Molecular Ecology (1998) **7**, 687–694

# Molecular genetic identification of southern hemisphere beaked whales (Cetacea: Ziphiidae)

M. L. DALEBOUT,* A. VAN HELDEN,† K. VAN WAEREBEEK‡ and C. S. BAKER*
*School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1000, New Zealand, †Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington, New Zealand, ‡Peruvian Centre for Cetacean Research (CEPEC), Jorge Chavez 302, Pucusana, Lima 20, Peru*

**Abstract**

**To assist in the species-level identification of stranded and hunted beaked whales, we compiled a database of 'reference' sequences from the mitochondrial DNA control region for 15 of the 20 described ziphiid species. Reference samples for eight species were obtained from stranded animals in New Zealand and South Australia. Sequences for a further seven species were obtained from a previously published report. This database was used to identify 20 'test' samples obtained from incompletely documented strandings around New Zealand. Analyses showed that four of these 'test' specimens (20%) had initially been misidentified. These included two animals of particular interest: (i) a Blainville's beaked whale (*Mesoplodon densirostris)*, the first record of this species in New Zealand waters; and, (ii) a juvenile Andrews' beaked whale (*Mesoplodon bowdoini*), a species known from just over 20 strandings worldwide. A published sequence from a beaked whale product purchased in the Republic of Korea was identified as a Cuvier's beaked whale (*Ziphius cavirostris*). Levels of intra- and interspecific variation were compared to determine the potential for misidentification when the database or taxonomy is incomplete. Intraspecific variation was generally < 2%, and interspecific divergence was generally > 4.7%. Exceptions were within-species variation in *Hyperoodon planifrons*, southern bottlenosed whale (4.12%), which exceeded the variation between the two species of *Berardius* (3.78%), and variation between the two specimens assigned to *M. hectori*, Hector's beaked whale (7.14%). The latter case appears to be an error in species identification, and could represent the discovery of a new species of beaked whale.**

*Keywords:* forensic, genetic diversity, mitochondrial DNA, phylogenetics, stranding

*Received 8 August 1997; revision received 9 December 1997; accepted 9 December 1997*

## Introduction

Beaked whales (Ziphiidae) are among the least known of all mammalian groups (Wilson 1992). The family consists of 20 described species in five genera: *Ziphius*, *Tasmacetus*, *Mesoplodon*, *Hyperoodon*, and *Berardius*. Most species are rarely seen at sea due to their preference for deep ocean waters, elusive habits and, in some cases, possible low abundance (Mead 1989). Many are known from only a small number of stranded individuals and fragmentary skeletal material collected over the last hundred years. For example, *Mesoplodon pacificus* is known from only two skulls and has never been seen alive (Baker 1990).

Correspondence: C. S. Baker. Fax: +64-9-373-7417; E-mail: cs.baker@auckland.ac.nz

At least 14 species of beaked whales are known to occur in the southern hemisphere. While several northern hemisphere species have been studied because of commercial whaling interest (Nishiwaki & Omura 1972; Benjaminsen & Christensen 1979; Kasuya 1986), or fisheries bycatch concerns (Barlow *et al*. 1995; Henshaw *et al*. 1997), most information on southern hemisphere species has been gathered from strandings. All species of beaked whales remain incompletely described, from basic life-history parameters, behaviour and distribution, to individual variation in morphology.

Determination of species identity is a critical requirement for collecting useful information from stranded animals. The primary diagnostic character for identification of beaked whale species is tooth morphology (Moore 1968). Most species have retained only a single pair of

functional teeth set in the lower jaw, and these erupt only in adult males. In females and juveniles, the teeth remain embedded in the gum, and must be extracted by dissection of the jaw to confirm identity. Many beaked whales are also very similar in overall appearance, especially those in the most speciose genus *Mesoplodon*. A great potential exists therefore for the misidentification of beaked whales, even when the whole animal is available for examination by experts (e.g. Moore 1968; Kitchener & Herman 1995).

Molecular genetics offers a powerful tool to assist in the accurate identification of beaked whales. Using the polymerase chain reaction (PCR), sufficient DNA for analysis can be amplified from small samples of skin or other tissue. These samples can be easily collected by field agents in attendance at a stranding. Sampling need not be invasive because sloughed skin from live stranded animals, which may yet be successfully refloated, can also yield DNA (Milinkovitch *et al*. 1994). Even tissue obtained from animals in advanced stages of decomposition, or from commercially processed meat products, can contain enough DNA for identification (Pääbo 1989; Cooper *et al*. 1992; Baker & Palumbi 1994).

To assist in the identification of beaked whales we have compiled a molecular genetic database of DNA reference sequences, including the majority of southern hemisphere species and available northern hemisphere species (Henshaw *et al*. 1997). We report here on the utility of this DNA database for the identification of stranded beaked whales from New Zealand's coasts, as well as whale meat products from the commercial markets of Asia.

## Materials and methods

### *Reference and test samples*

Samples were collected from all stranded beaked whales in New Zealand as part of a nationwide program coordinated by the Museum of New Zealand Te Papa Tongarewa and the Department of Conservation. Samples from *Mesoplodon bowdoini* and *M. hectori* were obtained from strandings in South Australia. A sample was included in the reference database if the specimen was examined by a scientist familiar with beaked whale morphology (A. van Helden, A. N. Baker or C. Kemper), and diagnostic skeletal material or extensive photographic evidence was obtained by either the Museum of New Zealand Te Papa Tongarewa or the South Australian Museum. Where a stranded animal was not available for examination, and/or was identified only by field agents, the sample was considered a 'test'. A beaked whale test sequence obtained from a whale meat product bought on the commercial markets of Korea (Baker *et al*. 1996) was also analysed to confirm species identity. Other beaked whale reference sequences were obtained from GenBank. These specimens were identified on the basis of skull morphology (Henshaw *et al*. 1997). As the distribution of most beaked whale species is incompletely known (Mead 1989), all available reference sequences from both northern and southern hemisphere beaked whales were included in our analyses (Table 1).

### *Sample collection, DNA extraction and sequencing*

Samples consisted of small amounts of skin collected from dead animals, stored in 70% ethanol or frozen prior to genetic analysis. Total genomic DNA was isolated using proteinase K, following standard methods (Davis *et al*. 1986), as modified by Baker *et al*. (1994), using less than a 100 µg sample of tissue from each individual. A 550 bp fragment of the mitochondrial (mt) DNA control region (D-loop) was amplified by PCR following standard protocols (Saiki *et al*. 1988; Palumbi 1996), and primers light-strand t-Pro whale (5'-TCACCCAAAGCTGRART-TCTA-3') and heavy-strand Dlp5 (5'-CCATCGWGATG-TCTTATTTAAGRGGAA-3'). We focused on this portion of the mitochondrial DNA control region because its rapid rate of divergence allows even closely related species to be easily distinguished (Arnason *et al*. 1993; Baker & Palumbi 1994; Baker *et al*. 1996). Following PCR amplification, the double-stranded DNA was 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 standard solid-phase protocols (Hultman *et al*. 1989). At least one individual per species was sequenced in both forward and reverse directions to confirm results. All reference sequences have been submitted to GenBank. Test sequences are available by E-mail from the authors.

### *Phylogenetic analysis of 'reference' sequences and identification of 'test' individuals*

Sequences were aligned using the program PILEUP, available in the GCG package (Deveraux *et al*. 1984), with initial gap penalty 2, and extension penalty 0.3. The multiple sequence files generated were then further checked and corrected for alignment inconsistencies by eye. Phylogenetic reconstruction methods using neighbour-joining distance algorithms, as implemented in the program MEGA (Kumar *et al*. 1993), and parsimony, as implemented in the program PAUP 3.1.1 (Swofford 1993), were used to determine the relationship between the reference sequences. The heuristic search option, with tree bisection-reconnection, was used for the parsimony analyses. For the neighbour-joining method, the Kimura 2-parameter distance correction option, available in MEGA (Kumar *et al*. 1993), was used to adjust for multiple

**Table 1** Details of beaked whale mtDNA control region (D-loop) reference sequences. Species recorded only from the northern or southern hemisphere, or both, are as indicated. All previously unpublished sequences were generated by M. L. Dalebout from samples collected from stranded animals. Other sequences were obtained from Genbank, as described by Henshaw et al. (1997)

| Scientific name | Common name | AUNZ code | Geographic origin | Museum code/Source | Genbank No. |
|---|---|---|---|---|---|
| Southern hemisphere | | | | | |
| *Mesoplodon bowdoini* | Andrews' beaked whale | m18047 | South Australia | SAM m18047 | AF036221 |
| *Mesoplodon grayi* | Gray's beaked whale | MgrH04 | New Zealand | NMNZ 2160 | AF036211 |
| | | Mgr02 | New Zealand | NMNZ 2234 | AF036212 |
| | | Mgr03 | New Zealand | NMNZ 2132 | AF036213 |
| | | Mgr07 | New Zealand | NMNZ in prep. | AF036214 |
| | | Mgr10 | New Zealand | NMNZ in prep. | AF036215 |
| *Mesoplodon layardii* | strap-toothed whale | Mlay01 | New Zealand | NMNZ files | AF036216 |
| | | Mlay04 | New Zealand | NMNZ in prep. | AF036217 |
| | | Mlay06 | New Zealand | NMNZ 2268 | AF036218 |
| | | Mlay07 | New Zealand | NMN in prep. | AF036219 |
| *Hyperoodon planifrons* | southern bottlenosed whale | Hpl01 | New Zealand | NMNZ 2214 | AF036224 |
| | | Hpl02 | New Zealand | NMNZ 2233 | AF036225 |
| *Tasmacetus shepherdi* | Shepherd's beaked whale | Tsh01 | New Zealand | NMNZ 2184 | AF036226 |
| | | Tsh02 | New Zealand | NMNZ 2183 | AF036227 |
| | | Tsh04 | New Zealand | NMNZ 2189 | AF236228 |
| | | Tsh69 | Argentina | Henshaw *et al*. (1997) | U70469 |
| *Berardius arnuxii* | Arnoux's beaked whale | Bar02 | New Zealand | NMNZ files | AF036229 |
| Northern hemisphere | | | | | |
| *Mesoplodon bidens* | Sowerby's beaked whale | Mbi56 | Florida, USA | Henshaw *et al*. (1997) | U70456 |
| | | Mbi57 | North Atlantic | Henshaw *et al*. (1997) | U70457 |
| | | Mbi58 | North Atlantic | Henshaw *et al*. (1997) | U70458 |
| | | Mbi59 | North Atlantic | Henshaw *et al*. (1997) | U70459 |
| *Mesoplodon carlhubbsi* | Hubb's beaked whale | McarSW | North Atlantic | Henshaw *et al*. (1997) | U70461 |
| *Mesoplodon europaeus* | Gervais' beaked whale | MeurSW | North Atlantic | Henshaw *et al*. (1997) | U70460 |
| *Mesoplodon stejnegeri* | Stejneger's beaked whale | Mste62 | North Pacific | Henshaw *et al*. (1997) | U70462 |
| | | Mste63 | North Pacific | Henshaw *et al*. (1997) | U70463 |
| *Berardius bairdii* | Baird's beaked whale | Bba67 | North Pacific | Henshaw *et al*. (1997) | U70467 |
| | | Bba68 | North Pacific | Henshaw *et al*. (1997) | U70468 |
| Northern & Southern hemisphere | | | | | |
| *Mesoplodon densirostris* | Blainville's beaked whale | MdenSW | North Pacific | Henshaw *et al*. (1997) | U70464 |
| *Mesoplodon hectori* | Hector's beaked whale | m16387 | South Australia | SAM m16387 | AF036220 |
| | | MhecSW | North Pacific | Henshaw *et al*. (1997) | U70466 |
| *Mesoplodon mirus* | True's beaked whale | MmirSW | North Atlantic | Henshaw *et al*. 91997) | U70465 |
| *Ziphius cavirostris* | Cuvier's beaked whale | Zca02 | New Zealand | NMNZ files | AF036222 |
| | | Zca04 | New Zealand | NMNZ files | AF036223 |
| | | Zca52 | North Pacific | Henshaw *et al*. (1997) | U70452 |
| | | Zca53 | North Atlantic? | Henshaw *et al*. (1997) | U70453 |
| | | Zca54 | North Pacific | Henshaw *et al*. (1997) | U70454 |
| | | Zca55 | North Atlantic | Henshaw *et al*. (1997) | U70455 |

AUNZ, University of Auckland; NMNZ, Museum of New Zealand Te Papa Tongarewa; SAM, South Australian Museum.

substitutions. All distances reported are Kimura 2-parameter corrected unless otherwise stated. The most parsimonious trees found were consistent with the neighbour-joining tree in all details relevant to the identification of test sequences. Only the results of the neighbour-joining analyses are shown here.

Test sequences were added to the reference database and analysed individually and as a group to establish

species identity. The statistical consistency of reference and test sequence groupings was evaluated by 1000 bootstrap resamplings of the data and neighbour-joining reconstructions. Although parsimony and maximum likelihood outperform neighbour joining for phylogenetic reconstruction under many conditions (Hillis *et al*. 1994), only the latter allowed bootstrap simulations with the large number of taxa used here.

The following outgroups were used in the phylogenetic analyses: pygmy right whale, *Caperea marginata*, Cma-CSB (Baker & Palumbi 1994); beluga whale, *Delphinapteras leucas*, Dle-WL, (Lillie *et al*. 1996); pygmy sperm whale, *Kogia breviceps*, Kbr-UA (Arnason *et al*. 1993); humpback whale, *Megaptera novaeangliae*, Mno-UA (Arnason *et al*. 1993); killer whale, *Orcinus orca*, Oor-RH (Hoelzel *et al*. 1991); harbour porpoise, *Phocoena phocoena*, Pho-PR (Rosel *et al*. 1995b); and sperm whale *Physeter macrocephalus*, Pma-UA (Arnason *et al*. 1993).

## Results

### *Phylogenetic relationships of reference sequences*

A 350-bp fragment of the mtDNA control region was sequenced for the 19 reference specimens obtained from strandings in New Zealand and South Australia, representing eight species from all five genera of beaked whales. A further 18 reference sequences from seven other beaked whale species were obtained from GenBank, giving a total of 37 reference sequences, representing 15 of the 20 described species of beaked whale (Table 1). Species missing from the database are: *Hyperoodon ampullatus*, northern bottlenosed whale; *M. bahamondi*, Bahamonde's beaked whale (Reyes *et al*. 1995); *M. ginkgodens*, ginkgo-toothed beaked whale; *M. pacificus*, Longman's beaked whale; and *M. peruvianus*, Peruvian beaked whale. Another putative species, *Mesoplodon* sp. 'A', is known from observations at sea (Jefferson *et al*. 1993), but a specimen has never been recovered. Alignment of these reference sequences with sequences published for other cetacean species showed that all beaked whales have a 50-bp deletion in this portion of the mtDNA control region, extending from position 16143 to position 16192, with reference to the fin whale mtDNA genome (Arnason *et al*. 1991a). This deletion event uniquely distinguishes the Ziphiidae from all other whales and dolphins. The phylogenetic reconstructions of the relationships between the reference sequences (Fig. 1) strongly supported the monophyly of the Ziphiidae (100% bootstrap value), but showed only weak support for other higher-order relationships within the family. All conspecific sequences grouped together consistently (> 95% bootstrap value), with the exception of the two putative *Mesoplodon hectori* sequences (arrows).

### *Identification of test specimens*

The target fragment of the mtDNA control region was successfully amplified and sequenced for 20 test samples obtained from incompletely documented strandings. Subsequent phylogenetic analyses, including other cetacean taxa as outgroups, unambiguously (100% bootstrap values) grouped all test sequences with beaked whale reference sequences. Sixteen of the test sequences grouped with reference sequences from the species of initial identification (reconstructions not shown). However, four test sequences grouped with reference sequences from other species (Fig. 2; arrows): (1) Mbow01*, initially identified as *Mesoplodon bowdoini*, grouped with *M. densirostris*; (2) Zca01*, initially identified as *Ziphius cavirostris*, grouped with *M. bowdoini*; (3) Mgr09*, initially identified as *M. grayi*, grouped with the *M. hectori* from South Australia; and, (4) Mlay03*, initially identified as *M. layardii*, grouped with *M. grayi*. The beaked whale sequence from the Korean market sample (KN1) was identified as *Z. cavirostris* (arrow 5).

### *Intra- and interspecific genetic variation*

To assess the possibility of misidentification due to an incomplete database and uncertainties in ziphiid taxonomy, pairwise sequence differences (%) were calculated for the nine beaked whale species for which more than one reference sequence was available. To minimize the possibility of underestimating variation by analysing related animals, only individuals from different strandings were used. These estimates of intraspecific variation were compared to the minimum interspecific differences based on pairwise comparisons for all beaked whale species (Fig. 3). Intraspecific variation was found to be generally less than 2%, while the interspecific differences were generally greater than 4.7%. There were three important exceptions to this general observation: (i) the two *M. hectori* sequences differed by 7.14%; (ii) the two *Hyperoodon planifrons* sequences differed by 4.12%; and (iii) *Berardius bairdii* differed from *B. arnuxii* by 3.78%.

## Discussion

Our results confirm the utility of molecular genetic techniques for the identification of stranded beaked whales (e.g. Henshaw *et al*. 1997). The genetic identity of the majority of the test specimens (80%) agreed with that initially determined by agents attending the strandings. However, the potential for the misidentification of beaked whales was highlighted by the four test specimens (20%) that were initially misclassified. This included two animals of particular importance: (i) a *Mesoplodon densirostris* specimen (AUNZ code: Mbow01*), the first record of this

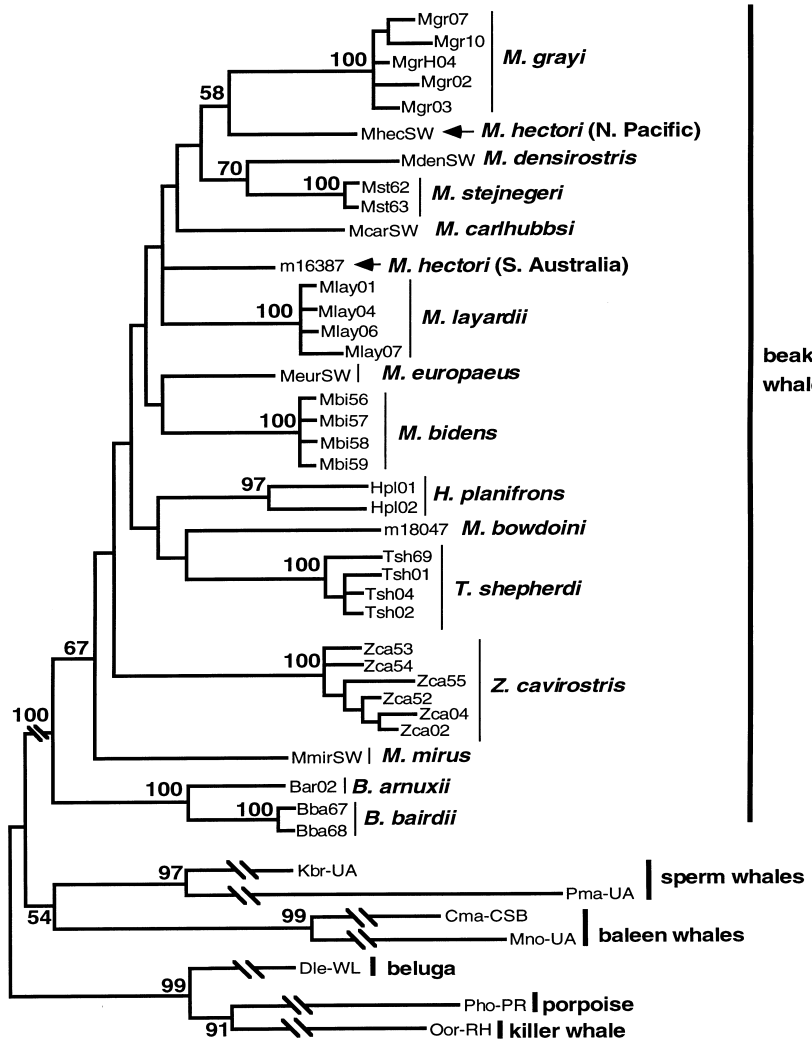


**Fig. 1** Phylogenetic reconstruction of beaked whale reference sequences, with other cetacean taxa as outgroups, using the neighbour-joining method. Bootstrap values > 50% based on 1000 resamplings of the data are shown at relevant nodes. Individual beaked whale sequences are labelled according to Table 1. The disparate positions of the South Australian and North Pacific *Mesoplodon hectori* sequences are indicated by arrows.

species from New Zealand waters; and, (ii) a juvenile *M. bowdoini* (AUNZ code: Zca01*), a species known from just over 20 strandings worldwide (Mead 1989). Without genetic identification, these occurrences would have passed undocumented and the information gathered attributed to other species. Our results also confirm the utility of these methods for identification of hunted beaked whales (e.g. Baker *et al*. 1996). The sale of meat from a *Z. cavirostris* specimen on the commercial market in the Republic of Korea suggests a possible threat to this species or stock from unregulated bycatch or direct hunting in this area.

### *Species identification*

Although molecular genetics is a powerful tool for species identification, there are circumstances in which the results of phylogenetic analysis could be ambiguous. A test specimen could be misidentified if: (i) the database of reference sequences is incomplete; or (ii) the taxonomy of the group in question is uncertain or incomplete (Baker *et al*. 1996). The first difficulty is known to be true in this study (five species are missing from the database), and the second is probable (Mead 1989). Evaluating levels of intra- and interspecific genetic difference in groups of interest can help to assess the potential for misidentification and indicate possible problem taxa. For example, a test sequence of a species not represented in the reference database will group with or basal to the reference sequences of the next most closely related species (a branching-order error). An unusually large divergence between the test and reference sequences could indicate that such an intermediate species or taxa is missing (Baker *et al*. 1996). This was generally not the case in the Ziphiidae. Less than 2% intraspecific pairwise sequence difference was found in seven of the nine species of beaked whales for which more than one reference sequence was available (Fig. 3). This is similar to the criterion of ≤10 bp difference (2.7–2.8%) for positive species identification suggested by Henshaw *et al*. (1997). These findings are also

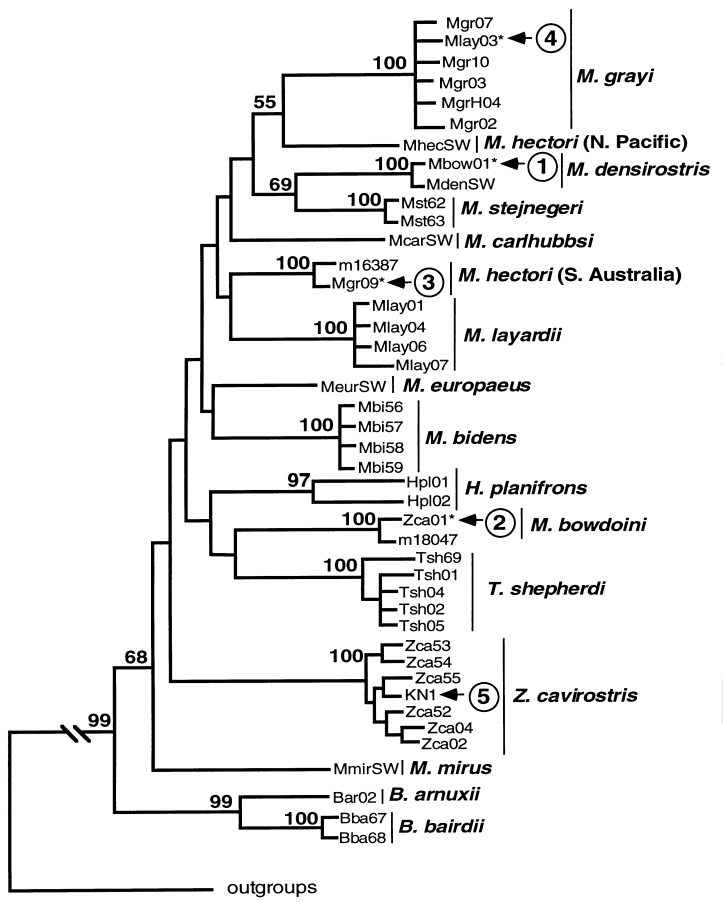


**Fig. 2** Phylogenetic reconstruction used to identify test sequences relative to reference sequences using the neighbour-joining method. Bootstrap values > 50% based on 1000 bootstrap resamplings of the data are shown at relevant nodes. High bootstrap values at the species level and poor resolution at higher order levels are typical of rapidly evolving sequences. Individual beaked whale sequences are labelled as per Table 1 and text. Outgroups are as shown in Fig. 1. Arrows 1–4 indicate the true identity of test sequences from animals initially misclassified on the basis of field reports from the strandings (20% of total test sequences analysed). Arrow 5 indicates the identity of a sequence from a whale-meat product from the commercial markets of Korea (Baker *et al*. 1996).

comparable to levels of variation found in this portion of the mtDNA control region in other cetacean species, e.g. North Atlantic and Antarctic minke whales, 0.65% and 1.66% (Bakke *et al*. 1996), humpback whales worldwide, 3.0% (Baker *et al*. 1993), North Atlantic and North Pacific harbour porpoises, 0.90% and 1.3% (Rosel *et al*. 1995a), and sperm whales worldwide, 1.7% (Lyrholm *et al*. 1996).

However, in two ziphiid species intraspecific variation was higher than that normally found in cetaceans. The two *Hyperoodon planifrons* sequences differed from each other by 4.12% (Fig. 3), slightly more than the difference seen between two other congeneric beaked whale species, *Berardius bairdii* and *B. arnuxii* (3.78%). The large divergence between the *H. planifrons* specimens could indicate a large effective population size or multiple evolutionary significant units (ESUs) within the recognized species (Moritz 1994). Conversely, the small divergence between the *B. bairdii* and *B. arnuxii* specimens suggests that these two forms may not warrant species-level distinction (see also Balcomb 1989). Both possibilities require larger population samples and the analysis of additional loci for further evaluation.

More intriguing was the 7.12% difference between the sequences of the two specimens assigned to *M. hectori* from South Australia (m16387) and the North Pacific (MhecSW) (Fig. 3). This is comparable to the difference seen between other recognized species of beaked whales. The relatively large difference between these two sequences, and their failure to group together in the phylogenetic analyses (e.g. Fig. 1; arrows), suggests that these animals are not the same species. Assuming that one of these animals is *M. hectori*, the other represents a species not included in the reference database. Although neither of these specimens have been examined by the present authors, the morphological descriptions of both appear inconsistent with any of the five species missing from the database (C. Kemper, personal communication; Mead 1981). These findings could suggest the molecular genetic discovery of a new species of beaked whale. Detailed morphological comparison of these specimens is needed to evaluate this possibility.

### *Phylogenetic relationships within the Ziphiidae*

Although the rapid rate of evolution of the mtDNA control region obscured many of the higher-order phylogenetic relationships in the Ziphiidae, two observations warrant comment. First, the close grouping of *B. bairdii* and *B. arnuxii* (Fig. 1), and the relatively low genetic difference

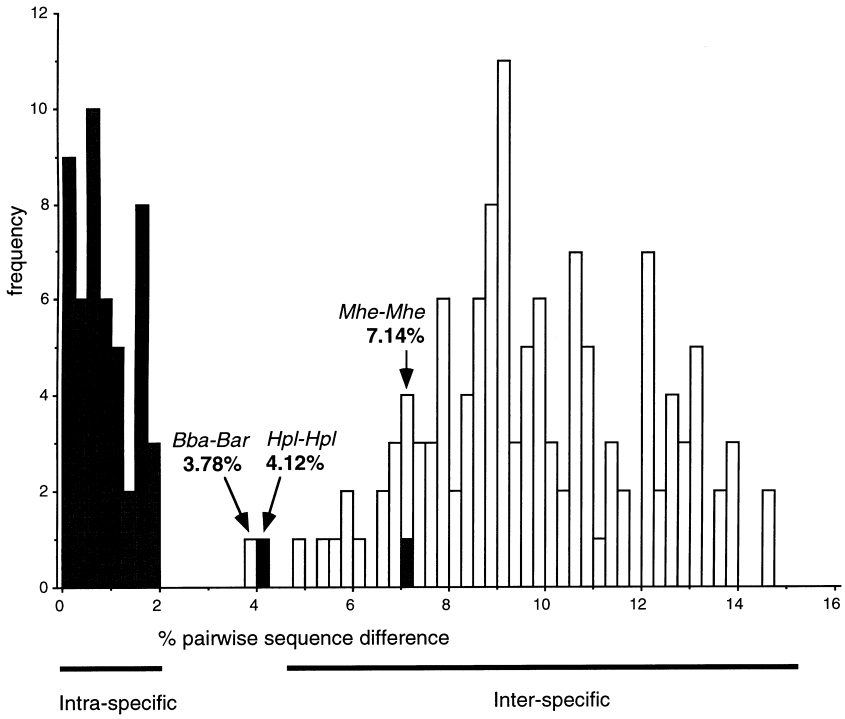


**Fig. 3** Frequency distribution of intra- and interspecific differences among beaked whales, based on pairwise comparisons of a 350-bp sequence of the cetacean mtDNA control region. Distances are adjusted for multiple substitutions using the Kimura 2-parameter method. All intraspecific distances (shaded bars) and the minimum interspecific distances (open bars) are shown. *Bba*, *Berardius bairdii*; *Bar*, *B. arnuxii*; *Hpl*, *Hyperoodon planifrons*; *Mhe*, *Mesoplodon hectori* (South Australian and North Pacific specimens).

seen between these species as discussed previously (Fig. 3), which is consistent with their morphological descriptions (Balcomb 1989). Second, *Mesoplodon carlhubbsi* failed to group with *M. bowdoini* in these analyses (Fig. 1) despite their reported morphological similarity and sister-species status (Mead 1989).

Further analyses of more slowly evolving mtDNA genes are necessary to obtain a confident reconstruction of the evolutionary relationships of the species within this family, and determine the relationship of this group as a whole to the rest of the Cetacea. Nuclear markers, such as intron sequences, could also be useful for systematic inference, as well as to investigate potential hybridization as has been documented in other cetacean families, e.g. 'blue-fin' hybrids (Arnason *et al*. 1991b). We cannot discount the possibility that these events could contribute to the uncertainty of morphological identification for these poorly described species.

## Acknowledgements

For the collection of test and reference samples from stranded beaked whales in New Zealand, we thank the field centre staff of the New Zealand Department of Conservation, the Massey University Cetacean Investigation Centre and Project Jonah. For reference samples from stranded beaked whales in South Australia, we thank C. Kemper of the South Australian Museum, Adelaide. For access to reference sequences from northern hemisphere beaked whales, we thank M. D. Henshaw, R. G. LeDuc, S. J. Chivers, and A. E. Dizon. We also thank A. N. Baker, A. E. Dizon, J. E. Heyning, J. G. Mead, and P. R. Wade for valuable discussion pertaining to this work, and R. Constantine, G. M. Lento, N. Patenaude, and F. B. Pichler for assistance and support in the laboratory. We would also like to thank F. Cipriano, G. Wallis and an anonymous reviewer for their constructive comments on the manuscript. This research is funded by the New Zealand Lotteries Board, the New Zealand Marsden Fund, the Auckland University Research Council, and the International Fund for Animal Welfare. M. L. Dalebout is supported by a New Zealand Vice-Chancellor's Committee William Georgetti Scholarship, and a University of Auckland Doctoral Scholarship. K. Van Waerebeek is partially supported by the Gesellschaft zum Schutz der Meeressaugetiere and Leopold III-Fonds.

## References

Arnason U, Gullberg A, Widegren B (1991a) The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus*. *Journal of Molecular Evolution*, **33**, 556–568.

Arnason U, Spilliaert R, Palsdottir A, Arnuson A (1991b) Molecular identification of hybrids between the two largest whale species, the blue whale (*Balaenoptera musculus*) and the fin whale (*B. physalus*). *Hereditas*, **115**, 183–189.

Arnason U, Gullberg A, Widegren B (1993) Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. *Molecular Biology and Evolution*, **10**, 960–970.

Baker AN (1990) *Whales and Dolphins of New Zealand and Australia: An Identification Guide*, Victoria University Press, Wellington.

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

Baker CS, Perry A, Bannister JL *et al*. (1993) Abundant mitochon-

drial 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.

Bakke I, Johansen S, Bakke O, El-Gewely MR (1996) Lack of population subdivision among minke whales (*Balaenoptera acutorostrata*) from Icelandic and Norwegian waters based on mitochondrial DNA sequences. *Marine Biology*, **125**, 1–9.

Balcomb KC III (1989) Baird's beaked whale, *Berardius bairdii* Stejneger, 1883: Arnoux's beaked whale, *Berardius arnuxii* Duvernoy, 1851. In: *Handbook of Marine Mammals* (eds Ridgway SH, Harrison R), pp. 261–288. Academic Press, London.

Barlow J, Swartz SL, Eagle TC, Wade PR (1995) *US Marine Mammal Stock Assessments: Guidelines for Preparation, Background, and a Summary of the 1995 Assessments*. US Department of Commerce, NOAA Technical Memorandum NMFS-OPR-6.

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

Cooper A, Mourer-Chauvire C, Chambers GK *et al.* (1992) Independent origins of New Zealand moas and kiwis. *Proceedings of the National Academy of Sciences, USA*, **89**, 8741–8744.

Davis LG, Dibner MD, Batteny JF (1986) *Basic Methods in Molecular Biology*, Elsevier, Amsterdam.

Deveraux JP, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Research*, **12**, 387–395.

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

Hillis DM, Huelsenbeck JP, Cunningham CW (1994) Application and accuracy of molecular phylogenies. *Science*, **264**, 671–677.

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

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 Nations Environment Programme, Food and Agriculture Organization of the United Nations, Rome.

Kasuya T (1986) Distribution and behaviour of Baird's beaked whales of the Pacific coast of Japan. *Scientific Reports of the Whales Research Institute*, **37**, 61–83.

Kitchener AC, Herman JS (1995) Re-identification of the supposed True's beaked whale, *Mesoplodon mirus*, from Scotland. *Journal of Zoology*, **236**, 353–357.

Kumar S, Tamura K, Nei M (1993) *MEGA: Molecular Evolutionary Genetics Analysis*, version 1.01. The Pennsylvania State University, University Park, PA.

Lillie WR, Brown-Gladden JG, Tretiak DN (1996) Amplification and sequencing of control region mitochondrial DNA from the beluga whale, *Delphinapteras leucas. Canadian Technical Reports of Fisheries and Aquatic Science*, **2080**, iv, 12pp.

Lyrholm T, Leimar O, Gyllensten U (1996) Low diversity and biased substitution patterns in the mitochondrial DNA control region of sperm whales: implications for estimates of time since common ancestry. *Molecular Biology and Evolution*, **13**, 1318–1326.

Mead JG (1981) First records of *Mesoplodon hectori* (Ziphiidae) from the northern hemisphere and a description of the adult male. *Journal of Mammalogy*, **62**, 430–432.

Mead JG (1989) Beaked whales of the genus *Mesoplodon*. In: *Handbook of Marine Mammals* (eds Ridgway SH, Harrison R), pp. 349–430. Academic Press, London.

Milinkovitch M, Dunn L, Powell JR (1994) Exfoliated cells as the most accessible DNA source for captive whales and dolphins. *Marine Mammal Science*, **10**, 125–128.

Moore JC (1968) Relationships among the living genera of beaked whales. *Fieldiana Zoology*, **53**, 209–298.

Moritz C (1994) Defining evolutionarily significant units for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

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

Pääbo S (1989) Ancient DNA: extraction, characterization, molecular cloning and enzymatic amplification. *Proceedings of the National Academy of Sciences USA*, **86**, 1939–1943.

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, MA.

Reyes JC, Van Waerebeek K, Cardenas JC, Yanez JL (1995) *Mesoplodon bahamondi* sp. n. (Cetacea, Ziphiidae), a living beaked whale from the Juan Fernandez Archipelago, Chile. *Boletin de Museo Nacional de Historia Natural, Chile*, **45**, 31–44.

Rosel PE, Dizon AE, Haywood MG (1995a) Variability of the mitochondrial DNA control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. *Canadian Journal of Fisheries and Aquatic Science*, **52**, 1210–1219.

Rosel PE, Haywood MG, Perrin WF (1995b) Phylogenetic relationships among the true porpoises (Cetacea: Phocoenidae). *Molecular Phylogeny and Evolution*, **4**, 463–474.

Saiki RK, Gelfand DH, Stoffel S *et al.* (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487–491.

Swofford DL (1993) *PAUP: Phylogenetic Analysis using Parsimony*, version 3.1.1. Illinois Natural History Survey, Champaign, Illinois.

Wilson EO (1992) *Diversity of Life*. Harvard University Press, Cambridge, Massachusetts.

---

This work is part of an ongoing study aimed at understanding the evolutionary relationships within the beaked whales, and determining the relationship of this family to other groups of cetaceans through molecular genetic analyses of both mitochondrial and nuclear loci. Merel Dalebout is a PhD student at the University of Auckland, Anton van Helden is the marine mammal collections manager at the Museum of New Zealand, Koen Van Waerebeek is the director of the Peruvian Centre for Cetacean Research, and Scott Baker is a senior lecturer in marine biology and molecular ecology at the University of Auckland.

---