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Worldwide Population Structure in Cuvier's Beaked Whales: Identification of Units for Conservation

by

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13. ABSTRACT ***(maximum 200 words)***

Small bone samples from over 500 Cuvier's beaked whales (*Ziphius cavirostris*) representing populations worldwide were collected from museums and other institutions. Genetic analysis of mitochondrial DNA (mtDNA) control region and cytochrome *b* diversity is underway. These data will be used for a robust statistical assessment of genetic differentiation between populations at regional and ocean basin levels and estimation of rates of dispersal, allowing units for conservation to be identified. Information on the mtDNA haplotype and sex of each specimen will also be provided. Preliminary median-spanning network reconstructions and analyses of molecular variance (AMOVA) for a subset of samples (control region—317 base pairs, n = 219; cytochrome *b*—326 base pairs, n = 190) revealed strong differentiation among the three ocean basins (North Atlantic, North Pacific, and Southern Hemisphere) at the haplotype and nucleotide level ($p < 0.0001$). There is also possible evidence of historical movements of these whales between the Atlantic and Pacific Oceans prior to the closure of the Isthmus of Panama approximately 3 million years ago. DNA extraction and sequencing are ongoing. The final deliverables for these contracts are expected in approximately 6 months' time.

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Worldwide population structure in Cuvier's beaked whales: identification of units for conservation

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The following report summarizes the research conducted under two contracts from the Southwest Fisheries Science Center using funding from the Office of Naval Operations, N45, and the Naval Postgraduate School, Monterey.

Contents

Final Report: Order # JG133F05SE6342

Period of work
10 August 2005 to 10 August 2006

Tasks summary

1. Establish contact with museum/institutional curators around the world to seek permission to take small samples of bone from specimens of Cuvier's beaked whales (*Ziphius cavirostris*).
2. Fly to each of 25 museums/institutions to collect genetic samples from bone using ultra-clean methods to avoid possible contamination.

3. All samples to be archived at the University of New South Wales (UNSW) and will be made available for future studies of population genetic structure.

Outcome summary

1. Over 50 museums, institutions, and individuals in over 40 countries were contacted, and subsequently contributed samples and provided access to specimens of Cuvier's beaked whales for this project. Due to their generosity, the number of specimens available for this project (n > 500) is at least double that envisioned in the original project proposal.
2. The majority of specimens were sampled during in-person visits by the contractor to 23 of these institutions in 12 countries between March and September 2006. Other institutions were able to send samples directly to the contractor at UNSW without the need for an in-person visit.
3. With the exception of samples from institutions in Argentina and Chile, for which negotiations to obtain permits to export this material to Australia are ongoing, all samples have been archived at UNSW and are available for future studies of population genetic structure.

Methods

Sampling of Cuvier's beaked whale skeletal material was largely non-destructive. The method employed has previously been used by the contractor with great success on other museum-held whale specimens. A hand-held electric drill with a 2 mm diameter drill bit was used to obtain ~ 0.05 gm of bone or tooth powder from one or more locations on each specimen. This amount of bone or tooth powder is sufficient for DNA extraction using the sensitive silica-based guanidine-thiocyanate (GUSCN) method. Approximately 3 small holes of approximately 5 – 10 mm' depth were made in one or more of the dense bones of the specimen – e.g., the mandibular rami of the jaw, the teeth, and/or the occipital condyles of the skull (Figure 1). As DNA degradation can differ markedly in different bones of such specimens, taking samples from several locations is highly recommended in order to maximise the likelihood of successful DNA extraction and enable cross-checks to confirm no contamination of native DNA has occurred.

Results

Approximately 500 Cuvier's beaked whales have been sampled for this project to date[1] representing populations throughout much of the range of this species. The specimens sampled include approximately 259 animals from the North Atlantic (including the Eastern North Atlantic 87, Eastern Tropical Atlantic [Canary Islands] 40, Mediterranean 41, Western North Atlantic 20, and Western Tropical Atlantic [Caribbean region] 69), approximately 104 animals from the North Pacific (including the Eastern North Pacific 64, Western North Pacific 16, Eastern Tropical Pacific [including the Galapagos] 13, and Central Tropical Pacific [Hawai'i] 10), approximately 135 animals from the Southern Hemisphere (including the Eastern South Atlantic-Western South Indian [South Africa] 24, Eastern South Pacific-Western South Atlantic [Tierra del Fuego] 50, Western South Pacific 31, and Western Tropical Pacific 13), and 13 animals from the North Indian Ocean.

[1] A small number of samples may represent duplicates from the same individuals.

Figure 1. Sampling of Cuvier's beaked whale specimens for genetic analysis. (Photos by MLD)

Final Report: Order # JG133F07SE2186

Period of work
1 May 2007 to 1 May 2008

Tasks

1. Conduct 320 genetic analyses (DNA extraction, mitochondrial DNA sequencing, and molecular sexing) of samples from Cuvier's beaked whales.
2. Conduct statistical analyses to detect population-level genetic differences between ocean basins and (where sample size permits) finer geographic stratifications.

Outcomes

1. Of the 434 Cuvier's beaked whale samples currently held at UNSW (as of April 2008)[2], approximately 70% consist of bone or tooth powder and 30% consist of fresh soft tissue. To date, DNA extractions have been conducted for 346 samples (80% of total). For 309 (89%) of these samples, mitochondrial (mt) DNA fragments from the control region and cytochrome *b* genes have been successfully amplified using the Polymerase Chain Reaction (PCR) and sequenced. Attempts to obtain molecular sexing information from the bone-tooth samples have been unsuccessful due to the low-quality degraded DNA yielded by this type of material. However, the sex of many of these specimens was able to be determined from their skull morphology at the time of sampling. Based on a combination of morphology and molecular sexing (fresh tissue only), the sex of 75% of the samples at UNSW is currently known (F = 150, M = 177).

2. DNA extraction and mtDNA sequencing of samples is ongoing. However, some preliminary median-joining network reconstructions and analyses of molecular variance (AMOVA) have been run to explore population patterns at the ocean-basin level.

Deliverables
Ultimately, the contractor will provide: a) a summary of the sex and mtDNA haplotypes (control region and cytochrome *b* genes) for each Cuvier's beaked whale sampled; and, b) a summary of the statistical tests performed and the levels of significance for between-population differences at the ocean-basin and regional level (where sample sizes permit). However, this information cannot be provided until the lab work component of this project has been completed. Lab work has taken longer than anticipated due to the unexpected generosity of institutions and individuals in providing access to samples and specimens (i.e., many more specimens were available for sampling than originally envisioned, allowing substantial expansion of the scope of the project) as well as some delays in setting up the UNSW School of BEES Ancient DNA Laboratory (part of the Molecular Ecology and Evolution Facility; MEEF). These deliverables will be provided in approximately 6 months' time.

Results from preliminary analyses looking at ocean-basin level patterns in a subset of the samples follow.

[2] Negotiation of import-export permits for samples from specimens in Argentina and Chile is ongoing.

Brief summary of interim findings

Datasets (as of April 2008)

	Control region	**cytochrome *b***
Sample size (no. of animals)		
North Atlantic	122	109
North Pacific	50	38
Southern Hemisphere	47	43
Total	219	190
Sequence length (base pairs, bp)	317	326
No. of haplotypes	38	50
Heterozygosity	26 variable sites, of which 17 are phylogenetically informative $h = 0.91$	40 variable sites, of which 28 are phylogenetically informative $h = 0.90$

Note that in contrast to the usual mammalian pattern, the cytochrome *b* is more variable than the control region in beaked whales (Ziphiidae) (Dalebout *et al.* 2004, Dalebout *et al.* 2007). At the control region, the majority of variable sites are clustered in the first 200 bp of the 5' end, while the rest of the locus is relatively conserved. At the cytochrome *b*, variable sites are distributed relatively evenly throughout the locus.

Control region

Previous analyses (Dalebout *et al.* 2005) were based on 87 Cuvier's beaked whales (mostly from New Zealand and the US west coast) and a 290 bp fragment of the control region. The present analysis of a substantially larger dataset, including more comprehensive sampling from other parts of the range of this species, confirms many of the patterns observed by Dalebout *et al.* (2005). Two main haplotype clusters were observed: one consisting of haplotypes found almost exclusively in the North Atlantic, and the other dominated by haplotypes found predominantly in the Southern Hemisphere and North Pacific (Figure 2). Within the North Atlantic cluster, several haplotypes appear to be specific to the Mediterranean. Interestingly, almost all North Atlantic animals possessing haplotypes in the North Pacific-Southern Hemisphere cluster hailed from the Western Tropical Atlantic (Caribbean Sea), and may be evidence of historic movement between these oceans before the final closure of the Panama isthmus approximately 3 million years ago. In the North Pacific, most animals from Hawaii appear to possess the same unique haplotype.

Cytochrome b

Very similar patterns were revealed by the cytochrome *b* dataset. Here, three main haplotype clusters were observed: one consisting of haplotypes found almost exclusively in the North Atlantic, and the other two dominated by haplotypes from the Southern Hemisphere and North Pacific (Figure 3). Within the North Atlantic cluster, several haplotypes again appear to be specific to the Mediterranean. And, as also found in the control region, almost all North Atlantic animals possessing haplotypes in the North Pacific-Southern Hemisphere cluster hailed from the Western

Tropical Atlantic. Most animals from Hawaii appear to possess one of a small cluster of unique haplotypes.

Preliminary assessment of genetic differentiation among ocean basins

Preliminary statistical analyses to detect genetic differences among ocean basins have now been conducted based on fragments of the mitochondrial DNA control region (317 bp) and cytochrome *b* (326 bp). Analyses of molecular variance (AMOVA) revealed strong differentiation among the three ocean basins (North Atlantic, North Pacific, Southern Hemisphere) at the haplotype and nucleotide level for both the control region (F_{ST} = 0.149; Φ_{ST} = 0.384; $p<0.0001$) and cytochrome *b* (F_{ST} = 0.174; Φ_{ST} = 0.298; $p<0.0001$). Pairwise comparisons confirmed that all three ocean basins were significantly different from one another (Table 1). For the control region, similar levels of significant differentiation were found by Dalebout *et al.* (2005) based on a total sample size of 87 animals. The North Indian Ocean was not included in these analyses due to small sample size available for this area to date.

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Table 1.

Analysis of molecular variance (AMOVA) among the three main ocean basins, plus pairwise comparisons. Kimura 2-parameter (K2P) distances used for Φ_{ST} assessments.

	control region				**cytochrome *b***			
	Haplotype difference		Nucleotide distance		Haplotype difference		Nucleotide distance	
	variance %	probability	variance %	probability	variance %	probability	variance %	probability
Overall - 3 ocean basins	F_{ST} = 14.9	**0.0000**	Φ_{ST} = 38.4	**0.0000**	F_{ST} = 17.4	**0.0000**	Φ_{ST} = 29.8	**0.0000**
North Atlantic vs North Pacific	14.7	**0.0000**	39.8	**0.0000**	17.6	**0.0000**	26.6	**0.0000**
North Atlantic vs Southern Hemisphere	15.0	**0.0000**	40.8	**0.0000**	21.0	**0.0000**	35.9	**0.0000**
North Pacific vs Southern Hemisphere	14.8	**0.0000**	26.2	**0.0000**	5.8	**0.0023**	23.6	**0.0000**

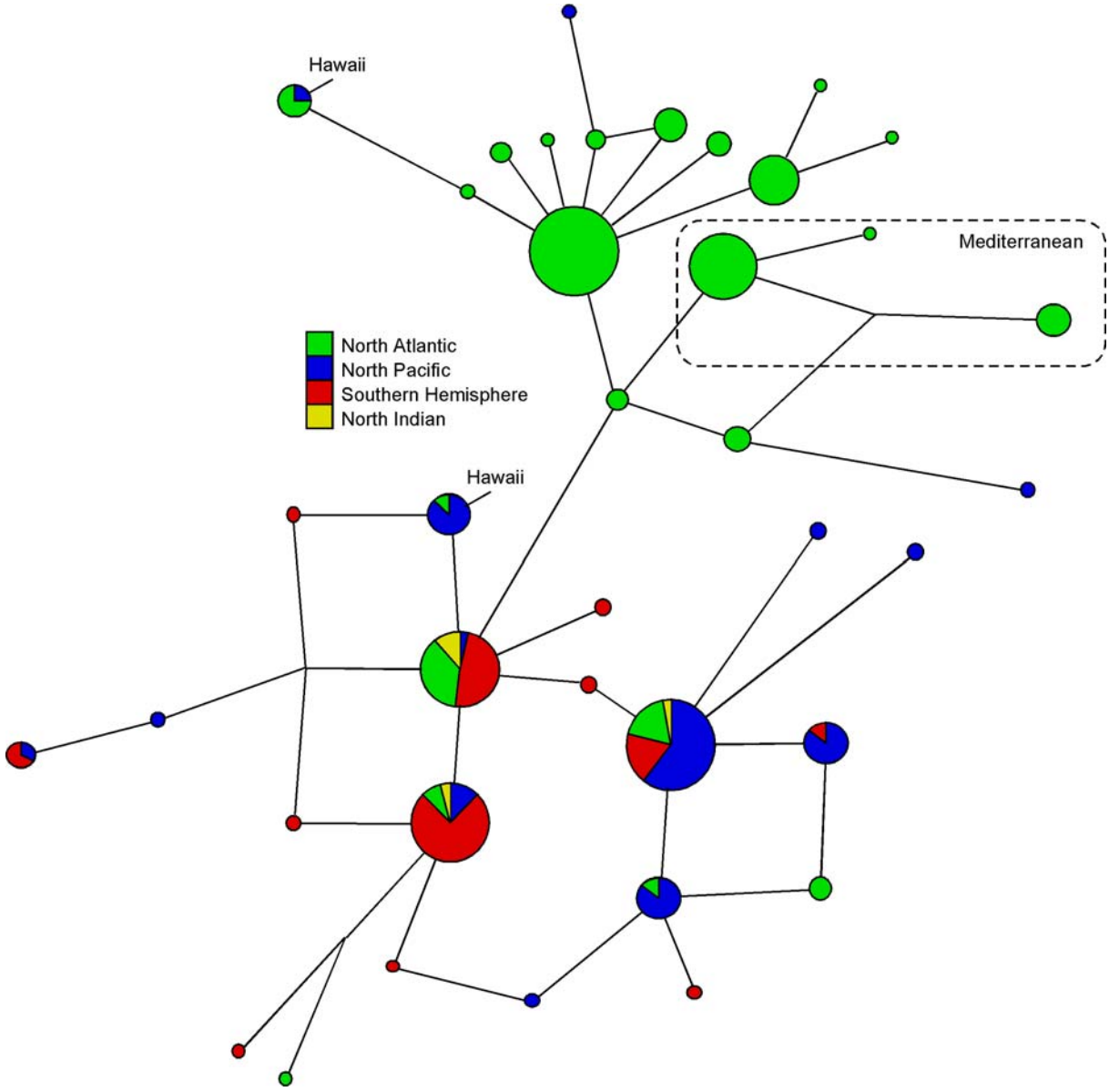

Figure 2.

Mitochondrial DNA control region median-spanning network. Nodes represent haplotypes scaled to the frequency of their occurrence in the sample. Branch lengths are scaled approximately to the number of nucleotide substitutions between haplotypes. The majority of haplotypes differ by a single substitution. Note that this is a rough first pass analysis only and the robustness of haplotype groupings has not been assessed. Variable sites, haplotype affinities, and the provenance of all specimens also need to be rechecked. Notes on Hawaii haplotypes – preliminary assessment only: one animal appears to have a haplotype in the "North Atlantic" cluster (same pattern/same animal for cytochrome *b*). All other Hawaiian animals (n = 5) appear to share the same haplotype, which falls within the "Southern Hemisphere-North Pacific" cluster. In the North Pacific, two other animals also appear to represent this haplotype; one from Taiwan and one from California. (Same animal has "Hawaiian" haplotype in cytochrome *b*.) In the North Atlantic, one animal from Scotland appears to represent this haplotype as well.

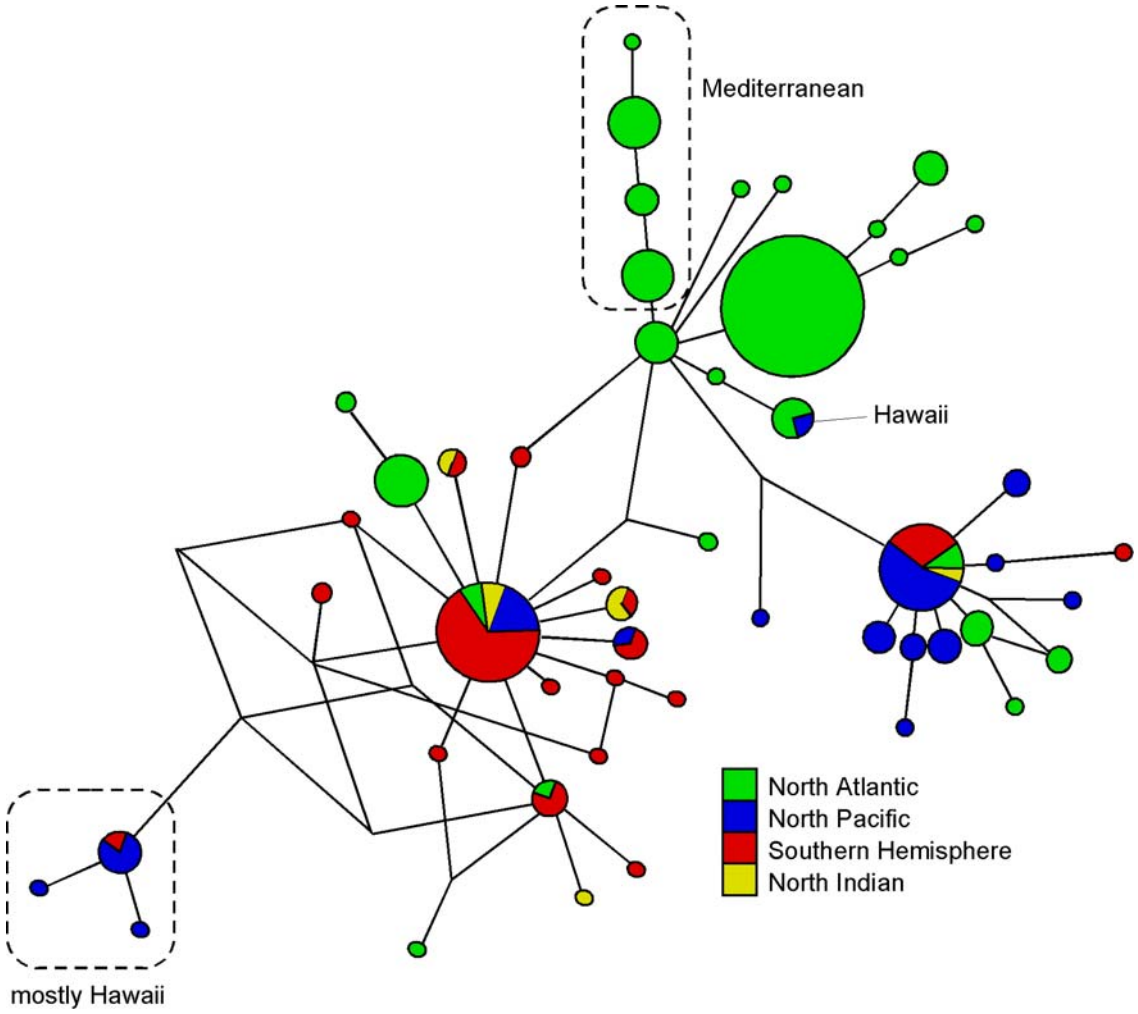

Figure 3.

Mitochondrial DNA Cytochrome *b* median-spanning network. Nodes represent haplotypes scaled to the frequency of their occurrence in the sample. Branch lengths are scaled approximately to the number of nucleotide substitutions between haplotypes. The majority of haplotypes differ by a single substitution. Note that this is a rough first pass analysis only and the robustness of haplotype groupings has not been assessed. Variable sites, haplotype affinities, and the provenance of all specimens also need to be rechecked. Notes on Hawaii haplotypes – preliminary assessment only: one animal appears to have a haplotype in the "North Atlantic" cluster (same pattern/same animal for control region). All other Hawaiian animals (n = 5) represent three closely related haplotypes, which fall within the "Southern Hemisphere-North Pacific" cluster. In the North Pacific, one other animal from California also appears to represent the most common of these haplotypes. (Same animal has "Hawaiian" haplotype in control region.) In the Southern Hemisphere, one animal from south-eastern Australia appears to represent this haplotype as well.

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