ACCESS MACQUARIE LIMITED
ABN 59 003 849 198

FINAL REPORT

GENETIC DIFFERENTIATION BETWEEN THE TWO AUSTRALIAN FEEDING AGGREGATIONS OF BLUE WHALES

MAY 2008

DR LUCIANA MÖLLER
MS CATHERINE ATTARD
DR LUCIANO BEHEREGARAY

MACQUARIE UNIVERSITY ~ SYDNEY AUSTRALIA

TABLE OF CONTEXT

PROJECT TITLE

Novel Genetic Markers for Stock Identification of Blue Whales and Differentiation Between the Two Main Australian Feeding Aggregations.

SUMMARY

This report includes information about a manuscript on the genetic differentiation of the two main Australian feeding aggregations, development of the new microsatellite library and additional sample collection attempted at the Bonney Upwelling Region.

Manuscript Preparation A manuscript entitled Genetic structure of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations (by Catherine Attard, Luciano B. Beheregaray, Curt Jenner, Peter Gill, Naohisa Kanda, Micheline Jenner, Margaret Morrice, John Bannister, Rick LeDuc, Luciana Möller) has been submitted to publication as a short communication in the Journal *Conservation Genetics*. The main findings from this study, which were based on six nuclear microsatellite loci and the mitochondrial DNA control region gene, revealed high gene flow between the two blue whale feeding aggregations at the Bonney Upwelling Region (southern Australia) and Perth Canyon (WA), strongly indicating they constitute one genetic population. This was especially evident when Australian samples were compared to Antarctic samples, suggesting low gene flow between Australian blue whales and the Antarctic subspecies of blue whales (*B. m. intermedia*). Please refer to manuscript submitted (below) for detailed information on findings from this study.

Microsatellite library development We have developed another microsatellite library using the enrichment protocol (Fischer, Bachmann, 1998) modified as in Saltonstall (2003), using the pCR 2.1-TOPO vector (Invitrogen). Please see Progress Report (February 2008) for detailed information on this method. We have sequenced so far

92 microsatellite enriched fragments and 18 unique sequences have been found. For unique sequences that contain microsatellite loci with sufficient flanking regions, primers will be designed using PRIMER3 (Rozen, Skaletsky, 1997). The development of this marker system will allow identification of blue whale stocks and, potentially, differentiation of blue whale subspecies.

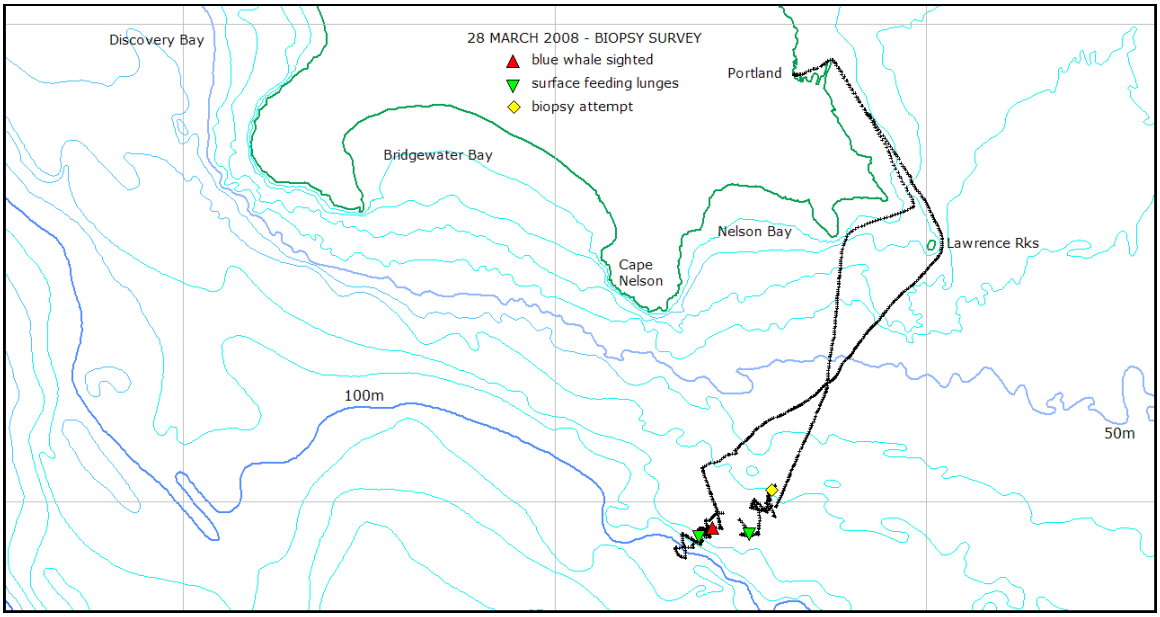
Sample collection at Bonney Upwelling Sampling of additional blue whale samples was attempted at the Bonney Upwelling Region, around Portland (VIC) between 24/03 and 30/03 with Dr Peter Gill from the Blue Whale Study. Severe weather conditions precluded boat surveys to be carried out in all but one day. On 28/03 with marginal conditions (10-12kt NW wind, 3-4m swell) a survey was conducted between 08:00 and 13:00. The survey was curtailed due to a gale warning. During the survey two blue whales were sighted (one photo-identified by P. Gill, Blue Whale Study, fig.1). However, due to the weather conditions and whales' evasive behaviour, it was not possible to approach whales at close range for successful biopsying. Only one biopsy attempt was made but the dart dropped short. Figure 2 shows the survey track and positioning of whale sighting (provided by P. Gill, Blue Whale Study).

Figure #1

Left hand side of blue whale sighted on 28/03/08 in the Bonney Upwelling Region (photo by P. Gill, Blue Whale Study).

Figure #2

Survey track on 28/03/08 in the Bonney Upwelling Region, showing position of blue whale sighting and sampling attempt (provided by P. Gill, Blue Whale Study).



Genetic structure of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations

Catherine Attard, Luciano B. Beheregaray, Curt Jenner, Peter Gill, Naohisa Kanda, Micheline Jenner, Margaret Morrice, John Bannister, Rick LeDuc, Luciana Möller*.

Running Title: Genetic structure of blue whales

Keywords: blue whale, *Balaenoptera musculus,* microsatellite, mitochondrial DNA, Australia

Catherine Attard, Luciano Beheregaray
Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

Curt Jenner, Micheline Jenner
Centre for Whale Research, P.O. Box 1622, Fremantle, WA 6959, Australia

Peter Gill, Margaret Morrice
Blue Whale Study, c/- Post Office, Narrawong, VIC 3285, Australia

John Bannister
The Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia

Naohisa Kanda
The Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo 104-0055, Japan

Margaret Morrice
School of Life and Environmental Sciences, Deakin University, PO Box 423, Warrnambool, VIC, 3280

John Bannister
The Western Australian Museum, Locked Bag 49, Welshpool DC, WA 6986, Australia

Rick LeDuc
Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, CA 92037-1508, USA

Luciana Möller
Graduate School of the Environment, Macquarie University, Sydney, NSW 2109, Australia

*Corresponding author, Luciana Möller, Graduate School of the Environment, Macquarie University, Sydney, NSW 2109, Australia. Phone: 61 (2) 9850 7998 Fax: 61 (2) 9850 7972
E-mail: luciana.moller@gse.mq.edu.au

Abstract

The worldwide distribution of blue whales (*Balaenoptera musculus*) has not prevented this iconic species from endangerment due to past whaling practices. In Australia there are two known feeding aggregations of blue whales, most likely to be of the pygmy blue whale subspecies (*B. m. brevicauda*). It is unknown whether individuals from these feeding aggregations migrate to different locations to breed. Six microsatellite loci and mitochondrial DNA control region markers were utilised to investigate the genetic structure of the Australian feeding aggregations. Both sets of markers revealed high gene flow between the two aggregations, strongly indicating they constitute one genetic population. This is especially evident when compared to low gene flow detected between Australian blue whales and the Antarctic subspecies of blue whales (*B. m. intermedia*). Our study provides key indicators to be incorporated into models for measuring recovery and status of blue whale populations in Australian waters.

Introduction

Whaling has reduced the abundance of the largest extant animal, the blue whale (*Balaenoptera musculus*), to a small fraction of their original numbers (Clapham et al. 1999; Branch et al. 2007). There are currently two recognised subspecies in the Southern Hemisphere: the 'pygmy' blue whale (*B. m. brevicauda*) and the Antarctic 'true' blue whale (*B. m. intermedia*). During the feeding season the former is found north of 54^{o}S (Ichihara 1966) and the latter in higher latitudes (Rice 1998). Most whaling effort was applied to the Antarctic subspecies. High prey concentrations, such as densities that occur in upwelling regions, can induce the solitary blue whales to aggregate in certain areas (Branch et al. 2007). Australia has two known feeding aggregations of presumably pygmy blue whales. These are supported by upwelling systems: the Bonney Upwelling and adjacent waters in South Australia and Victoria (Gill 2002; Middleton and Bye 2007) and the Perth Canyon in Western Australia (McCauley et al. 2000; McCauley et al. 2004; Rennie et al. 2006). Soviet whaling records from the mid-1960s showed an almost continuous distribution of pygmy blue whales from Tasmania west to Madagascar (Mikhalev 2000).

The classic hypothesis for baleen whale migration (Kellogg 1929) suggests that Australian blue whales migrate between temperate Australian feeding grounds during warmer months to lower latitude breeding grounds during colder months. Blue whale monthly distribution, as determined using catch, sighting, stranding and acoustic records, suggests that individuals from Australia may migrate along the coast of Western Australia to Indonesia where they potentially breed (Branch et al. 2007). Blue whales have also been sighted off the Solomon Islands in winter (Ohsumi and Shigemune 1993), suggesting that they may also migrate up Australia's east coast to

breed. However, migratory connections to breeding grounds for these two feeding aggregations have not yet been confirmed.

Pygmy blue whales have a potential characteristic vocalisation consisting of three call components (McCauley et al. 2000), however call types vary across pygmy blue whale distribution (McDonald et al. 2006). The same call type has been recorded from the Bonney Upwelling (R. D. McCauley personal communication) and the Perth Canyon (McCauley et al. 2000). Nonetheless, when 25 photo-identified individuals from the Bonney Upwelling were matched with 208 individuals from the Perth Canyon, only one resight was found (C. Jenner, M. Morrice and P. Gill, unpublished data).

Genetic studies of blue whales using microsatellite, mtDNA control region and intron markers between ocean basins have shown marked genetic differentiation (Conway 2005; LeDuc et al. 2007). These studies, however, were limited in their ability to perform analysis within ocean basins due to small sample sizes. The objectives of the current study were to assess the genetic structure and levels of gene flow between two known Australian blue whale feeding aggregations using microsatellite and mtDNA control region markers. We also compare results with DNA data obtained from samples of Antarctic blue whales. This study aids in conservation and management by carrying out an objective of the Australian Recovery Plan for Blue Whales (Department of the Environment and Heritage 2005), to provide indicators of population structure to be incorporated into models for measuring recovery and status of blue whale populations utilising Australian waters.

Methods

Sample collection

Samples were collected from the Bonney Upwelling (N = 22), the Perth Canyon (N = 78) and Antarctica (N = 16) (Fig. 1). The Antarctic samples, presumably from the Antarctic subspecies (*B. m. intermedia*), were utilised for comparative purposes. Australian samples were obtained from the Centre for Whale Research (Perth Canyon) and the Blue Whale Study (Bonney Upwelling), and Antarctic samples from the Institute of Cetacean Research in Japan. Samples were procured as strandings, biopsies, sloughed skin, extracted DNA or genome amplified DNA. Sampling performed in collaboration with the Blue Whale Study in the Bonney Upwelling used a Paxarms biopsy system (Timaru, New Zealand), a pole similar to that described by Bilgmann et al. (2007), or sloughed skin removed from the suction cup of dive logger tags. Tissue samples were preserved in 20% DMSO saturated with NaCl as suggested by Amos and Hoelzel (1991) or 100% ethanol.

Genetic methods

NOAA Southwest Fisheries Science Center (USA) provided genetic information for some of the Australian samples; 64 mtDNA control region haplotypes, and additionally for 29 of these samples, the multilocus microsatellite genotypes at four of the six loci analysed. DNA was extracted from tissue using a modified salting-out protocol (Sunnucks and Hales 1996). The microsatellite loci analysed were GATA028, GATA098, GATA417 (Palsboll et al. 1997) and GT023 (Berube et al. 2000) from humpback whales, and BM032 and BM261 developed specifically for blue whales (Attard et al. unpublished data). Microsatellite fluorescent-labelled PCR products were produced based on Schuelke (2000). Amplifications were performed in a 10 µl reaction containing ~50-100 ng template DNA, 2 pmol fluorescent-labelled

M13(-21) primer, 0.4 pmol forward primer and 2 pmol reverse primer, 200 μM each dNTP, 2 mM $MgCl_2$ with the exception of 2.5 mM $MgCl_2$ for GATA098, 0.05 μL 10% BSA, 0.5 U GoTaq Flexi DNA polymerase and its reaction buffer (Promega). The temperature profile was a touchdown PCR; 94°C for 3 min, then 35 cycles of 94°C for 20 s, 63°C for 45 s reducing by 2°C every cycle till stabilising at 53°C for 30 cycles, and 72°C for 1 min, then a final extension of 72°C for 10 min. PCR products were run on the ABI 3130xl (Applied Biosystems) and scored using GENEMAPPER 4.0 (Applied Biosystems).

Sequence data for a 394 bp fragment of mtDNA control region was obtained using the temperature profile described in Möller and Beheregaray (2001) with the primers Dlp-1.5 (Baker et al. 1998) and Dlp-5 (Baker et al. 1993). The mtDNA control region was amplified in a 30 μL reaction containing ~50-100 ng of template DNA, 48 pmol each primer, 160 μM each dNTP, 2.25 mM $MgCl_2$, 1.5 U *Taq* DNA polymerase and associated reaction buffer (Quiagen). PCR products were purified using an UltraClean 15 DNA Purification Kit (Mo Bio Laboratories), sequenced using an ABI 377 automated DNA sequencer (Applied Biosystems), and cleaned and aligned using the program SEQUENCHER 3.0 (Gene Codes Corporation, Ann Arbor, MI). Sequences of novel blue whale mtDNA haplotypes were deposited in GenBank.

Microsatellite data analysis

Duplicate samples were tested by identifying unique microsatellite multilocus genotypes using the program EXCEL MICROSATELLITE TOOLKIT 3.1 (Park 2001), and confirmed when duplicates had the same mtDNA haplotype. The second entry of duplicate samples was removed from the data set. Genotyping or scoring

errors, caused by null alleles, stuttering and short allele dominance, were checked for each putative population using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004).

Intra-population genetic variation was estimated by calculating the number of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_E) for each locus using the EXCEL MICROSATELLITE TOOLKIT 3.1 (Park 2001). Allelic richness was determined using FSTAT 2.9.3.2 (Goudet 1995). Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were tested using GENEPOP 3.4 (Raymond and Rousset 1995). Significance values for multiple, simultaneous comparisons on the same data were adjusted by Bonferroni correction (Rice 1989).

Genetic differentiation was tested with the fixation indices F_{ST} (Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) using the program ARLEQUIN 3.11 (Excoffier *et al.* 2005) (significance assessed by 10000 permutations). A Bayesian analysis to test for population structure was performed using STRUCTURE 2.2 (Pritchard *et al.* 2000) (burn-in 100000 iterations then runs of 10^6, K inferred from five independent runs of K = 1-3). Analyses were performed with the admixture model of ancestry and correlated allele frequency model, and without prior sampling location information. An exclusion test (Cornuet *et al.* 1999) was performed in GENECLASS 2.0 (Piry *et al.* 2004). This tests whether an individual can be excluded from each sampled population, and unlike STRUCTURE does not assume all source populations have been sampled. The Bayesian criteria of Rannala and Mountain (1997) and the Monte-Carlo resampling method of Paetkau *et al.* (2004), with 10000 simulated multilocus genotypes and the 'leave one out' procedure (Efron 1983), was used.

Mitochondrial DNA Data Analysis

Intra-population genetic variation was determined by calculating haplotype diversity (*h*) (Nei 1987) and nucleotide diversity (π) (Tajima 1983), and genetic differentiation using the fixation indices F_{ST} (Weir and Cockerham 1984) and Φ_{ST} (Excoffier *et al.* 1992) (significance assessed by 1000 permuations). The above tests were carried out with ARLEQUIN using a Kimura 2-parameter (Kimura 1980) genetic distance model and a gamma distribution of 0.5 where relevant. Genealogical relationships were determined by assembling a haplotype network using a parsimony method (Templeton *et al.* 1992) implemented in the program TCS 1.21 (Clement *et al.* 2000) with a 95% confidence level.

Results

Seven duplicate samples were identified in our sample of 87 blue whales. Five of the duplicate samples were recaptures within the same season and there were no resightings between feeding aggregations. No evidence for null alleles, stutter bands or short allele dominance was found in the microsatellite data set. No significant deviations from Hardy-Weinberg equilibrium or evidence of linkage disequilibrium were detected at any locus and putative population. Due to the array of tissue qualities, data were not able to be obtained for 29 samples at any microsatellite loci and 11 samples for the mtDNA control region.

Genetic Variation

Microsatellite variation was greater for Antarctica when compared with the Bonney Upwelling and the Perth Canyon. The mtDNA 394 bp fragment had 26 variable sites defining 26 unique haplotypes among 105 blue whales. Haplotypic and nucleotide diversity was relatively high in Antarctica compared to the Australian feeding aggregations (Table 1a).

Genetic Differentiation

Microsatellite based F_{ST} results showed no genetic differentiation between the Australian feeding aggregations but highly significant ($P < 0.001$) differentiation between each Australian feeding aggregation and Antarctica. R_{ST} revealed no significant differentiation between Australian feeding aggregations, significant ($P < 0.05$) differentiation between the Bonney Upwelling and Antarctica, and marginally non-significant ($P = 0.059$) differentiation between the Perth Canyon and Antarctica (Table 1b).

STRUCTURE estimated two genetic clusters (Fig. 2a); one encompassing Antarctica, and a second cluster encompassing the two Australian feeding aggregations. An analysis excluding the Antarctic samples did not provide evidence for subdivision in Australia. Therefore, for the GENECLASS exclusion test, Australian feeding aggregations were combined into one population. GENECLASS excluded ($P < 0.05$) all except one Antarctic individual (#13186) from the Australian population. In the Australian population, 14 out of the 55 individuals were excluded from the Antarctic population. No individuals were excluded from their sample population.

Mitochondrial fixation indices showed no significant differentiation between the two Australian feeding aggregations, but highly significant ($P < 0.001$) differentiation between each of the two Australian feeding aggregations and Antarctica (Table 1b). The haplotype network revealed that all the Bonney Upwelling haplotypes were also found in the Perth Canyon, however there were no haplotypes shared between Australian feeding aggregations and Antarctica (Fig. 2b).

Discussion

Blue whales in the Southern Hemisphere have shown surprising resilience at a genetic level to severe whaling practices in the 20th century, as evidenced by the relatively high levels of variability revealed in this study. We detected high gene flow between the two Australian feeding aggregations, to the extent that they may be classified as one genetic population. This means it is more likely that Australian blue whales are able to maintain their genetic diversity over time than if individuals from the feeding aggregations were from two separate populations. Our analysis emphasised the high gene flow between the two Australian feeding aggregations particularly when compared to the negligible and low gene flow between the two recognised Southern Hemisphere subspecies at mtDNA and microsatellite loci, respectively.

The maintenance of genetic variation despite severe whaling has also been observed in other baleen whales. For instance, whaling of humpback whales may have reduced their abundance to less than 10% of original numbers (Clapham et al. 1999), however they have maintained regional levels of genetic variation at mtDNA (Baker et al. 1993) and microsatellite (Valsecchi et al. 1997) loci similar to those found in this study of blue whales. Gray whales in the eastern North Pacific have mtDNA diversity levels comparable to those of the Antarctic blue whales (Steeves et al. 2001; LeDuc et al. 2002) despite whaling reducing their historical abundance to a few hundred individuals (Clapham et al. 1999). Western North Pacific sei whales also showed levels of genetic variation at microsatellite loci comparable to the Australian blue whales and no evidence of a genetic bottleneck (Kanda et al. 2006). LeDuc et al. (2007) previously revealed the high genetic variation of Antarctic blue whales compared to other Southern Hemisphere populations, despite that most whaling effort was applied to the Antarctic subspecies. This has also been observed in humpback

whales at microsatellite loci when comparing Antarctica and other worldwide populations (Valsecchi et al. 1997). These findings may be due to large historic population sizes or metapopulation structure.

The low sociality of blue whales and other baleen whales (Hoelzel 1994) may be a factor involved in the maintenance of mtDNA diversity compared to matrilineal whales and dolphins. Matrilineal taxa may have mtDNA selected based on cultural traits that are passed on to offspring in the natal group (Milius 1998; Whitehead 1998). Baker et al. (1993) suggests reasons for maintenance of mtDNA diversity in humpback whales; a long life-span, overlapping generations, and protection of the species. However, Nabholz et al. (2008) found that life history traits in mammals have little, if any, effect on mtDNA diversity. Instead, taxonomy accounts for a large amount of variation in diversity between species, potentially due to differences in mutation rates between taxonomic groups.

Based on previous circumstantial evidence regarding the structure of Australian blue whales (McCauley et al. 2000; Branch et al. 2007; C. Jenner, M. Morrice and P. Gill, unpublished data), it was perhaps likely that the two feeding aggregations composed a single genetic population. However, despite the relatively close proximity of the two Australian feeding aggregations and high mobility of many marine mammal species, other studies have shown remarkable differentiation between geographically close populations. Examples include southern right whales in Australia and New Zealand (Baker et al. 1999; Patenaude et al. 2007), beluga whales at different summer grounds in Alaska (O'Corry-Crowe et al. 1997) and long-finned pilot whales in four geographic areas of the North Atlantic (Fullard et al. 2000).

Humpback whales, a baleen whale extensively studied in Australia, show segregation in breeding and feeding grounds that was not observed in this study of blue whales. Humpback whales feed during warmer months in IWC Management Area IV and Area V as defined by Donovan (1991), then travel along migratory corridors in western Australia and eastern Australia respectively, to breed as far north as in the Kimberley in Western Australia and New Caledonia when migrating along the Australian east coast (Bannister 2005). Genetic studies have shown differentiation between western and eastern Australia (Baker et al. 1994; Baker et al. 1998), and Area IV and Area V (Loo et al. 2007). However, it is unknown whether humpback whales in South Australia migrate from Area IV or Area V (Kemper 2005).

Blue whales have also been observed by Burton et al. (2007) at Geographe Bay in Western Australia between September and January. This differs from the Bonney Upwelling and the Perth Canyon where blue whales are observed from November to May (Butler et al. 2002; Curt et al. 2002; Gill and Morrice 2003; McCauley et al. 2004). The role of Geographe Bay to the blue whales in uncertain, however Burton et al. (2007) has observed a blue whale excrete fecal matter in the bay, which is indicative of feeding, and blue whales travelling south and then west along the coast's contour.

A low recapture rate was observed between the Bonney Upwelling and the Perth Canyon using photo-ID (C. Jenner, M. Morrice and P. Gill, unpublished data) and, in the current study, there were no genetic recaptures between feeding aggregations. Burton et al. (2007) has also utilised photo-ID when studying blue whales at Geographe Bay in Western Australia. Even though Geographe Bay is approximately 200 km south of the Perth Canyon, only four resightings between the Perth Canyon

and Geographe Bay and three within Geographe Bay have been made from 473 animals photo-identified. No resightings were made within the same season. Burton et al. (2007) explained two potential reasons for a low resighting rate; a large population, or a low proportion of individuals travelling to both areas. The difference in when blue whales are observed in Perth Canyon and Geographe Bay must also be considered. The same two possible reasons apply to the low resights found between the two known Australian feeding aggregations. However, in this study it may also be accountable to the comparatively low level of current individual identification effort in the Bonney Upwelling.

Further research is required to elucidate the genetic identity of animals observed in Geographe Bay and to determine the importance of the bay to blue whales. Genetic and tagging studies also should be carried out to determine whether the Australian blue whales migrate to Indonesia, the Solomon Islands or elsewhere for breeding, and whether there is genetic continuity between blue whales found off Australia, Madagascar and New Zealand. Defining genetic stocks will be extremely important to future management and conservation efforts of blue whales.

Acknowledgements

This study was funded by the Australian Centre for Applied Marine Mammal Science (ACAMMS). Thank you to the Centre for Whale Research Western Australia, Australocetus Research, the Institute of Cetacean Research and Southwest Fisheries Science Center for providing blue whale samples or genetic data. Sampling at the Bonney Upwelling was conducted under Deakin University Animal Welfare Committee approval (#A19/2006) and a permit supplied by the Department of Environment, Water, Heritage and the Arts (EPBC permit #E2003-48230). Tissue and DNA samples were imported under permit from the Australian Quarantine and Inspection Service (AQUIS) and Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (#A60).

References

Amos W, Hoelzel AR (1991): Long-term preservation of whale skin for DNA analysis. Rep Int Whal Comm (Special Issue) 13:99-104

Baker CS, Florez-Gonazalez L, Abernethy B, Rosenbaum HC, Slade RW, Capella J, Bannister JL (1998): Mitochondrial DNA variation and maternal gene flow among humpback whales of the Southern Hemisphere. Mar Mamm Sci 14:721-737

Baker CS, Patenaude NJ, Bannister JL, Robins J, Kato H (1999): Distribution and diversity of mtDNA lineages among southern right whales (*Eubalaena australis*) from Australia and New Zealand. Mar Biol 134:1-7

Baker CS, Perry A, Bannister JL, Weinrich MT, Abernethy RB, Calambokidis J, Lien J, Lambersten RH, Urban Ramirez J, Vasquez O, Clapham PJ, Alling A, O'Brien SJ, Palumbi SR (1993): Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. Proc R Soc Lond, Ser B: Biol Sci 90:8239-8243

Baker CS, Slade RW, Bannister JL, Abernethy RB, Weinrich MT, Lien J, Urban J, Corkeron P, Calmabokidis J, Vasquez O, Palumbi SR (1994): Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, worldwide. Mol Ecol 3:313-327

Bannister JL (2005): Intercessional working group on Southern Hemisphere humpback whales: Revised table by breeding stocks (as at 1 May 2005). Document SC/57/SH11 presented to the IWC Scientific Committee, Ulsan, South Korea, May 2005 (unpublished)

Berube M, Jorgensen H, Mcewing R, Palsboll PJ (2000): Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. Mol Ecol 9:2155-2234

Bilgmann K, Griffiths OJ, Allen SJ, Möller LM (2007): A biopsy pole system for bow-riding dolphins: Sampling success, behavioural responses, and test for sampling bias. Mar Mamm Sci 23:218-225

Branch TA, Stafford KM, Palacios DM, Allison C, Bannister JL, Burton CLK, Cabrera E, Carlson CA, Galletti Vernazzani B, Gill PC, Hucke-Gaete R, Jenner KCS, Jenner M-NM, Matsuoka K, Mikhalev YA, Miyashita T, Morrice MG, Nishiwaki S, Sturrock VJ, Tormosov D, Anderson RC, Baker AN, Best PB, Borsa P, Brownell RLJ, Childerhouse S, Findlay KP, Gerrodette T, Ilangakoon AD, Joergensen M, Kahn B, Ljungblad DK, Maughan B, McCauley RD, McKay S, Norris KS, Group OWaDR, Rankin S, Samaran F, Thiele D, Van Waerebeek K, Warneke RM (2007): Past and present distribution, densities and movements of blue whales *Balaenoptera musculus* in the Southern Hemisphere and northern Indian Ocean. Mamm Rev 37:116-175

Burton CLK, Salgado-Kent CP, Glencross RG, Sturrock VJ, Jenner C, Jenner M-N (2007): Distribution and relative abundance of blue whales (*Balaenoptera musculus*) in Geographe Bay, Western Australia. Poster presented at the 17th Biennial Conference on the Biology of Marine Mammals, Cape Town, South Africa, 29 Nov - 3 Dec 2007

Butler A, Althaus F, Furlani D, Ridgway K (2002): Assessment of the conservation values of the Bonney Upwelling area: A component of the Commonwealth Marine Conservation Assessment Program 2002-2004: Report to Environment Australia. CSIRO Marine Research, Hobart, Australia

Clapham PJ, Young SB, Brownell RLJ (1999): Baleen whales: Conservation issues and the status of the most endangered populations. Mamm Rev 29:35-60

Clement M, Posada D, Crandall KA (2000): TCS: A computer program to estimate gene genealogies. Mol Ecol 9:1657-1659

Conway CA (2005): Global population structure of blue whales, *Balaenoptera musculus* spp., based on nuclear genetic variation. Dissertation, University of California

Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000

Curt K, Jenner S, Wilson SG, Hunt YM, Jenner M-N (2002): Evidence of blue whale feeding in the Perth Canyon, Western Australia (unpublished).

Department of the Environment and Heritage (2005): Blue, fin and sei whale recovery plan 2005-2010. Department of the Environment and Heritage, Canberra, Australia

Donovan GP (1991) A review of IWC stock boundaries. Rep Int Whale Comm. (Special Issue) 13:39-68

Efron B (1983) Estimating the error rate of a prediction rule: Improvement on cross-validation. J Am Stat Assoc 78:316-331

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetic analysis. Evol Bioinform Online 1:47-50

Excoffier L, Smouse PE, Quattro JM (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491

Fullard KJ, Early G, Heide-Jorgensen MP, Bloch D, Rosing-Asvid A, Amos W (2000) Population structure of long-finned pilot whales in the North Atlantic: A correlation with sea surface temperature? Mol Ecol 9:949-958

Gill PC (2002): A blue whale (*Balaenoptera musculus*) feeding ground in a southern Australian coastal upwelling zone. J Cetacean Res Manage 4:179-184

Gill PC, Morrice MG (2003): Blue whale research in the Bonney Upwelling, south-east Australia - current information. Deakin University, School of Ecology and Environment, Technical Paper 2003/1

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. J Hered 86:485-486

Hoelzel AR (1994): Genetics and ecology of whales and dolphins. Annu Rev Ecol Syst 25:377-399

Ichihara T (1966): The pygmy blue whale, *Balaenoptera musculus brevicauda*, a new subspecies from the Antarctic. In: Norris KS (ed) Whales, Dolphins and Porpoises. University of California Press, Berkeley

Kanda N, Goto M, Pastene LA (2006): Genetic characteristics of western North Pacific sei whales, *Balaenoptera borealis*, as revealed by microsatellites. Mar Biotechnol 8:86-93

Kellogg R (1929): What is known of the migration of some of the whalebone whales. Smithsonian Institution Annual Reports for 1928, pp. 467-494

Kemper CM (2005): Records of humpback whales *Megaptera novaeangliae* in South Australia. Trans R Soc S Aust 129:53-58

Kimura M (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111-120

LeDuc RG, Dizon AE, Goto M, Pastene LA, Kato H, Nishiwaki S, Leduc CA, Brownell RLJ (2007): Patterns of genetic variation in the southern hemisphere blue whales and the use of assignment test to detect mixing on the feeding grounds. J Cetacean Res Manage 9:73-80

LeDuc RG, Weller DW, Hyde J, Burdin AM, Rosel PE, Brownell RLJ, Wursig B, Dizon AE (2002): Genetic differences between western and eastern gray whales (*Eschrichtius robustus*). J Cetacean Res Manage 4:1-5

Loo J, Pomilla C, Olavarria C, Aguayo A, Thiele D, Bakere S, Ensor P, Rosenbaum H (2007): Genetic structure of feeding aggregations of humpback whales in the Southern Ocean. Paper presented at the 17th Biennial Conference on the Biology of Marine Mammals, Cape Town, South Africa, 29 Nov - 3 Dec 2007

McCauley RD, Bannister JL, Burton C, Jenner C, Rennie S, Kent CS (2004): Western Australian exercise area. Blue whale project. Final summary report. Milestone 6. Centre for Marine Science and Technology Report R2004-29, Project - 350

McCauley RD, Jenner C, Bannister JL, Cato DH, Duncan A (2000): Blue whale calling in the Rottnest trench, Western Australia, and low frequency sea noise. Paper presented at the Australian Acoustical Society Conference, Joondalup, Australia, 15-17 November 2000

McDonald MA, Mesnick SL, Hildebrand JA (2006): Biogeographic characterisation of blue whale song worldwide: Using song to identify populations. J Cetacean Res Manage 8:55-65

Middleton JF, Bye JT (2007): A review of the shelf-slope circulation along Australia's southern shelves: Cape Leeuwin to Portland. Prog Oceanogr 75

Mikhalev YA (2000): Whaling in the Arabian Sea by the whaling fleets *Slava* and *Sovetskaya Ukraina*. In: Yablokov AV, Zemsky VA (eds) Soviet whaling data. Center for Russian Environmental Policy, Moscow

Milius S (1998): Cultural life of whales may cut diversity. Science News 154:342

Möller LM, Beheregaray LB (2001) Coastal bottlenose dolphins from southeastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. Mar Mamm Sci 17:249-263

Nabholz B, Mauffrey J-F, Bazin E, Galtier N, Glemin S (2008): Determination of mitochondrial genetic diversity in mammals. Genetics 178:351-361

Nei M (1987): Molecular Evolutionary Genetics. Columbia University Press, New York.

O'Corry-Crowe GM, Suydam RS, Rosenberg A, Frost KJ, Dizon AE (1997): Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. Mol Ecol 6:955-970

Ohsumi S, Shigemune H (1993): A sightings survey of larger whales in lower latitudinal waters of the Pacific in austral winter, with special reference to the blue whale. Paper Blue/WP6 presented to the *ad hoc* Steering Group Intersessional Meeting on Research related to Conservation of Large Baleen Whales - Blue Whales - in the Southern Ocean, Tokyo, October (unpublished)

Paetkau D, Slade RW, Burden M, Estoup A (2004): Genetic assignment methods for the direct, real-time estimation of migration rate: A simulation-based exploration of accuracy and power. Mol Ecol 13

Palsboll PJ, Berube M, Larson AH, Jorgensen H (1997): Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. Mol Ecol 6:893-895

Park SDE (2001): Trypanotolerance in west African cattle and the population genetic effects of selection. Dissertation, University of Dublin

Patenaude NJ, Portway VA, Schaeff CM, Bannister JL, Best PB, Payne RS, Rowntree VJ, Rivarola M, Baker CS (2007): Mitochondrial DNA diversity and population structure among southern right whales (*Eubalaena australis)*. J Hered 98:147-157

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004): GENECLASS2: A software for genetic assignment and first-generation migrant detection. J Hered 95:536-539

Pritchard JK, Stephens M, Donnelly P (2000): Inference of population structure using multilocus genotype data. Genetics 155:945-959

Rannala B, Mountain JL (1997): Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94:9197-9201

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. J Hered 86:248-249

Rennie SJ, McCauley RD, Pattiaratchi CB (2006): Thermal structure above the Perth Canyon reveals Leeuwin current, undercurrent and weather influences and the potential for upwelling. Marine and Freshwater Research 57:849-861

Rice DW (1998): Marine Mammals of the World: Systematics and Distribution. Special Publication No. 4. The Society for Marine Mammalogy, Lawrence, Kansas

Rice WR (1989): Analyzing tables of statistical tests. Evolution 43:223-225

Schuelke M (2000): An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233-234

Slatkin M (1995): A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462

Steeves TE, Darling JD, Rosel PE, Schaeff CM, Fleischer RC (2001): Preliminary analysis of mitochondrial DNA variation in a southern feeding group of eastern North Pacific gray whales. Conserv Genet 2:379-384

Sunnucks P, Hales DF (1996): Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol Biol Evol 13:510-524

Tajima F (1983): Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437-460

Templeton AR, Crandall KA, Sing CF (1992): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619-633

Valsecchi E, Palsboll P, Hale P, Glocknere-Ferrari D, Ferrari M, Clapham P, Larsen F, Mattila D, Sears R, Sigurjonsson J, Brown M, Corkeron P, Amos B (1997): Microsatellite genetic distances between oceanic populations of the humpback whale (*Megaptera novaeangliae*). Mol Biol Evol 14:355-362

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004): MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535-538

Weir BS, Cockerham CC (1984): Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370

Whitehead H (1998): Cultural selection and genetic diversity in matrilineal whales. Science 282:1708-1711

Figure Legends and Table Captions

Figure #3 Sample locations of blue whales showing the (a) Southern Hemisphere, including IWC management areas, and (b) a close-up of Australia. Sample locations include potential duplicate samples. Due to unknown GPS location, six samples from the Bonney Upwellng and three samples from the Perth Canyon are not shown.

Figure #4 (a) Clustering results of STRUCTURE analysis with no prior population information estimated two genetic clusters (K = 2). The length occupied by each colour in a column is proportional to that individual's membership probability to the associated cluster. (b) Minimum spanning network of 26 unique mtDNA control region haplotypes. The size of each circle reflects the frequency that the haplotype was observed. The proportion coloured within each circle reflects the frequency that the haplotype was observed in the associated putative population. Each line represents one unique mutational event. Each black oval represents haplotypes that were not sampled or are extinct.

Table #1 (a) Summary of genetic variability in blue whales based on six microsatellite loci and mtDNA control region sequences and (b) pairwise comparisons between three blue whale putative populations in the Southern Hemisphere based on six microsatellite loci. Microsatellites and mtDNA fixation indices are below and above the diagonals respectively. R_{ST} and Φ_{ST} estimates are presented in the upper matrix and F_{ST} in the lower matrix. Significant results are marked with an asterisk.

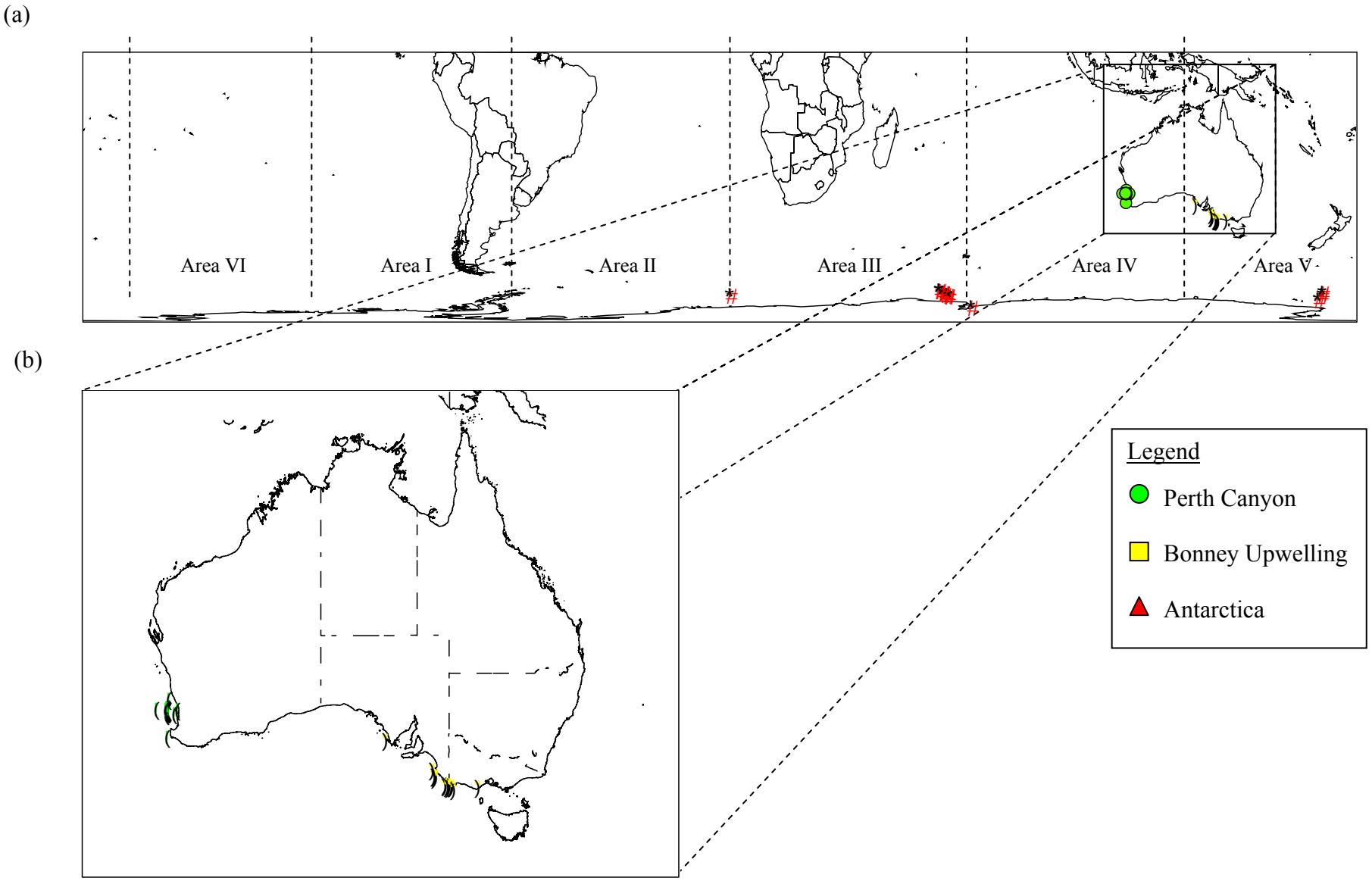
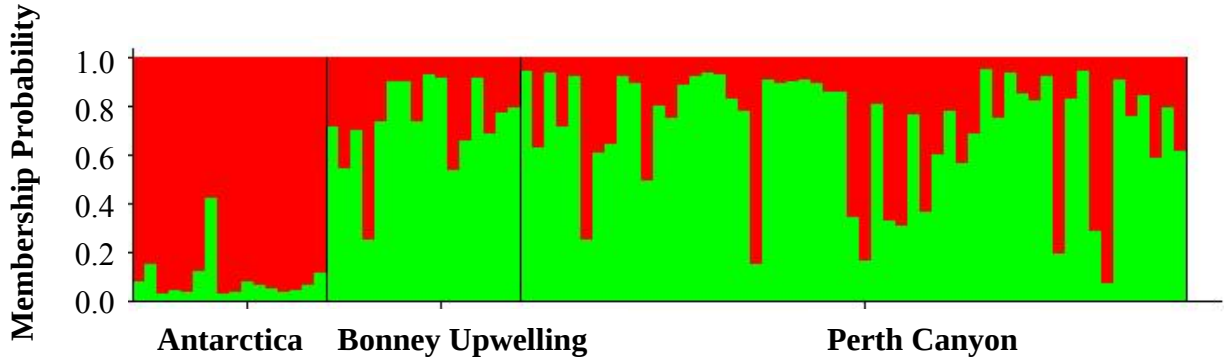

(a)
Area VI
Area I
Area II
Area III
Area IV
Area V
(b)
Legend
Perth Canyon
Bonney Upwelling
Antarctica

Figure #3

(a)


Membership Probability
1.0
0.8
0.6
0.4
0.2
0.0
Antarctica
Bonney Upwelling
Perth Canyon

(b)

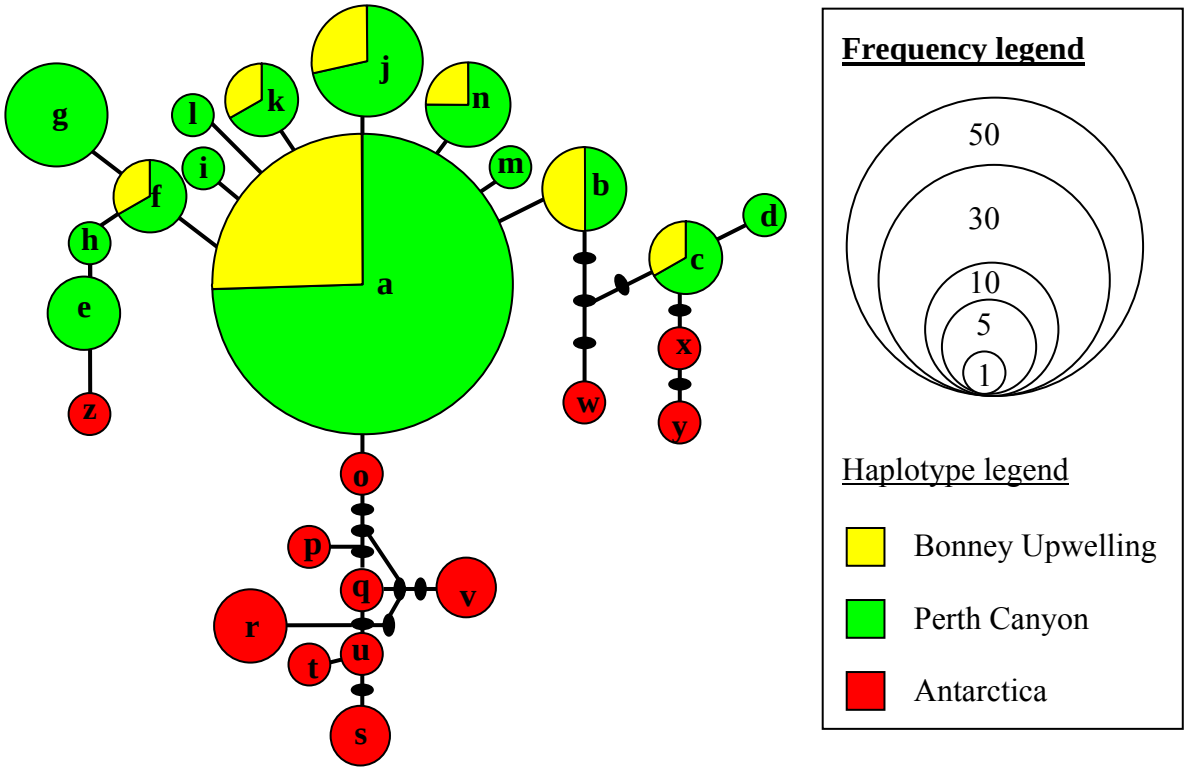

Frequency legend
50
30
10
5
1
Haplotype legend
Bonney Upwelling
Perth Canyon
Antarctica
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x
y
z

Figure #4

Table #1

(a)

	Microsatellites					mtDNA control region			
	ns	NA	H_O	H_E	AR	*ns*	NH	*h*	π
Antarctica	16	6.83 (2.56)	0.723 (0.046)	0.750 (0.073)	6.656	16	12	0.958 (0.036)	0.014 (0.008)
Bonney Upwelling	16	5.17 (2.48)	0.632 (0.050)	0.640 (0.064)	4.958	21	7	0.619 (0.118)	0.002 (0.002)
Perth Canyon	55	6.17 (3.49)	0.661 (0.026)	0.672 (0.052)	4.891	68	14	0.676 (0.062)	0.003 (0.002)

ns = number of samples; NA = mean number of alleles; Ho = mean observed heterozygosity; HE = mean expected heterozygosity; AR = mean allelic richness; NH = number of haplotypes; *h* = haplotypic diversity; p = nucleotide diversity. Standard deviation shown in parentheses

(b)

	Antarctica	Bonney Upwelling	Perth Canyon
Antarctica		0.051* / 0.400***	0.040 / 0.511***
Bonney Upwelling	0.101*** / 0.219***		-0.018 / -0.011
Perth Canyon	0.094*** / 0.208***	-0.004 / -0.016	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$