MITOCHONDRIAL DNA DIVERSITY AND POPULATION STRUCTURE OF HUMPBACK WHALES FROM THEIR WINTERING AREAS IN THE INDIAN AND SOUTH ATLANTIC OCEANS (WINTERING REGIONS A, B C, AND X).

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ABSTRACT

Humpback whales in the Southern Hemisphere are separated by the International Whaling Commission (IWC) into seven wintering Regions (A-G) based on tropical distribution. To better evaluate the significance of these stock subdivisions, an analysis of mtDNA was conducted for the eastern and western South Atlantic (Regions A and B), the southwestern Indian Ocean (Region C) and the northern Indian Ocean (Region X). A total of 1,416 individual whales representing eleven sampling sites within the four wintering Regions were sequenced for a portion of the mtDNA control region. A hierarchical analysis of molecular variance (AMOVA) using Φ_{ST} and F_{ST} supported the division of wintering Regions based on IWC designated boundaries of A, B, C, and X. Pairwise comparisons further confirmed the A, B and C divisions, although varying degrees of heterogeneity (particularly molecular distances) were detected for proposed sub-divisions within Regions B and C. Overall, this large-scale mtDNA analysis for humpback whales in the Indian and South Atlantic Oceans supports wintering Region designations by the IWC. However, additional analyses and consideration of biological parameters such as gene flow are needed so that 'within-region' genetic analyses can help evaluate population structure and recovery in a management context.

INTRODUCTION

In the Southern Oceans, humpback whale distribution on feeding grounds occurs within six primary areas that have subsequently been used for stock or sub-population identity (Mackintosh, 1942; Gambell, 1976, IWC 1997). The migration of these stocks from their feeding grounds is stratified for

distributions to seven low-latitude wintering Regions (breeding grounds and migratory corridors) termed A-G (IWC, 2001). At the Scientific Committee of the IWC in 2003, it was decided that northern Indian Ocean populations (ie humpback whales of Oman and the Arabian Sea) would be considered by the Southern Hemisphere sub-committee as Region X. For the most part, it has been presumed that the migratory cycle of whales in these seven statistical areas and wintering Regions occurs latitudinally (within divided longitudinal regions) from high-latitude feeding regions to tropical wintering grounds and back (refer to Appendix 1 for the use of terminology). Once numbering over 100,000 whales, it has been suggested that the entire population in the Southern Hemisphere may have been reduced to as few as 3,000 individuals by commercial exploitation (Chapman, 1974).

Several recent studies have examined the components of humpback whale stock structure among wintering Regions in the South Pacific (Baker *et al*. 1998; Rosenbaum *et al.* 1998; Olavarria, 2002). These analyses have been conducted primarily using tissue biopsy samples collected from humpback whales on the wintering grounds of Regions E, F, and G ranging from Eastern Australia to Colombia (IWC, 2001). In the most recent study from Olavarria *et al.* (2003), a large scale analysis of more than 1000 samples based on mtDNA control region sequences investigated the genetic relationship among components of the F stock (the Cook Islands and French Polynesia breeding grounds) compared to other South Pacific breeding grounds and stocks. The results presented in that study suggest that humpback whales show strong population structure on their breeding grounds in the South Pacific, a finding that did not necessarily agree with historical stock designations for these wintering regions (IWC 2001).

There have increasingly been additional studies contrasting the genetic relationships of humpback whales among the wintering Regions of the southwestern Atlantic Ocean (Region A), the southeastern Atlantic (Region B), the southwestern Indian Ocean (Region C), and the northern Indian Ocean (Region X) (Rosenbaum et al., 1998, 2000, 2001, 2002) (Figure 1). Preliminary mtDNA data reported by Rosenbaum *et al.* (2000, 2001) show that significant pairwise differences in haplotype frequencies alone (traditional F-statistics) were found among comparisons involving breeding grounds and migratory corridors sampled from Regions A, B, C (Rosenbaum *et al.,* 2000; Rosenbaum *et al.,* 2001). Significant genetic differentiation using both molecular information and haplotype frequencies was also found between whales off Oman and whales of wintering Region C of the southwestern Indian Ocean. These latter results suggest that if inter-hemisphere gene flow does occur, the populations are certainly not panmictic (Rosenbaum *et al.,* 2002).

While these recent reports have provided valuable information on population structure of humpback whales from these wintering regions where none previously existed, sample sizes for components or even entire wintering Regions were not included in these previous reports. With respect to maternal gene flow and population structure, the relationships between (and within) wintering Regions A, B and C and X needs to be revisited and evaluated using a multi-year and large-scale representative sampling approach.

In this report we describe the results of a genetic analysis based on mtDNA control region for 1,416 individual whales sampled at eleven different locations distributed in the four wintering Regions A, B, C, and X. These results will help elucidate stock definitions and the patterns of gene flow for these four managements units distributed within two ocean basins and both Hemispheres.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 1,416 samples were collected from individual humpback whales representing eleven sampling sites within the South Atlantic (Regions A and B), the southwestern Indian (Region C), and Northern Indian Ocean (Region X) (Table 1, Fig. 1). Skin tissues were mostly obtained using the biopsy dart procedure (Lambertsen 1987) or as sloughed skin when available. Samples were preserved in the field in 95% Ethanol or salt saturated 20% Dimethyl Sulfoxide solution (DMSO) and later stored at –20ºC until processed. Additional information on samples are detailed in Engel *et al.* (2003), Findlay *et al*. (1994), Best *et al*. (1998), Rosenbaum *et al.* (2000), Rosenbaum *et al.* (2001), and Rosenbaum et al. (2002).

Total genomic DNA was extracted from the epidermal layer of biopsies using proteinase K digestion followed by a standard Phenol/Chloroform extraction method (Sambrook et al. 1989) or using DNAeasy tissue kit (Qiagen).

PCR Amplification and DNA Sequencing

A 520 bp fragment within the mtDNA control region (Kocher *et al*., 1989; Baker *et al*., 1993) was amplified using Polymerase Chain Reaction (PCR). From this, a 486 bp region that contains the majority of variable nucleotide positions in the mtDNA control region of humpback whales was generated for all samples (Baker *et al*., 1993). PCR products were cycle-sequenced with dye-labeled terminators using conditions recommended by the manufacturer (Applied Biosystems). Sequence reactions were analyzed using an ABI-Prism model 377, 3100 or 3700 Genetic Analyzer (PE Applied Biosystem®, Foster City, CA). A number of samples were removed from the final analysis based on duplicated sampling, either determined from sampling history or genotype identity using the procedure detailed by Pomilla *et al.* (2004).

Data analysis

DNA sequence variation patterns were characterized into haplotype definitions for this species. Sequences for this portion of the mtDNA control region were maintained for each individual in MacClade v. 4.01 (Maddison and Maddison, 2000) and Sequencher v. 4.1. The diversity and geographic variation of haplotypes were quantified using the Analysis of Molecular Variance procedure (AMOVA; Excoffier et al., 1992) as implemented in the software Arlequin 2.0 (Schneider *et al*., 2000). This procedure calculates standard variance components and an array of haplotypic correlation measures for population structure, referred to as Φ-statistics. The Φ_{ST} is analogous to Wright's (1951) F-statistic and to other genotype correlations used for the study of population structure (e.g., Hudson *et al*., 1992; Weir and Cockerham, 1984; Takahata and Palumbi, 1985).

The significance of the observed Φ- or F-statistics was tested using the null distribution generated from 1,000 non-parametric random permutations of the data matrix variables. Significance of Φ_{ST} and F_{ST} values for pairwise comparisons between the four regions (A, B, C, and X) was tested against the null hypothesis that no inter-site (intra-site) differences exist in all pairwise comparisons using the distribution of pairwise Φ_{ST} or F_{ST} obtained by randomly permuting haplotypes for 1,000 replicates. The P value was determined by the proportion of permutations with a test statistic value greater than or equal to the one observed. Following the recommendation of Hudson *et al.* (1992), the traditional F_{ST} of Wright (1951) was calculated using haplotype (i.e., nucleotype, Nei, 1987) frequencies and the same permutation procedure described above for Φ_{ST}. This statistic considers only the binomial difference (i.e., 0,1) between identified haplotypes and does not include the contribution of molecular distances.

The diversity of humpback whale mtDNA sequences was estimated at both the haplotype and nucleotide level (Nei, 1987) using *Arlequin 2.0*. At the haplotype level, diversity and its standard error were calculated without reference to the genetic distance (i.e., number of nucleotide substitutions) between two mtDNA sequences. At the nucleotide level, diversity (Nei, 1987) and its standard error for both sampling and stochastic processes (Nei and Jin, 1989) were calculated from the pairwise differences between the mtDNA sequences.

RESULTS

Table 1 illustrates the sample sizes for each sampling site within the wintering Regions A, B, C, and X. A consensus region of 486 bp of the mtDNA control region was assembled in which 180 maternal haplotypes were detected from 23-68 polymorphic sites (Table 1). Haplotype diversity ranged from 0.974-0.978 for breeding grounds or migratory corridors in wintering Regions A, B, and C to a lowered haplotype diversity of 0.66 for Oman in Region X. Nucleotide diversities ranged between 0.022 and 0.17 for all four regions.

For the AMOVA, significant differences were found among and within the four wintering Regions A, B, C, and X only for both $\mathbf{\Phi}_{ST}$ and F_{ST}, although the 'among-Region' variance ($\mathbf{\Phi}_{CT}$ and F_{CT}) was significance just at the p=0.05 level (Table 2). Nearly all the molecular variance was however attributed to differences among sites within Regions, as well as to 'within-site' variation detected for both test statistics.

Among all the pairwise comparisons using $\mathbf{\Phi}_{ST}$ the following were statistically significant: Antongil Bay (BA) vs. Gabon (GA), west South Africa (WZ), and Mozambique (MZ), MZ vs. all populations with exception of southern Madagascar (MG) and Mayotte (MY); Oman was significantly different from all sampled breeding ground and migratory corridors within A, B, and C (Table 3).

Using haplotype frequencies only (F_{ST}), many pairwise comparisons of breeding populations across wintering Regions generally showed statistical significance (i.e. comparisons of breeding populations or migratory corridors of A vs. B, vs. C vs. X etc...). Many 'within-Region' comparisons also showed statistical significance, and often occurred where sub-regions for management have been proposed (i.e. C1 vs. C2. vs. C3). Interestingly, significant heterogeneity was found between animals sampled off Gabon (B1) and WZ (B2), while no significant differences were found among animals sampled along the C1 migratory corridor of east South Africa (EZ) and low-latitude breeding grounds in Mozambique (MZ) (Table 4).

DISCUSSION

Overall, the tests for population differentiation based on haplotype frequencies and molecular information showed significant differences across the South Atlantic (Regions A and B), southwestern Indian (Region C), and northern Indian Oceans (Region X).

A previous analysis of mtDNA control region sequences showed a general lack of significant differences between the South Atlantic and the southwestern Indian Ocean basins using either Φ_{ST} or F_{ST} test statistics. The overall lack of resolution provided by the mtDNA sequences in Rosenbaum *et al.* (2000) most likely results from a combination of factors, including lack of representative sample size for many of the populations examined and the retention of ancestral polymorphisms masking underlying geographic structure. With respect to the less significant differentiation among pairwise comparisons using molecular distances, it is possible that the lack of structure is due to a retained common ancestry. However, it is difficult to differentiate between retention of ancestral polymorphism and levels of recent gene flow. Since the designations of these subdivided Regions was originally based on whaling records and more recent records and patterns of whale distribution, the amount of 'within-Region' gene flow tolerated to still consider these as distinct management units still need to be defined by the IWC, so that recovery within each of these sub-divided Regions can be evaluated in a management context.

The present analysis included a larger and (presumably) more representative samples from the Gabon wintering ground in Region B, from the C1 population off Mozambique, as well as from the migratory regions off the west and east South African coasts. Additionally, the present analysis also included samples from Oman. The AMOVA and pairwise test results presented here generally support the division of A, B, C, and X humpback whale wintering regions as four strongly structured regions typically used for stock definition.

For pairwise comparisons of sampling sites from different Regions, Oman (both Φ_{ST} and F_{ST}) was the most differentiated from all other sites. The significant differentiation of whales sampled from Oman compared to the expanded sampling within Regions A, B, and C lends additional support to the possible non-migratory and potentially reproductively-isolated nature of humpback whales off the coast of Oman. Our analysis of mtDNA control region sequences showed that maternal lineages that exist among wintering populations of the southern Indian Ocean are also present in the whales sampled off the coast of Oman. Shared haplotypes between Northern and Southern Hemisphere populations may be the result of ancestral polymorphism among humpback whales from historical population expansion and subsequent subdivision in the Indian Ocean and throughout the Southern Hemisphere. Alternatively a moderate amount of inter-hemisphere gene flow may exist. Several individual maternal lineages may migrate between southwestern Indian Ocean wintering grounds and the waters off the coast of Oman.

The animals sampled off the coast of Brazil were significantly different from the other South Atlantic populations based on haplotype frequencies. However, the molecular distance statistics from Brazil were not significant when compared to other wintering Regions. This lack of significant variation using Φ_{ST} statistics may be indicative of shared common ancestry of Southern Hemisphere humpback whales. Some preliminary but striking acoustic similarities suggest some degree of contact between humpback whales in the eastern and western South Atlantic on their breeding, feeding grounds, or migratory corridors (Darling and Sousa-Lima, 2003). Yet a recent photographic matching exercise between Brazilian breeding grounds (n=2000 photographs) and Gabon (n=1000 photographs) failed to yield any matches (Pacheco et al., 2004). These intriguing molecular and non-molecular results are being examined with expanded sampling for both mtDNA and microsatellite loci in order to further examine population-level heterogeneity and movements of individual animals.

Several populations had such small sample sizes (i.e. Angola and Benin) making interpretation for pairwise comparisons very difficult. However, these samples were extremely valuable contributions for the regional analysis from areas where samples are difficult to obtain. Certain other 'within-region' subdivisions and connectivity between low-latitude breeding grounds and migratory corridors (ie populations sampled within C1 and within C3 showing no significant differentiation, but significant heterogeneity in haplotype frequencies between C1 and C3) were supported by our mtDNA data. The C2 population off Mayotte generally differs, but not entirely consistently in terms of haplotype frequencies from other areas sampled within Region C. This is partially similar in nature to results found at nuclear loci by Pomilla et al. (2004), as significant differentiation was found between C1 and C2, and C1 and C3 with the exception of comparisons to whales sampled off southern Madagascar (C3) and Mozambique (C1) based on mtDNA. Two photographic matches between Antongil Bay and Mayotte (Avolio *et al.* 2002; Rosenbaum *et al.* 2002) and potential genetic matches (Pomilla, *per. comm*.) provide some evidence for migratory movements between these populations. Additional samples from Mayotte have been obtained and will be analyzed to increase resolution on this issue.

A proposed division of Region B into sub-Regions B1 (Gabon) and B2 (Angola, west South Africa) (IWC 2000) was not supported by nuclear DNA analysis (Pomilla *et al*. 2004), as not a single significant pairwise difference was found within this region either using F_{ST} or R_{ST}. However, statistical differences in haplotype frequencies were found in our mtDNA preliminary study between low latitude areas in B1 (Gabon) with sampling of animals off west South Africa (Rosenbaum *et al*. 2001).

It was thought that samples sizes were considerably smaller in that study and the west South Africa sample included over-summering animals (M/C pairs) that may not have been representative of animals from this area. However, an expanded sampling in both areas in the present study supported this intriguing result, suggesting that animals migrating along the west South African coast may be different in overall maternal lineage composition than those that are found in the tropical breeding grounds off Gabon. These differences in results between molecular markers may be indicative of differential migration patterns and maternal fidelity to low-latitude breeding grounds within Region B. Furthermore, animals that are far offshore of west South Africa may be underrepresented in the samples from Region B due to difficulties in sampling animals at great distance from the coast (Best, *per. comm*.). Several animals that departed the Gabon breeding grounds and were satellite tagged, migrated great distances offshore of west South Africa and would likely not have been sampled on the southbound migration past this area (Mate and Rosenbaum, *in prep.*). An additional stratification of southbound and northbound migrating animals from west South Africa compared to animals off the coast of Gabon will be conducted.

Finally, maternal lineage diversity was relatively high for all populations sampled in A, B, and C but considerably lower for whales off Oman. Although other factors may reduce genetic variation, haplotype diversity is typically a function of population size over time. The finding that whales from Oman have lower haplotype and nucleotide diversities suggests that recent exploitation could have played a role in decreasing overall population size and genetic diversity in what is now thought to be a smaller and potentially genetically-isolated population. Alternatively, population size and genetic diversity could have been low historically. This would be compounded by reduced gene flow with other populations or lineage-specific directed fidelity to areas off Oman. Without knowledge of the levels of genetic diversity that existed before the 1960s and a more thorough sampling of the extant population, it is difficult to draw conclusions about loss of genetic diversity due to hunting.

Our mtDNA results represent an interesting contrast to the large-scale analyses of many of the same wintering grounds presented in Pomilla *et al.* (2004). These data need to be more carefully analyzed to evaluate the extent to which maternally directed fidelity to migratory destinations and male-mediated gene flow effect our abilities to detect population structure among and within these wintering Regions. Additional analyses will include feeding ground comparisons and expanded sampling, where indicated, to bring the most representative scientific data and analyses to management decision-making for these populations of humpback whales and their critical habitats and migratory corridors.

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LITERATURE CITED

Avolio, M., P. Ersts, C. Pomilla, M. Vely, J. J. Bastid, B. Wendling, R. Seitre, J. Seitre, P. Darmmangeat, V. Collin-Omnes, Y. Razafindrakoto, K. Findlay, and H. C. Rosenbaum. 2002. Humpback whale distribution and marine mammal diversity in the waters of Mayotte, (Comoros Archipelago) in the Mozambique Channel. Pp. 10. Paper SC/54/H18 presented to the IWC Scientific Committee, (unpublished).

Ayres, K. L., and D. J. Overall. 2004. api-calc 1.0: a computer program for calculating the average probability of identity allowing for substructure, inbreeding and the presence of close relatives. Molecular Ecology Notes 4:315-318.

Baker, C. S., L. Medrano-Gonzalez, J. Calambokidis, A. Perry, F. B. Pichler, H. Rosenbaum, J. M. Straley, J. Urban-Ramirez, M. Yamaguchi, and O. V. Ziegesar. 1998. Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. Molecular Ecology 7:695-707.

Baker, C. S., J. Murrell, S. Caballero, L. Florez-Gonzalez, J. Capella, C. Garrigue, J. Greaves, C. Jenner, M. Jenner, H. C. Rosenbaum, and J. Bannister. 2000. Differentiation of humpback whales mitochondrial DNA lineages from wintering grounds of the South Pacific and Indian Oceans. Paper presented to the IWC The Scientific Committee, 2000 (unpublished).

Best, P. B., K. P. Findlay, K. Sekiguchi, V. M. Peddemors, B. Rakotonirian, A. Rossouw, and D. Grove. 1998. Winter distribution and possible migration routes of humpback whales (*Megaptera novaeangliae*) in the southwest Indian Ocean. Marine Ecology Progress Series 162:287-299.

Best, P. B., D. Reeb, M. Morais, and A. Baird. 1999. A preliminary investigation of humpback whales off northern Angola. Paper SC/51/CAWS33 presented to the IWC Scientific Committee, 1999 (unpublished).

Best, P. B., K. Sekiguchi, and K. P. Findlay. 1995. A suspended migration of humpback whales *Megaptera novaeangliae* on the west coast of South Africa. Marine Ecology Progress Series 118:1-12.

Chapman, D.G. 1974. Status of Antarctic rorqual stocks. Pages 218-238. *In*: W.E. Schevill (ed.) *The Whale Problem.* Harvard University Press, Cambridge. 419pp

Darling, J. and Sousa-Lima, R. 2003. Abstract of The Biennial Conference on The Biology of Marine Mammals. Greensboro, North Carolina.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Gambell, R. 1976. World whale stocks. Mammal Review 5:41-53.

Hudson, R. R., D. D. Boos, and N. Kaplan. 1992. A statistical test for detecting geographic subdivisions. Molecular Biology and Evolution 9:138-151

.IWC. 1997. Report of the scientific committee. Report of the International Whaling Commission 47.

IWC. 2000. report of the scientific committee. Report of the International Whaling Commission 52.

Lambertsen, R. H. 1987. A biopsy system for large whales and its use for cytogenetics. Journal of Mammalogy 68:443-445.
Mackintosh, N. A. 1942. The southern stocks of whalebone whales. Discovery Reports 22:197-300.
Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
Olavarria, C. et al. 2003. Population differentiation of humpback whales from far Polynesia (Group F breeding grounds) based on mitochondrial DNA sequences. SC/55/SH11.
Godoy, L et al., 2004. Paper presented at this meeting.
Palsbøll, P. J., P. J. Clapham, D. K. Mattila, F. Larsen, R. Sears, H. R. Siegismund, J. Sigurjonsson, O. Vasquez, and P. Arctander. 1995. Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behavior on population structure. Marine Ecology Progress Series 116:1-10.
Park, S. 2001. MS_Toolkit: Excel add-in tool package for microsatellite data. Genetics Department, Trinity College, Dublin.
Pomilla C., Best P.B., Findlay K., Kotze P.J.H., Engel M.H., Barendse J., and Rosenbaum H.C. 2004. Population structure of Southern Hemisphere humpback whales from wintering Regions A, B, and C based on nuclear microsatellite variation, pp. 12, Paper SC/56/SH4 presented to the IWC the Scientific Committee, (unpublished).
Rosenbaum, H. C., P. B. Best, K. P. Findlay, M. H. Engel, C. Pomilla, Y. Razafindrakoto, M. E. Morete, A. C. Freitas, C. S. Baker, C. Jenner, M.-N. Jenner, and J. Bannister. 2000. Mitochondrial DNA variation among humpback whales from the wintering grounds in the South Atlantic and Southwestern Indian Oceans. Pp. 13. Paper SC/52/IA11 presented to the IWC The Scientific Committee, 2000 (unpublished).
Rosenbaum, H. C., P. B. Best, and C. Pomilla. 2001. A preliminary analysis of mtDNA variation among humpback whales of the southeastern Atlantic Ocean from the wintering grounds along the coast of West Africa. Pp. 8. Paper SC/53/IA32 presented to the IWC Scientific Committee, (unpublished).
Rosenbaum, H. C., P. Ersts, Y. Razafindrakoto, G. Sounguet, C. Pomilla, S. Ngouessono, V. Rasoamampianina, and L. White. 2002. Population characteristics, distribution, and relative abundance of humpback whales off the coasts of Madagascar and Gabon: an update on recent and planned research. Pp. 13. Paper SC/54/H20 presented to the IWC Scientific Committee, (unpublished).
Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.
Schlötterer, C., B. Amos, and D. Tautz. 1991. Conservation of polymorphic simple sequence loci in cetacean species. Nature 354:63-65.
Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN (Version 2.000): A software for population genetics data analysis. University of Geneva, Geneva, Switzerland.
Takahata, N., and S R. Palumbi. 1985. Extranuclear differentiation and gene flow in the finite island model. *Genetic* 109:441-457.
Waits, L., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249-256.
Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Figure 1. IWC boundaries for wintering and feeding grounds in the South Atlantic and Indian Oceans (Rosenbaum et al. 2000).

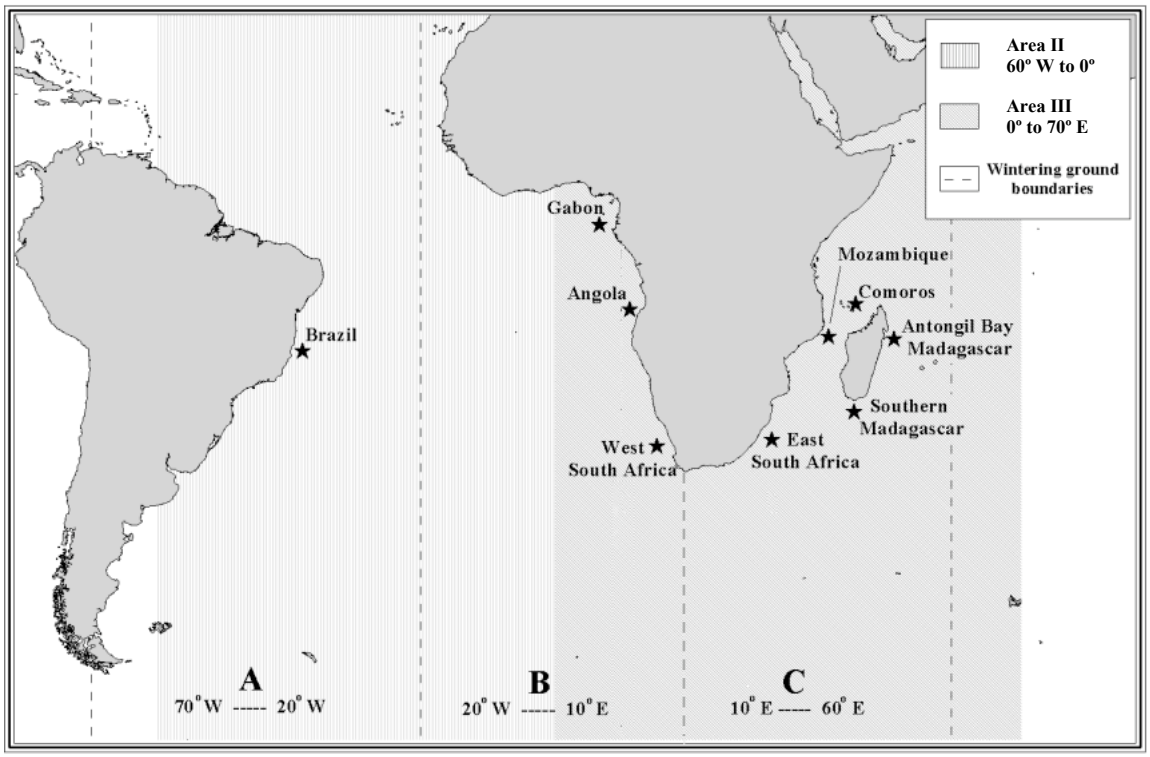

Table 1. Sample location, size, and information pertaining to the variability detected in the mtDNA control region for eleven breeding grounds and migratory corridors of Southern Hemisphere humpback whales. Haplotype (h) and nucleotide (π) diversities, as well as their standard deviations are provided below.

Wintering Region, Breeding Ground or Corridor	Sample Size	# of haplotypes	Polymorphic sites	h ± SD	π ± SD
Southwestern Indian Ocean (C)					
Antongil Bay, Madagascar (BA) C3	493	89	68	0.9774 +/- 0.0015	0.0207 +/- 0.0105
South Madagascar (MG) C3	30	19	46	0.9586 +/- 0.0209	0.0200 +/- 0.0105
Mozambique (MZ) C1	60	36	46	0.9712 +/- 0.0103	0.0174 +/- 0.0091
East South Africa (EZ) C1	87	46	51	0.9778 +/- 0.0057	0.0202 +/- 0.0104
Mayotte, Comoros (MY) C2	35	21	43	0.9546 +/- 0.0202	0.0206 +/- 0.0108
Northern Indian Ocean (X)					
Oman (OM) X	46	8	25	0.6618 +/- 0.0523	0.0170 +/- 0.0089
Southeastern Atlantic Ocean (B)					
West South Africa (WZ) B2	119	52	59	0.9742 +/- 0.0055	0.0203 +/- 0.0103
Angola (AG) B2	10	9	32	0.9778 +/- 0.0540	0.0223 +/- 0.0125
Gabon (GA) B1	483	97	71	0.9782 +/- 0.0016	0.0209 +/- 0.0106
Benin (BE)	5	5	23	1.0000 +/- 0.1265	0.0223 +/- 0.0143
Southwestern Atlantic Ocean (A)					
Abrolhos, Brazil (BR) A	48	30	45	0.9743 +/- 0.0096	0.0199 +/- 0.0103

Table 2: Results of the AMOVA for four wintering regions and eleven breeding grounds or migratory corridors of Southern Hemisphere humpback whales using molecular distances (**Φ**) and nucleotide diversity (*F*). The P-value is the probability of a more extreme variance component or Phi-value (*F*-value) than that observed, in comparison to a null distribution of these values on 1,000 random permutations of the data matrix. *F-CT* and the between region variance component involves the permutation of whole sites among regions. The *F-SC* and *F-ST* are tests against random permutations of the respective level under 'Source of variation'.

	Φ -*STATISTICS*				**F-*STATISTICS***			
Source of variation	**d.f**	**Percentage of total variation**	**Φ**	**P**	**d.f**	**Percentage of total variation**	**F**	**P**
Among Regions (A, B, C & X)	3	1.27	ΦCT: 0.01269	0.0567	3	1.87	*F-CT*: 0.01868	0.0596
Among Sites within Regions	7	0.3	ΦSC: 0.00307	0.0000	7	0.55	*F-SC*: 0.00559	0.0000
Within Sites	1405	98.43	ΦST: 0.01572	0.0000	1405	97.58	*F-ST*: 0.02416	0.0000

Table 3. The differentiation of maternal lineage sequences (Phi-ST) between all pairwise comparisons of eleven breeding grounds or migratory corridors. Values shown in bold are significant (P≤0.05) as estimated from 1000 random permutations.

	BA	GA	MY	OM	MG	WZ	EZ	BR	BE	MZ	AG
BA	0										
GA	**0.00418**	0									
MY	-0.00018	0.00774	0								
OM	**0.07696**	**0.09390**	**0.08435**	0							
MG	-0.00357	0.00827	-0.01326	**0.05808**	0						
WZ	**0.00555**	0.00217	-0.00061	**0.11431**	0.01076	0					
EZ	-0.00297	0.00371	0.00197	**0.08900**	-0.00228	0.00364	0				
BR	0.00117	0.00511	0.00483	**0.08712**	-0.00998	0.00710	-0.00068	0			
BE	0.04642	0.04893	0.07204	**0.15494**	0.06184	0.07068	0.03931	0.03856	0		
MZ	**0.01754**	**0.02197**	0.00978	**0.10795**	-0.00245	**0.02056**	**0.01813**	**0.02159**	**0.15180**	0	
AG	-0.01848	-0.02862	-0.02199	**0.09036**	-0.01262	-0.02479	-0.01827	-0.01425	0.07320	0.00077	0

Table 4. Conventional F-Statistics from haplotype frequencies for all pairwise comparison between eleven breeding grounds or migratory corridors. Values shown in bold are significant (P≤0.05) as estimated from 1000 random permutations.

	BA	GA	MY	OM	MG	WZ	EZ	BR	BE	MZ	AG
BA	0										
GA	**0.00708**	0									
MY	**0.00853**	**0.01812**	0								
OM	**0.12658**	**0.14058**	**0.10219**	0							
MG	0.00657	**0.01424**	-0.00344	**0.09771**	0						
WZ	**0.00886**	**0.00618**	**0.02117**	**0.15368**	**0.02095**	0					
EZ	**0.00240**	**0.00752**	**0.01769**	**0.14971**	**0.01680**	**0.01129**	0				
BR	**0.01164**	**0.00623**	**0.02790**	**0.17600**	**0.01286**	**0.01226**	**0.01401**	0			
BE	0.00303	-0.00529	0.02706	**0.22472**	-0.00252	0.01049	-0.00532	-0.01892	0		
MZ	**0.00713**	**0.00709**	**0.01155**	**0.13551**	0.00627	**0.01150**	0.00392	**0.01426**	-0.00300	0	
AG	0.00217	-0.00698	0.00334	**0.18515**	0.01277	-0.00804	0.00968	0.00335	0.01235	0.01266	0