

Population structure and possible migratory links of common minke whales, *Balaenoptera acutorostrata*, in the Southern Hemisphere

Luis A. Pastene · Jorge Acevedo · Mutsuo Goto ·
Alexandre N. Zerbini · Paola Acuña · Anelio Aguayo-Lobo

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Abstract Mitochondrial DNA control region sequences were analyzed to investigate population structure and possible migratory links of common minke whales (*Balaenoptera acutorostrata*) in two ocean basins: western South Atlantic (WSA) and western South Pacific (WSP). The results of several different phylogenetic estimations consistently grouped all haplotypes but one ($n = 1$) from these two ocean basins into two separate clades. South and North Atlantic haplotypes were more closely related to each other than either was to haplotypes from the WSP. The interpopulation genetic distance between WSA and WSP whales was similar to that reported between North Pacific and North Atlantic common minke whales (0.0234). The migration rate between the two ocean basins was estimated at near-zero using MDIV. The genetic evidence

presented here was consistent with the hypothesis of migratory links among Brazil, Chilean Patagonia and the Antarctic Peninsula, and between low-latitude and Antarctic waters of the WSP. The results suggest multiple populations of common minke whales in the Southern Hemisphere, which may have conservation as well as taxonomic implications. Our single locus results should be corroborated by additional analyses in a larger number of samples and at more genetic markers.

Keywords *Balaenoptera acutorostrata* · Southern Hemisphere · mtDNA · Genetic diversity · Phylogeny

Introduction

Rice (1998) reviewed both morphological (e.g., Omura 1975; Best 1985; Arnold et al. 1987) and genetic (e.g., Wada et al. 1991; Arnason et al. 1993; Pastene et al. 1994) data collected from extant minke whale populations and recognized two species; the larger Antarctic minke whale (*Balaenoptera bonaerensis*), which is restricted to the Southern Hemisphere, and the cosmopolitan common minke whale (*B. acutorostrata*). Further, he recognized three sub-species of the common minke whale: North Atlantic *B. a. acutorostrata*, North Pacific *B. a. scammoni* and Southern Hemisphere *B. a. subsp.* The common minke whale in the Southern Hemisphere is commonly referred to as the “diminutive” or “dwarf” minke whale (Arnold et al. 1987; Best 1985) and has been considered an undescribed subspecies (Rice 1998). In the Southern Hemisphere common minke whales have been reported for western South Atlantic (WSA) waters off Brazil (DaRocha and Braga 1982; Zerbini et al. 1996; 1997) and Chilean

L. A. Pastene (✉) · M. Goto
Institute of Cetacean Research, Toyomi-cho 4-5, Chuo-ku,
Tokyo 104-0055, Japan
e-mail: pastene@cetacean.jp

J. Acevedo · P. Acuña · A. Aguayo-Lobo
Centro de Estudios del Cuaternario Fuego-Patagonia y
Antarctica (CEQUA), Avenida Bulnes 01890, Punta Arenas,
Chile

A. N. Zerbini
National Marine Mammal Laboratory, Alaska Fisheries Science
Center, 7600 Sand Point Way N.E. F/AKC3, Seattle,
WA 98115-6349, USA

A. N. Zerbini
Instituto Aqualie, Projeto Monitoramento de Baleias por Satelite,
R. Edgard Werneck 428/32, Rio de Janeiro, RJ 22763-010,
Brazil

A. Aguayo-Lobo
Instituto Antártico Chileno, Plaza Muñoz Gamero
1055, Punta Arenas, Chile

Patagonia (Acevedo et al. 2006), western South Pacific (WSP) waters off New Zealand (Baker 1983) and central and northern Great Barrier Reef in Australia (Arnold et al. 1987; Arnold 1997), and western Indian Ocean waters off Durban in South Africa (Best 1985).

Little is known about the population genetic structure and migratory links for the common minke whale in the Southern Hemisphere. In the WSA, Zerbini et al. (1996, 1997) noted that common minke whales are observed in Brazil throughout the year but that most individuals are recorded in Brazil from June to August and in Uruguay and northern Argentina in April and May (Baldas and Castello 1986). These observations suggest that common minke whales present some degree of seasonal north/south movement. Acevedo et al. (2006) suggested the possibility that the population of common minke whale off Brazil may distribute much farther south in April, some into the Chilean Patagonia Channels. They postulated that common minke whales from Brazil and their reported animals from Patagonia belong to the same population. In addition these common minke whales might be related to whales observed in summer around the Antarctic Peninsula (Acevedo et al. 2007).

In the WSP, Arnold (1997) examined a total of 181 sighting of this species from the central and northern Great Barrier Reef, Australia, observed mainly in the austral winter months from May to September with 79.5% of the sightings in June and July. These common minke whales might be related to whales summering in Antarctic waters south of New Zealand and Australia, where they have been reported in the latitudinal range between 55° and 62°S (although one individual was caught at 65°S; Kato et al.

1989, 1990; Fujise et al. 1993; Kasamatsu et al. 1993). These observations are consistent with north–south seasonal movement.

In this study, published and newly obtained mitochondrial DNA (mtDNA) control region sequences were analyzed to investigate the population structure and possible migratory links of common minke whales in the Southern Hemisphere. Specifically the analyses were focused to investigate whether or not whales from the WSA and WSP are structured genetically.

Materials and methods

In the WSA the genetic survey involved low-latitude waters off Brazil ($n = 8$; mtDNA control region sequences published in Pastene et al. 2007) and mid-latitude waters off Chilean Patagonia ($n = 3$) (new collected samples) and the Antarctic Peninsula (AP) ($n = 1$) (new collected sample; Fig. 1). In Brazil, samples were from animals stranded mainly in winter months; in Patagonia, samples were from animals stranded on 12–19 April 2004 (Acevedo et al. 2006). The AP sample was obtained from a biopsy collected at 62°28'S, 59°43'W on 16 January 2007 during the 43rd Chilean Antarctic Expedition.

In the WSP, the genetic survey involved low-latitude waters off New Zealand (sequence available in GenBank with access number DQ145048) and Tonga (sequence available in GenBank with access number EU285375). In the Antarctic, samples were from 15 animals caught in summer months by the JARPA (Japanese Whale Research Program under Special Permit in the Antarctic) in the

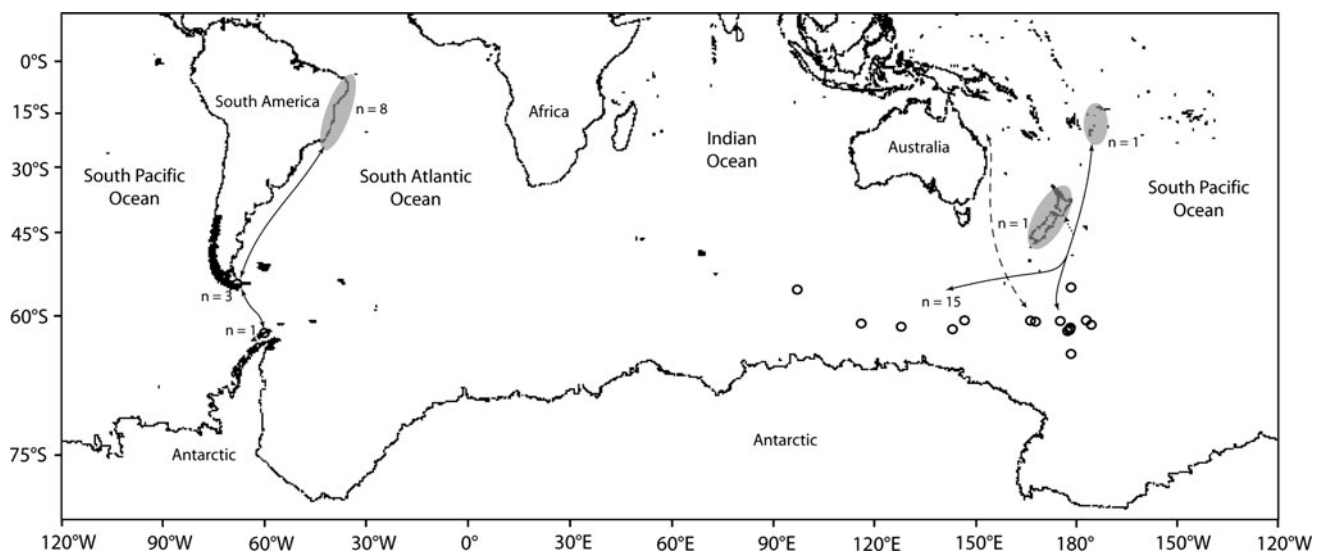


Fig. 1 Geographical position of Southern Hemisphere common minke whale samples used in the present genetic study. *Solid lines and arrows* indicate possible migration routes based on the location of

the samples used in this study. *Dashed line* indicates a possible connection between whales recorded in the Great Barrier Reef (Arnold 1997) and Antarctic

longitudinal range 90°E–170°W (Fig. 1; sequences published in Pastene et al. 2007).

For the newly collected tissue samples (three from Chilean Patagonia and one from Antarctic Peninsula) laboratory work on DNA extraction and sequencing was as described in Pastene et al. (2007). The genealogy of the mtDNA haplotypes was estimated using the Neighbor-Joining method (Saitou and Nei 1987) as implemented in the program PHYLIP (Felsenstein 1993). Genetic distances among haplotypes were estimated using the program DNADIST of PHYLIP, based on Kimura's 2-parameter model (Kimura 1980). A transition-transversion ratio of 5:1 was used. For comparative purposes, mtDNA control region sequences of common and Antarctic minke whales worldwide published by Pastene et al. (2007) were included in the estimation. The genealogy was rooted using the homologous sequence from nine baleen whale species (Arnason et al. 1993). To estimate support for each node a total of 1,000 bootstrap simulations were conducted and the majority-rule consensus genealogy estimated.

To further evaluate their population identity and differentiation, the mtDNA composition of WSA common minke whales was compared statistically to that of whales in the WSP. Within and between genetic diversities were estimated following Nei (1987), and a homogeneity test was conducted using Hudson et al. (1992)'s Hst and Kst* statistics. The probability of the observed values of Hst and Kst* was estimated from 10,000 permutations of the original data. To evaluate the relative effects of divergence and migration between WSA and WSP whales the approach of Nielsen and Wakeley (2001) modified for a finite mutation level (HKY; Palsbøll et al. 2004), was used. The approach was implemented in the computer program MDIV. In this approach, a coalescent model is established with three parameters: diversity θ , divergence time T and migration rate M . The maximum value of θ was set as the default; for T and M two different sets of the maximum value were used, 10 and 50. For each estimate, 10 million chains, with 500,000 for burn-in were run.

Results and discussion

The final data set included the first 327 nucleotides of the mtDNA control region. In the sample from WSA and WSP ($n = 29$) a total of 15 haplotypes was found (derived from 23 segregating sites). In the total sample of twelve animals from the WSA, five different haplotypes were detected. Haplotype "PAT2" was detected in four Brazilian samples and one Chilean Patagonian sample; haplotype "152" was detected in one Brazilian sample; haplotype "PAT3" was detected in three Brazilian samples and one Chilean

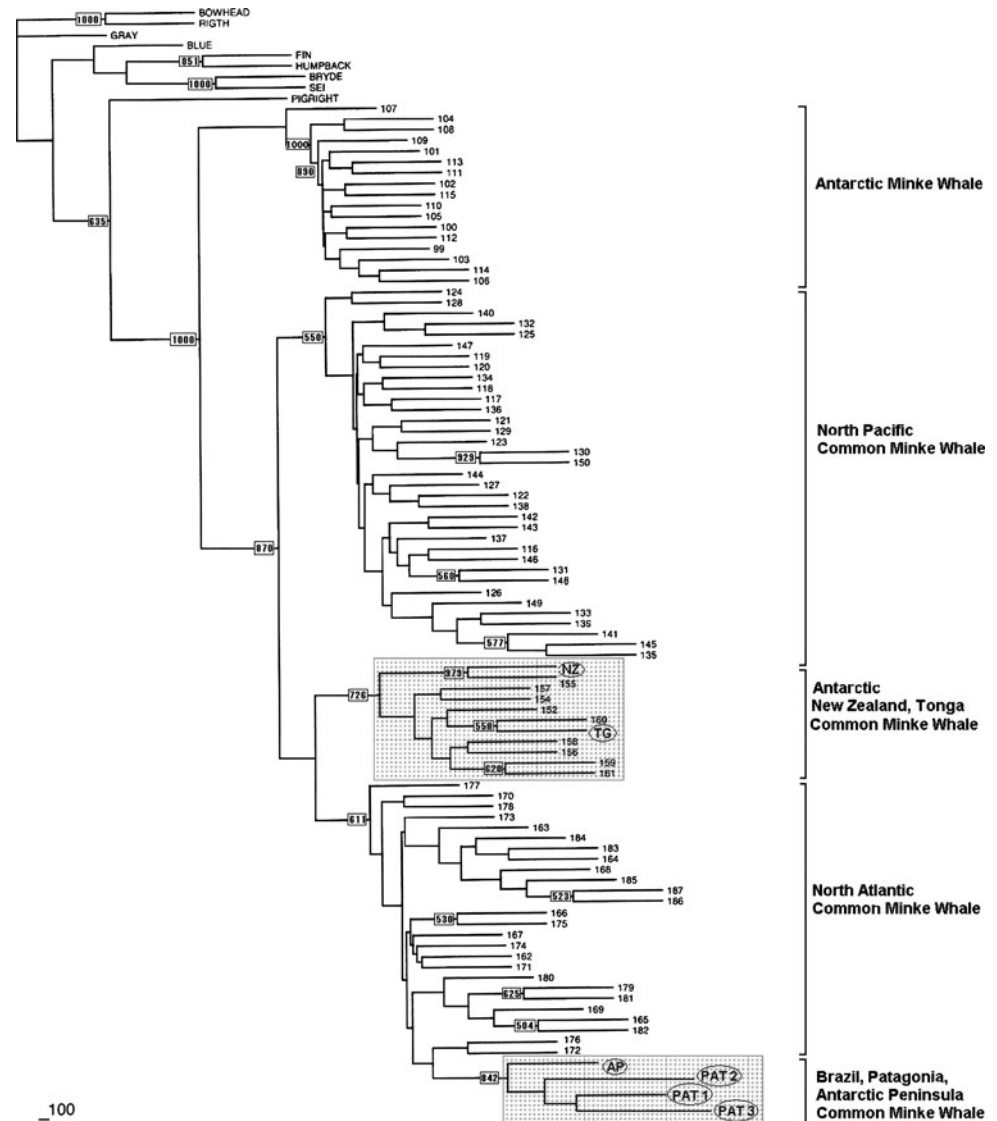
Patagonian sample; haplotype "PAT1" was detected in one Chilean Patagonian sample and finally haplotype "AP" was detected in the Antarctic Peninsula sample. The previously undetected haplotypes "PAT1" and "AP" have been deposited in GenBank under accession numbers FJ905903 and FJ905904, respectively. "PAT2" and "PAT3" corresponded to haplotypes "151" and "153", respectively, in Pastene et al. (2007), and are already present in GenBank.

In the total sample of 17 animals from the WSP, ten haplotypes were identified. Haplotypes "157" and "160" were detected in each three Antarctic samples, haplotypes "154", "155" and "156" were detected in each two Antarctic samples; and haplotypes "158", "159" and "161" were detected in each one Antarctic sample. The single sequence from New Zealand and that from Tonga corresponded to previously undetected haplotypes in the data set of Pastene et al. (2007), "NZ" and "TG", which corresponded to GeneBank accession numbers DQ145048 and EU285375, respectively.

Figure 2 shows the neighbor-joining-based genealogy estimated from the mtDNA haplotypes in this study as well as those haplotypes from Antarctic and common minke whales worldwide previously published by Pastene et al. (2007). Brazilian, Chilean Patagonian and AP (all WSA) haplotypes (except Brazilian Haplotype "152"), clustered in a single clade, supported by a bootstrap value of 84%. This clade is nested within the North Atlantic clade. New Zealand, Tonga and Antarctic (all from WSP) haplotypes clustered in a different clade supported by a bootstrap value of 73%. The Brazilian Haplotype "152" was part of the WSP clade. Haplotypes from the WSA whales share a more recent common ancestor with the North Atlantic minke whales than they do with the WSP minke whales. Phylogenetic inferences derived from minimum evolution (ME), maximum likelihood (ML) and maximum parsimony (MP) were consistent with the NJ tree in all these aspects (unpublished data).

Estimates of genetic diversity in the common minke whales from the WSA and WSP were similar (WSA, $n = 12$, $K = 5$, $\pi = 0.0098$ SE:0.0048; WSP, $n = 17$, $K = 10$, $\pi = 0.0084$ SE: 0.0015; total, $n = 29$, $K = 15$, $\pi = 0.0204$ SE: 0.0014). The overall diversity is higher than that observed in North Pacific Bryde's whales, *Balaenoptera brydei* (Kanda et al. 2007) and North Atlantic fin whales, *B. physalus* (Bérubé et al. 1998), and similar to that observed in humpback whales, *Megaptera novaeangliae* (Palsbøll et al. 1995). The net interpopulation distance between whales from Brazil and Chilean Patagonia was zero (point estimate was at -0.0007), while the distance between WSA and WSP populations was estimated at 0.0234. A homogeneity test detected a statistically significant degree of divergence

Fig. 2 Tree of minke whales mtDNA haplotypes based on the neighbor-joining method. The populations on which this study was focused (WSA and WSP) are shown in gray. Terminology for haplotypes in the Brazilian sample and that for haplotypes of the common minke whales from the North Pacific, North Atlantic, Southern Hemisphere and Antarctic minke whales, are the same as in Pastene et al. (2007). Sequences from the Patagonia and Antarctic Peninsula whales are indicated as “PAT” and “AP”, respectively (*in circle*). Sequences from New Zealand and Tonga are indicated as “NZ” and “TG”, respectively (*in circle*). “PAT1”, “AP”, “NZ” and “TG” correspond to new haplotypes. “PAT2” and “PAT3” correspond to haplotypes “151” and “153”, respectively, in Pastene et al. (2007). Clades supported by over 50% in 1,000 bootstrap simulations are also indicated



between these two populations: Hst: 0.0826 ($P = 0.0001$) and Kst*: 0.3729 ($P = 0.0001$).

The posterior probability distribution of θ , T and M are shown in Figs. 3, 4 and 5, respectively. The maximum likelihood was well defined in the estimation of θ with most probability mass surrounding $\theta = 3.0$ (Fig. 3). The posterior probability distribution of T reached a maximum at $T = 1$ (Fig. 4). The most relevant information for the present study was the posterior probability distribution of M , which point to a near-zero migration rate between the two populations (Fig. 5). No significant change of the posterior distributions was observed when the maximum values of M and T increased from 10 to 50.

As the M denotes the number of migrants by generation, the low value estimated in this study suggests that the occurrence of the Brazilian haplotype ‘152’ within the WSP clade (Fig. 2), is unlikely to be a result of migration, but rather due to incomplete lineage sorting.

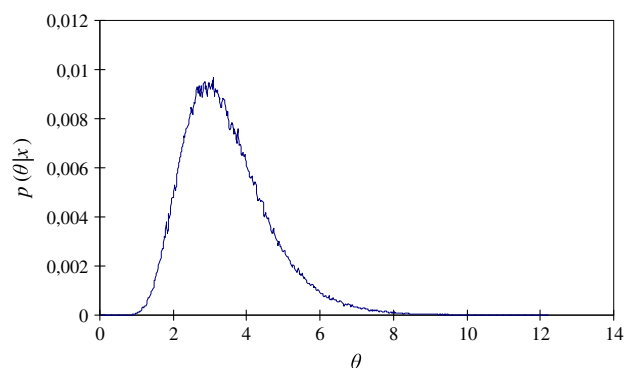


Fig. 3 The posterior probability distribution of diversity θ . The x axis denotes θ , the y axis the posterior probability

The results of the genetic analysis presented here suggest a high degree of maternal genetic differentiation between common minke whales within the Southern Hemisphere. Although the present genetic analysis used all

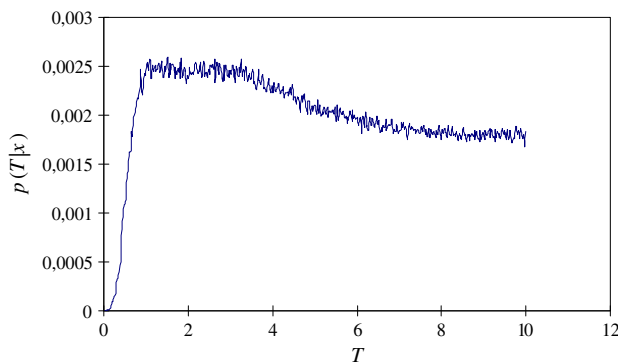


Fig. 4 The posterior probability distribution of divergence time T . The x axis denotes T , the y axis the posterior probability

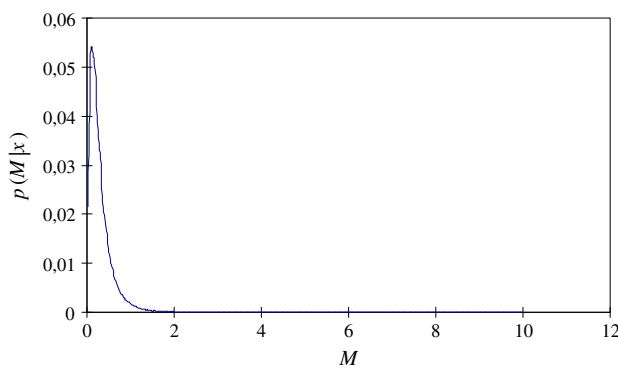


Fig. 5 The posterior probability distribution of migration rate M . The x axis denotes M , the y axis the posterior probability

known genetic samples of common minke whales in the Southern Hemisphere, the sample sizes are still small, and additional samples will be important to verifying these initial results. In particular adding samples from common minke whales from the western Indian Ocean (Best 1985), and Western Australia (J. Bannister, personal communication) is needed. Nonetheless, the mtDNA results presented in this study suggest a deep population division of this species in the Southern Hemisphere. Haplotypes from the WSA and WSP grouped clearly into two different clades (except haplotype “152”) and the degree of genetic divergence was similar than that estimated between common minke whales from the North Pacific and the North Atlantic (data not shown), and the migration rate, as estimated by MDIV, was at near zero.

Recently Acevedo et al. (2007) suggested a migratory connection between whales at low-latitude waters off Brazil and Chilean Patagonia and whales in high-latitude feeding grounds on the western side of the Antarctic Peninsula. Although phylogenetic analysis is inadequate to track individual movements or fine scale population structure and identity, the close phylogenetic relationship among whales from Brazil, Chilean Patagonia and AP (in comparison to other common minke whales in the Southern

Hemisphere) is consistent with this hypothesis. A similar situation could be the case for the WSP population given the close phylogenetic relationship between New Zealand and Tonga whales, with those whales sampled in a similar longitudinal range in the Antarctic (Fig. 1).

In the context of conservation, the results of the present study are highly relevant for the taxonomy of the common minke whale. Whales from WSA and WSP should be considered different Evolutionary Significant Units (ESUs) sensu Moritz (1994) as whales from these two populations are characterized by complete monophyly. Furthermore, the results of this mtDNA study showed that South and North Atlantic common minke whales haplotypes share a more recent common ancestor than either do with the common minke whales from the WSP. This observation has implications for the current putative sub-specific status of common minke whales (Rice 1998) which group Southern Hemisphere common minke whales in the same sub-species at the exclusion of North Atlantic minke whales. Our study suggests this is incorrect and the sub-species status need be further examined by analysis of additional samples, morphological characters and nuclear loci.

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