

# GENETIC CHARACTERISTICS AND POPULATION STRUCTURE OF HUMPBACK WHALES IN THE ANTARCTIC FEEDING GROUND AS REVEALED BY MITOCHONDRIAL DNA CONTROL REGION SEQUENCING AND MICROSATELLITE ANALYSES

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## ABSTRACT

Biopsy samples from 411 humpback whales obtained during surveys of the Japanese Whale Research Program under Special Permit in the Antarctic and International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research were analyzed in order to describe their genetic population structure in the Antarctic feeding grounds. Samples were obtained from International Whaling Commission (IWC)-designated management Areas III (n= 81), IV (n= 172), V (n= 97) and VI (n= 61), and were examined for i) sex determination, ii) the sequence variation of the first 334bp nucleotides of the mtDNA control region and iii) genetic variation at the genotypes of six microsatellite loci. Duplicated samples were excluded from the analysis. The level of genetic diversity in the Antarctic was high for both genomes: the nucleotide diversity at the mtDNA was estimated at 0.0263 and the mean expected heterozygosity at the nuclear loci at 0.7820 for the total samples. In general results based on both mtDNA and microsatellites were similar and they suggest population structure of humpback whales in the Antarctic feeding grounds. These genetic results are consistent with the previous view based on non-genetic data that Areas III, IV, V and VI are occupied by different populations. The most plausible pattern of structure in the Southern Hemisphere therefore is multiple breeding and feeding grounds with some extent of site fidelity. Marked differences were found between whales in Areas IV and V for both mtDNA and microsatellites, and the same pattern was found for both sexes. Results of the other pair-wise comparisons among Areas showed more subdivisions in females than in males. An explanation for this result is that the difference is due to the lower sample sizes for males in these comparisons. The possibility of intermingling of populations in bordering sectors can not be discarded yet and a comprehensive analysis that involves genetic data from low and high latitude, as recommended by the Scientific Committee, is necessary to solve this issue.

KEYWORDS: ANTARCTIC, FEEDING GROUNDS, GENETICS, HUMPBACK WHALE

## INTRODUCTION

Humpback whales, *Megaptera novaeangliae*, are found worldwide in all major oceans. Like other Balaenopterid species, humpback whales migrate between summer feeding grounds in mid- and high latitudinal waters and winter breeding grounds in tropical or subtropical waters. Animals occur primarily in coastal and continental shelf waters.

Three oceanic populations of the humpback whale occur in the North Atlantic, North Pacific and Southern Hemisphere, and different patterns of structure have been reported within each major oceanic population. In the western North Atlantic, for example, whales congregate to mate and calve in a single breeding area of the West Indies during the winter. In summer they congregate in several high latitude

feeding areas. Genetic studies suggest limited exchange among the summer feeding areas, and mixing in the winter breeding area (Palsboll *et al.*, 1997a). The pattern of structure here is single breeding grounds and multiple feeding grounds. In the North Pacific the situation is much more complex. Whales show a strong fidelity to winter breeding grounds in Costa Rica, Mexico, Hawaii, Southeast Asia, and wide, but philopatric, dispersal to feeding grounds in temperate and high latitudinal waters. Some movements of animals between low latitudinal breeding areas have been reported (Darling and McSweeney, 1985; Darling and Mori, 1993). Genetic studies have shown significant differences between whales distributed in central and eastern North Pacific (Baker *et al.*, 1998a). The pattern of structure here is single breeding grounds and multiple feeding grounds but the case of multiple breeding grounds using a single feeding ground has also been suggested. These phenomena seem to be related with differences in topography among ocean basins and as a consequence, the breeding grounds in the North Atlantic are few, those in the North Pacific are larger in number and those in the Southern Hemisphere are many.

Regarding the Southern Hemisphere, Mackintosh (1965) showed that humpback whales tend to gather into five or six distinct feeding concentrations in the Antarctic during the austral summer season. These feeding concentrations were denominated as Groups I-V (with a Group IIa and IIb) corresponding roughly to IWC Management Areas I-VI. The Groups most documented are Groups IV and V. Omura (1953) examined the distribution of humpback whale in the feeding grounds of Areas IV and V based on catch data. Based on catch information he suggested that two populations occur in these Areas with a boundary around 130°-142°E. He did not discard the possibility of intermingling between these two populations in the feeding ground. He also examined the pattern of distribution by month and suggested that for the month where more data were available (November-March) the boundary between these two populations changed from 120°-130°E in November to eastside of 140°E in December and to 120°-140°E in January. Dawbin (1966) summarize the distribution and seasonal migratory movement of humpback whales from Groups IV and V, as demonstrated by mark-recapture data (Discovery-type marks). Whales from Group IV move mainly between Antarctic Area IV and Western Australia while whales from Group V move between Antarctic Area V and Eastern Australia and along the coast of New Zealand and southwest Pacific islands. Interchange of a few individuals between Groups IV and V was reported. Dawbin (1966) also reported that the boundary of Groups IV and V in the Antarctic do not correspond to the actual boundary between Areas IV and V and that some whales marked in Area VI were recovered in eastern Australia.

These migratory corridors between low latitudinal breeding grounds and high latitudinal feeding grounds have been confirmed by analysis of photo-identified whales. Gill and Burton (1995) reported the movement of a photo-identified whale between Western Australia and Antarctic Area IV and Kaufman *et al.* (1990) that between Eastern Australia and the boundary between Areas V and VI. More recently the matches of three individual humpback whales between Eastern Australia and Antarctic Area V, were reported (Rock *et al.*, in press).

Genetic analyses based on mitochondrial DNA (mtDNA) and samples from low latitude waters (Baker *et al.*, 1998b) showed significant genetic differences between Western Australia, Eastern Australia and Colombia humpback whales, supporting the previous view that different populations occur in those regions (corresponding to Groups IV, V and I mentioned above). Little is known, however, on the genetic diversity and population structure of humpback whales in the Antarctic feeding grounds. The present paper addresses this issue by examining samples obtained in Areas IV and V and their adjacent Areas III and VI. As noted above, previous non-genetics studies had suggested that these Areas are occupied by different populations. Here the hypothesis of different populations occurring in these Areas is tested using genetic data. The analysis is also conducted to investigate the current status of genetic variability of humpback whales.

The analysis is based on the maternal inherited mtDNA and bi-parental inherited microsatellites. Further the analysis is conducted for total samples as well for each sex separately. Previous studies based on mark-recapture suggested a high fidelity of animals to feeding areas (identified approximately by the boundaries of the current Areas) and particular breeding areas. Therefore genetic differences among Areas are expected. There is the possibility, however, of a sex-differentiated pattern of dispersal in the feeding ground. This could be investigated by the genetic analysis conducted separately for males and females.

## MATERIALS AND METHODS

### Samples

Skin biopsy samples were obtained from free-ranging whales along the sighting surveys of the JARPA (Japanese Whale Research Program under Special Permit in the Antarctic) and IDCR/SOWER (International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research) surveys in Areas III, IV, V and VI (Figure 1), on an opportunistic basis. Biopsy samples in JARPA were collected using an air gun described in Kasamatsu *et al.* (1991) and more recently using a Paxarm system. Biopsy samples in IDCR/SOWER were collected using several methods including Paxarm system and crossbows. At the laboratory all biopsy samples were checked for the possibility of re-sampling (two or more samples taken from a same individual) by comparing the genotype profiles produced by a set of six microsatellites.

Re-sampling rates were higher for IDCR/SOWER than for JARPA samples. For the former it varied from 0% in Area V to 15.0% in Area VI (sample size in Area V was only seven). In the case of JARPA it varied from 4.3% in Area VI to 10.0% in Area III. Taylor and Chivers (1997) demonstrated by simulations that if duplicated samples are not recognized, results of genetic analyses on population structure will be biased. Therefore in the present study duplicates were excluded from all analyses.

When mother/calf pairs were sampled, only the genetic information of the mother was used for the analysis (nine cases in Area V).

Table 1 shows the actual number of samples used in the analysis by IWC management Area, sex, and kind of surveys, and Figure 1 shows the geographical distribution of samples in the feeding grounds of Areas III, IV, V and VI, by sex. A total of 411 samples were used.

### Molecular genetic analysis

#### *Extraction of DNA*

Genomic DNA was extracted from approximately 0.05g of the outer epidermal layer of the skin biopsy, by standard phenol/chloroform extractions (Sambrook *et al.*, 1989). Extracted DNAs were stored in TE buffer (10mM Tris-HCl, 1mM EDTA, pH8.0).

#### *Sex determination*

Sex of the whales sampled was determined following the method of Abe *et al.* (2001), which uses a set of oligonucleotide primers to detect the presence/absence of the SRY gene from the Y chromosome with a microsatellite locus (GATA 417) as an internal control.

#### *Mitochondrial DNA control region sequencing*

The first 334 nucleotides at the 5' end of the mitochondrial control region were amplified by the polymerase chain reaction (PCR, Mullis and Faloona 1987). The oligo-nucleotides employed in the PCR amplification were MT4 (Arnason *et al.* 1993) and P2R (5'-GAA GAG GGA TCC CTG CCA AGC GG-3'). Reactions were carried out in 50  $\mu$ L volumes containing 100 mM KCl, 20 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT; 0.5% Tween 20, 0.5% Nonidet P-40, 200  $\mu$ M dNTPs, 2.5 pM of each oligo-nucleotide and one unit of Taq DNA polymerase. After an initial denaturation step at 95°C for 5 minutes, a PCR amplification cycle of 30 seconds at 94°C, followed by 30 seconds at 50°C and 30 seconds at 72°C was repeated 30 times. The amplification was completed with a final extension step of 10 minutes at 72°C. Subsequent cycle sequencing reactions were performed with 100ng of products generated in the above PCR amplifications using the Prism™ dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). The oligo-nucleotides used to prime the cycle sequencing reaction were the same as employed in the initial PCR amplification listed above. A total of 25 cycles with 10 seconds at 96°C, 20 seconds at 56°C and four minutes at 60°C were performed. The nucleotide sequence of each cycle sequencing reaction was determined by electrophoresis through a 5% Long Ranger™ (FMC, Inc.) denaturing polyacrylamide matrix on a DNA Prism™ 377 DNA Sequencer (Applied Biosystems, Inc.) under standard conditions. Both strand samples were sequenced in their entirety for all samples.

#### *Microsatellites*

Six microsatellite loci were used in this study: four tetranucleotide motifs (GATA417, GATA28, GATA98, GATA53) (Palsbøll *et al.* 1997b), one trinucleotide motifs (TAA31) (Palsbøll *et al.* 1997b),

and one dinucleotide motifs (GT23) (Bérubé *et al.* 2000). PCR amplifications were performed in 15 $\mu$ l reaction mixtures containing 10 to 100ng of DNA, 5 $\mu$ mol of each primer, 0.625 units of Ex Taq DNA polymerase (Takara Shuzo), 2mM of each dNTP, and 10x reaction buffer containing 20mM MgCl<sub>2</sub> (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) were run on a 6% polyacrylamide denaturing gel (Long Ranger) using BaseStation100 DNA fragment analyzer (BioRad). Although alleles were visualized using Cartographer software specifically designed for the BaseStation, allelic sizes were determined manually in relation to the internal size standard and humpback whale's DNA of known size that were rerun on each gel.

### **Grouping of the samples**

The samples were grouped according to the boundaries of IWC Management Areas III (0°-70°E), IV (70°-130°E), V (130°E-170°W) and VI (170°-120°W). With regard Area III, biopsy samples were available only for the eastern part of this Area (34°-70°E) (Figure 1).

For the analysis samples collected in different austral summer seasons were pooled under the assumptions that the patterns of seasonal movement are the same for a given breeding population in different years and that lateral movement on feeding grounds and pattern of mixing are similar between years. These assumptions are discussed later.

### **Data analysis**

#### *mtDNA*

Genetic distances among unique sequences (haplotypes) were estimated using the Kimura's two parameters method (Kimura, 1980). The degree of mtDNA diversity within each Area was estimated using the nucleotide diversity (Nei and Li, 1979), and the nucleon diversity (Nei and Tajima, 1981).

Heterogeneity tests among Areas were conducted as described in Hudson *et al.* (1992), using the chi-square and the Kst\* statistics. The level of statistical significance was estimated from 10,000 Monte Carlo simulations as the proportion of simulations in which a similar or more extreme value of chi-square or Kst\* was observed. Tests were conducted for all samples combined as well for males and females separately. When multiple tests were conducted, the Rice (1989) correction for multiple tests was performed.

#### *Microsatellites*

Allele frequencies, the number of alleles per locus, allelic richness, heterozygosity at the six microsatellite loci and Fst values were computed using FSTAT 2.9.3 (Goudet, 1995). The program GENEPOP 3.2 (Raymond and Rousset, 1995) was used to test for deviations from expected Hardy-Weinberg genotypic proportions at each of the loci.

Heterogeneity tests among Areas were conducted using the probability test (or Fisher's exact test) with Markov chain method implemented in the GENEPOP. Tests were conducted for all samples combined as well for males and females separately. Statistical significance was determined using the chi-square value obtained from summing the negative logarithm of P-values over the six microsatellite loci (Sokal and Rohlf, 1995). When multiple tests were conducted, the Rice (1989) correction for multiple tests was performed.

The Bayesian clustering approach was conducted using STRUCTURE version 2.0 (Pritchard *et al.*, 2000) to determine the number of genetically distinct populations present in the samples. Three independent runs for each value of K between 1 (panmictic) and 5 with no prior information (i.e. only genetic information was considered) were conducted. All of the simulations were based on a burn-in period of 50,000 iterations and runs of 500,000 iterations. The ancestry model used for the simulation was the admixture model, which assumes individuals may have mixed ancestry. The allele frequency model used was the correlated allele frequencies model, which assumes frequencies in the different populations are likely to be similar due to migration or shared ancestry.

## RESULTS

### Sex ratio

The male proportion by Area was as follow: Areas III: 38.3%, IV: 52.9%, V: 47.4% and VI: 59.0%.

### Genetic variability

#### *mtDNA*

A segment of 334bp of the mtDNA control region was sequenced for all samples. A total of 65 variable sites defined a total of 98 unique sequences (haplotypes) in the sample of 411 humpback whales. Level of mtDNA diversity based on nucleotide and nucleon diversity is shown in Table 2. Both indices showed a high and similar degree of mtDNA diversity through the Areas. The nucleotide diversity at mtDNA for the total samples was estimated at 0.0263.

#### *Microsatellites*

All microsatellite loci were polymorphic in all the samples (Table 3). Five of the six loci had more than 10 alleles with an overall average of 15.3 over the all samples. The number of alleles per locus per Area varied between 10.7 and 12.7 with smaller differences in allelic richness values. Average expected heterozygosity was 0.782 over the Areas and ranged from 0.767 to 0.799 per Area. No evidence of significant deviation from expected Hardy-Weinberg expected genotypic proportions was observed in each of the Areas for over all loci tests. The P-value smaller than 0.05 observed in the Areas V and VI samples at GATA417 is most likely due to a chance effect. However the power of this analysis could be low.

### Genetic divergence

#### *mtDNA*

The results of the heterogeneity test are shown in Table 4. The chi-square test resulted more sensitive to detect differences than the Kst\* test. Below is a summary of the results for the former test.

Males: Statistically significant difference was detected across all the loci for the overall Areas. Two of the six pair-wise comparisons showed statistically significant differences after correction for multiple tests (Areas III/IV and Areas IV/V).

Females: Statistically significant difference was detected across all the loci for the overall Areas. Four of the six pair-wise comparisons showed statistically significant differences after correction for multiple tests (Areas III/IV, Areas III/V, Areas III/VI and Areas IV/V).

Total: Statistically significant difference was detected across all the loci for the overall Areas. Five of the six pair-wise comparisons showed statistically significant differences after correction for multiple tests. The only exception was Areas V/VI.

Kst\* values shown in Table 4 indicated low level of mtDNA divergence.

#### *Microsatellites*

The results of the heterogeneity test are shown in Table 5.

Males: Statistically significant difference was detected across the loci for the overall Areas. Only one of the six pair-wise comparisons showed statistically significant differences after corrections for the multiple tests (Areas IV/V).

Females: Statistically significant difference was detected across the loci for the overall Areas. Three of the six pair-wise comparisons showed statistically significant differences after corrections for the multiple tests (Areas III/IV, Areas III/V and Areas IV/V).

Total: Statistically highly significant difference was detected across the loci for the overall areas. Four of the six pair-wise comparisons showed statistically significant differences after corrections for the multiple tests. The only exceptions were Areas III/VI and Areas V/VI.

Bayesian clustering analyses conducted without information on geographic sampling origins presented the lowest likelihood probability at K=5 (Ln probability= -11,951) and the highest likelihood probability at K= 1 (Ln probability= -9,723).

Overall the Fst values indicated low level of genetic divergence. The overall Fst value for males was 0.008, for females 0.004 and for all samples combined 0.005. Ninety-five % confidence interval for the overall Fst indicated it was significantly different from zero.

## **DISCUSSION**

### **Genetic variability**

Level of genetic variability found in humpback whales in the Antarctic feeding grounds was high, for both mtDNA and microsatellites, and it was similar through the Areas. This is consistent with previous results obtained for the humpback whale worldwide for both markers, mtDNA (Baker *et al.*, 1993; Palsboll *et al.*, 1995) and microsatellites (Valsecchi *et al.*, 1997).

### **Pattern of stock structure**

In general results based on both mtDNA and microsatellites were similar and they suggest population structure of humpback whales in the Antarctic feeding grounds. Genetic differentiation among Areas based on both markers suggests a strong fidelity of humpback whales to migratory destinations. In particular marked differences were found between whales in Areas IV and V for both mtDNA and microsatellites, and the same pattern was found for both sexes. The pattern of structure in the Southern Hemisphere seems to be multiple breeding grounds and multiple feeding grounds as opposed to the pattern found in the North Pacific and North Atlantic.

Although the samples used in the present study were taken on an opportunistic basis along JARPA and IDCR/SOWER surveys, sampling had covered most of the longitudinal sectors of Areas III, IV, V and VI. The exceptions are a sector in Area VI and the western part of Area V for which samples of both sexes were limited (Figure 1). In general these samples were useful to respond the question of whether population structure exists in those Areas. It should be noted that for both markers and total samples, discrimination between Areas V and VI is less in evidence than the other differences. This could be reflecting the distribution of sampling in Area VI where most of the samples used from this Area were obtained in the IWC boundary separating Areas V and VI (Figure 1).

The mtDNA analysis showed a marked degree of genetic heterogeneity suggesting population structure of the humpback whales in the Antarctic feeding grounds. Populations appear to be structured longitudinally. It should be noted that despite the marked mtDNA heterogeneity detected, the level of genetic divergence found (as suggested by  $K_{st}^*$ ) was low. Likewise, the microsatellite analysis detected that the individuals in the samples came from genetically different populations of humpback whales that appeared to be distributed longitudinally. The overall Fst was low but significant, supporting the existence of multiple populations. STRUCTURE, however, failed to detect the evidence of population differentiation among the samples. Considering the low level of genetic differentiations (Fst), this could have been caused by the low power of the analysis due to the low number of microsatellite loci used.

### **Comparison of results between sexes**

The analyses were conducted for both sexes separately in order to check the possibility a sex-differentiated pattern of dispersal in the feeding ground. The mtDNA analysis showed a higher degree of differentiation among Areas for females (four of six pair-wise comparisons were significant statistically) than males (two of six pair-wise comparisons resulted in significant differences). Same as in the mtDNA analysis, the microsatellite results showed more subdivisions in females than in males, three and one significant differences in six pair-wise comparisons, respectively. These results suggest the possibility that males move wider than females in the Antarctic feeding grounds.

In the comparison between Areas IV and V, however, where the larger sample sizes were available, results for both sexes were the same for both genetic markers. Then the alternative explanation that differences are just due to the lower sample size available for males in the other comparisons is preferred

because the apparent differences between sexes are not consistent with populations structured longitudinally in the feeding ground.

#### **Yearly variation in the pattern of structure**

As noted earlier for the analysis samples collected in different austral summer seasons were pooled under the assumptions that the patterns of seasonal movement are the same for a given breeding population in different years and that lateral movement on feeding grounds and pattern of mixing are similar between years. The assumptions can be examined by comparing the mtDNA composition of humpback whales taken in different years in a same Area. A preliminary analysis of yearly variation was conducted for Area IV, the Area supported by the larger sample size. In this Area JARPA samples from six different years were involved. Both chi-square ( $P=0.6950$ ) and  $K_{st}^*$  (0.431) tests failed to find significant genetic differences among years suggesting that whales from the same populations come to this Area in each year. Similar analyses are desirable for the other Areas.

In general these genetic results support the previous view based on non-genetic information that the Areas are occupied by different populations during the feeding season. Our analysis based on samples from the feeding grounds can not reject yet the possibility that different populations mix to each other in some particular longitudinal sectors. A more accurate longitudinal delineation of populations in the Antarctic feeding grounds and the identification of intermingle sectors can be accomplished by a comprehensive analysis that combines DNA data available from low latitude breeding grounds and DNA data from high latitude feeding grounds. The IWC Scientific Committee is conducting an in-depth assessment of southern humpback whale stocks. The Committee agreed in 2006 that assessment of Stock D should be considered preliminary due to potential for exchange with breeding Stock E on the feeding ground. The Committee noted that the status of Stock D should be re-evaluated in the future, but that this will first require analyses to clarify the structure of the E breeding sub-stocks and the extent of mixing at high latitudes. The Committee endorsed the Hobart workshop recommendation that every effort be made for scientists to share data and carry out such analyses under the IWC Data Availability Agreement (IWC, 2006).

#### **Factors influencing the distribution and separation of populations in feeding grounds**

What factor(s) is responsible for the population separation in the feeding ground with lack of obvious physical barriers? Humpback whales are also capable of traveling long distance. The spatial and temporal separation of the whale populations in the feeding grounds is influenced by the ability of their site fidelity, the distribution of their prey species, water current relating to temperature gradient, and underwater topography (Berta and Sumich, 1999), so that we infer one or a combinations of these factors was responsible for the separation of the humpback whale populations in the feeding ground. Humpback whales appeared to largely mimic the distribution of the prey species which is dependent of the high phytoplankton biomass coinciding with Antarctic Circumpolar Current (ACC; Tynan 1998). Upwelling of deep, nutrient-rich water supports very high summertime primary production. Murase *et al.* (2002) described the relationship between the distribution of the prey species, euphausiids, and baleen whales in the Antarctic from 35°E to 145°W. The authors found that the distributions of humpback whales were correlated with high euphausiid density zone, regardless of the bottom topographic features through simultaneous whale sighting and hydroacoustic surveys conducted during JARPA surveys in 1998/1999 an 1999/2000 austral summer. Both sighting frequency and distribution of the prey species was low in the area between the Areas IV and V.

#### **ACKNOWLEDGEMENTS**

We thank all the researchers and crew members of JARPA and IDCR/SOWER surveys involved in the sampling of the biopsy samples used in this study. T. Tamura (ICR) assisted us by producing Figure 1.

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Table 1: Number of samples of the humpback whale used in the present genetic study, by management Area and sex (M:males, F:females).

Area	M	F	Total
Area IIIE	31	50	81 (JARPA: 50; SOWER: 31)
Area IV	91	81	172 (JARPA: 126; SOWER: 46)
Area V	46	51	97 (JARPA: 90; SOWER: 7)
Area VI	36	25	61 (JARPA: 44; SOWER: 17)

Table 2: Nucleotide diversity (upper figure) and nucleon diversity (lower figures) of humpback whales in the Antarctic feeding grounds, by management Area.

	Nucleon diversity	Nucleotide diversity (SE)
Area IIIE	0.9688	0.0246 (SE:0.0008)
Area IV	0.9701	0.0262 (SE:0.0006)
Area V	0.9712	0.0274 (SE: 0.0008)
Area VI	0.9670	0.0268 (SE: 0.0009)

Table 3. Number of alleles (A), allelic richness (AR), expected heterozygosity (He), and test result for expected Hardy-Weinberg genotypic proportion (HW) (p-value) at six microsatellite loci for humpback whales in the Antarctic feeding grounds, by management Area (n= number of individuals)

	Area III (n=81)				Area IV (n=172)				Area V (n=97)				Area VI (n=61)				All (n=411)		
	A	AR	He	HW	A	AR	He	HW	A	AR	He	HW	A	AR	He	HW	A	AR	He
GATA417	14	13.4	0.910	0.9880	12	12.0	0.906	0.5219	17	15.5	0.901	0.0254	14	14.0	0.917	0.0340	19	13.6	0.908
GATA28	10	9.7	0.477	0.8042	11	10.1	0.541	0.9245	12	11.6	0.632	0.5244	11	11.0	0.462	0.5894	14	11.4	0.528
GATA98	9	8.9	0.727	0.0909	13	11.1	0.794	0.1982	10	9.6	0.793	0.3135	8	8.0	0.732	0.6225	13	10.2	0.762
TAA31	14	13.2	0.874	0.4767	19	14.5	0.888	0.7771	15	14.4	0.906	0.7323	13	13.0	0.871	0.6365	24	14.5	0.885
GATA53	9	8.7	0.820	0.5894	11	9.4	0.830	0.6396	13	11.1	0.803	0.6028	10	10.0	0.837	0.9861	13	9.8	0.822
GT023	8	7.9	0.793	0.1704	9	8.3	0.797	0.1104	9	8.6	0.759	0.7344	9	9.0	0.802	0.7133	9	8.3	0.788
Avg.	10.7	10.3	0.767	0.5005	12.5	10.9	0.793	0.5722	12.7	11.8	0.799	0.3537	10.8	10.8	0.770	0.5829	15.3	11.3	0.782

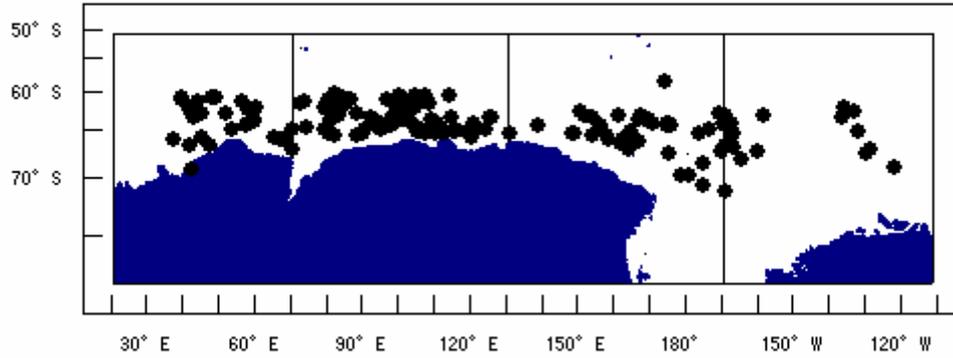
Table 4. Results of the heterogeneity test comparing mtDNA haplotype frequencies of humpback whales, among Antarctic management Areas (sample sizes in parenthesis). An asterisk indicates those comparisons that resulted in significant statistically differences after Bonferroni corrections.

	$\chi^2$ P-value	Kst*	P-value
<b>Males</b>			
III/IV (31, 91)	0.0040*	-0.0009	0.5830
III/V (31, 46)	0.0310	-0.0001	0.4470
III/VI (31, 36)	0.0300	0.0034	0.2180
IV/V (91, 46)	0.0000*	0.0049	0.0360
IV/VI (91, 36)	0.0500	0.0021	0.1890
V/VI (46, 36)	0.1570	-0.0002	0.4520
<b>Females</b>			
III/IV (50, 81)	0.0000*	0.0058	0.0400
III/V (50, 51)	0.0000*	0.0083	0.0190
III/VI (50, 25)	0.0030*	0.0028	0.2280
IV/V (81, 51)	0.0000*	0.0049	0.0600
IV/VI (81, 25)	0.2910	-0.0027	0.8070
V/VI (51, 25)	0.7980	-0.0030	0.7380
<b>Males+Females</b>			
III/IV (81, 172)	0.0000*	0.0019	0.0920
III/V (81, 97)	0.0000*	0.0071	0.0040*
III/VI (81, 61)	0.0000*	0.0065	0.0190
IV/V (172, 97)	0.0000*	0.0046	0.0040*
IV/VI (172, 61)	0.0020*	0.0012	0.1670
V/VI (97, 61)	0.0590	0.0008	0.2800

Table 5. Results of the heterogeneity test comparing allele frequencies of six microsatellite loci of humpback whales, among Antarctic management Areas (sample sizes in parenthesis). An asterisk indicates those comparisons that resulted in significant statistically differences after Bonferroni corrections.

	Males	Females	Males+Females
All Areas	0.00369	0.00002	High. Sig.
III/IV	0.43737	0.01129*	0.00471*
III/V	0.04608	0.00647*	0.00000*
III/VI	0.23073	0.15472	0.21129
IV/V	0.00075*	0.00621*	0.00000*
IV/VI	0.02619	0.19756	0.00427*
V/VI	0.01843	0.36140	0.09334

## MALES



## FEMALES

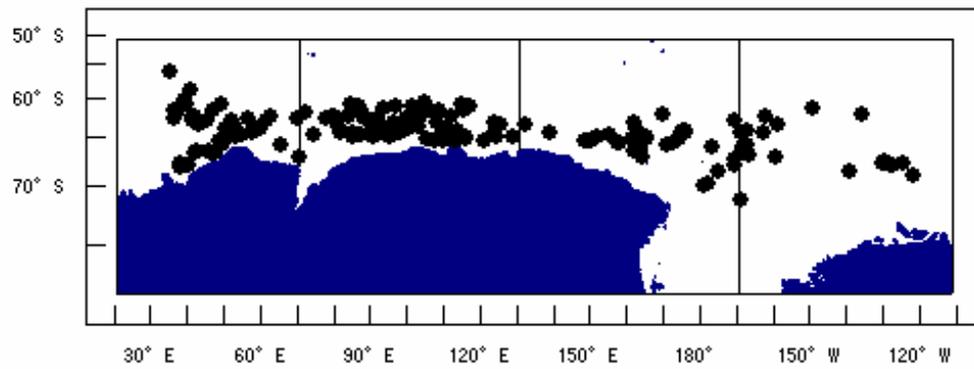


Figure 1: Geographical distribution of humpback whales examined in this study (upper figure: males; lower figure: female).