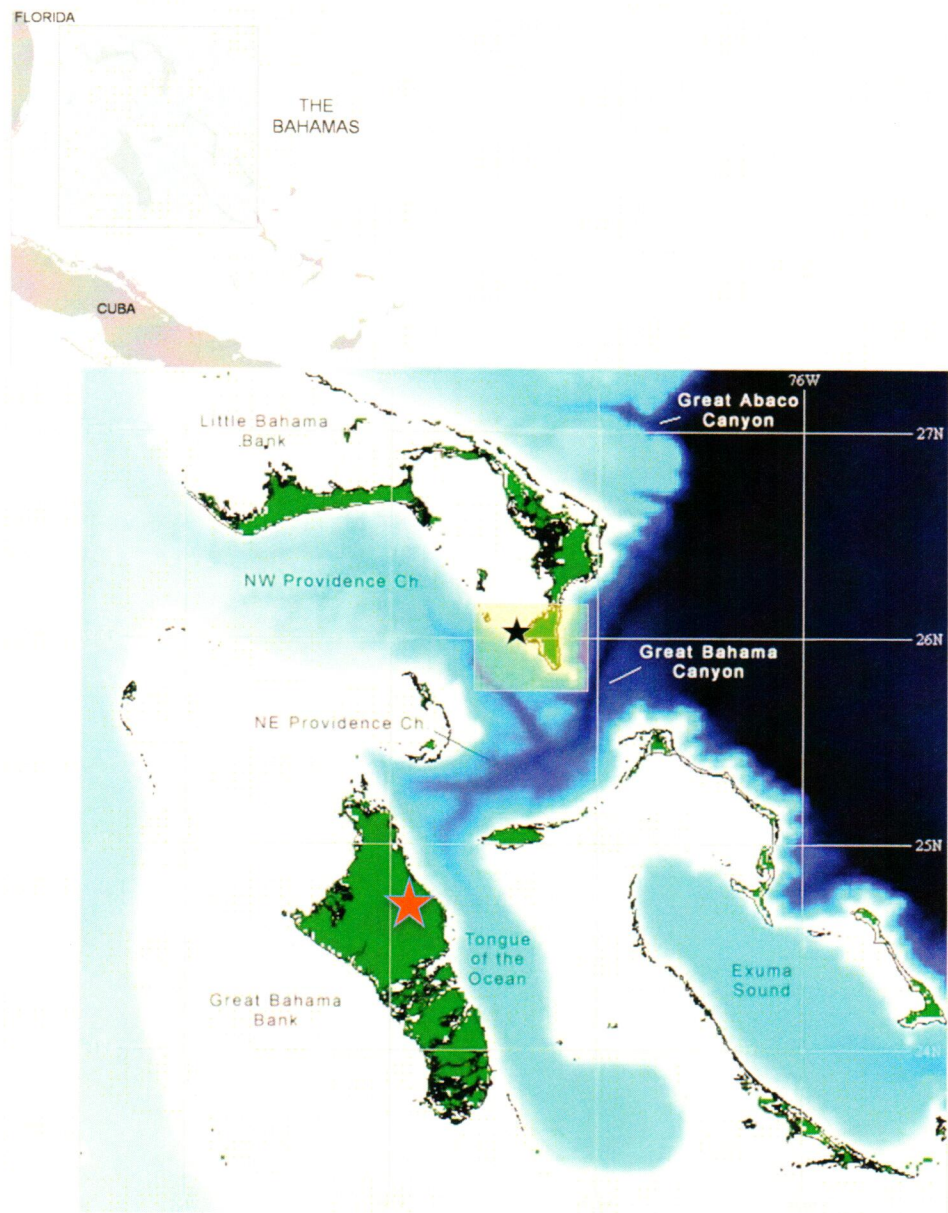


Figure 1: Map of northern Bahamas showing locations of AUTECH (red star) and Bahamas Marine Mammal Research Organization study site (blue star) (NOAA).

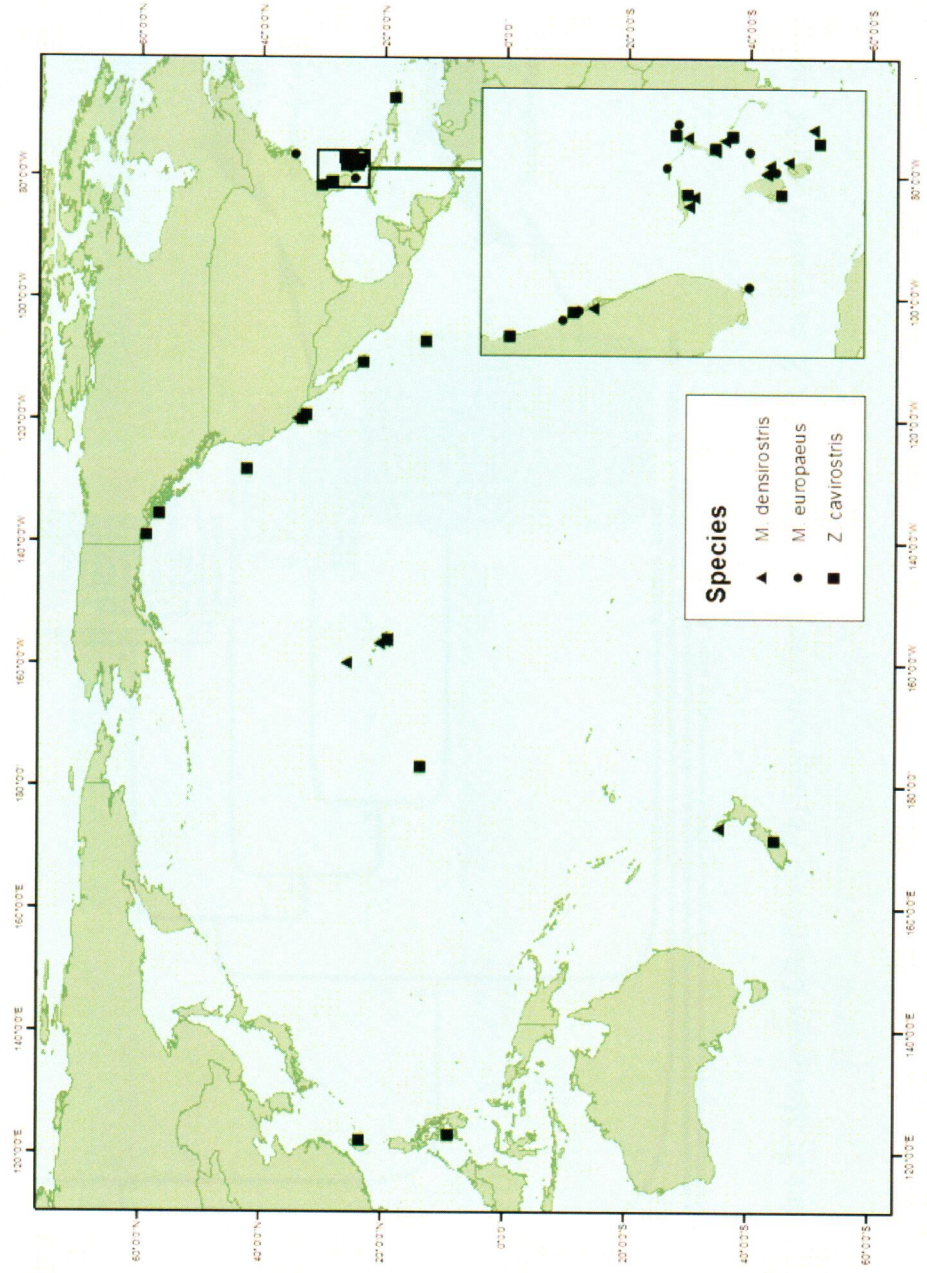


Figure 2: Map indicating locations where individuals were sampled (Morin et al., 2012).

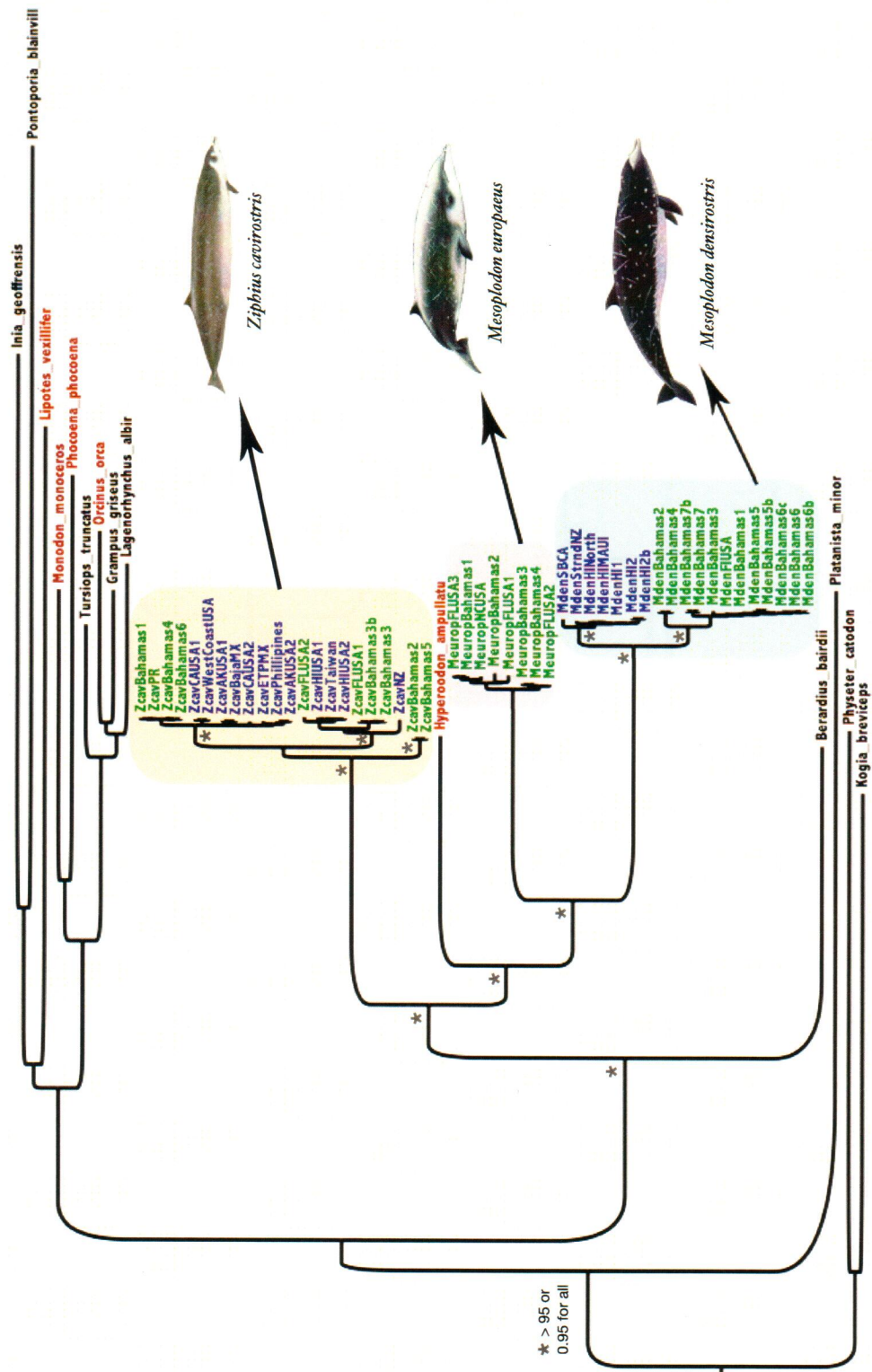


Figure 3: Maximum Likelihood Phylogenetic tree for *M. densirostris*, *Z. cavirostris* and *M. europaeus*.

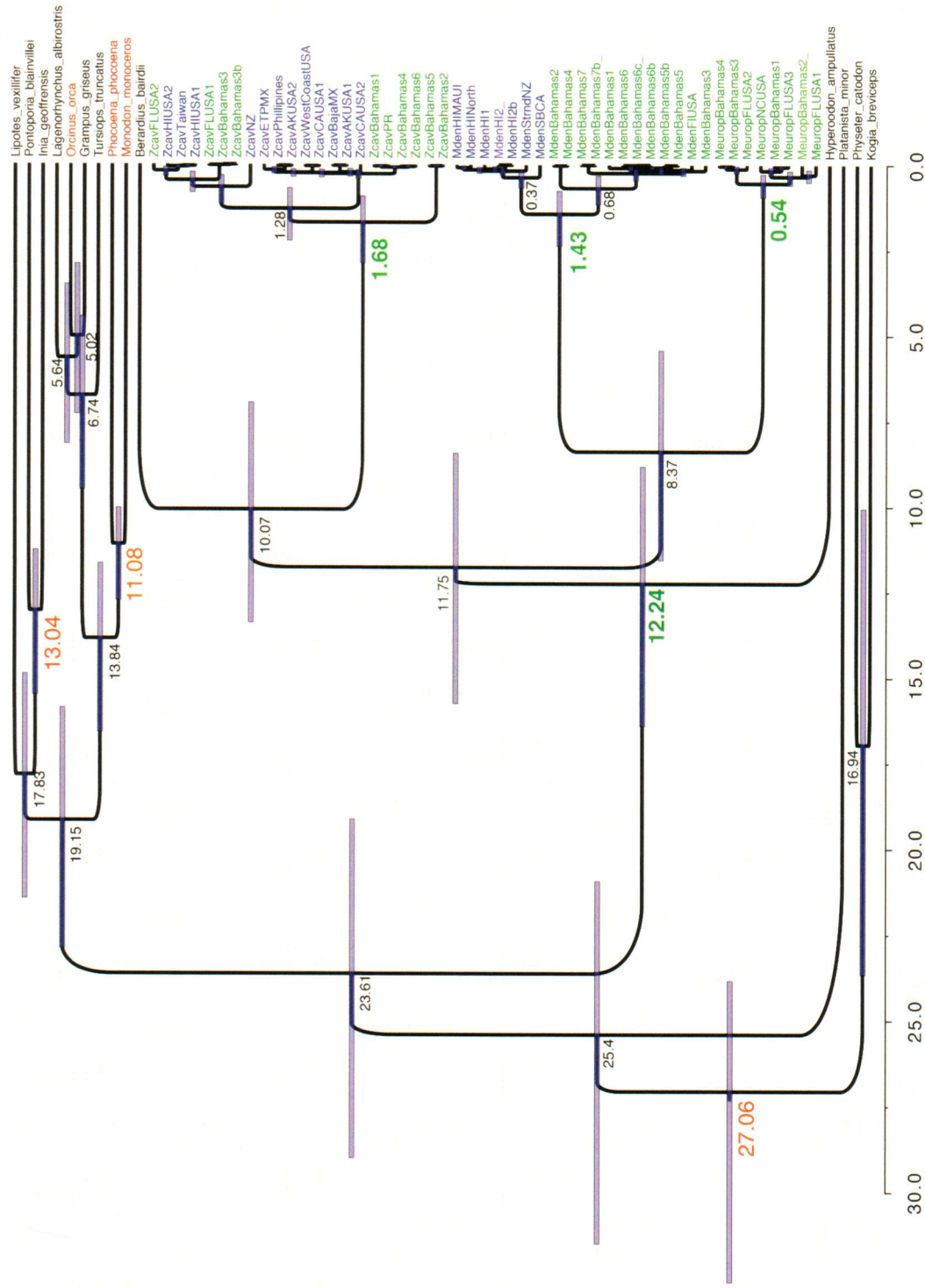


Figure 4: Bayesian phylogenetic tree showing divergence times for Analysis 2.

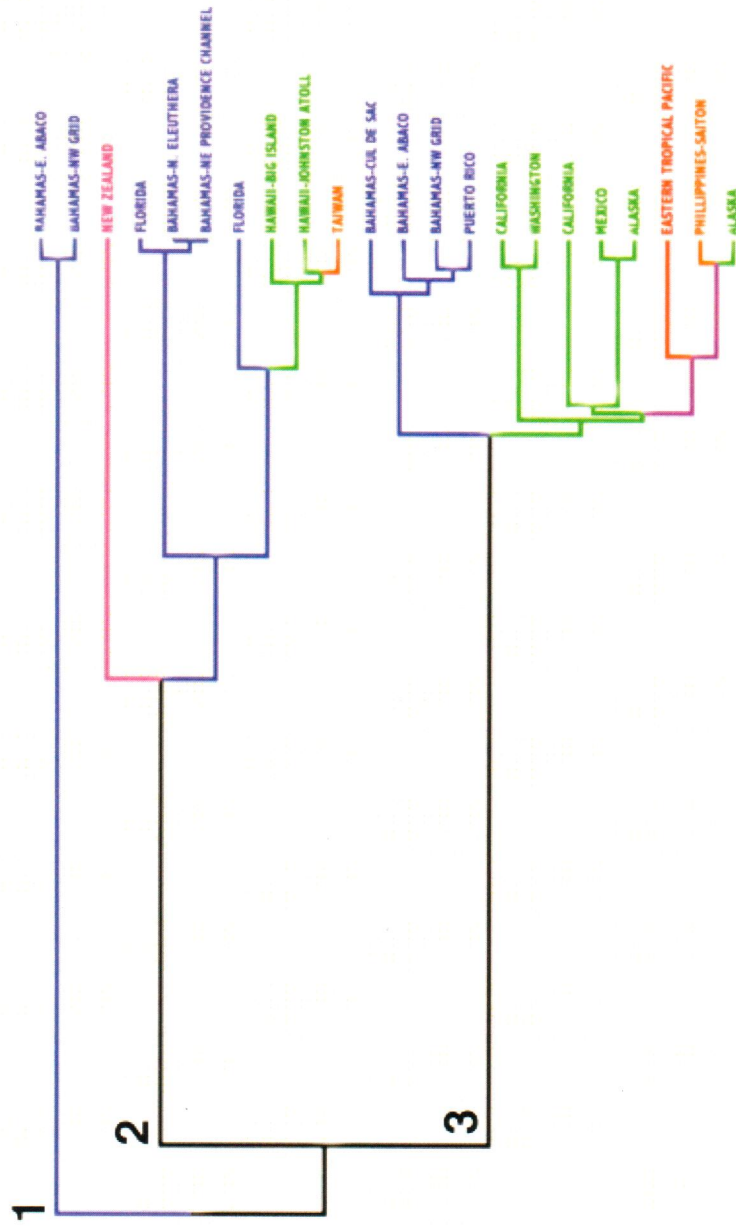


Figure 5: Maximum likelihood phylogenetic tree for *Ziphius cavirostris* showing three diverse clades.

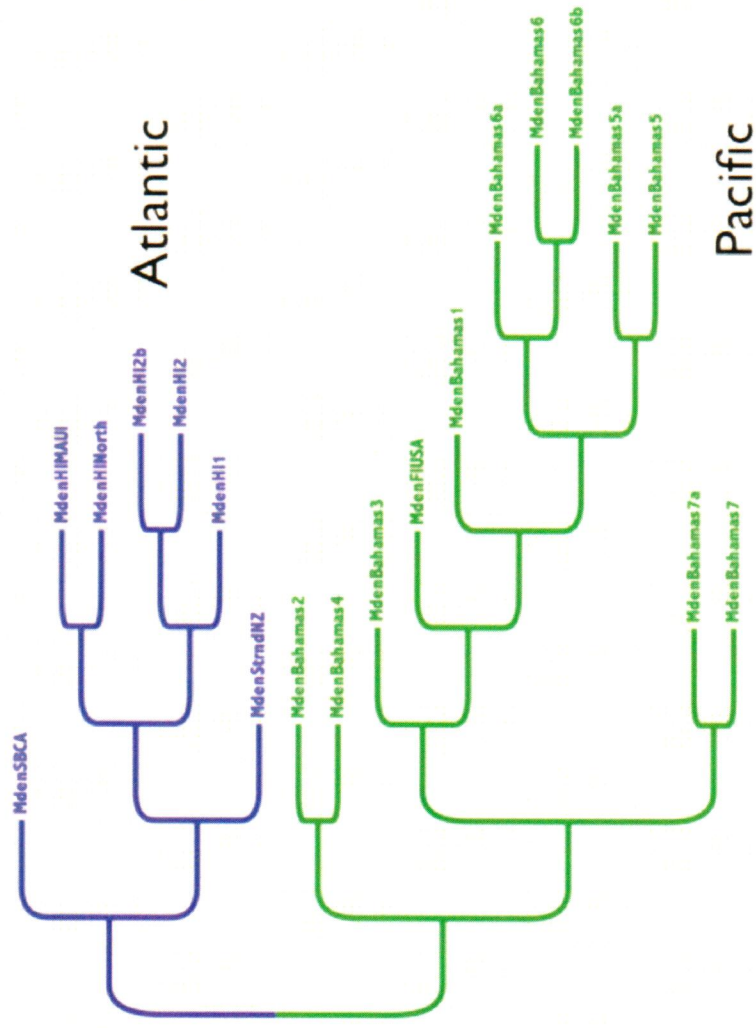


Figure 6: Maximum likelihood phylogenetic tree for *Mesoplodon densirostris* showing Atlantic and Pacific clades.

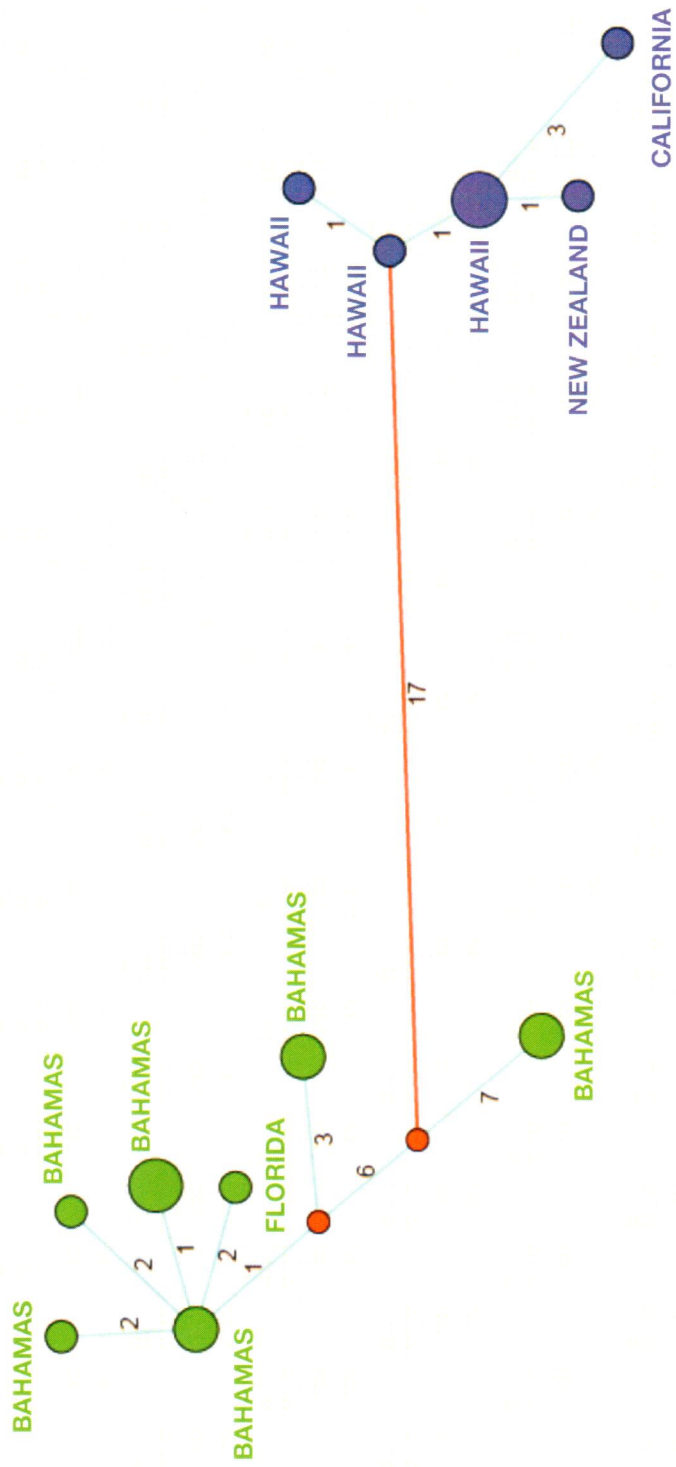


Figure 7: *Mesopodon densirostris* haplotype network based on COI gene.

References

- Balcomb, KC III | Claridge, DE. 2001. A mass stranding of cetaceans caused by naval sonar in the Bahamas. *Bahamas Journal of Science [Bahamas J. Sci.]*. Vol. 8, no. 2, pp. 2-12.
- Cox, T. M., Ragen, T. J., Read, A. J., *et al.* 2006. Understanding the impacts of anthropogenic sound on beaked whales. *J. Cetacean Res. Manag.* 7, 177-187.
- Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 4, 331-346.
- D'Amico, A. D., Gisiner, R., Ketten, D. R., *et al.* 2009. Beaked whale strandings and naval exercises. *Aquat. Mamm.* 35, 452-472.
- Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nuc. Acids Res.* 32, 1792-1797.
- Fernandez, A., Edwards, J.F., Rodriguez, F., *et al.* 2005. Gas and Fat Embolic Syndrome Involving a Mass Stranding of Beaked Whales (Family Ziphiidae) Exposed to Anthropogenic Sonar Signals." *Vet. Pathol.* 42: 446-457.
- Frantzis, A. 1998. Does acoustic testing strand whales? *Nature* 392(6671):29.
- Hoelzel, R., editor. 2002. *Marine Mammal Biology an Evolutionary Approach*. Malden, MA: Blackwell Publishing.
- Hooker, S.K., Baird, R.W., and Fahlman, A. 2009. Could beaked whales get the bends? Effects of diving behaviour and physiology on modeled gas exchange for three species: *Ziphius cavirostris*, *Mesoplodon densirostris* and *Hyperoodon ampullatus*. *Respiratory Physiology & Neurobiology*. 167: 235-246.
- Jepson, P.D., Arbelo, M., Deaville, R., *et al.*, 2003. Gas-bubble lesions in stranded cetaceans. *Nature*. 425: 575-576.
- Kvadsheim P.H, Miller P. J.O, Tyack P *et al.*, 2012. Estimated tissue and blood N₂ levels and risk of decompression sickness in deep-, intermediate-, and shallow-diving toothed whales during exposure to naval sonar. *Frontiers in Physiology*.
- Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75 <http://mesquiteproject.org>.
- Morin, P. Archer, F., Foote, A. Vilstrup, J., *et al.*, 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species.

Genome Research. 20: 908-916.

Moriarty, T. Navy Continues its Commitment to Science through Marine Mammal Studies. Navy.mil, August 10 2009. Accessed May 2, 2012.
<http://www.navy.mil/Search/print.asp?story_id=47536&VIRIN=74634&imagetype=1&page=1>.

Nowacek, D., Thorne, L., Johnston, D., Tyack, P. 2007. Responses of cetaceans to anthropogenic noise. *Mammal Rev.*, 37(2): 81-115.

NAVSEA AUTECT Accessed May 13, 2012.
<<http://www.navsea.navy.mil/nuwc/newport/autec/Pages/Environment.aspx>>

Simmonds, M.P. and Lopez-Jurado, L.F. 1991. Whales and the military. *Nature*. 337: 448.

Taylor, B., Barlow, J., Pitman, R., Ballance, *et al.*, 2004. A call for research to assess risk of acoustic impact on beaked whale populations.

The Government of the Bahamas. Accessed May 2, 2012. <www.bahamas.gov.bs>.

Taylor, B.L., Baird, R., Barlow, J., *et al.* 2008. *Ziphius cavirostris*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 12 May 2012.

Taylor, B.L., Baird, R., Barlow, J., *et al.* 2008. *Mesoplodon europaeus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 13 May 2012.

Taylor, B.L., Baird, R., Barlow, J., *et al.* 2008. *Mesoplodon densirostris*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 13 May 2012.

Tyack, P. L., Johnson, M., Soto, N. A., Sturlese, A., and Madsen, P. T. 2006. Extreme diving of beaked whales. *J. Exp. Biol.* 209, 4238–4253.

Tyack, P. L., Zimmer, W. M., Moretti, D., *et al.* 2011. Beaked whales respond to simulated and actual navy sonar. *PLoS ONE* 6, e17009.