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Worldwide structure of mtDNA diversity among Cuvier's beaked whales (*Ziphius cavirostris*): implications for threatened populations

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Abstract

We present the first description of phylogeographic structure among Cuvier's beaked whales (*Ziphius cavirostris*) worldwide using mitochondrial DNA (mtDNA) control region sequences obtained from strandings ($n = 70$), incidental fisheries takes ($n = 11$), biopsy ($n = 1$), and whale-meat markets ($n = 5$). Over a 290-base pair fragment, 23 variable sites defined 33 unique haplotypes among the total of 87 samples. Nucleotide diversity at the control region was relatively low ($\pi = 1.27\% \pm 0.723\%$) compared to wide-ranging baleen whales, but higher than strongly matrifocal sperm, pilot and killer whales. Phylogenetic reconstruction using maximum likelihood revealed four distinct haplotype groups, each of which displayed strong frequency differences among ocean basins, but no reciprocal monophyly or fixed character differences. Consistent with this phylogeographic pattern, an analysis of molecular variance showed high levels of differentiation among ocean basins ($F_{ST} = 0.14$, $\Phi_{ST} = 0.42$; $P < 0.001$). Estimated rates of female migration among ocean basins were low (generally ≤ 2 individuals per generation). Regional sample sizes were too small to detect subdivisions within oceans except in the North Atlantic, where the Mediterranean Sea ($n = 12$) was highly differentiated due to the presence of two private haplotypes. One market product purchased in South Korea grouped with other haplotypes found only in the North Atlantic, suggesting a violation of current agreements banning international trade in cetacean species. Together, these results demonstrate a high degree of isolation and low maternal gene flow among oceanic, and in some cases, regional populations of Cuvier's beaked whales. This has important implications for understanding the threats of human impact, including fisheries by-catch, direct hunting, and disturbance or mortality from anthropogenic sound.

Keywords: cetacean, genetic diversity, mitochondrial DNA, odontocete, phylogeography, Ziphiidae

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Introduction

Although the marine environment lacks obvious geographical barriers, most cetaceans (whales, dolphins and porpoises) are confined to a single ocean basin. The better known exceptions include some baleen whales, sperm whales (*Physeter macrocephalus*) and killer whales (*Orcinus orca*), which are found worldwide. Among the beaked whales (Ziphiidae), a speciose but little known family of odontocetes (toothed whales), only Cuvier's beaked whale (*Ziphius cavirostris*) and the densebeaked whale (*Mesoplodon densirostris*) have worldwide distributions. Of these, Cuvier's beaked whale (referred to here as *Ziphius*) has a more extensive range and is found in the deep offshore waters of all oceans from the tropics to cold-temperate zones, between *c*. 60°N and 55°S (Jefferson *et al*. 1993).

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Ziphius is a monotypic genus and is considered to constitute a single global species (Rice 1998). Although the widespread occurrence of *Ziphius* is well known, there is little information on local distribution and population structure. A great deal of morphological variation has been documented, including apparent regional differences in pigmentation patterns and cranial osteological features (Heyning 1989), suggesting that locally distinct populations may exist. While sightings of *Ziphius* at sea are relatively rare, more sightings and strandings have been reported for this species than for any other ziphiid and it may be among the most common of all beaked whales (Heyning 2002). Abundance estimates are currently available only for US waters and may be unreliable due to the general rarity of sightings and deep-diving behaviour of this species (Carretta *et al*. 2004). In some regions however, the abundance of *Ziphius* could exceed that of sperm whales [e.g. estimates of 12 728 (CV = 0.83) vs. 7082 (CV = 0.30), respectively, in Hawaiian waters; Barlow 2003].

Historically, *Ziphius* was taken opportunistically off the Pacific coast of Japan as part of an ongoing hunt for Baird's beaked whales (*Berardius bairdii*; Omura & Kimura 1955; Nishiwaki & Oguro 1972). Although no longer hunted commercially, products from this species are still found for sale on the markets of Japan and South Korea (Dalebout *et al*. 1998), indicating that *Ziphius* suffers from by-catch or undocumented direct exploitation in this region. *Ziphius* has been hunted in small numbers in the Lesser Antilles, Indonesia, Peru and Chile (Reeves 1988; Jefferson *et al*. 1993; Rudolph *et al*. 1997; Van Waerebeek *et al*. 1999), and taken incidentally in fisheries (by-catch) off the Pacific coast of the USA (Julian & Beeson 1998).

Recent atypical mass strandings of beaked whales have been linked to high-powered navy sonar and seismic exploration (Simmonds & Lopez-Jurado 1991; Mignucci-Giannoni 1996; Frantzis 1998; Rosario-Delestre *et al*. 1999; Evans *et al*. 2001; Anonymous 2003; Jepson *et al*. 2003; Frantzis 2004). For reasons that are still unclear, beaked whales appear to be more susceptible to anthropogenic sound than other marine mammals. Deployment of military sonar has led to strandings of beaked whales suffering from chronic and acute tissue damage due to the *in vivo* formation of gas bubbles, which may be the result of decompression sickness (Jepson *et al*. 2003). Several beaked whale species have been involved in these atypical mass strandings, but *Ziphius* is the most commonly affected.

Here we examine population genetic structure among *Ziphius* worldwide based on a 290-base pair (bp) fragment of the mitochondrial DNA (mtDNA) control region from 87 animals. These data allowed us to assess the following: (i) the possibility that multiple taxa exist within what is currently recognized as a monotypic genus; (ii) the potential for isolation and differentiation among the three ocean basins — the North Atlantic, North Pacific, and Southern Hemisphere; and (iii) the potential for isolation and differentiation among regions within ocean basins. To evaluate the risk of depletion of local populations of *Ziphius* from human activities, information on population structure and gene flow among geographical regions is required. If these activities were to displace or extirpate *Ziphius* in one locale, it is not known whether a distinct genetic unit would be irreversibly affected or whales from other areas would move in to recolonize. If linked by seasonal migration, a mortality event in one area could also affect populations in multiple regions (Baker *et al*. 1990; Bowen *et al*. 1995).

Materials and methods

Sample collection

A total of 87 *Ziphius* from the North Atlantic, North Pacific, and Southern Hemisphere were sampled, representing much of this species' worldwide distribution (Fig. 1). The majority of samples consisted of fresh tissue obtained from dead stranded animals ($n = 61$) or by-catch ($n = 11$). Nine samples consisted of bone, teeth or dried skin from stranded specimens held in museums or other collections. One sample was a biopsy from a free-swimming animal. Five samples were whale-meat products purchased from the commercial markets of Japan and Korea, as part of the ongoing molecular monitoring of cetaceans for sale on these markets (e.g. Baker & Palumbi 1994; Baker *et al*. 2000). Given the ban on the international trade of cetaceans under CITES (Convention on International Trade in Endangered Species), market products were assumed to originate from coastal by-catch around Japan or Korea (although analysis of one product suggested otherwise; see Results). See Appendix for a full list of specimens. Known cow — calf pairs, inferred from size/age, sex and reproductive status (i.e. lactating adult female accompanied by a juvenile animal of a size unlikely to be independent) were counted as a single sample for these mtDNA-based analyses.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was isolated from fresh tissue samples using either proteinase K digestion and phenol–chloroform following standard methods (Sambrook *et al*. 1989), as modified by Baker *et al*. (1994), or the Chelex method (Walsh *et al*. 1991). For historical samples (bone, tooth and dried skin), all work including preparation of reagents was conducted in a separate 'ancient DNA' laboratory in which no work on modern cetacean tissue had been conducted. The silica-based method (Höss & Pääbo 1993), as modified by Matisoo-Smith *et al*. (1997), was used to extract DNA from these specimens (see Pichler *et al*. 2001 for details).

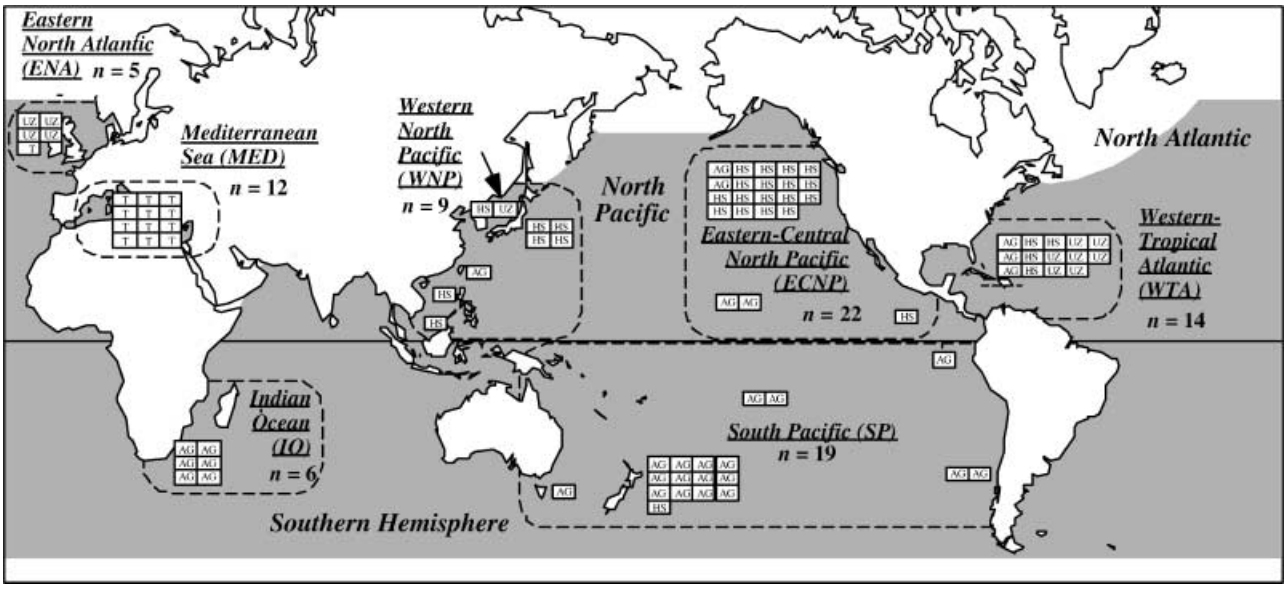

Fig. 1 Number and source locations of *Ziphius* samples (n = 87) available for this study. Blocks indicate haplotype group affinity (AG, HS, T or UZ) of samples by location (see Results). Dashed lines indicate regional groupings within ocean basins. Grey shading shows approximate range of this species. See Appendix for sample details.

Samples were amplified and sequenced as they were collected or received over a period of *c*. 10 years. As a result, amplification conditions, primers and sequencing methods varied. Most fresh tissue samples collected prior to the year 2000 are represented by a 500-bp fragment of the variable 5′ end of the mtDNA control region, amplified using the primers, M13Dlp1.5 [t-pro-whale]-L (5′-TGTAAAACGACGGCCAGTTCACCCAAAGCTGRARTTCTA-3′) and Dlp5-H (5′-CCATCGWGATGTCTTATTTAAGRGGAA-3′; Dalebout *et al*. 1998). Samples from Greece are represented by a 1000-bp control region fragment amplified using the universal primers of Hoelzel *et al*. (1991); 1-L (5′-TTCCCCGGTCTTGTAAACC-3′) and 2-H (5′-ATTTTCAGTGTCTTGCTTT-3′). The majority of fresh tissue samples collected after 2000 are represented by a 750-bp fragment of the control region amplified using the primers, M13-Dlp1.5 and Dlp8G (5′-GGAGTACTATGTCCTGTAACCA-3′; Lento *et al*. 1997). For historical material, only a smaller fragment of the control region (*c*. 300 bp) was amplified successfully in most cases, as expected from uncontaminated extractions from such material (Höss *et al*. 1996). This smaller fragment was amplified using the primers, M13Dlp1.5-L and Dlp4-H (5′-GCGGGWTRYTGRTTTCACG-3′; C. S. Baker, unpublished). The latter primer nests within Dlp5-H. A small number of stranded specimens were also represented only by this smaller fragment due to extensive decomposition. For a primer map see Dalebout *et al*. (2004) or visit http://www.DNA-surveillance.auckland.ac.nz. PCR (polymerase chain reaction) amplification from fresh tissue samples followed standard protocols (Palumbi 1996). Bovine serum albumin (BSA) solution was added to amplifications of DNA from osteological and degraded samples (final concentration; 0.5–1 mg/mL) to overcome the effects of inhibitors that often accumulate in such material (Pääbo 1990).

PCR products were sequenced either manually, with 35S radio-labelling of nucleotide sequenase terminators (fresh tissue samples obtained prior to 1997), or by automation using fluorescent chemistry. For manual sequencing, double-stranded PCR products were bound to streptavidin-coated, paramagnetic beads (Dynal Corp.) by a biotin group attached to the 5′ end of one of the primers. The unbound strand was stripped with 0.1 M NaOH, and the attached strand was sequenced using Sequenase™ and standard solid-phase protocols (Hultman *et al*. 1989). For automated sequencing, BigDye™ Dye Terminator Chemistry was used, and reactions were run on either an ABI 377, modified ABI 373, or ABI 3100 Automated Sequencer (Applied Biosystems, Inc.). A proportion of samples (27%) were sequenced in both directions, or twice in the same direction, to confirm variable sites.

Molecular sexing

The sex of specimens was identified from morphological features or via molecular sexing. For molecular sexing, one or more of the following protocols was used for each specimen: (i) the ZFX-ZFY method of Palsbøll *et al*. (1992) with *Taq*I digestion; (ii) the SRY method of Richard *et al*. (1994), for which a fragment of mtDNA was co-amplified as a positive control (Gowans *et al*. 2000); or (iii) the SRY plus ZFX-ZFY method of Gilson *et al*. (1998).

Phylogenetic reconstruction and analysis of population structure

The program SEQUENCHER 3.1.1 (Gene Codes Corp.) was used to align sequences and confirm polymorphic sites. A Nexus file of checked sequences was exported to MACCLADE version 4.0 (Maddison & Maddison 1992) to determine the position and number of polymorphic sites on the basis of which haplotypes were designated. Twenty-six haplotypes (A–Z) were defined in previous analyses using a smaller number of samples (Dalebout 2002). The seven new haplotypes found among the additional samples available for the current analyses were named based on their similarity to haplotypes identified previously.

Phylogenetic relationships among haplotypes were reconstructed using maximum likelihood (ML), neighbour joining (NJ), and maximum parsimony (MP), as implemented in PAUP* (Swofford 1999), using homologous sequences from related ziphiid species as an outgroup. For ML, an HKY + G model of sequence evolution was used, as derived by MODELTEST 3.06 (Posada & Crandall 1998), with estimated nucleotide frequencies A = 0.3378, C = 0.2211, G = 0.1202, and T = 0.3209, transition/transversion ratio = 4.8094, and g(alpha) shape parameter = 0.1831. For comparison, ML analyses were also conducted using a GTR + G + I model estimating all parameters. Heuristic search conditions for ML used starting trees obtained by stepwise addition with 10 random sequence addition replicates and tree-bisection–reconnection (TBR) branch swapping. For NJ, Kimura 2-parameter distances were used to correct for multiple substitutions, using minimum evolution as the default optimality criterion. For MP, heuristic search conditions were as for ML but with searches limited to 1 000 000 rearrangements for each replicate. The robustness of phylogenetic groupings was assessed by bootstrap resampling (replicates: NJ—1000 NJ; MP—1000 fast stepwise; ML—200 fast stepwise). Bremer support was calculated using TREEROT version 2a (Sorensen 1999) based on the MP strict consensus tree. A minimum-spanning network of haplotype relationships based on uncorrected '*p*' distances was constructed using the program, MINSPNET (Excoffier 1993), as implemented in ARLEQUIN version 2.000 (Schneider *et al*. 2000).

To investigate phylogeographic structure, specimens were grouped by ocean basin and region within ocean basin as follows: Southern Hemisphere (SH), $n = 25$ [South Pacific (SP), $n = 19$; Indian Ocean (IO), $n = 6$]; North Pacific (NP), $n = 31$ [Eastern-Central (ECNP), $n = 22$; Western (WNP), $n = 9$]; North Atlantic (NA), $n = 31$ [Eastern (ENA), $n = 5$; Mediterranean (MED), $n = 12$; Western-Tropical (WTA), $n = 14$; Fig. 1]. Standard indices of genetic variation (nucleotide diversity, π, and haplotype diversity, h) were calculated over all available samples, for each ocean basin, and for each region within ocean basin using ARLEQUIN. Analyses of molecular variance (AMOVA) incorporating both Φ_{ST} and F_{ST} (Weir & Cockerham 1984) were used to investigate differentiation among ocean basins and regional groupings. The Φ_{ST} takes into account the relationships between haplotypes based on molecular distance, while the F_{ST} is based only on the difference in overall haplotype frequencies (Excoffier *et al*. 1992). Due to the relatively low levels of intraspecific diversity observed, uncorrected '*p*' distances were used for Φ_{ST} analyses. The statistical significance of these values was tested by 20 000 permutations of the data. All statistical analyses of population structure were carried out using ARLEQUIN.

Due to our reliance on opportunistic methods of sample collection from strandings and by-catch, available samples were not random with respect to the known distribution of *Ziphius*. A disproportionately large number of samples were collected in the Mediterranean, where 10 of the 12 animals sampled stranded together in May 1996 in Greece (Frantzis 1998), and in the ECNP, where 10 of the 22 animals sampled were taken as by-catch in the California–Oregon thresher shark and swordfish gillnet fishery between August 1992 and December 1995 (Julian & Beeson 1998). Recognizing that clumped sampling could bias our results, additional analyses were run at the ocean basin and regional level to explore this problem. First, only the unique matrilines among the Greece animals were counted ($n = 2$), reducing the sample size for the Mediterranean to $n = 4$ overall. Second, the California by-catch animals were similarly reduced to unique matrilines ($n = 4$), reducing the sample size for the ECNP to $n = 16$ overall. Third, to further assess the validity of combining these latter samples with those from other sources in the ECNP, the genetic profile of the California by-catch sample was compared to that of stranded animals from the same region ($n = 11$).

Estimates of effective population size and migration between ocean basins

To estimate long-term effective population size and migration rates (i.e. effective number of female migrants per generation) among ocean basins, we used the ML coalescent approach implemented in the program MIGRATE version 2.0.3 (Beerli & Felsenstein 1999, 2001). MIGRATE simultaneously estimates θ, the product of effective population size and mutation rate ($2N_e\mu$ for mtDNA, where μ is the per-generation mutation rate), and $N_e m(f)$, the product of effective population size and migration rate. Default parameters were used, with transition/transversion ratio = 4.8 (estimated via MODELTEST), starting estimates for θ based on F_{ST} calculations, burn-in = 100 000 trees, 10 short chains, with a total of 100 000 genealogies, and 10 long chains, with a total of 1 000 000 genealogies. Mean values and lower- and upper-profile likelihood percentiles (0.025 and 0.975, which approximate the 95% confidence intervals; Beerli & Felsenstein 1999) for $N_{e(f)}$ and

$N_e m(f)$ are reported from calculations based on the results from 10 replicate runs. In addition, mean estimates of θ derived by the program were used as starting values for an additional run to check the consistency of results.

No estimates of female generation time or control region mutation rate are available for *Ziphius*. Beaked whales first appear in the fossil record in the early mid-Miocene [~20 million years ago (Ma)], but the origins of the modern genera are not clear (Mead 2002). The Ziphiidae likely diverged from the Platanistidae (river dolphins) between 20 and 40 Ma, after the ancestor of both these groups diverged from the Physeteridae (sperm whales; Cassens *et al*. 2000). As such, only a rough estimate can be made for the origin of *Ziphius* (20–40 Ma). Given an HKY distance between *Ziphius* and susu (*Platanista minor*, AJ554058; Arnason *et al*. 2004) of 0.4867, we estimated the mtDNA control region mutation rate to be $0.6–1.2 \times 10^{-8}$ bp per year. This is comparable to the estimate of $0.7–1.0 \times 10^{-8}$ bp per year for baleen whales of Baker *et al*. (1993), but somewhat lower than the estimate of 1.5×10^{-8} bp per year based on the diversification of *Balaenoptera* (Pesole *et al*. 1999) or 2.0×10^{-8} bp per year based on the diversification of *Balaena–Eubalaena* (Rooney *et al*. 2001).

The maximum recorded age of female *Ziphius* based on tooth growth layer groups (GLGs) is 30 years (Mead 1984), but no data are available for age of sexual maturity. In the related ziphiid species, the northern bottlenose whale (*Hyperoodon ampullatus*), the minimum age of female sexual maturity is 7 years (Benjaminsen & Christensen 1979). We therefore used 15 years as an estimate of generation time (the average age of mothers giving birth if fecundity remains constant with age) for *Ziphius*.

Results

Genetic diversity and haplotype distribution

A fragment of the 5′ end of the mtDNA control region was successfully amplified from all 87 *Ziphius* samples available for this study. Due to differences in sample quality and changes in methodology over time, the total length of sequence obtained varied among samples, ranging from *c*. 300 bp to 750 bp. For the analyses presented here, all sequences were truncated to the length of the shortest sequence obtained, 290 bp. Although comparatively short, this segment encompasses the most variable portion of the ziphiid control region (Dalebout *et al*. 2004). Examination of this 290-bp segment revealed 23 variable sites defining 33 unique haplotypes (Table 1).[1] Of these variable sites, 17 were transition substitutions, three were transversions, two incorporated both substitution types, and one was insertion-deletion. Haplotypes differed from one another by 1 to 8 bp (0.34% to 2.98%), with a mean of 4.7 bp (1.62%). The most common haplotype (L) was shared by 19.54% of whales sampled ($n = 17$), with second most common haplotype (A) shared by 13.79% of whales sampled ($n = 12$). Worldwide, haplotype diversity (h) was 0.926 ± 0.0154, and nucleotide diversity (π) was 1.27% ± 0.723%. Similar but slightly lower levels of genetic diversity were observed within each ocean basin (Table 2). Sequences representing all 33 haplotypes have been deposited in GenBank (Accession nos DQ068216–DQ068248).

[1]Examination of longer sequences (435 bp) held for a subset of individuals revealed only three additional variable sites beyond position 290 which split haplotype A into three new haplotypes but generated no other changes.

Phylogenetic relationships among haplotypes

In phylogenetic reconstructions, all *Ziphius* haplotypes grouped together to the exclusion of sequences from related ziphiids confirming the monophyly of mtDNA lineages for this worldwide species (bootstrap score, 100%; Fig. 2). Below the species level, phylogenetic reconstructions showed strong frequency differences in the oceanic distribution of haplotypes but no pattern of reciprocal monophyly or fixed character differences among oceans. ML analyses revealed four haplotype groups (labelled AG, HS, T and UZ; Fig. 2). Identical trees were obtained from analyses using HKY + G and GTR + G + I models. Very similar topologies were also recovered by NJ and MP, and by the unrooted minimum-spanning network (Fig. 3). All methods recovered the same haplotype groupings, though UZ was nested within T in NJ and MP trees. Bootstrap support for these haplotype groups was low (< 50%), but the HS and 'T + UZ' groups received some Bremer support (1) in MP reconstructions.

The frequencies of the four haplotype groups showed strong phylogeographic patterns among ocean basins. Of whales sampled in the Southern Hemisphere, 80% possessed AG haplotypes. In the North Pacific, only 14% possessed AG haplotypes, while 81% possessed HS haplotypes. All four haplotype groups were represented among animals sampled from the North Atlantic, with T and UZ haplotypes predominating (39% and 36% of whales sampled, respectively). Frequency patterns of the most common haplotype in each ocean basin followed that of the haplotype groups (Fig. 3): haplotype A was the most common in the Southern Hemisphere (44.4% of whales sampled); haplotype L was the most common in the North Pacific (41.9% of whales sampled); and, haplotype T3 was the most common in the North Atlantic (29% of animals sampled), followed by haplotype V (16% of animals sampled). Only four haplotypes were shared among ocean basins, and each ocean basin possessed a similar number of private haplotypes (North Atlantic, $n = 9$; North Pacific, $n = 11$; Southern Hemisphere, $n = 9$; Figs 2 and 3).

Table 1 Positions of the 23 variable sites within the 290 bp consensus segment of the *Ziphius* mtDNA control region, defining the 33 unique haplotypes used in these analyses. Dots indicate identity to the top sequence (haplotype A)

Haplotype	9 3	1 8	2 9	3 4	4 1	6 0	8 6	8 3	9 5	9 6	9 8	9 0 1	1 0 2	1 0 6	1 3 3	1 6 6	1 2 2	1 5 5	2 4 9	2 6 2	2 8 8	2 8 9
A	C	T	A	G	G	A	C	C	A	T	A	G	T	A	G	T	G	T	A	A	C	T	T
A2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
B2	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C
C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.
C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
D	?	?	?	?	?	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.
E	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.
F	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	C	.	.
F2	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	G	.	.	C	.	.
G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	G	.	C	.	.
H	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.
I	.	.	.	.	.	.	.	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.
J	.	.	.	A	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C
K	.	.	.	A	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
L	.	.	.	A	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.
M	T	.	.	A	.	–	T	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.
N	.	.	.	A	.	.	T	.	T	.	.	.	.	.	A	.	.	.	.	.	C	.	.
O	T	.	.	A	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.
P	A	.	.	A	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.
Q	.	.	.	A	.	.	T	.	T	.	.	.	.	.	.	.	.	.	G	.	C	.	.
R	?	?	.	A	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	.	G	C	.
S	.	.	.	A	.	.	T	T	T	.	G	.	.	.	.	.	.	.	.	.	C	.	.
T	.	C	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	C	.	.
T2	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	C	.	.
T3	.	.	.	.	.	.	.	.	T	.	G	.	.	T	.	.	.	.	.	.	C	.	.
T4	.	.	.	.	.	.	.	.	T	.	G	.	C	T	A	.	.	.	.	.	C	.	.
U	.	.	.	.	.	.	.	.	T	C	.	.	.	T	.	.	.	C	.	.	C	.	.
V	.	.	.	.	.	.	.	.	T	C	.	.	.	T	.	.	.	.	.	.	C	.	.
W	.	.	G	.	.	.	.	.	T	C	.	.	.	T	.	.	.	.	.	.	C	.	.
X	?	.	.	.	A	.	.	.	T	C	.	.	.	T	.	C	.	.	.	.	C	.	.
Y	.	.	.	.	A	.	.	.	T	C	.	.	.	T	.	.	.	.	.	.	C	.	.
Z	.	.	.	.	C	.	.	.	T	C	.	.	.	T	.	.	.	.	.	.	C	.	.

Table 2 Comparative haplotype (*h*) and percent nucleotide diversity (π) among *Ziphius* in different ocean basins and regions. Sex information is included for comparison. See Fig. 1 for regions abbreviated. ?, sex unknown

Level	*n*	*h*	Nuc. diversity percentage	Sex M	F	?
Ocean basin						
Southern Hemisphere	25	0.80 ± 0.078	0.77 ± 0.489	17	7	1
North Pacific	31	0.81 ± 0.065	0.87 ± 0.541	12	13	6
North Atlantic	31	0.89 ± 0.040	0.95 ± 0.574	20	11	0
Region within ocean basin						
Southern Hemisphere						
SP	19	0.82 ± 0.084	0.82 ± 0.521	13	6	0
IO	6	0.80 ± 0.172	0.78 ± 0.599	4	1	1
North Pacific						
ECNP	22	0.77 ± 0.080	0.77 ± 0.490	10	10	2
WNP	9	0.92 ± 0.092	1.34 ± 0.853	2	3	4
North Atlantic						
ENA	5	0.70 ± 0.218	0.28 ± 0.275	2	3	0
MED	12	0.41 ± 0.133	0.28 ± 0.244	9	3	0
WTA	14	0.95 ± 0.038	1.01 ± 0.678	9	5	0
	87			49	31	7

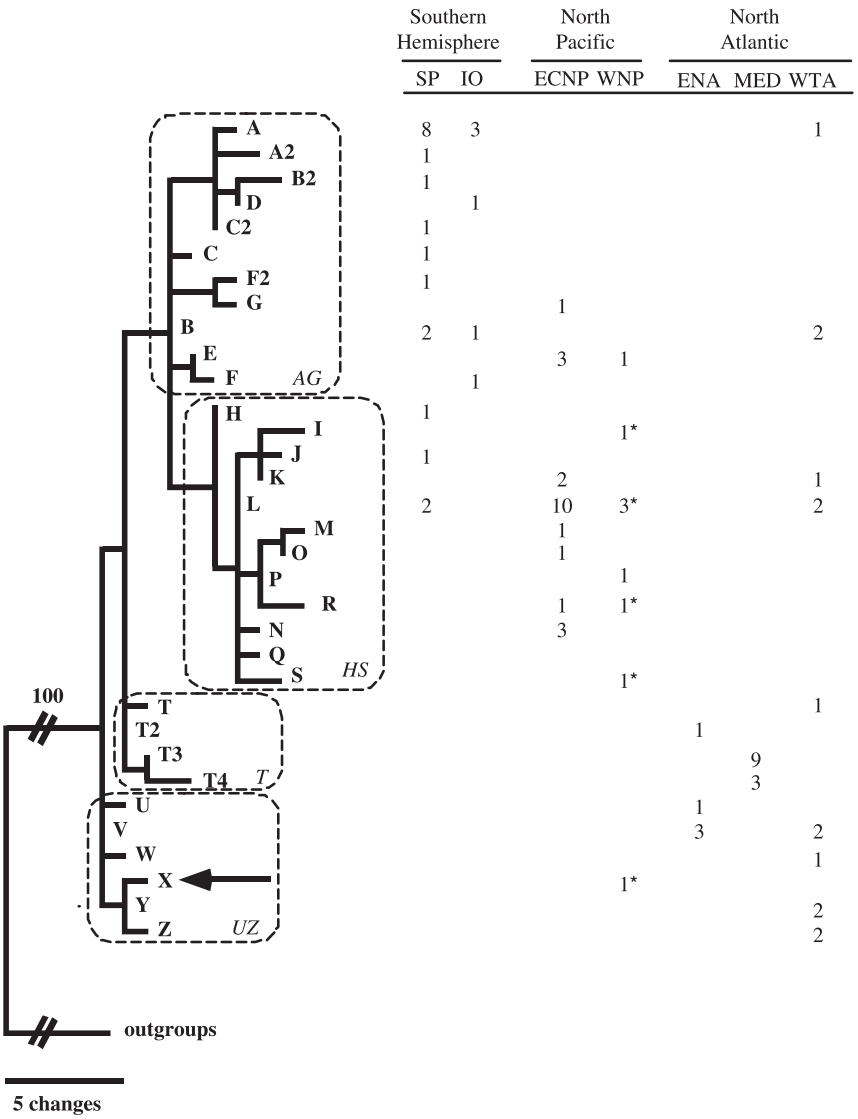

Fig. 2 Phylogenetic relationships among the 33 *Ziphius* haplotypes defined by the 290-bp consensus segment of the mtDNA control region based on ML analyses (–Ln likelihood score = 989.892). Representatives of other ziphiid genera (*Berardius bairdii*, *Hyperoodon ampullatus*, *Hyperoodon planifrons*, and *Tasmacetus shepherdi*) were used as an outgroup. Branch termini represent haplotypes labelled as in Table 1. Dashed-line boxes indicate the four main haplotype groups. Haplotype frequencies by region for each ocean basin are shown on the right. The arrow highlights haplotype X in the North Atlantic UZ group, which is represented by a single sample from the North Pacific, a whale-meat product purchased on the commercial markets of Korea. Asterisks highlight haplotypes represented by market products ($n = 5$).

Provenance of market products

Although whale-meat market products were assumed to originate from by-catch in coastal waters of Japan and Korea, the haplotype of one product was discordant with the phylogeographic pattern of other samples. Haplotype X, represented by a product purchased in Korea in 1994, grouped with UZ haplotypes otherwise found exclusively in the North Atlantic (Fig. 3). We considered three hypotheses for this: (i) UZ haplotypes also occur in the North Pacific but due to our small sample size, only one animal representing this haplotype group was found; (ii) reconstruction of relationships among haplotypes was not robust, and as such haplotype X grouped among the UZ haplotypes by chance; or (iii) the Korean market product originated from a North Atlantic animal. The first hypothesis is difficult to exclude without additional geographical sampling. The second hypothesis, however, was discounted by the results of a Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999). Forcing haplotype X into haplotype groups found in the Southern Hemisphere or North Pacific (AG or HS) resulted in a significantly less-likely tree ($P > 0.05$; Table 3). Further, haplotype X possesses a C at position 95, found only among UZ haplotypes, and a T at position 102, found only among UZ and T haplotypes (Table 1).

In phylogenetic reconstruction, ancestral haplotypes occupy central positions, have the highest frequencies, and the greatest number of mutational links to less common haplotypes (Donnelly & Tavare 1986; Crandall & Templeton 1993). Older alleles in subdivided populations also have wider distributions (Takahata 1988). The AG and HS haplotypes that are shared among ocean basins possess these characteristics. Following this predicted pattern, the UZ or T haplotypes most likely to be found in other ocean basins would be V, or T2 and T3, respectively. In contrast, haplotype X is not central or common, and has only a single

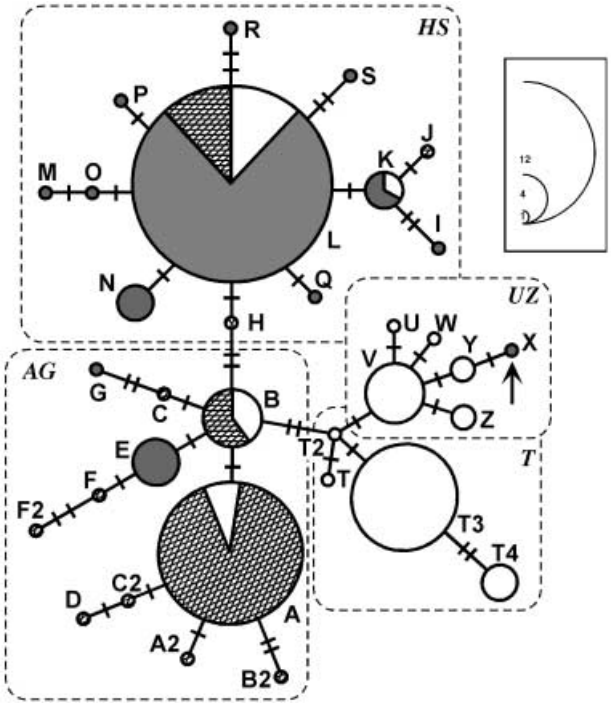

Fig. 3 Minimum spanning network of relationships among the 33 *Ziphius* haplotypes. Circles (nodes) represent haplotypes labelled as in Table 1. Haplotype nodes are scaled to overall frequency of occurrence. Each crossbar indicates a single nucleotide substitution. Dashed-line boxes indicate the four main haplotype groups. No alternative connections linking haplotypes from different groups were found. Node shading indicates frequency by ocean basin: North Atlantic, white; North Pacific, grey; Southern Hemisphere, hatched. The arrow highlights the position of haplotype X (see Results).

link to another haplotype (Y) which is also not common (Fig. 3).

Overall, these observations add support to the hypothesis that haplotype X likely originated in the North Atlantic, as do other *UZ* haplotypes. Given the possibility that this Korean market sample may therefore not represent a local Western North Pacific animal, it was excluded from subsequent phylogeographic comparisons. The other market samples (Japan, $n = 3$; Korea, $n = 1$) represent *HS* group haplotypes (Fig. 2) and, as such, were assumed to represent local by-catch.

Phylogeographic patterns among ocean basins and regions

The AMOVA showed strong differentiation among the three ocean basins at both the haplotype and nucleotide level ($F_{ST} = 0.14$; $\Phi_{ST} = 0.42$; $P < 0.0001$; Table 4).[2] Pairwise comparisons confirmed that all three ocean basins were significantly different from one another. Hierarchical AMOVAs grouping animals by region within ocean basin revealed significant structure at both levels (Table 4), but pairwise comparisons revealed that the regional effect was driven largely by the Mediterranean sample. The Mediterranean differed significantly from the Eastern North Atlantic ($F_{ST} = 0.49$, $P < 0.001$) and Western Tropical Atlantic ($F_{ST} = 0.31$, $P < 0.001$), but the latter two regions did not differ significantly from one another ($F_{ST} = 0.08$, $P = 0.0971$). The large effect size of these comparisons was due partly to the low diversity of the Mediterranean sample, which was approximately half that observed in other regions ($h = 0.41 \pm 0.13$, $\pi = 0.28\% \pm 0.244\%$; Table 2). The majority of Mediterranean animals (75%) shared a single haplotype (T3; Fig. 2).

Additional analyses conducted with a reduced sample size for the Mediterranean and Eastern-Central North Pacific were consistent with results from the full analysis. Reduction of the Greece mass stranding sample ($n = 10$) to two unique matrilines (i.e. Mediterranean, $n = 4$ matrilines overall) had little affect on comparisons among ocean basins ($F_{ST} = 0.13$, $\Phi_{ST} = 0.40$; $P < 0.0001$). Overall genetic variance among regions within oceans was reduced but remained significant ($F_{ST} = 0.05$, $P = 0.0384$; $\Phi_{ST} = 0.06$, $P = 0.0184$). For pairwise comparisons within the North Atlantic, levels of differentiation were also lower but still significant (MED vs. ENA — $F_{ST} = 0.39$, $P = 0.047$; MED vs. WTA — $F_{ST} = 0.21$, $P = 0.002$), though these comparisons may not be valid given the small sample sizes. Reduction of the California by-catch sample ($n = 10$) to four unique matrilines (i.e. ECNP, $n = 16$ matrilines overall), in addition to reduction of the Mediterranean sample, gave similar results at the ocean basin level ($F_{ST} = 0.11$, $\Phi_{ST} = 0.37$; $P < 0.0001$). Overall genetic variance among regions within oceans was also further reduced but still significant ($F_{ST} = 0.05$, $P = 0.0426$; $\Phi_{ST} = 0.06$, $P = 0.0231$).

Comparisons among California gillnet by-catch animals and those stranded on the US West Coast ($n = 11$) did not reveal a significant difference at the haplotype level ($F_{ST} = 0.01$, $P = 0.380$). In both groups, close to half of the animals represent haplotype L (*HS* group), from which the other haplotypes differ by only 1–2 bp (Figs 2 and 3). However, four stranded animals possessed *AG* group haplotypes, while all by-catch animals represent *HS* group haplotypes. It is likely that this was responsible for the borderline significant result obtained from Φ_{ST} comparisons ($\Phi_{ST} = 0.17$, $P = 0.049$). Additional samples will be required to investigate these potential differences.

Sex effects and sex-biased dispersal

The worldwide sample of 87 animals consisted of 49 males and 31 females, with seven animals of unknown sex

[2]Exclusion of all market products ($n = 5$) from the AMOVA did not affect the strength of differentiation among ocean basins ($F_{ST} = 0.15$; $\Phi_{ST} = 0.43$; $P < 0.0001$). Pairwise comparisons were similarly unaffected (all P values < 0.0001).

Table 3 Shimodaira–Hasegawa test scores comparing the best maximum-likelihood (ML) tree with haplotype X as a member of the *UZ* haplotype group to alternative hypotheses of relationships among haplotype X and other haplotype groups. Analyses were run as a one-tailed test, with estimated log-likelihood resampling (RELL) and 10 000 bootstrap replicates (Goldman *et al*. 2000), as implemented in PAUP*. Significant *P* values (< 0.05) are in bold. –ln L, likelihood score

Tree	–ln L	Difference in –ln L	*P*
1 — best ML tree	989.8924	(best)	
2 — hap X is basal in *T* haplotype group	997.3522	7.4598	0.3422
3 — hap X is basal in *HS* haplotype group	1013.4247	23.5324	**0.0076**
4 — hap X in *HS* group, among haplotypes with pos. 93 = T	1008.9523	19.0599	**0.0174**
5 — hap X is basal in *AG* haplotype group	1007.4710	17.5786	**0.0292**
6 — hap X is derived in *AG* haplotype group	1013.0292	23.1368	**0.0144**

Table 4 Hierarchical and pairwise analyses of molecular variance (AMOVA) for mtDNA control region sequences among and between oceanic populations of *Ziphius*. Significant *P* values (< 0.05) based on 20 000 permutations are shown in bold. See Fig. 1 for regions abbreviated

		Haplotype difference		Nucleotide distance	
Level	d.f.	Variance %	Probability	Variance %	Probability
Worldwide hierarchical					
Among oceans by region [SH, 2; NP, 2; NA, 3]	2	7.7	**0.0369**	35.2	**0.0088**
Among regions within oceans	4	12.8	**0.0000**	13.4	**0.0000**
Pairwise comparisons among oceans		F_{ST}%		Φ_{ST}%	
Among all three oceans	2	14.4	**0.0000**	42.2	**0.0000**
SH vs. NP		17.1	**0.0000**	41.1	**0.0000**
SH vs. NA		13.1	**0.0000**	42.4	**0.0000**
NP vs. NA		13.2	**0.0000**	42.8	**0.0000**
Pairwise comparisons among regions within oceans					
Southern Hemisphere (SH)					
SP vs. IO		< 1	0.9499	< 1	0.7851
North Pacific (NP)					
ECNP vs. WNP		< 1	0.6263	< 1	0.6044
North Atlantic (NA)					
ENA vs. MED		48.8	**0.0008**	67.5	**0.0002**
ENA vs. WTA		7.8	0.0971	6.7	0.1603
MED vs. WTA		31.4	**0.0000**	43.6	**0.0000**

(Table 2). In the Southern Hemisphere and North Atlantic, animals sampled were predominantly male but the sex ratio was approximately equal in the North Pacific (Table 2). Males predominated in three of the four haplotype groups (*AG*, *HS*, and *T*), but the sex ratio was approximately equal in the *UZ* group (1.2 to 1, with one animal of unknown sex). An AMOVA showed no significant differences between sexes at the haplotype (F_{ST} = –0.01, P = 0.847) or nucleotide level (Φ_{ST} = –0.01, P = 0.827). In a hierarchical AMOVA grouping animals by sex within ocean basin, sex explained < 1% of molecular variance and was not significant (P > 0.05).

In comparisons of genetic differentiation among ocean basins by sex, F_{ST} scores for males were higher (F_{ST} = 0.208, P < 0.001) than those for females (F_{ST} = 0.059, P = 0.015). This pattern was less pronounced at the nucleotide level (males, Φ_{ST} = 0.381, P < 0.001; females, Φ_{ST} = 0.451, P < 0.001). All pairwise comparisons were nonsignificant for females (P > 0.05), but significant for males (NA vs. SH, F_{ST} = 0.143; NA vs. NP, F_{ST} = 0.236; SH vs. NP, F_{ST} = 0.279; all P < 0.001).

Comparative effective population size and migration rates among ocean basins

Estimates of long-term $N_{e(f)}$ suggested some differences among ocean basins (SH, 51 000–102 000; NP, 28 000–57 000;

and NA, 35 000–70 000), but CIs were wide and overlapping (Table 5). Estimates of effective $Nm(f)$ per generation also differed by ocean basin and direction of migration, but overall migration among ocean basins was low (Table 5). Some general trends were apparent: (i) similar low rates of female migration from the SH to the NP and NA; (ii) similar low/negligible rates of female migration from the NA to the SH and NP; and (iii) rates of female migration from the NP to the SH potentially several-fold higher than migration from the NP to the NA.

Discussion

Monophyly and monotypy

Studies of phylogeographic patterns often take it for granted that the species under consideration represent genetically and reproductively independent lineages, for which the gene trees will be accurate approximations of the species trees (i.e. organismal history; Funk & Omland 2003). That is, it is assumed that all DNA lineages represented in a species are more closely related to one another than to lineages that exist in any other species. The hypothesis of species-level monophyly of mtDNA lineages among closely related taxa can only be tested through comprehensive surveys of population-level sequence variation. Although *Ziphius* has a widespread cosmopolitan distribution and apparent regional variation in some morphological features (Heyning 1989), all mtDNA sequences were found to be monophyletic with respect to related ziphiid genera (*Hyperoodon*, *Tasmacetus*, and *Berardius*) in phylogenetic reconstruction, confirming that *Ziphius* worldwide represents a single, independent genetic entity. A preliminary survey of nuclear actin intron diversity among *Ziphius* and other ziphiids has revealed similar patterns (Dalebout *et al*. 2004).

For widespread organisms, it is possible that gene flow between populations has in fact ceased and isolated units have evolved into distinct species or subspecies, even in the absence of obvious morphological differentiation. For example, right whales (*Eubalaena* spp.) were long considered to constitute an antitropical species pair. Recently, genetic evaluation revealed an absence of female-mediated gene flow among the three ocean basins, leading Rosenbaum *et al*. (2000) to propose that right whales worldwide should in fact be considered three distinct species. Similarly, it is possible that *Ziphius* could constitute a monophyletic but polytypic genus harbouring multiple, distinct taxa. Strong frequency differences were found among ocean basins in the distribution of haplotype groups, but these groups were not reciprocally monophyletic and there were no fixed differences. In this regard, *Ziphius* is more similar to humpback whales (*Megaptera novaeangliae*; Baker *et al*. 1993). Both the monophyly and phylogeography of *Ziphius* mtDNA lineages support its accepted classification as a single, globally distributed species.

Comparative mtDNA diversity and predictions for social structure

The mtDNA control region diversity among *Ziphius* worldwide was relatively low ($\pi = 1.27\% \pm 0.723\%$). This is just under half that observed among humpback whales worldwide (2.57%) based on analyses using a similar number of animals and length of control region sequence ($n = 90$, 283 bp; Baker *et al*. 1993). Right whales in the Southern Hemisphere and North Pacific also possess higher levels of mtDNA diversity ($\pi = 2.68\%$ and 2.10%, respectively; Rosenbaum *et al*. 2000), though that of the endangered North Atlantic species is considerably lower ($\pi = 0.60\%$; Malik *et al*. 1999). Among some worldwide odontocetes such as sperm whales and killer whales, mtDNA diversity is lower still ($\pi = 0.39\%$; Lyrholm & Gyllensten 1998, and 0.52%; Hoelzel *et al*. 2002, respectively). It has been proposed that the extremely low mtDNA variation observed in these latter species could result from their matrilineal social structure (where female offspring remain with their mother and do not disperse from their natal group) due to cultural hitch-hiking and group selection (Whitehead 1998), or variance in female reproductive success if animals in a group have correlated fitness (Tiedemann & Milinkovitch 1999). The model of Whitehead (1998) requires strong matrilineal transmission (> 99%) of a cultural trait for substantial reduction in mtDNA diversity to occur, which may be an unrealistic expectation given that even sperm whale groups are comprised of a mix of related and unrelated individuals (i.e. multiple matrilines; Richard *et al*. 1996; Mesnick *et al*. 1999). However, Tiedemann & Milinkovitch (1999) demonstrated that simple stochastic heterogeneity in fecundity is sufficient to cause a drastic reduction in mtDNA diversity in matrilineal populations. The social organization of *Ziphius* is not known. However, the intermediate levels of mtDNA diversity observed in this species suggest that social groups in *Ziphius* are perhaps unlikely to be strongly matrifocal.

Strong phylogeographic structure among ocean basins

Strong mtDNA differentiation was observed among *Ziphius* worldwide, with over 42% of the total molecular variance attributable to variation among the three ocean basins. Similar mtDNA divergence has been found among humpback whales in different ocean basins (38%; Baker & Medrano 2002). In humpbacks, differentiation at both the oceanic and regional level is the result of strong female philopatry to low-latitude winter breeding grounds and the seasonality of annual migrations in different hemispheres (Baker *et al*. 1990). Males also show strong philopatry but male-mediated gene flow is several-fold greater than that of females for some regional populations (Baker *et al*. 1998). It is not known if *Ziphius* undertakes

Table 5 Estimates of *Ziphius* long-term female effective population size, $N_{e(f)}$, and effective migration rate of females per generation, $N_e m(f)$, among ocean basins. Estimates are averages from 10 replicate runs. Confidence intervals (95%) derived from estimates of θ are shown. NA, North Atlantic; NP, North Pacific; SH, Southern Hemisphere

Ocean basin	$N_{e(f)}$ (thousands) (95% CI)
SH	
min	51 (26–95)
max	102 (52–189)
NP	
min	28 (19–123)
max	57 (39–246)
NA	
min	35 (19–83)
max	70 (38–166)
	$N_e m(f)$ per generation (95% CI)
From SH	
to NP	1.9 (1.0–3.7)
to NA	1.5 (0.4–4.1)
From NP	
to SH	4.8 (1.6–10.2)
to NA	0.9 (0.2–2.8)
From NA	
to SH	0.3 (0.0–1.5)
to NP	0.2 (0.0–1.1)

seasonal migrations, or whether the sexes differ temporally or spatially in their distribution (e.g. as in sperm whales; Whitehead 2002a), but this species' worldwide mitochondrial phylogeography has more in common with that of humpback whales than other widespread odontocetes. Killer and sperm whales worldwide show comparatively little geographical structure in mtDNA diversity. In sperm whales, haplotype frequency differences among ocean basins were only 3.0–4.8% (Lyrholm & Gyllensten 1998) compared to 14.4% found here for *Ziphius*. Sperm whales and *Ziphius* occupy a similar ecological niche. Both are found in deep oceanic waters at the edge of the continental shelf and feed on meso- and benthypelagic squid (Heyning 1989; Whitehead 2002a), yet geographical location and philopatry appear to have played a far greater role in shaping patterns of mtDNA diversity in *Ziphius* than in sperm whales.

Lack of strong signal for sex-biased dispersal

Analysis of mtDNA haplotypes showed no evidence of sex-biased dispersal among ocean basins. As the haplotypes of dispersing males are not passed onto their offspring and are available for sampling only during an animal's lifetime, this does not preclude some degree of long-term, sex-biased dispersal of males. However, given the common mammalian pattern of male-biased dispersal (Greenwood 1980), it is surprising that differentiation in haplotype frequencies was greater for males (20.8%) than for females (5.9%). Analysis of male-specific or biparentally inherited markers and larger sample sizes from adjacent geographical regions will be needed to confirm this apparent lack of strong male-biased dispersal in *Ziphius*.

International trade in Ziphius *products?*

The strong phylogeographic structure of *Ziphius* allowed us to estimate the geographical provenance of market products, at least at the ocean basin level. This has potential application in conservation and enforcement of international agreements banning trade in cetacean products. Almost two decades after the international moratorium on whaling came into effect in 1986, whale-meat markets in the southeastern coastal cities of Korea continue to thrive from the sale of animals taken incidentally as fisheries by-catch and undocumented direct exploitation (Baker *et al*. 1996, 2000; Mills *et al*. 1997). Our finding suggests that the demand for whale meat maintained by these markets poses a threat to cetaceans far beyond those in local Western North Pacific waters. Large far-seas fisheries vessels are fully capable of processing small- to medium-size cetaceans and returning these products to domestic markets. The discordant phylogeography of the Korean market product suggests a case of such 'importation from the high seas'. Confirmation of this violation will require additional samples from the North Pacific and improved statistical estimates of assignment based on mtDNA phylogeography.

Long-term effective population size and migration rates among ocean basins

Given both population and locus sampling limitations, the limitations of the program MIGRATE itself (Abdo *et al*. 2004), the lack of robust fossil dates for the origin of *Ziphius*, and the lack of accurate estimates of life history parameters, our estimates of long-term female effective population size, $N_{e(f)}$, and migration rates, $N_e m(f)$, must be interpreted with caution. However, some general trends are worth noting. The estimated $N_{e(f)}$ was similar for each of the three ocean basins, though may be slightly higher in the Southern Hemisphere. By pooling ocean basin $N_{e(f)}$ estimates and assuming a sex ratio of approximately 1:1, we can obtain a rough estimate of the total number of breeding adults (N_T), following Roman & Palumbi (2003). In most populations with constant population size, the ratio of N_T:N_e approaches 2.0 (Nunney 1993). This two-step conversion yields an

estimate of 456–916 thousand breeding adults worldwide, suggesting that *Ziphius* is a relatively abundant species, though not as abundant as some baleen whales or sperm whales prior to exploitation (Whitehead 2002b). However, *Ziphius* has not been subjected to large-scale exploitation in the past and its current abundance could exceed these exploited species. If so, the ecological role of *Ziphius* requires further consideration.

$N_em(f)$ among ocean basins was relatively low; on the order of one to two individuals per generation or less in most cases. The rates of interchange were not symmetrical, with migration from the North Pacific to the Southern Hemisphere potentially several-fold greater than that in the opposite direction. This differs from humpback whales where the centre of diversity appears to be the Southern Hemisphere, and migration seems to have been from this centre into the North Pacific and North Atlantic, perhaps during periods of global cooling (Baker & Medrano 2002). Other constraints may operate on the beaked whales.

Differentiation among regions within ocean basins

Small sample size and the lack of directed sampling design constrained our analyses of regional differentiation within oceans. Nonetheless, the Mediterranean was found to be highly distinct from the neighbouring Eastern North Atlantic. This is consistent with the high levels of endemism observed among other marine organisms in the Mediterranean, and its biological distinctiveness from the adjacent Atlantic Ocean (Fredj *et al*. 1992). Haplotype diversity of *Ziphius* was also lower in the Mediterranean than in other regions, suggesting that this population could be both isolated and relatively small. However, we cannot exclude the potential for some bias in this sample due to kinship. Ten of the 12 animals sampled in the Mediterranean stranded together on the coast of Greece on 12–13 May 1996, likely as a direct result of nearby navy acoustic testing (Frantzis 1998). Under natural circumstances, social odontocetes that strand together are often closely related (e.g. Robson 1984; Evans *et al*. 2002). However, the Greece stranding was not natural and the whales were spread along 38 km of the coast and separated by a mean distance of 3.5 km (Frantzis 1998). As such, these whales may not represent a single, closely related social unit. The other two animals from the Mediterranean were single strandings from the Croatian coast of the Adriatic Sea, both representing haplotype T3. Neither the T3 or T4 haplotypes were found outside the Mediterranean. Thus, despite the potential for inclusion of some related individuals in the sample, the Mediterranean appears to be comparatively isolated. This pattern of isolation was supported by AMOVA even when the Greece mass-stranding sample was reduced to unique matrilines. As such, it is recommended that *Ziphius* in the Mediterranean be considered a separate evolutionarily significant unit (ESU) distinct from other populations in the greater North Atlantic until more data can be collected.

Few conclusions can be drawn about the possible existence of regional divisions among *Ziphius* within other ocean basins until more comprehensive sampling is conducted. This should focus on areas that are under- or unrepresented in the present study, such as Hawaii, the Eastern Tropical Pacific, Sea of Japan, Sea of Cortez, tropical Indo-Pacific, northern Indian Ocean, Bay of Biscay, Canary Islands, Bahamas, and the Gulf of Mexico. Particular attention should be given to areas of known marine endemism (e.g. Sea of Japan, Sea of Cortez, Caribbean Sea), where navy sonar and seismic activity, and fisheries by-catch or directed hunting are known or suspected to occur.

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References

Abdo Z, Crandall KA, Joyce P (2004) Evaluating the performance of likelihood methods for detecting population structure and migration. *Molecular Ecology*, **13**, 837–851.

Anonymous (2003) Whales beach seismic research. *Geotimes*, January 2003, pp. 8–9. Available at http://www.geotimes.org/jan03/NN_whales.html.

Arnason U, Gullberg A, Janke A (2004) Mitogenomic analyses provide new insights into cetacean origin and evolution. *Gene*, **333**, 27–34.

Baker CS, Medrano-Gonzalez L (2002) World-wide distribution and diversity of humpback whale mitochondrial DNA lineages. In: *Cell and Molecular Biology of Marine Mammals* (ed. Pfeiffer CJ), pp. 84–99. Krieger Publishing Co., Malabar, Florida.

Baker CS, Palumbi SR (1994) Which whales are hunted? A molecular genetic approach to monitoring whaling. *Science*, **265**, 1538–1539.

Baker CS, Palumbi SR, Lambertsen RH *et al.* (1990) Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. *Nature*, **344**, 238–240.

Baker CS, Perry A, Bannister JL *et al.* (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences, USA*, **90**, 8239–8243.

Baker CS, Slade RW, Bannister JL *et al.* (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales *Megaptera novaeangliae*, world-wide. *Molecular Ecology*, **3**, 313–327.

Baker CS, Cipriano F, Palumbi SR (1996) Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. *Molecular Ecology*, **5**, 671–685.

Baker CS, Medrano-Gonzalez L, Calambokidis J *et al.* (1998) Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. *Molecular Ecology*, **7**, 695–707.

Baker CS, Lento GM, Cipriano F, Dalebout M, Palumbi SR (2000) Scientific whaling: source of illegal products for market? *Science*, **290**, 1695.

Barlow J (2003) *Cetacean Abundance in Hawaiian Waters During Summer/Fall 2002*. Administrative Report LJ-03–13. NOAA National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, California.

Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, **152**, 763–773.

Beerli P, Felsenstein J (2001) Maximum-likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences, USA*, **98**, 4563–4568.

Benjaminsen T, Christensen I (1979) The natural history of the bottlenose whale, *Hyperoodon ampullatus* (Forster). In: *Behaviour of Marine Animals*, vol. 3. *Cetaceans* (eds Winn HE, Olla BL), pp. 143–164. Plenum Press, New York.

Bowen BW, Abreu-Grobois FA, Balaz GH *et al.* (1995) Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial markers. *Proceedings of the National Academy of Sciences, USA*, **92**, 3731–3734.

Carretta JV, Forney KA, Muto MM *et al.* (2004) US Pacific Marine Mammal Stock Assessments: 2003. In: *NOAA-TM-NMFS-SWFSC-358*. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Centre.

Cassens I, Vicario S, Waddell VG *et al.* (2000) Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. *Proceedings of the National Academy of Sciences, USA*, **97**, 11343–11347.

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, **134**, 959–969.

Dalebout ML (2002) *Species identity, genetic diversity and molecular systematic relationships among the Ziphiidae (beaked whales)*. PhD Thesis, School of Biological Sciences, University of Auckland, Auckland, New Zealand.

Dalebout ML, van Helden A, Van Waerebeek K, Baker CS (1998) Molecular genetic identification of Southern Hemisphere beaked whales (Cetacea: Ziphiidae). *Molecular Ecology*, **7**, 687–694.

Dalebout ML, Baker CS, Cockcroft VG, Mead JG, Yamada TK (2004) A comprehensive and validated molecular taxonomy of beaked whales, family Ziphiidae. *Journal of Heredity*, **95**, 459–473.

Donnelly P, Tavare S (1986) The ages of alleles and a coalescent. *Advances in Applied Probability*, **18**, 1–19.

Evans DL, England GR, Lautenbacher CC *et al.* (2001) *Joint interim report — Bahamas marine mammal stranding event, March 14–16, 2000*. National Oceanic and Atmospheric Administration. Unpublished report released by the US Department of Commerce and the Secretary of the Navy. Available at http://www.nmfs.noaa.gv/prot_res/overview/Interim_Bahamas_Report.pdf.

Evans K, Morrice M, Hindell M, Thiele D (2002) Three mass strandings of sperm whales (*Physeter macrocephalus*) in southern Australian waters. *Marine Mammal Science*, **18**, 622–643.

Excoffier L (1993) *MINSPNET*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland. Available as freeware for PC from http://cmpg.unibe.ch/excoffier/.

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

Fertl D, Schrio AJ, Collier S, Worthy GAF (1997) Stranding of a Cuvier's beaked whale (*Ziphius cavirostris*) in Southern Texas with comments on stomach contents. *Gulf of Mexico Science*, **2**, 92–93.

Frantzis A (1998) Does acoustic testing strand whales? *Nature*, **392**, 29.

Frantzis A (2004) The first mass stranding that was associated with the use of active sonar (Kyparissiakos Gulf, Greece, 1996). In: *Proceedings of the Workshop on Active Sonar and Cetaceans* (eds Evans PGH, Miller LA), pp. 14–20. European Cetacean Society (ECS), Newsletter no. 42, Special issue.

Fredj G, Bellan-Santini D, Meinardi M (1992) Etat des connaissances sur la faune marine mediterranienne. *Bulletin de l'Institut Oceanographique, Monaco*, **no Special 9**, 133–145.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematics*, **34**, 397–423.

Gilson A, Syvanen M, Levine K, Banks J (1998) Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game*, **84**, 159–169.

Goldman N, Anderson JP, Rodrigo AG (2000) Likelihood-based tests of topologies in phylogenetics. *Systematic Biology*, **49**, 652–670.

Gowans S, Dalebout ML, Hooker SH, Whitehead H (2000) Reliability of photographic and molecular techniques for sexing bottlenose whales, *Hyperoodon ampullatus*. *Canadian Journal of Zoology*, **78**, 1224–1229.

Greenwood PJ (1980) Mating systems, philopatry, and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Henshaw MD, LeDuc RG, Chivers SJ, Dizon AE (1997) Identification of beaked whales (family Ziphiidae) using mtDNA sequences. *Marine Mammal Science*, **13**, 487–495.

Heyning JE (1989) Cuvier's beaked whale, *Ziphius cavirostris* G. Cuvier, 1823. In: *Handbook of Marine Mammals* (eds Ridgway SH, Harrison R), pp. 289–307. Academic Press, London.

Heyning JE (2002) Cuvier's beaked whale, *Ziphius cavirostris*. In: *Encyclopedia of Marine Mammals* (eds Perrin WF, Wursig B, Thewissen JGM), pp. 305–307. Academic Press, San Diego.

Hoelzel AR, Natoli A, Dahlheim M *et al.* (2002) Low world-wide genetic diversity in the killer whale (*Orcinus orca*); implications for demographic history. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 1467–1475.

Hoelzel RA, Hancock JM, Dover GA (1991) Evolution of the cetacean mitochondrial D-loop region. *Molecular Biology and Evolution*, **8**, 475–493.

Höss M, Pääbo S (1993) DNA purification from Pleistocene bones by a silica-based purification method. *Nucleic Acids Research*, **21**, 3913–3914.

Höss M, Jaruga P, Zastawny TH, Dizdaroglu M, Pääbo S (1996) DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Research*, **24**, 1304–1307.

Hultman T, Stahl S, Hornes E, Uhlen M (1989) Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Research*, **17**, 4937–4946.

Jefferson TA, Leatherwood S, Webber MA (1993) *FAO Species Identification Guide: Marine Mammals of the World*. United States Environment Programme; Food and Agriculture Organization of the United Nations (FAO), Rome.

Jepson PD, Arbelo M, Deaville R *et al.* (2003) Gas-bubble lesions in stranded cetaceans. *Nature*, **425**, 575–576.

Julian F, Beeson M (1998) Estimates of marine mammal, turtle and seabird mortality for two Californian gillnet fisheries: 1990–1995. *Fisheries Bulletin*, **96**, 271–284.

Lento GM, Patenaude NJ, Baker CS (1997) *Molecular genetic identification of whale and dolphin products for sale in Japan and Korea, 1995–1997*. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/49/O21). Available from Red House, 135 Station Road, Impington, Cambridge CB4 9NP, UK.

Lyrholm T, Gyllensten U (1998) Global matrilineal population structure in sperm whales as indicated by mitochondrial DNA sequences. *Proceedings of the Royal Society London. Series B, Biological Sciences*, **265**, 1679–1684.

Maddison WP, Maddison DR (1992) MACCLADE *(Release version 3.04): Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Malik S, Brown MW, Kraus SD *et al.* (1999) Assessment of genetic structuring and habitat philopatry in the North Atlantic right whale. *Canadian Journal of Zoology*, **77**, 1217–1122.

Matisoo-Smith E, Allen JS, Ladefoged TN, Roberts RM, Lambert DM (1997) Ancient DNA from Polynesian rats: extraction, amplification and sequence from single small bones. *Electrophoresis*, **18**, 1534–1537.

Mead JG (1984) Survey of reproductive data for the beaked whales (Ziphiidae). *Reports of the International Whaling Commission, Special Issue*, **6**, 91–96.

Mead JG (2002) Beaked whales, overview. In: *Encyclopedia of Marine Mammals* (eds Perrin WF, Wursig B, Thewissen JGM), pp. 81–84. Academic Press, San Diego, California.

Mesnick SL, Taylor BL, Le Duc RG *et al.* (1999) Culture and genetic evolution in whales: reply to Whitehead. *Science* **284**, 2055a.

Mignucci-Giannoni AA (1996) *Marine mammal strandings in Puerto Rico and the United States and British Virgin Islands*, PhD Thesis, Department of Marine Sciences, University of Puerto Rico, Mayaguez, Puerto Rico.

Mills J, Ishihara A, Sakaguchi I *et al.* (1997) *Whale Meat Trade in East Asia: A Review of the Markets in 1997*. Traffic International, Cambridge, UK.

Nishiwaki M, Oguro N (1972) Catch of Cuvier's beaked whales off Japan in recent years. *Scientific Reports of the Whales Research Institute, Tokyo*, **24**, 35–41.

Nunney L (1993) The influence of mating system and overlapping generations on effective population size. *Evolution*, **47**, 1329–1341.

Omura H, Kimura S (1955) Beaked whale *Berardius bairdii* of Japan, with notes on *Ziphius cavirostris*. *Scientific Reports of the Whales Research Institute, Tokyo*, **10**, 89–132.

Pääbo S (1990) Amplifying ancient DNA. In: *PCR Protocols: A Guide to Methods and Applications* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp. 159–166. Academic Press, San Diego, California.

Palsbøll PJ, Vader A, Bakke I, Raafat El-Gewely M (1992) Determination of gender in cetaceans by the polymerase chain reaction. *Canadian Journal of Zoology*, **70**, 2166–2170.

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: *Molecular Systematics* (eds Hillis DM, Moritz C, Mable BK), pp. 205–247. Sinauer Associates, Sunderland, Massachusetts.

Pesole G, Gissi C, De Chirico A, Saccone J (1999) Nucleotide substitution rate of mammalian mitochondrial genomes. *Journal of Molecular Evolution*, **48**, 427–434.

Pichler FB, Dalebout ML, Baker CS (2001) Non-destructive DNA extraction from sperm whale teeth and scrimshaw. *Molecular Ecology Notes*, **1**, 106–109.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics Applications Note*, **14**, 817–818.

Reeves RR (1988) *Exploitation of cetaceans in St. Lucia, Lesser Antilles, January 1987*. Unpublished Report to the Scientific Committee of the International Whaling Commission (SC/39/O1). Available from Red House, 135 Station Road, Impington, Cambridge CB4 9NP, UK.

Rice DW (1998) *Marine Mammals of the World: Systematics and Distribution*. Special Publication Number 4. The Society for Marine Mammalogy, Lawrence, Kansas.

Richard KR, McCarrey SW, Wright JM (1994) DNA sequence from the SRY gene of the sperm whale (*Physeter macrocephalus*) for use in molecular sexing. *Canadian Journal of Zoology*, **72**, 873–877.

Richard KR, Dillon MC, Whitehead H, Wright JM (1996) Patterns of kinship in groups of free-living sperm whales (*Physeter macrocephalus*) revealed by multiple molecular genetic analyses. *Proceedings of the National Academy of Sciences, USA*, **93**, 8792–8795.

Robson FD (1984) *Strandings* Science Press, Johannesburg, p. 124.

Roman J, Palumbi SR (2003) Whales before whaling in the North Atlantic. *Science*, **301**, 508–510.

Rooney AP, Honeycutt RL, Derr JN (2001) Historical population size change of bowhead whales inferred from DNA sequence polymorphism data. *Evolution*, **55**, 1678–1685.

Rosario-Delestre RJ, Muñoz-Hincapié MF, Mignucci-Giannoni AA (1999) New mass stranding of Cuvier's beaked whales for Puerto Rico. In: *Abstracts, 13th Biennial Conference on the Biology of Marine Mammals, Maui, Hawaii, 28 Nov–1 Dec, 1999*, p. 162. Society for Marine Mammalogy, Orlando, Florida (available from http://www.marinemammalogy.org/publications.htm Abstract).

Rosenbaum HC, Brownell RL Jr, Brown MW *et al.* (2000) World-wide genetic differentiation of *Eubalaena*: questioning the number of right whale species. *Molecular Ecology*, **9**, 1793–1802.

Ross GJB (1984) The smaller cetaceans of the south east coast of Southern Africa. *Annals of the Cape Provincial Museum (Natural History)*, **15**, 173–410.

Ross GJB, Tietz RM (1972) Records of Cuvier's beaked whales from South America. *Annals of the Cape Provincial Museum (Natural History)*, **9**, 1–10.

Rudolph P, Smeenk C, Leatherwood S (1997) Preliminary checklist of Cetacea in the Indonesian Archipelago and adjacent waters. *ZoologischeVerhandelingen, Leiden*, **312**, 1–47.

Sambrook E, Fritsch F, Maniatis T (1989) *Molecular Cloning*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, *(Version 2.000): Software for population genetics analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland. Available from http://anthropologie.unige.ch/arlequin.

Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**, 1114–1116.

Simmonds MP, Lopez-Jurado LF (1991) Whales and the military. *Nature*, **351**, 448.

Sorensen MD (1999) TREEROT, *Version 2*. Boston University, Boston, Massachusetts. Available from http://people.bu.edu/msoren/TreeRot.html.

Swofford DL (1999) PAUP*: *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

Takahata M (1988) The coalescent in two partially isolated diffusion populations. *Genetics Research*, **52**, 213–222.

Tiedemann R, Milinkovitch MC (1999) Technical comment: response to Whitehead (1999) culture and genetic evolution in whales. *Science*, **284**, 2055a.

Van Waerebeek K, Van Bressem M-F, Alfaro-Shigueto J *et al*. (1999) *A Preliminary Analysis of Recent Captures of Small Cetaceans in Peru and Chile*. Unpublished Report to the Scientific Committee of the International Whaling Commission (SC/51/SM17). Available from Red House, 135 Station Road, Impington, Cambridge CB4 9NP, UK.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506–513.

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.

Whitehead H (2002a) *Sperm Whales: Social Evolution in the Ocean*. University of Chicago Press, London.

Whitehead H (2002b) Estimates of the current global population size and historical trajectory for sperm whales. *Marine Ecology Progress Series*, **242**, 295–304.

Willis PM, Baird RW (1998) Sightings and strandings of beaked whales on the west coast of Canada. *Aquatic Mammals*, **24**, 21–25.

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Appendix

Sample data for all *Ziphius* specimens. Samples were obtained from stranded animals unless otherwise indicated. Hap., haplotype

Code	Country, state or province	Location	Collection date	Hap.	Hap. Group	Sex	Method	Source
Southern Hemisphere								
South Pacific Ocean								
ZcaNZ02	New Zealand	Timaru, Otago	18 Sept 1992	B	AG	M	morph.	1
ZcaNZ03	New Zealand	Gisborne, Hawke's Bay	7 June 1995	A	AG	M	morph.	1
ZcaNZ04	New Zealand	Huia, Auckland	14 Feb 1995	C	AG	M	morph.	1
ZcaNZ05	New Zealand	Coromandel, South Auckland	30 May 1996	B	AG	F	mol.	1
ZcaNZ06	New Zealand	Wanganui, Taranaki	25 Oct 1997	A	AG	M	morph.	1
ZcaNZ07	New Zealand	Nelson	April 1998	A	AG	M	mol.	1
ZcaNZ08/09 cow-calf	New Zealand	Dunedin, Otago	12 Mar 1998	L	HS	F/M	morph.	1
ZcaNZ11	New Zealand	Papamoa, Bay of Plenty	1 Apr 1999	J	HS	M	mol.	1
ZcaNZ12	New Zealand	Maniapoto, Te Kuiti	20 Jun 1999	A	AG	M	morph.	1
ZcaNZ13	New Zealand	Port Levy, Bank's Peninsula	3 Mar 2001	B2	AG	M	morph.	1
ZcaNZ14	New Zealand	Mahia Beach, Hawke's Bay	6 May 2002	A	AG	M	morph.	1
ZcaNZ15	New Zealand	Rodgers Road Beach, Tauranga	12 Jan 2002	C2	AG	M	morph.	1
ZcaNZ16/17 cow-calf	New Zealand	Haast Beach, Southwestland	3–4 Sept 2003	A	AG	M/F	morph.	1
ZcaTMAG-A1875	Australia, Tasmania	Sandy Cape Beach, Ordinance Pt., West Coast	3 Nov 2003	A2	AG	F	mol.	2
*ZcaCO67-1	Chile	Valparaiso	1967	F2	HS	M	mol.	3
ZcaCO99-1	Chile	Aconcagua River Estuary, Concón	May 1999	A	AG	F	mol.	3
*ZcaCI10-Raro	Cook Islands	Rarotonga	1990	H	HS	M	morph.	4/5
*ZcaCI Manuae-A	Cook Islands	Manuae	1998	L	HS	M	morph.	6
ZcaSW8503	Galapagos Islands	—	5 Mar 1994	A	AG	F	mol.	7
Indian Ocean								
*ZcaPEM50[a]	South Africa	Maitland/Seaview, Cape Province	7 Feb 1966	D	AG	F	morph.	8
*ZcaPEM272[a]	South Africa	Waterloo Bay, Cape Province (Fish River Pt.)	23 Mar 1976	F	AG	M	morph.	8
*ZcaPEM387	South Africa	Boknes, Cape Province	20 Jan 1979	A	AG	M	morph.	8
*ZcaPEM430[a]	South Africa	Gulu River Mouth, East London, Cape Province	6 Jun 1967	A	AG	M	morph.	8
*ZcaPEM1353	South Africa	Goukamma, Cape Province	?	B	AG	?	—	8
*ZcaPEM1494	South Africa	Boknes, Cape Province	10 May 1988	A	AG	M	morph.	8

Appendix *Continued*

Code	Country, state or province	Location	Collection date	Hap.	Hap. Group	Sex	Method	Source
North Pacific Ocean								
Western North Pacific								
ZcaJE9308	Japan	market sample — no data	1993	R	HS	?	—	9/10
ZcaJ95b78	Japan	market sample — Nagasaki	9 May 1995	I	HS	?	—	11
ZcaJa95b99	Japan	market sample — Fukuoka, Sea of Japan coast	10 May 1995	L	HS	?	—	11
ZcaTSM31331	Japan	Sizuoka, Pacific Coast	13 Feb 1997	L	HS	M	morph.	12
ZcaKo9401-SN[b]	South Korea	market sample — East Coast provinces	Sept 1994	X	UZ	?	—	10/11
ZcaK00-23	South Korea	market sample — East Coast provinces	28 May 2000	S	HS	M	mol.	11/13/14
ZcaSW7398	Malaysia	Tuaran, Sabah	11 Apr 1994	P	HS	F	morph.	7
ZcaSW5565	Philippines	Mlalbuham Saiton (by-catch)	?	L	HS	F	morph.	7
ZcaSW9561	Taiwan	Taiwan	?	E	AG	F	mol.	7/15
Eastern-Central North Pacific								
ZcaSW9234	USA, California	Moss Landing	1998	L	HS	F	morph.	7
ZcaSW4961/ LACM84111[c]	USA, California	Santa Monica	6 Aug 1988	N	HS	M	mol.	7/16
ZcaSW745	USA, California	driftnet by-catch	13 Aug 1992	M	HS	F	mol.	7
ZcaSW1120[c]	USA, California	gillnet by-catch	10 Nov 1992	K	HS	M	morph.	7
ZcaSW2160	USA, California	driftnet by-catch	26 Nov 1993	L	HS	M	morph.	7
ZcaSW2157	USA, California	driftnet by-catch	8 Jan 1994	L	HS	M	mol.	7
ZcaSW4777	USA, California	driftnet by-catch	3 Sept 1994	N	HS	F	morph.	7
ZcaSW3762	USA, California	driftnet by-catch	26 Nov 1994	L	HS	M	mol.	7
ZcaSW3763	USA, California	driftnet by-catch	26 Sep 1994	L	HS	M	mol.	7
ZcaSW3764	USA, California	driftnet by-catch	16 Dec 1994	L	HS	F	mol.	7
ZcaSW5005	USA, California	driftnet by-catch	17 Nov 1995	N	HS	F	morph.	7
ZcaSW5009	USA, California	driftnet by-catch	11 Dec 1995	K	HS	F	mol.	7
ZcaSW5319/SC	USA, California	Santa Cruz	23 Feb 1996	L	HS	F	mol.	7
ZcaSW8398	USA, California	Silver Strand	1 Oct 1997	L	HS	M	morph.	7
ZcaLACM-X	USA, California	—	?	E	AG	M	mol.	16
ZcaSW12708	USA, Washington	Oyehut	19 Nov 1998	L	HS	M	mol.	7/17
ZcaBC91-30[d]	Canada, British Columbia	North Langara Island	10 Jul 1991	G	AG	F	morph.	18
ZcaBC93-18[d]	Canada, British Columbia	Vancouver Island	17 May 1993	Q	HS	F	morph.	18
ZcaBC94-47[d]	Canada, British Columbia	Queen Charlotte Islands	15 July 1994	L	HS	M	morph.	18
ZcaSW4927	USA, Hawaii	Nanakuli	16 Jan 1996	E	AG	F	morph.	7
ZcaSW4967/ LACM91909[c]	USA, Hawaii	Johnston Atoll	?	E	AG	?	—	7
ZcaSW1212	Eastern Tropical Pacific	biopsy sample	1 Nov 1992	O	HS	?	—	7

Appendix *Continued*

Code	Country, state or province	Location	Collection date	Hap.	Hap. Group	Sex	Method	Source
Atlantic Ocean								
Eastern North Atlantic								
ZcaSAC0356	UK, Scotland	Hushinish, Western Isles	29 Jan 1993	U	UZ	M	morph.	19
ZcaSAC0446	UK, Scotland	North Uister, Western Isles	27 Feb 1999	V	UZ	F	morph.	19
ZcaIRL-1	Ireland	Ballyferriter, Co. Kerry	1 Mar 2000	V	UZ	F	morph.	20
ZcaIRL-X2	Ireland	Doonbeg, Co. Clare	25 Mar 2000	V	UZ	M	morph.	20
ZcaIRL-X3	Ireland	Doonbeg, Co. Clare	8 May 2001	T2	T	F	mol.	20
Mediterranean Sea								
ZcaGR1	Greece, SE Ionian Sea	Agrilos, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T3	T	M	mol.	21
ZcaGR2	Greece, SE Ionian Sea	Vathy, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T4	T	F	mol.	21
ZcaGR3	Greece, SE Ionian Sea	Gianitsaina, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T3	T	M	mol.	21
ZcaGR4	Greece, SE Ionian Sea	Kartelas, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T3	T	M	mol.	21
ZcaGR5	Greece, SE Ionian Sea	Kalo nero, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T3	T	F	morph.	21
ZcaGR6	Greece, SE Ionian Sea	Vounaki, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T4	T	M	morph.	21
ZcaGR7	Greece, SE Ionian Sea	Vounaki, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T3	T	M	morph.	21
ZcaGR8	Greece, SE Ionian Sea	Vounaki, Kyparissiakos Gulf, West Peloponnese	12 or 13 May 1996	T3	T	M	morph.	21
ZcaGR9	Greece, SE Ionian Sea	Giannitsochorio, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T3	T	M	morph.	21
ZcaGR10	Greece, SE Ionian Sea	Neochorio, Kyparissiakos Gulf, West Peloponnese	12 May 1996	T4	T	M	morph.	21
ZcaCRT58	Croatia, Adriatic Sea	Zupski zaljev, near Dubrovnik	Apr 2001	T3	T	F	morph.	22
ZcaCRT75	Croatia, Adriatic Sea	Pupnatska luka, Korcula Island	7 Feb 2002	T3	T	M	mol.	22
Western-Tropical Atlantic								
ZcaNC02-96	USA, North Carolina	North Carolina Aquarium at Fort Fisher (NCAFF)	1 Feb 1996	Z	UZ	F	mol.	23
ZcaVGT204	USA, North Carolina	1.8 miles west of Sportsman's Pier	31 May 1996	Z	UZ	M	mol.	23
ZcaSW3981	USA, Florida	—	1995	T	HS	M	mol.	7
ZcaSW4472	USA, Florida	North Talbot Island	3 Aug 1995	B	AG	M	morph.	7
ZcaNEPST382	Puerto Rico	Aguadilla	30 July 1998	L	HS	M	morph.	24
ZcaNEPST384	Puerto Rico	Aguadilla	30 July 1998	Y	UZ	F	morph.	24
ZcaNEPST385	Puerto Rico	Aguadilla	30 July 1998	A	AG	M	morph.	24

Appendix *Continued*

Code	Country, state or province	Location	Collection date	Hap.	Hap. Group	Sex	Method	Source
ZcaNEPST392	Puerto Rico	Aguadilla	30 July 1998	V	UZ	M	morph.	24
ZcaNEPST401	Puerto Rico	Aguada	30 July 1998	Y	UZ	M	morph.	24
ZacNEPST505	Puerto Rico	Aguada	25 Nov 1998	V	UZ	M	morph.	24
ZcaNEPST421	US Virgin Islands	Fish Bay, St. John	14 Apr 1999	K	HS	M	mol.	24
ZcaNEPST575	US Virgin Islands	St Thomas	3 Oct 1999	L	HS	F	morph.	24
ZcaNEPST576	US Virgin Islands	Coral Bay, St. John	3 Oct 1999	B	AG	F	morph.	24
ZcaSW3035[e]	USA, Texas	South Padre Island, Gulf of Mexico	2 Apr 1994	W	UZ	F	morph.	7

*Historical specimens represented only by bone, teeth, or dried skin for which 'ancient' DNA methods were used (see Methods).

[a]Discussed in Ross (1984) as PEM1514/08, ELM857, and PEM1520/01, respectively. First two specimens also discussed in Ross & Tietz (1972).

[b]Discussed in Baker & Palumbi (1994), Baker *et al*. (1996), and Dalebout *et al*. (1998).

[c]Shorter mtDNA control region sequence from these specimens originally published by Henshaw *et al*. (1997) (GenBank Accession nos: U70454, U70453 and U70452, respectively).

[d]Discussed in Willis & Baird (1998).

[e]Stomach contents of this specimen discussed in Fertl *et al*. (1997); a shorter mtDNA control region sequence was originally published by Henshaw *et al*. (1997; U70455).

Sample sources

1. University of Auckland collection (samples courtesy of field centre staff, New Zealand Department of Conservation; A. L. van Helden, National Museum of New Zealand Te Papa Tongarewa; and P. Duignan and P. Madie, Massey University Cetacean Investigation Centre, Palmerston North).
2. D. Pemberton and D. Robertson, Tasmanian Museum and Art Gallery, Hobart, Tasmania, Australia.
3. C. Olavarria B., Universidade de Valparaíso, Viña del Mar, Chile.
4. N. Hauser, Centre for Cetacean Research and Conservation (www.whaleresearch.org).
5. G. Wragg, Beach Road, R.D. 7, Ashburton, New Zealand.
6. K. Pollack, 62 Arawa Road, Whakatane, New Zealand.
7. A. E. Dizon, K. Robertson and R. L. Brownell, NOAA Southwest Fisheries Science Centre, La Jolla, California, USA.
8. V. G. Cockcroft and G. Watson, Port Elizabeth Museum (BayWorld), Port Elizabeth, South Africa.
9. F. Cipriano, Conservation Genetics Laboratory, Department of Biology, San Francisco State University, San Francisco, USA.
10. S. Galster and S. LeBudde, EarthTrust.
11. N. Funahashi, International Fund for Animal Welfare, Japan.
12. T. K. Yamada, National Science Museum, Okubo, Tokyo, Japan.
13. Korean Federation of Environmental Movement (KFEM), Seoul, Republic of (South) Korea.
14. GreenPeace International.
15. L. Siang-Chou, Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan.
16. J. E. Heyning, Los Angeles County Museum of Natural History, California, USA.
17. J. Calambokidis, Cascadia Research Collective, Olympia, Washington, USA.
18. R. W. Baird, Marine Mammal Research Group, Box 6244, Victoria, British Columbia, Canada.
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