

A BRIEF REVIEW OF THE GENETIC STUDIES ON DWARF MINKE WHALE BASED ON JARPA SAMPLES

Luis A. Pastene

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

ABSTRACT

Prior to the JARPA surveys 'dwarf' minke whales were only believed to be found between 7-41°S. However the locality of the JARPA catches in Areas IV and V was mainly between 55-62°S (one animal was caught at 65°S), showing that the 'dwarf' minke whale is found much further south than shown by the previous data. A total of 16 'dwarf' minke whales were sampled by JARPA and these animals were examined genetically with the purpose to investigate their relationship to the southern 'ordinary' minke whale and to minke whales in the Northern Hemisphere. Early studies showed that the degree of mtDNA divergence between both southern minke whales was large and similar to that found between southern 'ordinary' minke whale and Northern Hemisphere minke whales. Phylogenetic analyses showed that the 'dwarf' minke whale was more closely related to Northern Hemisphere minke whales. In 1993 the Scientific Committee recommended the inclusion of the 'dwarf' minke whale in the Schedule, so that catch limits for Antarctic minke whales recognise the distinction between the two southern minke whales. The genetic analysis conducted using JARPA samples of the southern 'ordinary' and 'dwarf' minke whales assisted greatly to the taxonomic review of minke whale conducted by Rice (1998), which confirmed the existence of two species, the larger, Antarctic minke whale *Balaenoptera bonaerensis* confined to the Southern Hemisphere, and the smaller, common minke whale *B. acutorostrata* distributed in both hemispheres. 'Dwarf' minke whale is sympatric to the Antarctic minke whale but in the review by Rice (1998) it was considered an unnamed sub-species of the common minke whale. Recent mtDNA analyses suggest that the sub-specific classification of common minke whales requires further consideration. Recent studies have showed that the 'dwarf' minke whales are genetically structured in the Southern Hemisphere.

INTRODUCTION

The first documented study that demonstrated marked morphological differences between 'diminutive' or 'dwarf' and 'ordinary' minke whales in the Southern Hemisphere was based on samples from South Africa (Best, 1985). Subsequently, Arnold *et al.* (1987) presented evidence for morphological differentiation along the Australian coast. Those authors described several morphological differences between the 'dwarf' and 'ordinary' minke whales in the Southern Hemisphere of which the most distinctive concerned the color of the body and baleen plates and the shape of the skull. The 'dwarf' minke whale has a predominantly light baleen series and a white base to the flippers, whilst the 'ordinary' minke whale has asymmetrically colored baleen plates. Although the flippers of the 'ordinary' minke whale may have one or two tones of gray, they are never white at the base, as in the 'dwarf' minke whale. In the 'dwarf' minke whale, the dark pigmentation in the neck region extends onto the ventral grooves whereas in the 'ordinary' minke whale it does not occur below eye level. The 'dwarf' minke whale also has anteriorly convex nasal bones as opposed to the concave nasal bones in the 'ordinary' minke whale. In most of these morphological characteristics the 'dwarf' minke whale more closely resembles Northern Hemisphere minke whales.

Kato and Fujise (2000) reported the morphology, growth and life history of 'dwarf' minke whales based on 16 animals sampled by JARPA in the Antarctic. Results of their analysis confirmed several of the morphological and morphometric characters reported previously. They reported the mean body length at physical maturity at 7.0m for females and 6.6m for males. According to this source the apparent pregnancy rate among mature females was 1.0 and the conception is highly concentrated in mid-winter. These authors also reported that the age and length at sexual maturity in females was 7-10 years and 6.0-6.5m, respectively.

The 'dwarf' minke whale has been identified from the Brazilian minke whale catches (da Rocha and Braga, 1982). Baker (1983) identified and illustrated this whale among minke whales stranded on the New Zealand coast. Zerbini *et al.* (1996) reviewed the records on 'dwarf' minke whale in Brazil, and their results suggest that this whale could be relatively common in that region. More recently Acevedo *et al.* (in press) reported the stranding of three 'dwarf' minke whales in Navarino Island, in the southern tip of the America continent.

Prior to the JARPA in the Antarctic (1987/88) the 'dwarf' minke whale was only believed to be found between 7-41°S (Best, 1985); no 'dwarf' minke whales had been identified in past commercial catches near the ice edge. The 'dwarf' minke whale was reported for Antarctic waters for the first time, on the basis of catches conducted during earlier JARPA surveys (Kato *et al.*, 1989; 1990; Fujise *et al.*, 1990; 1993; Kasamatsu *et al.*, 1993). Distribution of the JARPA catches in Areas IV and V was mainly between 55°-62°S (one individual was caught at 65°S), showing that the 'dwarf' minke whale is found much further south than shown by the previous catch data.

The objective of this paper is to summarize the genetic studies conducted on 'dwarf' minke whales and the implication for their taxonomy, management and conservation.

GENETIC STUDIES

Allozyme analysis

The first genetic study was carried out by Wada (1983) who analysed a single sample from a South Africa animal that presented similar morphological characteristics to the 'dwarf' minke whale. His allozyme study, however, found no significant differences between this sample and those from the southern 'ordinary' minke whale.

Mitochondrial DNA RFLP analysis

Wada *et al.* (1991) examined genetic diversity in minke whales from the North Pacific and the Antarctic using restriction fragment length polymorphism (RFLP) analysis of the whole mtDNA genome. The Antarctic sample included a single individual of the 'dwarf' minke whale, sampled during the first JARPA feasibility study in Area IV in the austral summer 1987/88 (Kato *et al.*, 1989). They digested mtDNAs with 14 six-base sequence recognition restriction enzymes and, based on the composite digestion patterns of a total 142 minke whales, 19 haplotypes were resolved. There was no shared haplotype among North Pacific, southern 'ordinary' and 'dwarf' minke whales. Furthermore an UPGMA-derived dendrogram based on genetic distances, showed that the 'dwarf' minke whale and minke whales from the North Pacific are more similar to each other than they are to the southern 'ordinary' minke whale.

It should be noted that the allozyme study of Wada (1983) and the mtDNA study of Wada *et al.* (1991) were based on a single 'dwarf' minke whale, which generated contradictory results. Using a larger number of samples of the 'dwarf' minke whale, Pastene *et al.* (1994) examined the differentiation of mtDNA between southern 'ordinary' and 'dwarf' minke whales. These authors conducted a RFLP analysis of the whole mtDNA molecule using 11 samples of the 'dwarf' and 18 of the 'ordinary' minke whales taken during JARPA surveys. MtDNAs extracted from liver samples were digested with 11 six-base restriction enzymes revealing a total of thirteen mtDNA haplotypes in the total sample. There were no shared haplotypes between the two southern minke whales. Three haplotypes were found in the 'dwarf' and ten in the 'ordinary' minke whale. The larger number of samples of the 'dwarf' minke whale enabled the degree of genetic variation within this form to be investigated. The index of nucleotide diversity (Nei and Li, 1979) was higher in the 'ordinary' minke whale than in the 'dwarf' minke whale. The degree of genetic diversity within each of these minke whales was similar or lower to that of other cetaceans (Pastene *et al.*, 1994). The net genetic distance between the two southern minke whales was estimated at 0.0524. A comparison with published information for other species revealed that the genetic distance between the 'dwarf' and 'ordinary' minke whales was larger than that for some recognised species of land and marine mammals.

Using RFLP data from Wada *et al.* (1991) for the North Pacific minke whale, Pastene *et al.* (1994) studied the genetic relationships among North Pacific, Antarctic 'ordinary' and 'dwarf' minke whales. There was no shared haplotypes among them (two were discriminated in the 'dwarf', three in the North Pacific and eight in the southern 'ordinary' minke whales). An UPGMA-derived dendrogram of mtDNA haplotypes based on genetic distances (Figure 1), confirmed that the dwarf and the North Pacific minke whales are more similar to each other than they are to the Antarctic 'ordinary' minke whale. Then the work by Pastene *et al.* (1994), which was based on a larger number of samples of the 'dwarf' minke whale, supported the previous finding of Wada *et al.* (1991).

Mitochondrial DNA control region sequencing

Hori *et al.* (1994) and Pastene *et al.* (1996) investigated the phylogenetic relationships of 'dwarf' minke whale with North Pacific, North Atlantic and Antarctic 'ordinary' minke whales. Antarctic 'dwarf' and 'ordinary' minke whales samples were taken during JARPA surveys. In the analysis, published sequence data of the North Atlantic minke whale were used. The method used was the amplification by the polymerase chain reaction (PCR)

of the non-coding control region of mtDNA followed by sequencing of 343 base-pairs. A neighbor-joining-based phylogenetic tree (Saitou and Nei, 1987) of 56 unique sequences (Figure 2) showed that southern 'dwarf', southern 'ordinary', North Pacific and North Atlantic minke whales are separated from each other, suggesting independent genetic populations. The tree also showed that 'dwarf' minke whales are more closely related to Northern Hemisphere minke whales than to the southern 'ordinary' minke whale. The 'dwarf' minke whale is clearly remote from the southern 'ordinary' minke whale despite the overlap in their distribution and apparent ecological niche.

Table 1 shows the nucleotide diversity and the net genetic distances among minke whales from different ocean basins based on the Kimura's two parameters method (Kimura, 1980). The larger distances are obtained in the pairwise comparisons involving the southern 'ordinary' minke whale. These values range from 0.082 to 0.088. The values among the other three minke whales range from 0.015 (comparison between Antarctic 'dwarf' and North Atlantic minke whales) to 0.025 (comparison between Antarctic 'dwarf' and North Pacific minke whales).

TAXONOMIC STATUS OF MINKE WHALES

Some authors (Omura, 1975; Rice, 1977) had proposed sub-specific status for the North Atlantic (*Balaenoptera acutorostrata acutorostrata*), North Pacific (*B. a. davidsoni*) and Antarctic 'ordinary' minke whales (*B. a. bonaerensis*). Arnason *et al.* (1993) suggested that given the large genetic distances between the southern 'ordinary' minke whale and minke whales from other oceanic basins the former should be given full species status *Balaenoptera bonaerensis*.

Rice (1998) reviewed both morphological (e.g., Omura, 1975; Best, 1985; Arnold *et al.*, 1987) and genetic (e.g., Pastene *et al.* 1994; Wada *et al.* 1991; Arnason *et al.*, 1993) data collected from extant minke whale populations and divided minke whales into two species; the larger Antarctic minke whale (*B. bonaerensis*), which is restricted only to the Southern Hemisphere, and the cosmopolitan common minke whale (*B. acutorostrata*). Furthermore he divided the common minke whale into three sub-species: North Atlantic *B. a. acutorostrata*, North Pacific, *B. a. scammoni* and Southern Hemisphere ('dwarf'), *B. a. subsp.* The sub-specific classification is controversial if we consider the phylogeny of common minke whales from different ocean basins. Recent genetic studies have shown a paraphyletic relationship between North Atlantic and southern 'dwarf' minke whales (see next section). The sub-specific classification of common minke whale should be re-considered in the light of results of additional genetic studies of minke whales based on nuclear DNA, and the completion of some on-going comparative morphological and morphometric analyses.

RECENT GENETIC STUDIES AND FUTURE WORK

Acevedo *et al.* (2005) studied the phylogenetic relationship of three 'dwarf' minke whales stranded in Navarino Island in the southern tip of the American continent, with 'dwarf' minke whales from the Antarctic (Areas IV and V, n=15) and Brazil (n=8), North Atlantic common minke whale, North Pacific common minke whale and Antarctic minke whale. A neighbor-joining-based tree of mtDNA control region sequences showed that, of three Brazilian haplotypes, one clustered with the Antarctic 'dwarf' minke whale clade and two with the North Atlantic common minke whale clade (Figure 3). The Chilean 'dwarf' minke whales were phylogenetically related to 'dwarf' minke whales from Brazil. No shared haplotypes were found between 'dwarf' minke whales from the Antarctic and Brazil, and significant level of genetic divergence (as measured by Kst) was found between them (data not shown).

A large collection of genetic samples from the 'dwarf' minke whale are necessary to further understand their genetic structure in the Southern Hemisphere and their relationship to other minke whales. Nuclear DNA analyses are necessary to complement the results found by the mtDNA analyses. This additional genetic analysis and the completion of on-going morphological and morphometric work are also important to clarify the taxonomy of the common minke whale.

MANAGEMENT IMPLICATIONS

On the basis on the morphological differences documented by Best (1985) and Arnold *et al.* (1987) and genetic differences (Wada *et al.*, 1991), the SC recognised the existence of two southern minke whales and agreed that the two minke whales in the Southern Hemisphere should definitely be considered separately for management purposes (IWC, 1991). In 1993, after examining the genetic information given by Pastene *et al.* (1994), the SC recommended the inclusion of the 'dwarf' minke whale in the Schedule, so that catch limits for Antarctic minke whales recognise the distinction between the two southern minke whales (IWC, 1994).

As mentioned above, the Committee had recommended the management of the two southern minke whale species separately. This had had practical implications for population assessment studies, and both Antarctic and 'dwarf' minke whales are recorded separately in JARPA and SOWER sighting cruise surveys. Experienced researcher and crew members involved in these surveys in the Antarctic indicated that there are distinct differences that can be detected at sea, e.g. body colouration, the white patch on the base of flipper and the swimming pattern. Some of these probably require good weather and observation conditions to be identified.

REFERENCES

- Acevedo, J., Aguayo-Lobo, A., Acuna, P., Goto, M., Zerbini, A. and Pastene, L.A. 2005. First record of the dwarf minke whale *Balaenoptera acutorostrata* in Chilean waters. Paper SC/57/IA10 presented to the IWC Scientific Committee. May 2005 (unpublished). 8pp.
- Acevedo, J., Aguayo-Lobo, A., Acuna, P. and Pastene, L.A. (in press). First record of the dwarf minke whale *Balaenoptera acutorostrata* in Chilean waters. *Journal Cetacean Res Manage* (in press).
- Arnold, P., Marsh, H. and Heinsohn, G. 1987. The occurrence of two forms of minke whales in east Australian waters with a description of external characters and skeleton of the diminutive or dwarf form. *Scientific Reports of the Whales Research Institute Tokyo* 38:1-46.
- Baker, A.N. (ed.). 1983. *Whales and dolphins of New Zealand and Australia. An Identification Guide*. Victoria University Press. 133pp
- Best, P.B. 1985. External characters of southern minke whales and the existence of a diminutive form. *Scientific Reports of the Whales Research Institute Tokyo* 36:1-33.
- da Rocha, J.M. and Braga, N.M.A. 1982. Brazil Progress report on cetacean research, June 1980 to May 1981. *Rep int. Whal. Commn* 32:155-9.
- Fujise, Y., Zenitani, R., Saino, S., Itoh, S., Kawasaki, M., Matsuoka, K. and Tamura, T. 1993. Cruise report of the 1992/93 Japanese research under the special permit for Southern Hemisphere minke whales. Paper SC/45/SHBa12 presented to the IWC Scientific Committee, April 1993 (unpublished). 39pp.
- Government of Japan. 1993. The 1993/94 research plan of whale resources in the Antarctic. Paper SC/45/SHBa10 presented to the IWC Scientific Committee, April 1993 (unpublished). 6pp.
- Hori, H., Bessho, Y., Kawabata, R., Watanabe, I., Koga, A. and Pastene, L.A. 1994. World-wide population structure of minke whales deduced from mitochondrial DNA control region sequences. Paper SC/46/SH14 presented to the IWC Scientific Committee, May 1994 (unpublished). 11pp.
- International Whaling Commission. 1991. Report of the Scientific Committee. *Rep. int. Whal. Commn* 41:51-89.
- International Whaling Commission. 1994. Report of the Scientific Committee. *Rep. int. Whal. Commn* 44:41-73.
- Kasamatsu F, Yamamoto Y, Zenitani R et al. 1993. Report of the 1990/91 southern minke whale research cruise under scientific permit in Area V. *Report of the International Whaling Commission* 43:505-522.
- Kato, H. and Fujise, Y. 2000. Dwarf minke whales; morphology, growth and life history with some analyses on morphometric variation among the different forms and regions. Paper SC/52/OS3 presented to the Scientific Committee. June 2000 (unpublished). 30pp.
- Kato, H., Hiroshima, H., Fujise, Y. and Ono, K. 1989. Preliminary report of the 1987/88 Japanese feasibility study of the special permit proposal for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 39: 235-248.
- Kato, H., Fujise, Y., Yoshida, H., Nakagawa, S., Ishida, M. and Tanifuji, S. 1990. Cruise report and preliminary analysis of the 1988/89 Japanese feasibility study of the special permit proposal for southern hemisphere minke whales. *Rep. int. Whal. Commn* 40: 289-300.

- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111-120.
- Nei, M, and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science, USA* 76:5269-5273.
- Omura, H. 1975. Osteological study of the minke whale from the Antarctic. *Scientific Reports of the Whales Research Institute* 27:1-36.
- Pastene, L.A., Fujise, Y. and Numachi, K. 1994. Differentiation of mitochondrial DNA between ordinary and dwarf forms of minke whale. *Report of the International Whaling Commission* 44:277-281.
- Pastene, L.A., Hori, H., Watanabe, K., Bessho, Y. and Goto, M. 1996. Phylogenetic relationships in the minke whale world-wide as revealed by two independent analyses of mitochondrial DNA. 7th Working Meeting of Specialists in Aquatic Mammals of South America, 22-25 October 1996, Viña del Mar, Chile. Supplement p.53.
- Rice, D.W. 1977. A list of the marine mammals of the world. *NOAA Tech. Rep.* 711:1-15.
- Rice, D.W. 1998. Marine Mammals of the World. Systematic and Distribution. *Marine Mammal Science* (Special Issue).
- Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic tree. *Mol. Biol. Evol.* 4(4):406-25.
- Wada, S. 1983. Genetic structure and taxonomic status of minke whales in the coastal waters of Japan. *Rep. int. Whal. Commn* 33:361-3.
- Wada, S., Kobayashi, T. and Numachi, K. 1991. Genetic variability and differentiation of mitochondrial DNA in minke whales. *Report of the International Whaling Commission* Special Issue 13:203-215.
- Zerbini, A.N., Secchi, E.R., Siciliano, S. and Simoes-Lopes, P.C. 1996. The dwarf form of the minke whale, *Balaenoptera acutorostrata* Lacepede, 1804, in Brazil. *Rep. int. Whal. Commn* 46:333- 340.

Table 1: Nucleotide diversity (diagonal) and net genetic distances, derived from mtDNA control region sequence data. AO= southern 'ordinary' minke whale, AD= southern dwarf minke whale, NP= North Pacific minke whale, NA= North Atlantic minke whale (from Pastene *et al.*, 1996).

	AO	AD	NP	NA
AO	0.025	0.086	0.082	0.088
AD		0.012	0.025	0.015
NP			0.014	0.020
NA				0.007

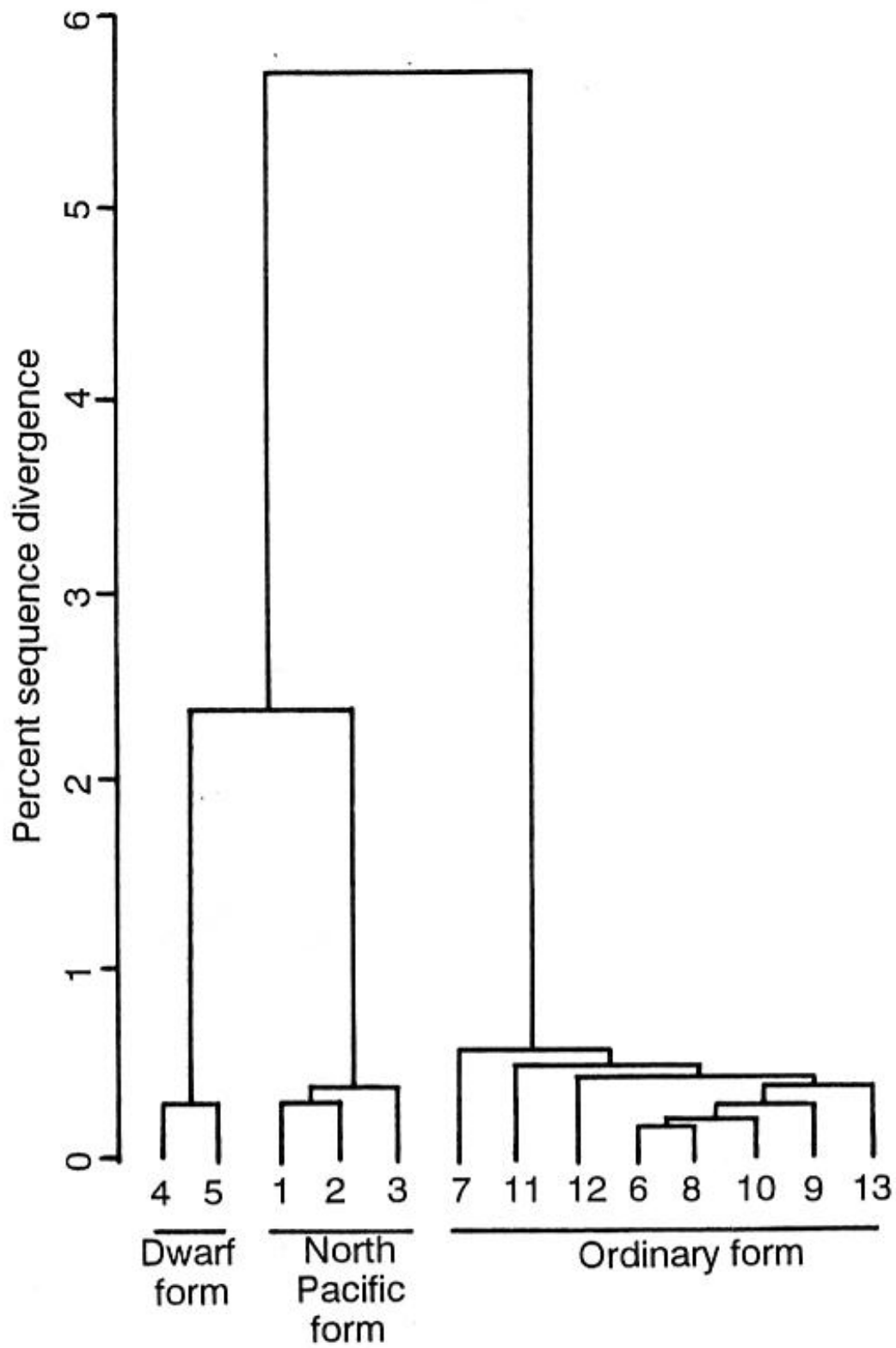


Figure 1: UPGM-derived dendrogram of 13 minke whale RFLP-derived mtDNA haplotype, based on sequence divergence among them (from Pastene *et al.*, 1994).

AO NP AD NA

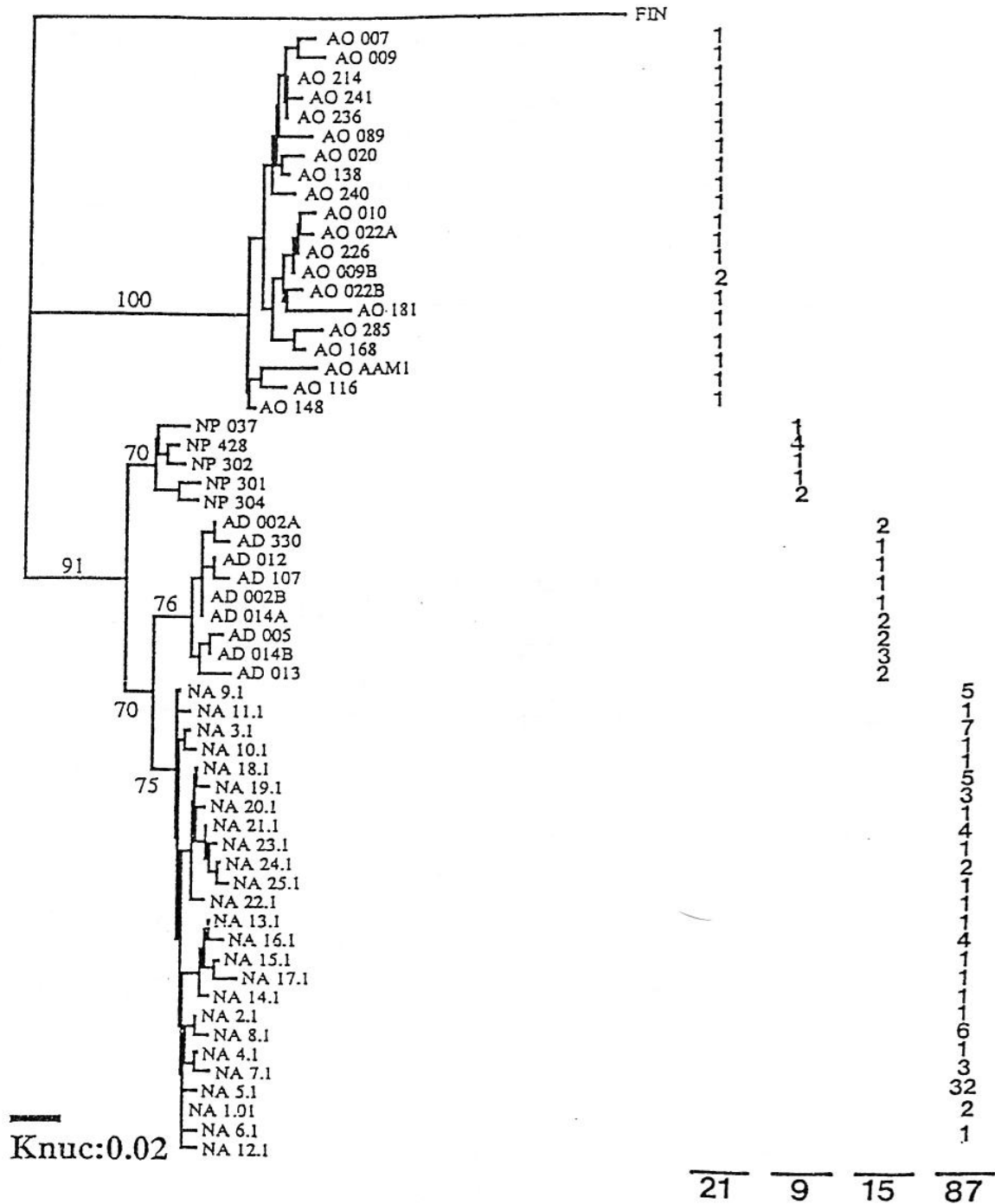


Figure 2: Neighbor-joining-derived tree of 56 unique mtDNA control region sequences in the minke whale AO= southern 'ordinary' minke whale, AD= southern dwarf minke whale, NP= North Pacific minke whale, NA= North Atlantic minke whale (from Pastene *et al.*, 1996).

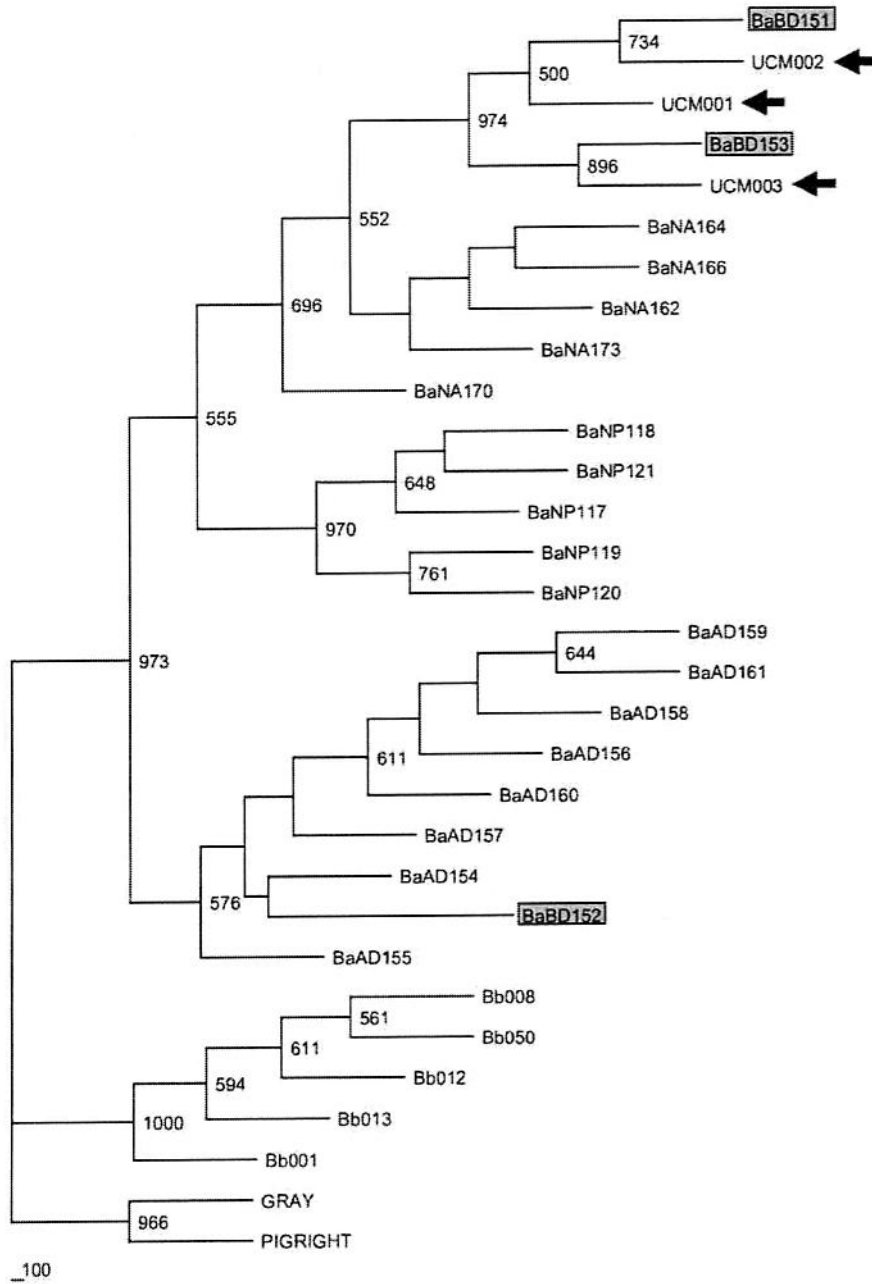


Figure 3: Neighbor-joining-derived tree of minke whale mtDNA control region sequences. Bb= *B. bonaerensis*; BaAD= *B. acutorostrata*, Antarctic dwarf; BaNP= *B. acutorostrata* North Pacific; BaNA= *B. acutorostrata* North Atlantic; BaBD= *B. acutorostrata* Brazil dwarf; UCM= *B. acutorostrata* Chile. Arrows shows the position of the three Chilean dwarf minke whale haplotypes. Brazilian dwarf haplotypes are indicated in squares. Figures show bootstrap values (in 1,000 simulations) (from Acevedo *et al.*, 2005).