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Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata*

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Abstract

How do populations of highly mobile species inhabiting open environments become reproductively isolated and evolve into new species? We test the hypothesis that elevated ocean-surface temperatures can facilitate allopatry among pelagic populations and thus promote speciation. Oceanographic modelling has shown that increasing surface temperatures cause localization and reduction of upwelling, leading to fragmentation of feeding areas critical to pelagic species. We test our hypothesis by genetic analyses of populations of two closely related baleen whales, the Antarctic minke whale (*Balaenoptera bonaerensis*) and common minke whale (*Balaenoptera acutorostrata*) whose current distributions and migration patterns extent are largely determined by areas of consistent upwelling with high primary production. Phylogeographic and population genetic analyses of mitochondrial DNA control-region nucleotide sequences collected from 467 whales sampled in four different ocean basins were employed to infer the evolutionary relationship among populations of *B. acutorostrata* by rooting an intraspecific phylogeny with a population of *B. bonaerensis*. Our findings suggest that the two species diverged in the Southern Hemisphere less than 5 million years ago (Ma). This estimate places the speciation event during a period of extended global warming in the Pliocene. We propose that elevated ocean temperatures in the period facilitated allopatric speciation by disrupting the continuous belt of upwelling maintained by the Antarctic Circumpolar Current. Our analyses revealed that the current populations of *B. acutorostrata* likely diverged after the Pliocene some 1.5 Ma when global temperatures had decreased and presumably coinciding with the re-establishment of the polar–equatorial temperature gradient that ultimately drives upwelling. In most population samples, we detected genetic signatures of exponential population expansions, consistent with the notion of increasing carrying capacity after the Pliocene. Our hypothesis that prolonged periods of global warming facilitate speciation in pelagic marine species that depend on upwelling should be tested by comparative analyses in other pelagic species.

Keywords: *Cetacea*, evolution, expansion, global warming, phylogeography, speciation

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Introduction

Most of baleen whales undertake extensive semi-annual migrations (Martin *et al*. 1984; Stone *et al*. 1990; Palsbøll *et al*. 1997; Stevick *et al*. 1998; Pomilla & Rosenbaum 2005) and thus posses a tremendous potential for dispersal in an environment that is relatively unobstructed by geographical barriers. This observation begs the question: how do populations of such highly mobile pelagic species in such an open environment become reproductively isolated from each other and evolve into new species?

Our current knowledge regarding the processes that govern the speciation and radiation in baleen whales is very limited. According to Fordyce (1977, 1980), baleen whales likely evolved in the South Pacific during the early Oligocene when the Antarctic Circumpolar Current (ACC) became established. The cooling of the oceans during Late Eocene and Oligocene produced areas where cold and nutrient-rich polar waters met and mixed with warmer less nutrient-rich water of equatorial origin resulted in areas of upwelling with high levels of primary production (Haywood *et al*. 2000; Raymo *et al*. 1996; Ravelo & Andreasen 2000; Ravelo *et al*. 2004). In the Southern Hemisphere, the density gradient between the ACC and its adjacent waters increased which led to the establishment of a continuous circumpolar belt of high primary productivity (the ACC), especially in areas where terrestrial-derived nutrients were available, such as off New Zealand (Fordyce 1977, 1980). Fordyce suggested that this change in upwelling intensity, and thus primary production, facilitated the environmental changes that eventually led to the evolution of the filter-feeding mode observed in baleen whales (Fordyce 1977, 1980). The hypothesis that speciation in baleen whales is closely tied to upwelling is logical, given the mode of foraging of this species group. The importance of intensive and seasonally persistent upwelling in high latitudes, which generates predictable seasonal pulses of primary production and support rich food webs, is also readily evident from the current distribution and migration patterns of all extant baleen whales.

A process that would fragment large continuous areas of upwelling, such as that associated with the ACC, could potentially result in reproductive isolation of conspecific baleen whale populations, which then might diverge genetically and evolve into different species given sufficient time. One such process is global warming which reduces the polar-to-equator temperature gradients that drive upwelling (e.g. shown by simulations using coupled models by Schmittner 2005). Under such circumstances, upwelling will be greatly reduced and significant upwelling will only persist in areas where local conditions (e.g. local bottom topography) facilitate upwelling. Hence, global warming would not only result in habitat fragmentation by breaking up larger continuous density gradients that drive upwelling but also reduce the overall amount of area where upwelling occurs, thereby reducing the overall carrying capacity and subsequently likely the abundance of the species that depend upon the high primary productivity facilitated by upwelling. Once conditions reverse (i.e. decreasing global temperatures), upwelling is re-established increasing connectivity among baleen whale populations. However, if the time of reduced upwelling is sufficiently prolonged, conspecific populations which became reproductively isolated may have diverged sufficiently to evolve into new species, which may exists in sympatry once upwelling is re-established.

While the modelling conducted by Schmittner (2005) was aimed at assessing the effects of rising temperatures on the thermohaline circulation (THC) in the North Atlantic, other studies have modelled the effect of global warming in the Southern Ocean (Hirst 1999; Bi *et al*. 2001). These modelling studies demonstrated a reduction in deepwater upwelling at the ACC under global warming conditions.

From our current knowledge of baleen whale migration patterns, it seems highly plausible that their habitat could be fragmented and populations of conspecifics evolve into new species during the conditions described above. Since the period of gestation in most extant cetaceans is about 1 year, both mating and calving occur in much lower latitudes. However, global geography is such that populations forced to feed in isolated patches around Antarctica, when heading north to breed could find themselves on different sides of ocean basins or even in different ocean basins altogether, thus becoming also reproductively isolated.

Most extant species of baleen whales are present in Antarctic waters during the summer, one of which is the minke whale. Recently, Rice (1998) reviewed both morphological (e.g. Omura 1975) and genetic (e.g. Wada *et al*. 1991; Pastene *et al*. 1994) data collected from extant minke whale populations and divided minke whales into two species; the larger Antarctic minke whale (*Balaenoptera bonaerensis*), which is restricted only to the Southern Hemisphere, and the cosmopolitan common minke whale (*Balaenoptera acutorostrata*). The main external morphological character that most readily distinguishes the two species is a white flipper patch that is only present in *B. acutorostrata* (Fig. 1). The cosmopolitan distributed *B. acutorostrata* has further been divided into several distinct forms or subspecies based upon geographical origin and minor morphological differences, mainly in the North Atlantic, North Pacific and Southern Hemisphere (Fig. 1). *B. acutorostrata* in the Southern Hemisphere are commonly referred to as the 'diminutive' or 'dwarf' minke whale (Arnold *et al*. 1987; Best 1985).

The two species of minke whales are both found in the Southern Hemisphere, where they have overlapping ranges (Best 1985; Arnold *et al*. 1987). While both species migrate to higher latitudes during the austral spring, *B. acutorostrata*

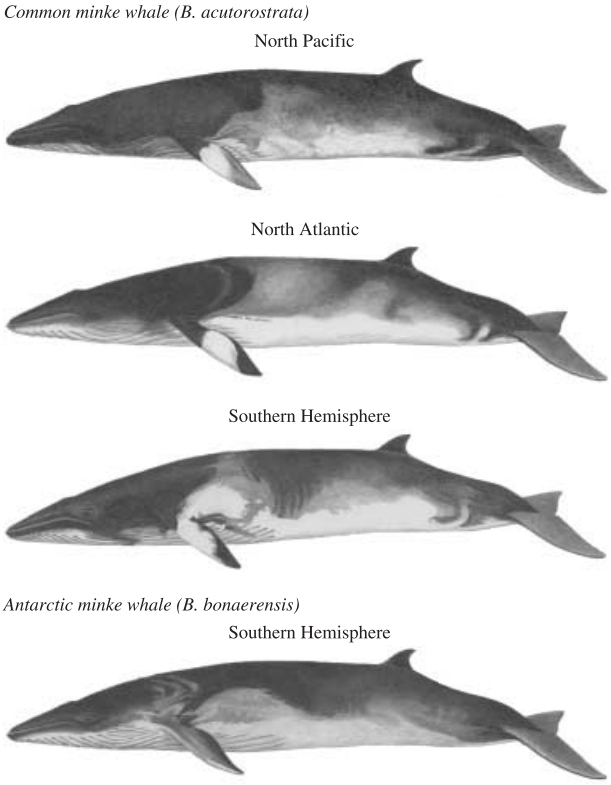

Fig. 1 Different morphological forms of *Balaenoptera acutorostrata* and *Balaenoptera bonaerensis*.

is only rarely observed above 60° South (Fig. 2). The two species also appear to have different diets; *B. bonaerensis* primarily feeds on krill, whereas *B. acutorostrata* has a more varied diet, primarily consisting of fish (Kasamatsu *et al*. 1993).

The average degree of sequence divergence between the two minke whale species is 9% in the mitochondrial DNA (mtDNA) control region (Table 1) which suggests a divergence time in the order of 4–5 million years ago (Ma) using a substitution rate of 2% per million years (Hoelzel *et al*. 1991). This rough and preliminary estimate of divergence time places this speciation event at some time during the Pliocene (5–2 Ma). The Pliocene was characterized by a warm climate with temperatures 3 °C higher than current temperatures (Raymo *et al*. 1996). The temperature gradient driving the continuous upwelling belt facilitated by the ACC would have been greatly reduced or absent during this extended period of global warming. Upwelling was thus likely highly localized resulting in a fragmentation of the ancestral minke whale species' summer feeding habitats and, as explained above, also of breeding grounds, potentially leading to reproductive isolation and subsequent speciation.

The observation that the two species coexist in the Southern Hemisphere and the fact that *B. bonaerensis* is only found in the Southern Hemisphere suggests that the place of origin of the two species was in the Southern Hemisphere. Once the climate cooled at the end of the Pliocene and the continuous belt of upwelling along ACC became re-established, the overall area of upwelling, and thus minke whale feeding habitat, would have increased significantly likely facilitating the expansion of the (now) two species of minke whales (i.e. *B. bonaerensis* and *B. acutorostrata*). *B. acutorostrata* could then have radiated to all the major oceans where it is observed today possibly facilitated by the broader prey base of this species, relative to *B. bonaerensis*.

The aim of this study was to use genetic analyses to test some of the predictions emerging from the hypothesis outlined above. We employed mtDNA control region nucleotide sequences collected from extant populations of *B. bonaerensis* and *B. acutorostrata*. Our study took advantage of the fact that the two minke whale species are closely related, making it possible to apply population level

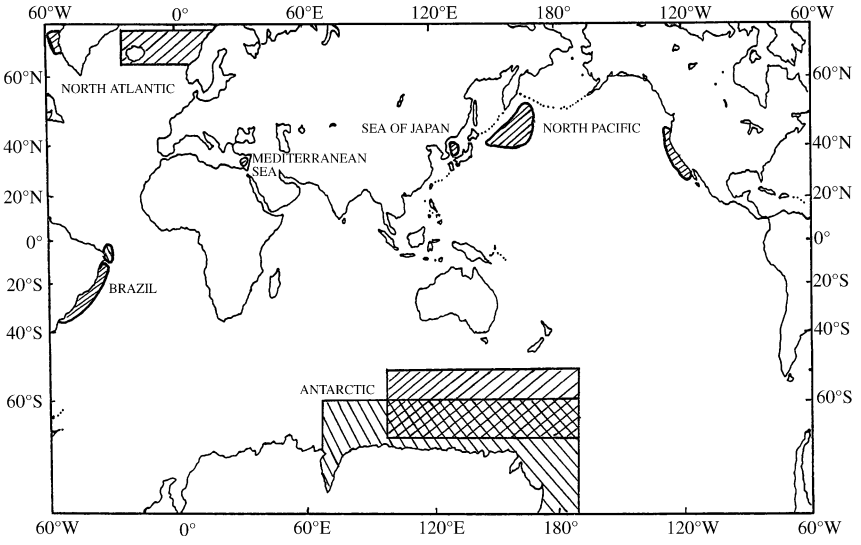

Fig. 2 Sampling sites by minke whale species. Notes: ▨ denotes sample localities of *Balaenoptera acutorostrata*. ▨ denotes sample localities of *Balaenoptera bonaerensis*.

Table 1 Estimates of genetic divergence between different balaenopterid species

Species	*B. acutorostrata*	*B. bonaerensis*	*B. borealis*	*B. edeni* Solomon Islands	*B. edeni* Kochi Japan	*B. edeni* Common type	*B. musculus*
Common minke whale *B. acutorostrata*	—						
Antarctic minke whale, *B. bonaerensis*	0.09	—					
Sei whale, *B. borealis*	0.21	0.24	—				
Bryde's whale, *B. edeni*, Solomon Islands	0.23	0.24	0.18	—			
Bryde's whale, *B. edeni*, Kochi, Japan	0.18	0.23	0.08	0.14	—		
Bryde's whale, *B. edeni*, common type	0.20	0.23	0.08	0.17	0.08	—	
Blue whale, *B. musculus*	0.16	0.19	0.18	0.19	0.16	0.16	—
Fin whale, *B. physalus*	0.17	0.19	0.16	0.19	0.14	0.14	0.13

Notes: genetic divergence was estimated as Kimura's 2 parameter distances. Data obtained from Arnason *et al.* (1993) and Yoshida & Kato (1999).

approaches to estimate population divergence time, which will improve the preliminary estimate of haplotype divergence times mentioned above.

Our hypothesis yields three predictions, each of which may be addressed by analyses of nucleotide sequences collected from populations of the same or closely related species: (i) the estimate of population divergence time should place the divergence of *B. bonaerensis* and *B. acutorostrata* in the Pliocene epoch; (ii) the global increase in upwelling areas as global temperatures decreased after the Pliocene should be evident as genetic signatures of population expansions in most minke whale populations; and (iii) the population divergence times and the spatial pattern of the current *B. acutorostrata* populations should be consistent with a radiation from the Southern Hemisphere after the Pliocene.

This hypothesis may be tested against the alternative vicariant speciation mechanism which would predict that the two species diverged and evolved in different hemispheres, aided in part by the anti-polar differences in breeding seasons tending to isolate northern and southern populations, and is prevalent in many extant, and some extinct, cetacean groups (Davies 1963; Barnes 1985).

In Fig. 3(A–C), we have illustrated the expected phylogenetic relationship among the extant populations of *B. acutorostrata* given different centres of origin for this species (the Southern Ocean, the North Pacific and the North Atlantic, respectively). These threes can be tested against a tree constructed using actual genetic data from this study.

Materials and methods

Samples

B. acutorostrata samples were collected from locations in the North Pacific (the Sea of Japan (n = 28), the western North Pacific (n = 127), and the eastern North Pacific (n = 6)); in the Atlantic (West Greenland coast (n = 15), eastern North Atlantic (n = 87), and the Brazilian coast (n = 8)); in the Mediterranean Sea (n = 1) and the Antarctic (n = 15) (Fig. 2). The data from the eastern North Atlantic include previously published mitochondrial control region nucleotide sequences collected from the Barents Sea (n = 46) as well as off the Icelandic coast (n = 41) (Bakke *et al.* 1996). Samples from *B. bonaerensis* were collected from Antarctic waters between 70°E and 170°W (n = 119), and off the coast of Brazil (n = 61). In total, data were obtained from 467 samples (see Appendix).

Laboratory analyses

Total-cell DNA was extracted from muscle, heart or liver stored at –20 °C, by standard phenol-chloroform extractions (Sambrook & Russell 2001). The first 500 nucleotides at the 5′ end of the mtDNA control region were amplified by polymerase chain reaction (PCR, Mullis & Faloona 1987). The oligo-nucleotides employed in the PCR amplification were MT4 (Arnason *et al.* 1993) and P2R (5′-GAAGAGGGATCCCTGCCAAGCGG-3′). Reactions were carried out in 50 μL 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 μM dNTPs, 2.5 pM of each oligonucleotide and 1 U of *Taq* DNA polymerase. After an initial denaturation step at 95 °C for 5 min, a PCR amplification cycle of 30 s at 94 °C, followed by 30 s at 50 °C and 30 s at 72 °C was repeated 30 times. The amplification was completed with a final extension step of 10 min at 72 °C. Subsequent cycle sequencing reactions were performed with 100 ng of products generated in the above PCR amplifications using the ABI PRISM dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems). The oligonucleotides 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 s at 96 °C, 20 s at 56 °C and 4 min at 60 °C were performed. The nucleotide

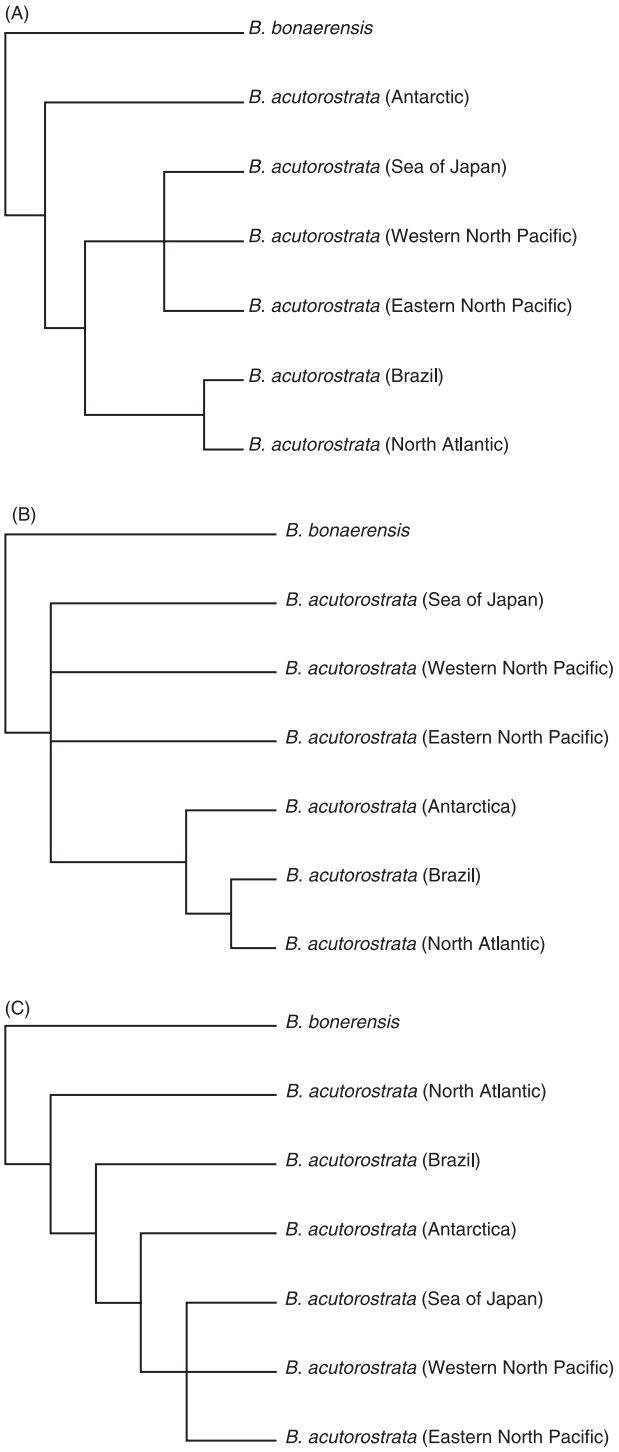

Fig. 3 The expected phylogenetic relationship among extant populations of *B. acutorostrata* assuming a Southern Ocean (3A), North Pacific (3B) and North Atlantic (3C) centre of origin.

sequence of each cycle sequencing reaction was determined by electrophoresis through a 5% Long Ranger (FMC denaturing polyacrylamide matrix on a DNA ABI PRISM· 377 DNA Sequencer (Applied Biosystems) under standard conditions. Both strand samples were sequenced in their entirety for all samples.

Data analyses

Estimation of phylogenetic relationships. Sequences were aligned by eye using Sequence Navigator (Applied Biosystems). First, approximate maximum likelihood (ML) trees were obtained by locally rearranging the tree topology starting from a neighbour-joining tree in search for topologies with the higher likelihoods. This preliminary assessment was conducted using all identified mtDNA haplotypes (187) and all baleen whale species as outgroup, as well using a subset of 89 mtDNA haplotypes in which the closely related sequences were excluded, and all baleen whale species as outgroup. Since it was impossible to conduct an effective and exhaustive search for the most likely topology with this many mtDNA haplotypes, a more exhaustive search for the ML topology was undertaken using an approximate method based upon a set of 60 haplotypes in total (again the very closely related sequences in the set of 89 were excluded).

The genealogical relationship among the sampled mtDNA control region haplotypes was estimated using the NUCML program in the MOLPHY computer package (version 2.3, Adachi & Hasegawa 1996); the BASEML program in the PAML computer package (version 3.1, Yang 1997) and the TREE-PUZZLE program (version 5) of the quartet-puzzling (QP) method (Strimmer & von Haeseler 1996). In the latter case, we employed the HKY85 model (Hasegawa *et al*. 1985). In using the BASEML and TREE-PUZZLE programs, the discrete gamma-distribution (with eight categories) to correct for site-heterogeneity was used (Yang 1996). The transition/transversion ratio of the HKY85 model and α, the shape parameter of the gamma-distribution, were optimized.

Estimation of haplotype divergence times. For this purpose, we used a data set of 48 sequences (the very closely related sequences in the set of 60 above were excluded). For this set, the likelihood was computed using BASEML for two models (assuming a molecular clock model or a nonclock model). The two models were compared employing a likelihood-ratio test (e.g. Hasegawa *et al*. 1993), which failed to reject the clock model. Given this result, we applied a molecular clock when estimating the divergence times within minke whales. As a calibration point, we assumed that minke whales and the grey whale (*Eschrichtius robustus*) separated 20 Ma. According to Nikaido *et al*. (2001), the minke whales lineage diverged from the lineage leading to the fin and humpback whales at 19.8 ± 4.7 ($\pm$: 1SE) Ma. This date was estimated from the SINE flanking sequences with the relaxed molecular clock method, which takes into account of rate differences among

different lineages (Thorne *et al*. 1998). Almost the same estimate was obtained for the divergence among minke/grey/fin and humpback whales from the mitochondrial genome sequences (Sasaki *et al*. 2005). Thus, the 20 Ma date we used in calibrating the clock might be reasonable, but this date can be older than the speciation date because we are dealing with the gene tree but not the species tree and the ancestral polymorphism generally tends to yield estimates for a branching in a gene tree that predates that of a species tree (Arbogast *et al*. 2002).

Estimation of within- and among-population diversity. The level of intrapopulation variation was estimated as the nucleotide diversity (Nei & Li 1979). The degree of divergence between different minke whale sampling localities was estimated as described by Hudson *et al*. (1992) employing K_{ST} as the test statistic. Hudson's K_{ST} was chosen as the test statistic following the recommendations outlined by Hudson *et al*. (1992), which are based upon the level of haplotypic diversity observed among the collected sequences. The probability of the observed values of K_{ST} was estimated from 10 000 permutations (between sample partitions) of the original data.

Phylogenetic relationship among populations. A phylogenetic relationship' among sampling localities was estimated employing K_{ST} as the measure of genetic divergence. Bootstrap support for each branch was estimated from 1000 bootstrap samples, each of which was obtained by resampling the observed number of mtDNA control region sequences with replacement from each sample partition in the original data set. A majority-rule consensus tree was estimated from the 1000 matrices of K_{ST} estimates using the neighbour-joining algorithm as implemented in the PHYLIP software package (version 3.52c, Felsenstein 1993).

Deviation from mutation-drift equilibrium. We employed mismatch distributions (Slatkin & Hudson 1991) and the FLUCTUATE program (Kuhner *et al*. 1998) to estimate and assess if the population growth rate (g) over evolutionary time deviated significantly from zero. The estimations were carried out with 10 short chains each of 2000 steps and two long chains, each of 20 000 steps. For this exercise, we employed the transition-transversion ratio of 5:1 (the observed ratio). Using FLUCTUATE, a maximum-likelihood estimate of θ ($2N_{ef}\mu$, where N_{ef} is the effective population size of females and μ the mutation rate) was obtained for a growth rate fixed at zero (θ_{g0}) as well as at the maximum-likelihood estimate of g (θ_{gmax}). We used the log likelihood ratio -2λ (where λ is the log ratio of the maximum-likelihood estimates of θ_{g0} and θ_{gmax} obtained from the same Markov chain) as the test statistic, assuming that -2λ was χ^2-distributed with 1 degree of freedom.

Estimation of population divergence times. The divergence time (T) between each pair of populations was estimated using the approach originally developed by Nielsen & Wakeley (2001), modified for an HKY mutation model (Palsbøll *et al*. 2004), as implemented in the computer program MDIV. For each estimate, we ran 10 million chains, with 500 000 for burn-in. The maximum value of θ was set as the default (an appropriate maximum is then defined by the software); for the divergence time at 4, 10 or 30 (in units of N_{ef} generations); and the migration rate Nm at zero (preliminary estimations yielded estimates of Nm at zero) for all pairwise comparisons except between samples from the North Pacific and Sea of Japan. A mutation rate was required in order to translate the divergence times estimated by the above approach to absolute time. Although the mutation rate of the cetacean mtDNA control region has been estimated by several different authors, these estimates were all based upon interspecific calibration points (i.e. fossils of known age). Such phylogenetic estimates of the mutation rate of a rapidly evolving DNA sequence (Lyrholm *et al*. 1996) are likely biased downward when applied to intraspecific data. Studies in humans have shown this bias to be one order of magnitude (Howell *et al*. 2003). We employed three mutation rates among those reported for the corresponding segment of the cetacean mtDNA control region; 7×10^{-8} (Harlin *et al*. 2003), 2×10^{-8} (Rooney *et al*. 2001) and 1×10^{-8} (Hoelzel 1993) per nucleotide per year. Since divergence times are estimated in units of N_{ef} generations then a generation time was required as well. Roman & Palumbi (2003) employed the average female age as generation time, which has been reported at 17 years in *B. bonaerensis* (Kishino *et al*. 1991). Skaug (2001) used a generation time of 7 years.

Results

Data

The final data set included the first 340 nucleotides of the mtDNA control region from a total of 467 specimens (287 of *B. acutorostrata* and 180 of *B. bonaerensis*). Eighty-seven nucleotide positions were polymorphic and defined 187 unique sequences (haplotypes, see Appendix). The sequences have been deposited in GenBank under Accession nos EF113709–EF113895. In addition to what we inferred as four different insertion/deletion events, we identified 99 segregating sites at which 80% of the inferred substitutions were transitions. The degree of divergence among haplotypes ranged from zero to 12.8%. Fixed differences in the nucleotide sequence between the *B. bonaerensis* and *B. acutorostrata* were observed at eight nucleotide positions (positions 71, 129, 289, 300, 302, 303, 304, and 312, see Appendix).

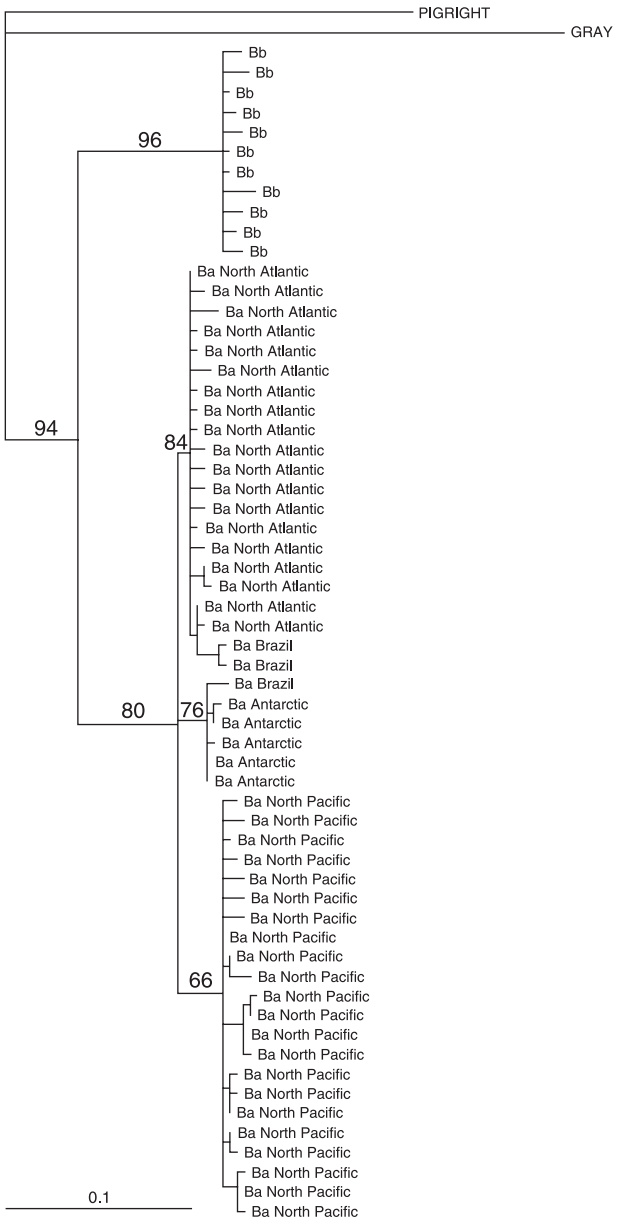

Fig. 4 Genealogy estimated from mitochondrial control region nucleotide sequences. Figures in the nodes are QP reliability values. Notes: Ba denotes *Balaenoptera acutorostrata*, and Bb *Balaenoptera bonaerensis*.

Phylogenetic estimations

Figure 4 shows the QP tree for the 60 haplotypes. *B. acutorostrata* samples collected in the North Atlantic, Antarctic and North Pacific were each grouped in independent clusters supported by QP reliability values of 84%, 76% and 66%, respectively. Two Brazilian haplotypes yielded a monophyletic clade supported by an 80% reliability value that fell within the North Atlantic clade. The other Brazilian haplotype fell within the Antarctic *B. acutorostrata*. The trichonomy which lead to *B. acutorostrata* haplotypes from the North Atlantic, Antarctic and North Pacific was supported by a reliability value of 80%. These relationships were also supported by the ML tree generated with the local rearrangements option of NUCML starting from the NJ tree, and irrespective of the number of sequences and outgroup species employed in the estimation (results not shown).

Table 2 Estimates of genetic diversity in oceanic minke whale samples

Species and sample locality	*n**	*K*†	π‡	SE(π)§
B. bonaerensis (Brazil)	61	47	0.016	0.001
B. bonaerensis (Antarctic)	119	83	0.015	0.001
B. acutorostrata (eastern North Pacific)	6	5	0.006	0.002
B. acutorostrata (western North Pacific)	127	34	0.010	0.0005
B. acutorostrata (Sea of Japan)	28	3	0.006	0.001
B. acutorostrata (Brazil)	8	3	0.012	0.006
B. acutorostrata (Antarctic)	15	8	0.007	0.001
B. acutorostrata (North Atlantic)	102	26	0.006	0.0005

Notes: *The number of mtDNA control region sampled. †The number of different mtDNA control region DNA sequences detected. ‡The nucleotide diversity (in units of $1/r$, where r is the mutation rate, Kuhner *et al*. 1998). §The standard error of the nucleotide diversity estimate.

Population diversity and divergence

Levels of variation within and among species and populations. The nucleotide diversity for the Brazilian and Antarctic populations of *B. bonaerensis* was estimated at 1.6% and 1.5%, respectively, and in *B. acutorostrata*, the nucleotide diversity ranged from 0.6 to 1.2% depending upon sampling locality (Table 2). The haplotype frequencies partitioned by species and sampling locality are listed in the Appendix. All haplotypes were unique to each minke whale species and most haplotypes were unique to each ocean.

In the case of *B. bonaerensis*, 15 haplotypes were shared between samples collected in the Antarctic and off Brazilian waters. A homogeneity test based on K_{ST} failed to detect any significant level of genetic heterogeneity between these localities. Hence, these samples were combined and treated as a single population sample in subsequent analyses. In the case of *B. acutorostrata*, the five haplotypes identified in the eastern North Pacific were also represented among the samples collected in the western North Pacific, albeit at different frequencies. Among the samples collected in the Sea of Japan, only three haplotypes were detected, two of which were also detected among the samples from the western North Pacific. However, the most common haplotype (haplotype no. 150 representing 21 of the 28 samples collected in the Sea of Japan) was not detected among the samples from the western

Table 3 Estimates of genetic divergence among minke whale species and populations

	B. bonaerensis (Antarctic & Brazil)	*B. acutorostrata* (North Pacific)	*B. acutorostrata* (Sea of Japan)	*B. acutorostrata* (Antarctic)	*B. acutorostrata* (Brazil)	*B. acutorostrata* (North Atlantic)
B. bonaerensis (Antarctic & Brazil)	—					
B. acutorostrata (North Pacific)	0.76	—				
B. acutorostrata (Sea of Japan)	0.57	0.19	—			
B. acutorostrata (Antarctic)	0.45	0.32	0.60	—		
B. acutorostrata (Brazil)	0.33	0.28	0.57	0.48	—	
B. acutorostrata (North Atlantic)	0.75	0.51	0.46	0.26	0.15	—

Note: estimate of K_{ST}. The probability of the observed values (assuming the two samples were collected from the same population) was in all instances estimated at less than 0.000001 (see text for details).

Table 4 Estimation of population growth rates from mtDNA control region nucleotide sequences

	Population size model					
	Constant		Exponentially expanding			
Sample	$\hat{\theta}_{g0}$	$\ln \Delta_{g0}$	$\hat{\theta}_{gmax}$	$\ln \Delta_{gmax}$	$\hat{g}$	-2λ
B. acutorostrata						
Sea of Japan	0.005	−0.55	0.003	0.0001	−180	$1.0^{n.s.}$
North Pacific	0.05	2.6	0.06	19	590	33**
North Atlantic	0.04	3.5	0.04	12	580	17**
Brazil	0.01	−0.31	0.008	0.0	−60	$0.62^{n.s.}$
Antarctic	0.01	−0.36	0.009	0.0002	180	$0.73^{n.s.}$
B. bonaerensis						
Antarctic & Brazil	0.2	−29	0.5	30	380	118**

Notes: ln Δ denotes the estimated maximum log likelihood values. g denotes the maximum likelihood estimate of the growth rate. λ equals the log likelihood ratio ($\ln \Delta_{g0} - \ln \Delta_{gmax}$) and is assumed to be χ^2-distributed with one degree of freedom. **$P < 0.0001$. $^{n.s.}$, $P > 0.05$.

North Pacific. The haplotype of the single sample collected in the Mediterranean Sea was identical to the most common haplotype observed among whales sampled in the North Atlantic (haplotype no. 162). No haplotypes were shared between whales sampled in Brazilian and Antarctic waters. Our homogeneity tests (using K_{ST} as test statistic) revealed significant levels ($P < 0.00001$) of genetic heterogeneity between the two minke whale species and among all oceanic populations of *B. acutorostrata*. However, no significant level of heterogeneity was detected within ocean basins aside from that between the western North Pacific and the Sea of Japan (Table 3). However, the lack of statistically significant levels of heterogeneity within ocean basins may simply be a result of the limited samples sizes and data (e.g. see Andersen *et al*. 2003).

Population expansions. The mismatch distributions in population samples from *B. bonaerensis* as well as *B. acutorostrata* from the North Pacific and the North Atlantic were consistent with exponential population expansions. In contrast, *B. acutorostrata* populations sampled from the Sea of Japan, as well as from Antarctic and Brazilian waters had multimodel mismatch distributions, which is inconsistent with exponential population expansions (Fig. 5). The assessment of the sequence data using the FLUCTUATE program confirmed the putative population expansion (indicated by the mismatch distributions) yielding estimates of growth rates that were significantly larger than zero in all population samples, except for the Sea of Japan, Antarctic and Brazil *B. acutorostrata* samples (see Table 4).

Phylogenetic relationships among common minke whale populations. The tree of the sampled populations of *B. acutorostrata* is depicted in Fig. 6. The tree was rooted with the *B. bonaerensis* samples collected in Antarctic and Brazilian waters (presumably part of the same population). The population of *B. acutorostrata* placed closest to the root was the Antarctic population, followed by a dichotomy leading to either North Pacific populations (including the Sea of Japan) or Atlantic populations. All branches were supported by bootstrap values above 80%, apart from the branch leading to North Pacific and Atlantic samples, where the

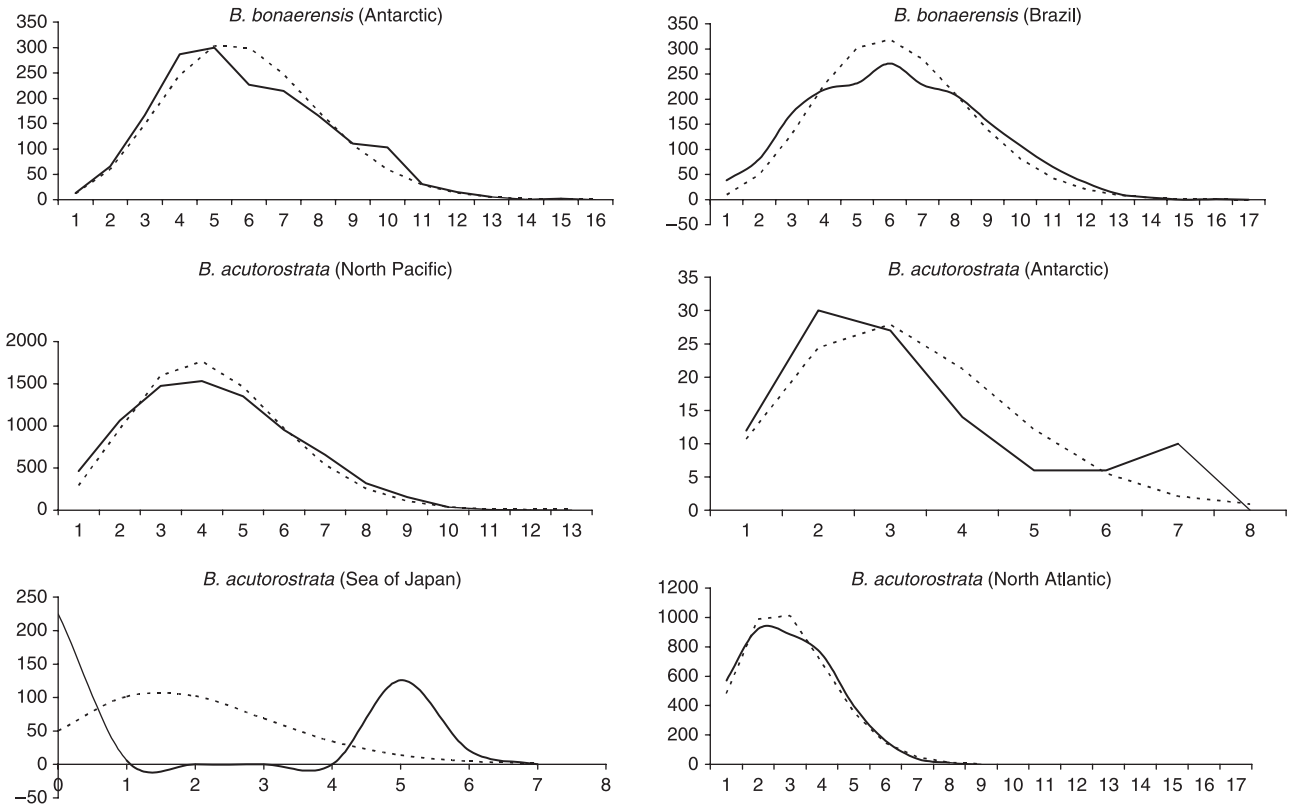

Fig. 5 Observed and expected (under a model of exponential growth) frequency distributions of the number of nucleotide differences among all pairs of sampled mtDNA control region sequences. Notes: solid line denotes observed distribution and broken line the expected Poisson-distribution assuming exponential population growth. The *x* axis is the number of substitutions, the *y* axis the frequencies of the pairwise comparisons.

bootstrap support was estimated at 44%. The Sea of Japan sample was located on the branch leading to the western and eastern North Pacific and basal to the North Pacific samples. Of the three North Pacific samples, the Sea of Japan sample was least divergent from the Antarctic population. The observed phylogeny and estimated phylogenetic relationship among the extant populations was similar to that illustrated in Fig. 3A, pointing towards an origin of the extant populations of *B. acutorostrata* in the Southern Ocean.

Divergence times

Haplotype divergence times. The divergence between *B. bonaerensis* and *B. acutorostrata* was estimated at 6.6 (SE: 2.9) million years (Ma) with a 95% confidence interval of 1.0–12.3 Ma. The split of the trifurcation leading to each major oceanic lineage of *B. acutorostrata* (see Fig. 4) was estimated at 1.2 (SE: 0.5) Ma with a 95% confidence interval ranging from 0.3 to 2.2 Ma. The time of diversification of the North Pacific lineages was estimated at 0.4 (SE: 0.2) Ma with a 95% confidence interval from 0.1 to 0.8 Ma (Fig. 4).

Population divergence times. The posterior probability distribution of θ and *T* for each pair of populations are shown in Figs 7 and 8, respectively. In general, the maximum likelihood was well defined for all pairwise estimations of θ (Fig. 7), but poorly defined with respect to *T* for those pairwise estimations that involved the Sea of Japan samples (Fig. 8). The estimates of population divergence times using MDIV show just how sensitive such estimates are to the assumptions of those parameter values that are necessary in order to translate relative divergence times into an absolute measure. The divergence between all *B. acutorostrata* population samples and the *B. bonaerensis* sample ranged from 4.4 to 4.9 Ma, assuming a mutation rate of 7×10^{-8} per nucleotide per year (Harlin *et al*. 2003) and a generation time of 7 years (Skaug 2001) (Table 5). Reducing the mutation rates to 2×10^{-8} and 1×10^{-8}, increased these same estimates of divergence times to ~16 Ma and ~32 Ma, respectively, preceding the estimated haplotype divergence times. In the same manner, employing a generation time of 17 years increased the estimates of divergence times between *B. acutorostrata* and *B. bonaerensis* to ~11 Ma, also exceeding the haplotype divergence time estimates. These results suggest that the combination of the

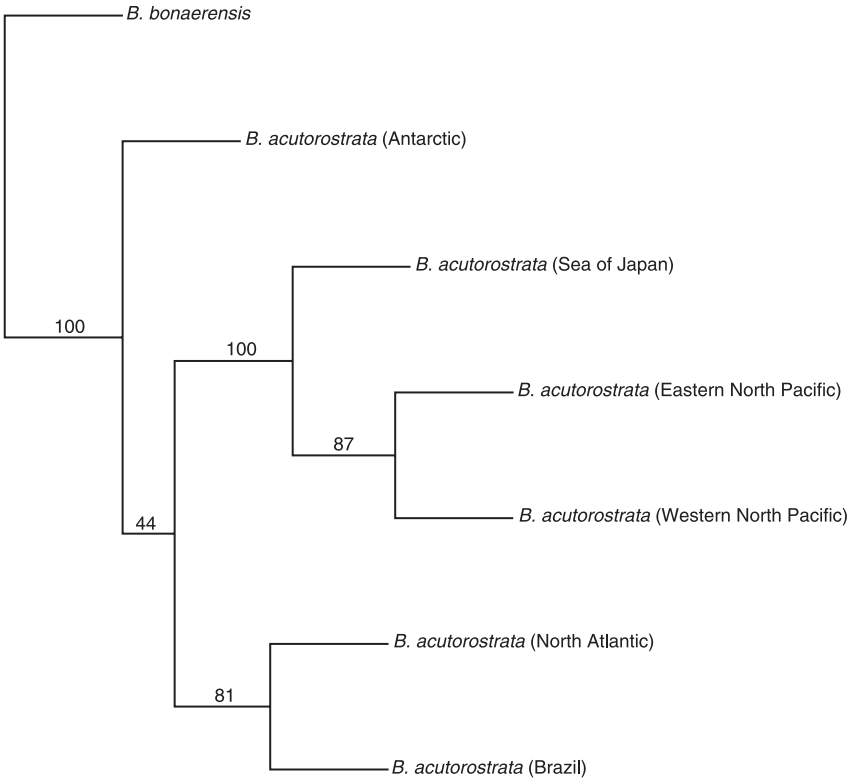

Fig. 6 The phylogenetic relationship among *B. acutorostrata* populations estimated from mtDNA control region nucleotide sequences.

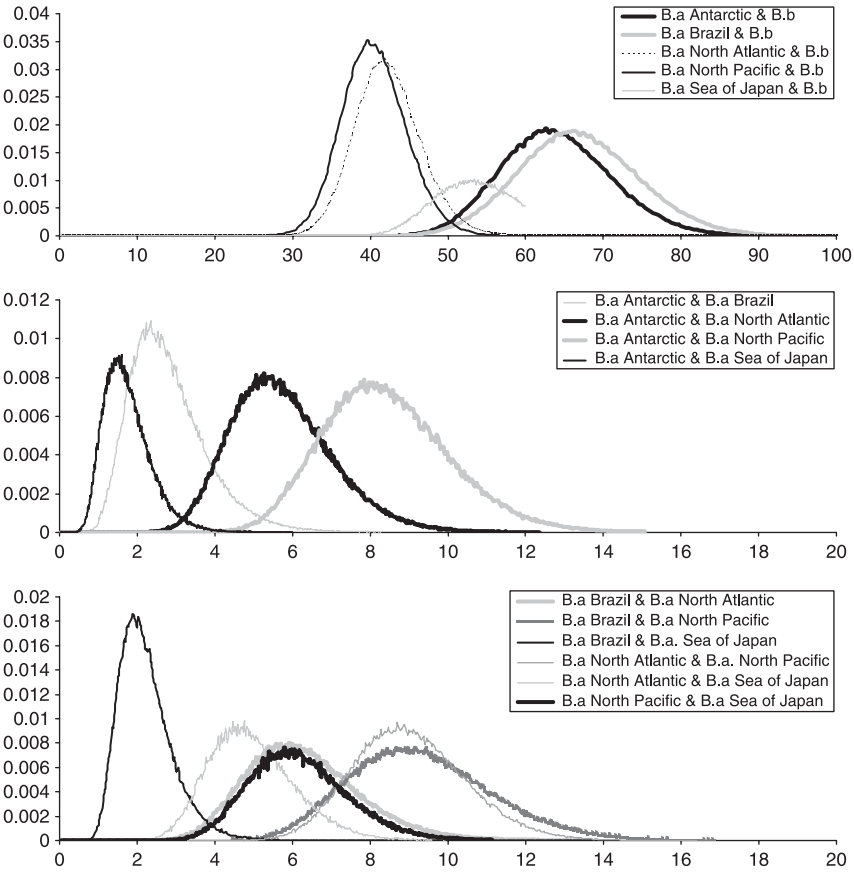

Fig. 7 Posterior probability distribution of θ for each pair of populations. Notes: B.a denotes *B. acutorostrata*, and B.b *B. bonaerensis*. The *x* axis denotes θ, the *y* axis the posterior probability.

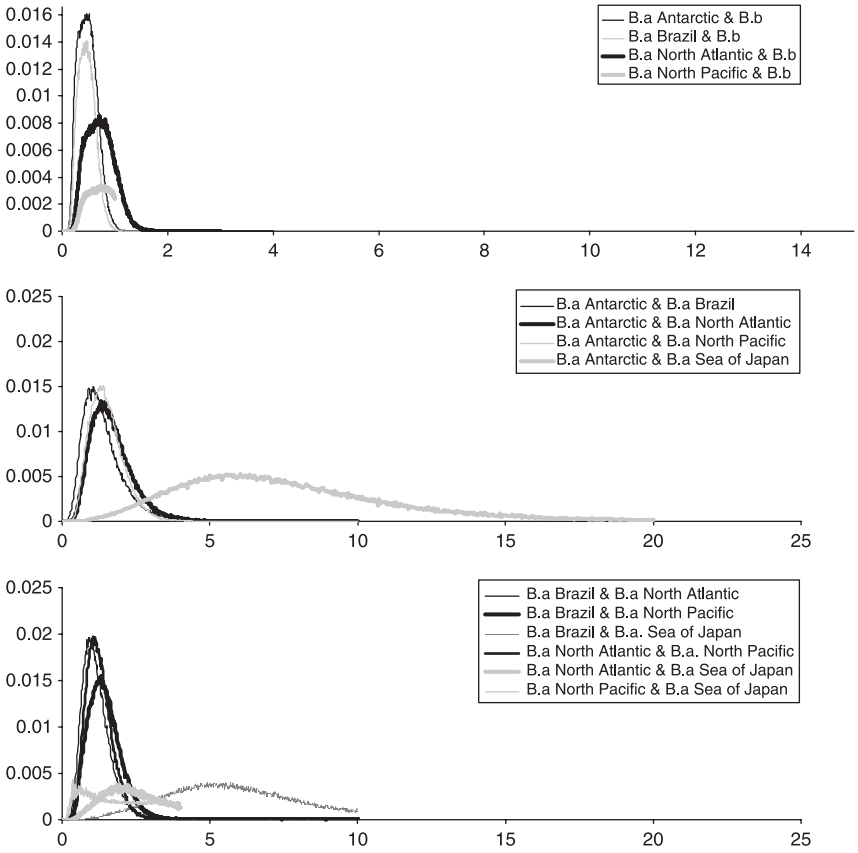

Fig. 8 Posterior probability distribution of T for each pair of populations. Notes: B.a denotes *B. acutorostrata*, and B.b *B. bonaerensis*. The x axis denotes T, the y axis the posterior probability.

Table 5 Estimates of divergence time among oceanic populations and species of the minke whale

		B. acutorostrata			
B. acutorostrata	*B. bonaerensis*	Antarctic	Brazil	North Atlantic	North Pacific
Antarctic	4.9				
Brazil	4.5	0.37			
North Atlantic	4.4	0.57	0.88		
North Pacific	4.6	1.6	1.7	1.4	
Sea of Japan	4.8	1.6	1.5	1.4	0.14

Notes: estimates are in units of millions of years. The estimates were obtained from the MDIV estimates obtained for θ and T assuming a mutation rate 7×10^{-8} per nucleotide per year (Harlin *et al*. 2003) and a generation time of 7 years (Skaug 2001). Please refer to Materials and methods for further details.

highest mutation rate (7×10^{-8}, Harlin *et al*. 2003) and lowest generation time (7 years) is most consistent with the notion that haplotype divergence precedes population divergence. Other parameter values yields divergence time estimates that approached or precedes the split of grey/fin and minke whales (see above). Based upon these parameter values, we estimated the divergence time of *B. acutorostrata* in the three ocean basins to approximately 1.5 Ma, and within the North Pacific (Sea of Japan and western North Pacific) at 0.14 Ma.

Discussion

The aim of our study was to test the hypothesis that periods of global warming facilitate speciation in pelagic species by fragmenting areas of upwelling by which conspecific baleen whales become divided into smaller reproductively isolated populations. This hypothesis would apply not only to baleen whales, but most pelagic species that are closely tied to the primary production that upwelling is responsible for.

In our study, we focused on the timing of the speciation and subsequent radiation of the common minke whale, *B. acutorostrata*. Given that its closest relative (*B. bonaerensis*) is only found in the Southern Hemisphere, we argued that the speciation event happened in the Southern Hemisphere. A preliminary estimate of the sequence divergence at the mtDNA control region between the two minke whale species at 0.09 suggested that the two species diverged some 4–5 Ma, which places the speciation event in the Pliocene, an epoch of global warming.

We proposed three predictions: (i) the estimate of population divergence time should place the divergence of *B. bonaerensis* and *B. acutorostrata* in the Pliocene epoch; (ii) we should detect genetic signatures of population expansions in most populations of both species; and (iii) the population divergence times and spatial pattern of the current *B. acutorostrata* populations should be consistent with a radiation from the Southern Hemisphere after the Pliocene.

Our analyses place the divergence of *B. bonaerensis* and *B. acutorostrata* at 4.7 Ma in the Early Pliocene. The subsequent radiation of the extant oceanic populations of *B. acutorostrata* was estimated at 1.5 Ma, some 3 Ma after the two species diverged and after the end of the Pliocene global warming period. During this time, most of the sampled populations (in both species) appear to have undergone significant population expansions consistent with an overall increase in carrying capacity as upwelling was re-established.

The radiation of *B. acutorostrata* into different oceanic basins appeared to have occurred rapidly, resulting in an unresolved trichotemy in the haplotype genealogy (Fig. 4). This unresolved trichotemy and the low bootstrap support in the basal part of the population tree make it difficult to speculate about the order of radiation events in these trees. However, the overall branching pattern was inconsistent with origin of *B. acutorostrata* in the Northern Hemisphere, rather pointing to an origin in the Southern Hemisphere (Fig. 3A). Since (and this is well supported by our data) the split of *B. bonaerensis* and *B. acutorostrata* precedes the divergence of the populations of *B. acutorostrata*, this implies that the bifurcation of *B. bonaerensis* and *B. acutorostrata* defines the root of the *B. acutorostrata* part of the tree. The original reason to include *B. bonaerensis* in the analysis was to obtain such directionality of the *B. acutorostrata* part of the sequence and population tree.

The alternative view of antitropical speciation 4.7 Ma from a previously continuous bihemispheric distribution of the ancestral form is not favoured in our case. First and as mentioned above, the overall branching pattern in the haplotype and population trees is not consistent with an origin of *B. acutorostrata* in the Northern Hemisphere. Second, the estimated population divergence time of ~1.5 Ma for the oceanic populations of *B. acutorostrata* in the Northern Hemisphere, dates their divergence well after the raise of the Panama Isthmus, about 3 Ma (Savage 1983; Duque-Caro 1990). Had *B. acutorostrata* been inhabiting the Northern Hemisphere ever since 4.7 Ma, one would expect the divergence time of the North Atlantic and North Pacific populations to date back 3 Myr, near the time of Panama uplift. It makes much more sense that the speciation occurred in the Southern Hemisphere and that *B. acutorostrata* radiated into the North Atlantic and the North Pacific, aided by the pronounced cooling of Late Pliocene/Early Pleistocene. The long-closed Panamanian Seaway effectively contributed to the isolation of these populations. This later radiation and the maintenance of distinct northern and southern populations of *B. acutorostrata*, happening during an epoch characterized by glacial cycles and intervening warm periods could actually be ascribed to antitropical distribution mechanisms.

Of the three *B. acutorostrata* haplotypes detected among the small South Atlantic (Brazilian) sample ($n = 8$), one clustered with the Antarctic *B. acutorostrata* clade and two with the North Atlantic *B. acutorostrata* clade (Fig. 4). This absence of a well-supported single South Atlantic clade could be due to recent gene flow, incomplete lineage sorting, or both. However, the estimates of migration rates (using MDIV) suggest a zero migration rate between the North and South Atlantic, and thus the incomplete lineage sorting seems the most likely explanation. A more complete sampling in terms of space, sample sizes and loci is required to clarify the order of radiation events in the Southern Hemisphere.

Our data provide no insights as to whether the current partly sympatric distribution in the Southern Hemisphere is primary or secondary following allopatric speciation. From Fig. 6, it could be speculated that the sympatric distribution was primary because if *B. acutorostrata* diverged from *B. bonaerensis* in the Southern Hemisphere, then crossed into the Northern Hemisphere only to return later to the Southern Hemisphere, then the Antarctic *B. acutorostrata* would be expected to be least related to *B. bonaerensis*, unlike the case in Fig. 6 (which matched our hypothesis 1 in Fig. 3A). The caveat here is that the bootstrap support of the basic part of this tree is low. As mentioned above the haplotype tree is not informative in this context due to the unresolved trichotemy in the genealogy (Fig. 4).

As was evident by our exploration of the possible range of values for just two parameters in this estimation (the mutation rate and generation time), the translation of divergence times into years is then subject to a great deal of uncertainty. In addition, several of the required parameter values are poorly known and the estimation of the divergence time is subject to a bias stemming from the choice of mutation and population model employed in our estimation. The approach implemented in the software used in this study (MDIV, Nielsen & Wakeley 2001; Palsbøll *et al.* 2004) assumes constant and equal population sizes, and

ignores rate heterogeneity among sites in the sample sequence. Our analyses revealed substantial deviation from drift-mutation equilibrium in several populations, which may bias the estimate of population divergence times. The estimate of genetic diversity differs substantially among the sampled populations, which suggests different effective population sizes. A further investigation of these two aspects (Palsbøll, unpublished results) suggested that these latter two deviations from the model assumptions result in upward biased estimates of divergence times.

Overall, the degree of genetic divergence was low within each ocean basin, and we were unable to detect any significant degree of spatial heterogeneity in the distribution of variation at the mtDNA control region among samples of *B. acutorostrata* within oceanic basins, except between the Sea of Japan and the North Pacific, which has been reported earlier (Goto & Pastene 1997). The analyses presented here reveal significant departures from mutation-drift equilibrium in most areas (including the North Atlantic), indicative of rapid population expansions over recent evolutionary times. The relative degrees of divergence among different classes of loci and modes of inheritance under a model of expansion will differ from the expectations in equilibrium.

There are several possible causes for the seemingly global population expansion (over evolutionary time) that we observed in several of the minke whale populations sampled in this study, such as: founder effects; range expansion after the Pleistocene glaciation; or perhaps interspecific dynamics. While in principle it is possible to estimate the time frame for expansions from nucleotide sequence data these estimations rely upon an infinite-site model of evolution (Lyrholm *et al*. 1996), which unfortunately does not apply to the fast-evolving mtDNA control region.

Our analysis presented here was based on a 340-bp segment of a single locus. A phylogeny estimated from a single locus might, for stochastic reasons alone, provide an inaccurate representation of the phylogenetic history. In this case, our findings are supported by an ongoing study of minke whale using multiple nuclear loci (SINEs, short interspersed repetitive elements, data not shown). In addition, our results are supported by earlier analyses of morphological and morphometric data in minke whales worldwide (Best 1985; Arnold *et al*. 1987).

Overall, our results are supportive of the hypothesis that global warming fragments upwelling areas, thereby leading to reproductive isolation and potentially speciation given sufficient time. The observation that the overall area of upwelling is reduced implies smaller effective population sizes and thus more rapid genetic drift and hence genetic divergence among conspecific populations. In addition, the reduction in primary production may also select for different prey preference. In our study, the more cosmopolitan of the two species, *B. acutorostrata*, does indeed forage on a much broader range of prey than *B. bonaerensis*. The evolutionary consequences of the increase in thermal conditions have been studied in other marine organisms. Kelly *et al*. (1998) focused on planktonic foraminifera assemblages preserved in the central equatorial Pacific. Their study revealed that those genera, which inhabited the near-surface mixed layer, diversified during the latest Palaeocene thermal maximum.

There have been a number of more substantial warming periods in the Antarctic during the Holocene (e.g. at the start of the Eemian interglacial, about 100K years ago). If these events had similar effects as proposed here, then additional population genetic structure should perhaps be evident in *B. bonaerensis* in the Antarctic. These periods of increased oceanic surface temperatures were brief in relation the period which is the focus of this study and hence not resulted in any significant population genetic structure, which are likely to be erased once the ACC is re-established. Hence, it would not be surprising that we see little or no effect in these populations. In fact using a larger sample size, small but significant genetic differences were detected in *B. bonaerensis* (Pastene *et al*. 1996).

Our hypothesis is testable as it should apply to other pelagic species in the Southern Hemisphere that depend strongly upon upwelling as well.

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The authors all share a common interest in minke whales, perhaps the least appreciated and most curious of the baleen whales.

Appendix MitochondrialDNA control region haplotype nucleotide sequences and sampling frequencies

	Nucleotide position									Area and frequency§								
	1111112	2334566677	1111 7788892222	1111111111 2344455566	1111222222 7789001112	2222222222 2233344445	2222222222 5555677777	2222222233 8899999900	3333333 0011233									
No	6790145670	3584805612	3623531268	9534508901	7977280232	3423627892	3567201689	5934567902	3425546	1	2	3	4	5	6	7	8	9
Bb001	A-GTGCA--G	CGCAGAGTTT	TTTCTCAGTA	TCAA-GATAC	GGTCGGACCA	CTTTATGGTT	CCTATCGAAT	ATATGAATCT	GCCCTTA	1	2							
Bb002	....A.....	....	.....T....	....	....	C	TT........	...CC..C..	....C..	1	1							
Bb003	....	....	....	....	.......T..	....	....	....C..C..	....C..	1								
Bb004	....	....	....	....T.....	....	....	.....T....	...CC..C..	....C..	1								
Bb005	....	....	.....T....	....	....	....	....	...CC.....	....C..	1								
Bb006	....A.....	....	.....T....	....	....	.....C...C	TT..C.....	...CC..C..	....C..	1								
Bb007	....	....	....	....	....	....G.....	.....T....	...CC..C..	....C..	1								
Bb008	....	....	....	....	....	....	....	...CC..C..	....C..	8	9							
Bb009	....	....	.....T....	....	....	....	....	...CC..C..	....	1	1							
Bb010	....A.....	....	....	....G.....	....	.....CA..C	TT...T....	...CC..C..	....C..	1								
Bb011	....	....	....	....	.........G	T.........	.....T....	....C..C..	....C..	1								
Bb012	....	....	....	...T.G....	.....A....	....	....	...CC..C..	....C..	2	1							
Bb013	....A.....	....	....	....	....	.....C...C	TT........	...CC..C..	....C..	2	1							
Bb014	....A.....	....	....	....	....	.C.......C	TT..C.....	...CC..C..	....C..	1								
Bb015	....	....	....	....	....	....	..C.......	...CC..C..	....C.G	1								
Bb016	....	....	....	....	....A.....	....	....	...CC..C..	....C..	4								
Bb017	....	....	....	....	.......T..	......A...	....	...CC..C..	....CC.	1								
Bb018	....	....	..C..T.A..	....	....	....	....	...CC..C..	....C..	1	1							
Bb019	....	....	....	....	.......T..	....	....	...CC.G...	....C..	1								
Bb020	....	....	.....T....	....	....	....	.....T....	...CC..C..	....	2	1							
Bb021	....	....	....C.....	....	....	....	.....T....	...CC..C..	....C..	1								
Bb022	....	....	....	....	....	....	.....T....	...CC.....	....C..	1	1							
Bb023	....	....	....	.........T	.......T..	....	....	...CC..C..	....C..	1	2							
Bb024	....	....	....C.....	....	....	AC........	....C.A...	...CC..C..	....C..	2								
Bb025	....	....	....C.....	....	....	AC........	......A...	...CC..C..	....C..	1								
Bb026	....A.....	....	....	....	....	.....C...C	TT..C.....	...CC..C..	....C..	1								
Bb027	....	....	....	....	........T.	T.........	....	....CG....	....CC.	1								
Bb028	....	....	.....T....	....	....	....	.....T....	...CC..C..	....C..	1								
Bb029	..A.......	....	....C.....	....	....	G.........	....	...CC..C..	....	1								
Bb030	....	....	....	....	A.........	....	T.........	....C..C..	....C..	1	1							
Bb031	....	....	....	....	....	....	....	...CC..C..	....C.G	1	2							
Bb032	....	....	.....T....	....	....	T.........	....	...CC..C..	....C..	1								
Bb033	....	....	.....T....	....	....	....G....C	.....T....	....C..C..	....C..	1								
Bb034	....	.....GA...	....C.....	....	....	G.........	....	...CC..C..	....C..	1	2							
Bb035	....	....	....	....	....	T.C.......	.....T....	....C.....	....C..	1								
Bb036	....	C	....	....	....	....	....	...CC..C..	....C..	1	1							
Bb037	-.........	....	.....T....	....	....	....	.....T....	...CC.....	....C..	1								
Bb038	....	....A.....	....	....	....	....G.....	.....T....	...CC..C..	....C..	1								

Appendix *Continued*

	Nucleotide position									Area and frequency§								
	1111112	2334566677	7788892222	2344455566	7789001112	2233344445	5555677777	8899999900	0011233									
No	6790145670	3584805612	3623531268	9534508901	7977280232	3423627892	3567201689	5934567902	3425546	1	2	3	4	5	6	7	8	9
Bb039	....A.....	....	....	....	....	C	TT...T....	...CC..C..	....C..	1								
Bb040	....	..T.......	.......A..	....	.......T..	......AC.C	..C.......	...CC..C..	....C..	1								
Bb041	....	....	....	....	....	....	T....T....	...CC..C..	....C..	1								
Bb042	....A.....	....	....	....	....	C	TT........	...CC..C..	....C..	1	1							
Bb043	....A.....	....	....	....	....	C	TT........	...CT..C..	....C..	1								
Bb044	....	....	....	....	....	....	....	....C..C..	....C.G	1								
Bb045	....	....	....	....	....	.C.....A..	....	....C..C..	....C..	1								
Bb046	....A.....	....	....C.....	.....A....	........T.	A.........	T...C.A...	...CC..C..	....C..	1								
Bb047	....	....	....	....	....	.C.......C	T....T....	...CC..C..	....C..	1								
Bb048	....	...G......	....	....	....	....	....	...CC..C..	....		1							
Bb049	....	....	.....T....	....	....	....	T...CT....	...CC..C..	....C..		1							
Bb050	....	....	....	....	....	....	.....T....	...CC..C..	....C..		7							
Bb051	....A.....	....	....C.....	....A.....	....	....	....	...CC..C..	....		1							
Bb052	....	....	....	....	....	....	....	...CC.....	....		2							
Bb053	....	....	....	....	........T.	....	.....T....	...CC..C..	....C..		1							
Bb054	....	....	....	....	........T.	....	....	....CG....	....C..		2							
Bb055	....	....	....	....	........T.	.C.....A..	....	....C..C..	....C..		1							
Bb056	....	....	..C.......	....	....	....	.....T....	...CC..C..	....C..		1							
Bb057	....	....	....	........G.	....	....	....	...CC..C..	....C..		1							
Bb058	....	....	....	....	..C.......	T........C	T....T....	...CC..C..	....C..		2							
Bb059	....	....	.....T....	....	....	C	.....T....	...CC..C..	....C..		1							
Bb060	....	....	....	....	........T.	T.........	....	...CC.....	....C..		4							
Bb061	....A.....	....	....	....	...T....T.	C	TT........	...CC..C..	....C..		1							
Bb062	....	....	.....T....	....	....	....	....	....C..C..	....C..		1							
Bb063	....	C	....	....	....	....	.....T....	...CC..C..	....		1							
Bb064	....	....	....	....	....	T.........	.....T....	...CC..C..	....C..		2							
Bb065	....	.A........	C.........	....	....	.......ACC	T....T....	...CC..C..	....C..		1							
Bb066	....	....	....	....	..C.......	....	T....T....	...CC..C..	....C..		2							
Bb067	....	.A........	....	....	....	....	....	...CC.....	....C..		1							
Bb068	....	....	....	....	....	T........C	.....T....	...CC..C..	....C..		1							
Bb069	....A.....	....	....C.....	.....A....	........T.	A.........	....C.A...	...CC..C..	....C..		1							
Bb070	....	....	....	....	....	....	....	...CC..C..	....		1							
Bb071	....	....	....	....	....	....	.....T..G.	...CC..C..	....C..		1							
Bb072	....	....	....	....	....	....	....C.....	...CC..C..	....C..		1							
Bb073	....	....	....	....	....	....	....	...CC.....	....C..		3							
Bb074	....A.....	....	.C........	....	....	....	.....T....	...CC..C..	....		1							
Bb075	....	....	....	....	....	....	.T........	...CC..C..	....C.G		1							
Bb076	....A.....	....	....	........T.	.....A....	C	T....T..G.	...CC..C..	....C..		1							

Appendix *Continued*

	Nucleotide position									Area and frequency§								
	1111112	2334566677	1111 7788892222	1111111111 2344455566	1111222222 7789001112	2222222222 2233344445	2222222222 5555677777	2222222233 8899999900	3333333 0011233									
No	6790145670	3584805612	3623531268	9534508901	7977280232	3423627892	3567201689	5934567902	3425546	1	2	3	4	5	6	7	8	9
Bb077	....	....	....	....	....	....	....	....C..C..	....C..		2							
Bb078	....	....	....	....	....	......A..C	T.........	...CC..C..	....C..		1							
Bb079	....	.....G....	....C.....	....	....	G.....A...	....	....C..C..	....C..		1							
Bb080	....	.A........	....	....	........T.	....	....C.....	....C..C..	....C..		1							
Bb081	....	....	....	....	.......T..	....	....	...CC.....	....C..		4							
Bb082	....	....	....CT....	....	....	AC........	....C.A.G.	...CC..C..	....C..		1							
Bb083	....	....	....C.....	....	....	G.........	....	...CC..C..	....C..		1							
Bb084	....A.....	....	....C.....	....	........T.	AC........	....C.A...	...CC..C..	....C..		1							
Bb085	....	....	....	....	....	T.........	....	...CC.....	....		1							
Bb086	....	....	....	....	....	.C........	....	...CC..C..	....C..		1							
Bb087	....	....	....C.....	....	.......T..	....	....	...CC..C..	....C..		1							
Bb088	....A.....	....	.....T..C.	....	....	C	TT........	...CC.GC..	....C..		1							
Bb089	..A.A.....	....	....C.....	....	....	A..C.....G	T.........	G..CC..C..	....C.G		1							
Bb090	....A.....	....	....	....	....	.....C...C	TT...T....	...CC..C..	....C..		1							
Bb091	....	....	....	....	....	....	.....T....	....C..C..	....C..		1							
Bb092	....	....	....	....	....	.....CA..C	T.........	...CC..C..	....C..		1							
Bb093	....	....	....	....	.......T..	....	....	...CC..C..	....C..		2							
Bb094	....A.....	....	....	....	.......T..	.....C...C	TT........	...CC..C..	....C..		1							
Bb095	....	....	.....T....	....	........T.	T.........	....	....CG....	....		1							
Bb096	....	....	....	....	....A.....	.C........	....	...CC..C..	....C..		2							
Bb097	....	....	....	.........T	A.........	....	....	....	....C..		1							
Bb098	....	....	....	....	....	....	T.........	...CC..C..	....		1							
Bb099	....	....	....	....	....	....G.....	.....T....	....C..C..	....C..		1							
Bb100	....A.....	....	....	....	....	C	T....T....	...CC..C..	....C..		1							
Bb101	....	....	....C.....	....	........T.	A....C....	T...CT....	....C..C..	....C.G		1							
Bb102	....	....	....CT....	...G.A....	....	....G.....	....	...CC..C..	....C..		1							
Bb103	....	.A........	C.........	....	....	....	.....T....	...CC..C..	....C..		2							
Bb104	....	....	....	....	.A........	......A...	T.........	...CC..C..	....C..		1							
Bb105	....	....	..C.......	....	....	....	....	...CC..C..	....C..		1							
Bb106	....	....	....	..G.......	....	....	.....T....	...CC.GC..	....C..		1							
Bb107	....A.....	..T.......	.....T....	....	....	.....CA..C	TT........	...CT..C..	....C..		1							
Bb108	....	....	....	....	....	......A...	....	...CC..C..	....C.G		1							
Bb109	....	....	....	....	.......T..	....	....	...CC.....	....		1							
Bb110	....	....	....	....	...T......	....	....	...CC..C..	....C..		1							
Bb111	....	....	....	....	........T.	....	....	...CC..C..	....C..		2							
Bb112	....A.....	....	....	....	....	.......A.C	TT........	...CC..C..	....C..		1							
Bb113	....	....	....	....	........T.	T.........	....	...CC..C..	....		1							
Bb114	G.........	....	....	....	....	....	.....T....	...CC..C..	....C..		1							

Appendix *Continued*

	Nucleotide position									Area and frequency§								
No	1111112 6790145670	2334566677 3584805612	1111 7788892222 3623531268	1111111111 2344455566 9534508901	1111222222 7789001112 7977280232	2222222222 2233344445 3423627892	2222222222 5555677777 3567201689	2222222233 8899999900 5934567902	3333333 0011233 3425546	1	2	3	4	5	6	7	8	9
Bb115	....	....	.....T....	....	....	T.........	....	....C..C..	....C..		1							
Ba116	-.A.TT....	..T.A.A.C.	..C....A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TAT....			1	8					
Ba117	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TAT....			1	12	1				
Ba118	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...			1	9					
Ba119	-.A.TT....	..T.A.A.C.	..CT...A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT..G			1	7					
Ba120	-.A.TT....	..T.A.A.C.	..CT...A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...			2	7					
Ba121	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AG.T...TA	TATT...				21					
Ba122	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TATT...				2					
Ba123	-.A.TT....	..T.A.A.C.	..CT...A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AG.T...TA	TATT...				8					
Ba124	-.A.TT....	..T.A.A.C.	..CTC..A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TAT....				1					
Ba125	-.A.TT....	..T.A.A.C.	..CT...A.G	C.........	.....A..T.	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...				5					
Ba126	-.A.TT....	..T.A.A.C.	..C....A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TAT....				2					
Ba127	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TAT....				4					
Ba128	-.A.TT....	T.T.A.A.C.	..CTC..A..	C.........	.....A....	A.....AA..	.T.....TC.	.AGCT...TA	TATT...				1					
Ba129	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AG.T...TA	TAT....				3					
Ba130	..A.TT...A	..T.A.A.C.	..CT...A..	C.........	....	A.....AA..	.T.G...TC.	.A..T...TG	TATT...				1					
Ba131	-.A.TT....	..T.A.A.C.	..C....A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TAT...G				5					
Ba132	-.A.TT....	..T.A.A.C.	..CT.T.A.G	C.........	.....A..T.	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...				3					
Ba133	..A.CT....	..T.A.A.C.	..C....A..	C.........	.....A....	AC....AA..	.T.G...TC.	.AGCT...TA	TAT....				3					
Ba134	-.ACTT....	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...				1					
Ba135	..A.CT....	..T.A.A.C.	..C....A..	C.........	.......T..	AC....AA..	.T.G...TC.	.AGCT...TA	TAT....				2					
Ba136	-.A.TT....	..T.A.A.C.	..CT.T.A..	CT........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TAT....				1					
Ba137	-.A.TT....	..T.A.A.C.	..C..T.A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TAT....				5					
Ba138	-.A.TT...A	..T.A.A.C.	..CT.T.A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TATT...				1					
Ba139	..ACCT....	..T.A.A.C.	..C....A..	C.........	.....A....	AC....AA..	.T.G...TC.	.AGCT...TA	TAT....				1					
Ba140	-.A.TT....	..T.A.A.C.	..CT.T.A..	C.........	.....A..T.	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...				3					
Ba141	..A.CT....	..T.A.A.C.	..C....A..	C.........	.....A.T..	AC....AA..	.T.G...CC.	.AGCT...TA	TAT....				2					
Ba142	-.A.TT....	..T.A.A.C.	..C....A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AG.T...TA	TATT...				1					
Ba143	-.A.TT....	..T.A.A.C.	..C....A..	C.........	.....A....	A.....AA..	.T.G...CC.	.AG.T...TA	TAT....				1					
Ba144	-.A.TT....	..T.A.A.C.	..CT...A..	C.........	A....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TATT...				1					
Ba145	..A.CT....	..T.A.A.C.	..C....A..	C.........	.....A.T..	AC....AA..	.T.G...TC.	.AGCT...TA	TAT....				1					
Ba146	-.A.TT....	..T.A.A.C.	..C....A..	....	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	TAT....				2					
Ba147	-.A.TT....	..T.A.A.C.	..CT...A..	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TAT....				1	6				
Ba148	-.A.TT....	..T.A.A.C.	..C....A..	C.........	.....A..T.	A.....AA..	.T.G...CC.	.AGCT...TA	TAT...G				1					
Ba149	..A.CT....	..T.A.A.C.	..C....A..	C.........	.....A..T.	AC....AA..	.T.G...TC.	.AGCT...TA	TAT....				1					
Ba150	..A.TT...A	..T.A.A.C.	..CT...A..	C.........	....	A.....AA..	.T.G...TC.	.AG.T...TG	TATT...					21				
Ba151	.GA.A--...	TC..A.A.C.	..CTC....G	C.........	A....A..T.	A.....AA..	.T.G...TCA	.AGCT...T.	CATT...						4			
Ba152	-.A.AT....	....A.A.C.	..CTC....G	C......C..	.....A....	T.....AA..	.TC....TC.	.A.CA...TA	TAT....						1			

Appendix *Continued*

No	Nucleotide position 1111112 6790145670	2334566677 3584805612	1111 7788892222 3623531268	1111111111 2344455566 9534508901	1111222222 7789001112 7977280232	2222222222 2233344445 3423627892	2222222222 5555677777 3567201689	2222222233 8899999900 5934567902	3333333 0011233 3425546	Area and frequency§ 1	2	3	4	5	6	7	8	9
Ba153	-GA.A--...	TC..A.A.C.	..CTC....G	C.........	.....A..T.	A.....AA..	.TCG...TCA	.AGCT...T.	CATT...						3			
Ba154	-.A.AT.TA.	....A.A.C.	..CTC....G	C......C..	A....A....	A.....AA..	.TC....TC.	.A.CA...TA	TATT...									2
Ba155	-.A.AT....	....A.A.C.	..CTCT...G	C......C..	A....A....	A.....AA..	TT.....TC.	.A.CA...TA	TATT...									2
Ba156	-.A.AT....	....A.A.C.	..CTC....G	C......C..	.....A....	A.....AA..	.TCG...TC.	.A.CA...TA	TATT...									2
Ba157	-.A.AT....	....A.A.C.	..CTC....G	C......C..	A....A....	A.....AA..	.TCG...TC.	.A.CA...TA	TATT...									3
Ba158	-.A.AT.TA.	....A.A.C.	..CTC....G	C......C..	.....A....	A.....AA..	.TCG...TC.	.A.CA...TA	TATT...									1
Ba159	-.A.AT....	....A.A.C.	..CTC....G	C......C..	.....A....	AC....AA..	.TCG...TC.	.A.CA...TA	TATT...									1
Ba160	-.A.AT....	....A.A.C.	..CTC....G	C......C..	.....A....	A.....AA..	..CG...TC.	.A.CA...TA	TATT...									3
Ba161	-.A.AT.TA.	....A.A.C.	..CTC....G	C......C..	.....A....	AC....AA..	.TCG...TC.	.A.CA...TA	TATT...									1
Ba162	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...							1	37	
Ba163	-.A.A--...	.C..A.A.C.	..CTC....G	C....A....	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	AATT...								2	
Ba164	-.A.A--...	.C..A.ACC.	..CTC....G	C....A....	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								6	
Ba165	-.A.A--...	.C..A.A.C.	..CTC.C..G	C.........	A....A..T.	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								1	
Ba166	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....AG...	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								7	
Ba167	..A.A--...	.C..A.A.C.	..CTC....G	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT..G								2	
Ba168	-.A.A--...	.C..A.A.C.	..CTC....G	C....A....	.....A....	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								2	
Ba169	-.A.A--...	.C..A.A.C.	..CTC.C..G	C.........	A....A....	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								5	
Ba170	-.A.AT....	.C..A.A.C.	..CTC....G	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...								7	
Ba171	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....A....	A.....AA..	...G...TC.	.AGCT...TA	CATT...								4	
Ba172	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....A..T.	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								1	
Ba173	..A.AT....	.C..A.A.C.	..CTCT...G	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								7	
Ba174	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....A....	A.....AA..	.T.G...CC.	.AGCT...TA	CATT...								2	
Ba175	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....AG...	AC....AA..	.T.G...TC.	.AGCT...TA	CATT...								1	
Ba176	..A.AT....	.C..A.A.C.	..CTC....G	C.........	.....A..T.	AC....AA..	.T.G...TC.	.AGCT...TA	CATT...								1	
Ba177	-.A.AT....	.C..A.A.C.	..CTCT...G	C.........	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...								1	
Ba178	-.A.AT....	.C..A.A.C.	..CTC....G	C.........	A....A....	A.....AA..	.T.G...TC.	.AGCT...TA	TATT...								1	
Ba179	-.A.A--...	.C..A.A.C.	..CTC.C..G	C.........	A....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								1	
Ba180	-.A.A--...	.C..A.A.C.	..CTC.C..G	C.........	.....A....	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								1	
Ba181	-.A.A--...	.C..A.A.C.	..CTC.C..G	C.........	A....A....	A.....AA..	.T.G..ATC.	.AGCT...TA	CATT...								1	
Ba182	-.A.A--...	.C..A.A.C.	..CTC.C..G	C.........	A....A..T.	A.....AA..	.T.G...TC.	.AG.T...TA	TATT...								1	
Ba183	-.A.A--...	.C..A.A.C.	..CTC....G	C....A....	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								1	
Ba184	-.A.A--...	.C..A.A.C.	..CTCT...G	C....A....	.....A....	A.....AA..	.T.G...TC.	.AGCT...TA	CATT...								3	
Ba185	-.A.A--...	.C..A.A.C.	..CTCT...G	C....A....	.....A....	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								4	
Ba186	-.A.A--...	.C..A.A.C.	..CTC....G	C....A....	.....A...G	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								1	
Ba187	-.A.A--...	.C..A.A.C.	..CTCT...G	C....A....	.....A...G	A.....AA..	.T.G...TC.	.AG.T...TA	CATT...								2	

Notes: §Area: 1: *B. bonaerensis* (Brazil), 2: *B. bonaerensis* (Antarctic), 3: *B. acutorostrata* (eastern North Pacific), 4: *B. acutorostrata* (western North Pacific), 5: *B. acutorostrata* (Sea of Japan), 6: *B. acutorostrata* (Brazil), 7: *B. acutorostrata* (Mediterranean Sea), 8: *B. acutorostrata* (Antarctic), 9: *B. acutorostrata* (North Atlantic).