HAPLOTYPE CHARACTERIZATION OF A STRANDED BALAENOPTERA PHYSALUS (LINNAEUS, 1758) FROM ANCONA (ADRIATIC SEA, CENTRAL ITALY)

VINCENZO CAPUTO*, MASSIMO GIOVANNOTTI

Dipartimento di Biochimica, Biologia e Genetica, Università Politecnica delle Marche, via Ranieri, 65, 60100 Ancona, Italy *Corresponding author, e-mail: v.caputo@univpm.it

Received 23 March 2009; accepted 6 May 2009

RIASSUNTO - *Caratterizzazione aplotipica di una balenottera comune spiaggiata presso Ancona (Mare Adriatico, Italia centrale)*. La sequenza della regione di controllo del DNA mitocondriale di un esemplare di balenottera comune (*Balaenoptera physalus*) spiaggiata presso Ancona (Mare Adriatico) viene descritta e confrontata con quelle note per la specie. L'aplotipo ottenuto è risultato identico a quello descritto per il Mar Ligure, permettendo di ipotizzare spostamenti fra l'area tirrenica e l'Adriatico centro-settentrionale.

Parole chiave: balenottera comune, Cetacea, Balaenopteridae, mtDNA, Mare Adriatico

Since the 1990s, genetic tagging with DNA markers has been considered a suitable tool for assessing the genetic composition, migratory movements, site fidelity and genetic effects of overexploitation of marine mammals (see Hoelzel et al., 2002). Concerning the fin whale (Balaenoptera physalus), genetic analyses performed on Mediterranean individuals have revealed the existence of a recently-diverged population, characterised by limited gene flow with North Atlantic conspecifics (Bérubé et al., 1998). Significant levels of divergence and heterogeneity in both nuclear and mitochondrial DNA have been reported and three private haplotypes have been found in the Mediterranean sample. The hypothesis that the Mediterranean population could be isolated is supported by sound evidence that breeding takes place inside the basin (Notarbartolo di Sciara et al., 2003). This population should be considered vulnerable because of the small number of individuals and their confinement in a partially degraded marine environment (Notarbartolo di Sciara *et al.*, 2003).

The fin whale regularly inhabits the western Mediterranean Sea, whilst, as most cetaceans (the only exception is the bottlenose dolphin, Tursiops truncatus), its numbers sharply decline in the eastern part of the basin (Lipej et al., 2004). Concerning the Adriatic Sea, fin whale occurrence is scarce, particularly in its shallow northern and central parts where appropriate habitat is lacking. In fact, depth is a critical parameter determining habitat choice by the fin whale, which is primarily observed in deep offshore waters (mean depth: 2248 m, Zanardelli et al., 1992). So far, in the Adriatic Sea 26 specimens have been reported, the majority of records being represented by sightings, followed by stranded animals and floating carcasses. Most observations occurred in summer and autumn along the eastern coast of the Adriatic basin (Fig. 1), the size of the stranded animals (7-15 meters) suggesting the predominance of juveni-



Figure 1. Records (black circles) of stranded fin whales in the North-central Adriatic Sea. 1, Sirolo beach (this paper); 2, Cesenatico (Notarbartolo di Sciara *et al.*, 2003); 3, Lido delle Nazioni (Notarbartolo di Sciara *et al.*, 2003); 4, Porto di Trieste (Lipej *et al.*, 2004); 5, Muggia (Lipej *et al.*, 2004); 6, Pirano (Lipej *et al.*, 2004); 7, Krk (Lipej *et al.*, 2004); 8, Pag (Lipej *et al.*, 2004); 9, Karinsko more (Notarbartolo di Sciara *et al.*, 2003).

les (Lipej *et al.*, 2004). On the 22^{nd} of November 2007 a fin whale was found stranded at Sirolo beach near Ancona ($43^{\circ}32'01.94''N 13^{\circ}35'38.19''E$, central Italy; Fig. 1). The specimen was an 18 m long subadult female and the causes of its death are unknown. In order to determine the possible provenience of this individual, we sequenced a portion of the mtDNA control region to compare the obtained haplotype to those described by Bérubé *et al.* (1998).

Genomic DNA was extracted from 25 mg of bone marrow using a standard phenolchloroform protocol (Sambrook *et al.*, 1989). PCR amplification of the whole mtDNA control region (*D-Loop*) was performed with primers MT3 and MT4 (Arnason *et al.*, 1993). PCR products were purified with the ExoSAP-IT Kit (Amersham Pharmacia Biotech) and cycle-sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequences were then resolved on an ABI310 Genetic Analyser (Applied Biosystems). The nucleotide polymorphisms were confirmed by sequencing PCR products in both directions. Sequences were aligned using the ClustalX program (Thompson *et al.*, 1997).

A total of 843 base pairs (bp) of the D-loop were sequenced. The nucleotide composition of this trait was: A: 28.8%; T: 31.6; C: 23.4%; G: 16.3% (GenBank accession number: FJ832130). The comparison of 288 bp of the control region of the fin whale from Ancona with the haplotypes available from other populations from the North Atlantic, Sea of Cortez and Mediterranean basin (Bérubé *et al.*, 1998) revealed that the haplotype of the stranded individual was identical to BP 46, reported as a private haplotype of the Mediterranean population. Particularly, BP 46 seems to be proper to the Ligurian Sea, considered not only the principal feeding ground, but also a main breeding ground for the fin whale in the Mediterranean Sea (Orsi Relini, 2000). The presence of this haplotype in the northern Adriatic Sea suggests the occurring of vast movements across the whole basin.

Considering that the North-central Adriatic Sea does not seem to be suitable for this species, the stranded individual might be considered a vagrant individual from the Ionian feeding grounds (see Notarbartolo di Sciara *et al.*, 2003). Alternatively, the increasing presence of other planctivorous vertebrates, like the humpback whale (*Megaptera novaeangliae*) and the basking shark (*Cetorhinus maximus*) (Lipej *et al.*, 2004), suggests that changes in zooplankton abundance over the continental shelf may induce fin whales to feed close to the coast (Panigada *et al.*, 2005).

ACKNOWLEDGEMENTS

We thank Gabriele Achille (Università di Camerino) for providing a tissue sample and Roberto Danovaro (Università Politecnica delle Marche) for having informed us about the age and sex of the stranded fin whale.

REFERENCES

- Arnason U., Gullberg A. and Wideqren B. 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. *Molec. Biol. Evol.*, 10: 960-970.
- Bérubé M., Aguilar A., Dendanto D., Larsen F., Notarbartolo di Sciara G., Sears R., Sigurjonsson J., Urbàn R. and Palsbøll P. 1998. Population genetic structure of North Atlantic, Mediterra-

nean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758): analysis of mitochondrial and nuclear loci. *Mol. Ecol.*, 7: 585–599.

- Hoelzel A.R., Goldsworthy S.D. and Fleischer R.C. 2002. Population genetic structure. In: Hoelzel A.R. (ed.), Marine Mammal biology. Blackwell Publishing, Malden, Mass., 325-352.
- Lipej L., Dulčić J. and Kryštufek B. 2004. On the occurrence of the fin whale (*Balaenoptera physalus*) in the northern Adriatic. *J. Mar. Biol. Ass.* U.K., 84: 861-862.
- Notarbartolo di Sciara G., Zanardelli M., Jahoda M., Panigada S. and Airoldi S. 2003. The fin whale, *Balaenoptera physalus* (L., 1758) in the Mediterranean Sea. *Mammal. Rev.*, 33:105-50.
- Orsi Relini L. 2000. The cetacean sanctuary in the Ligurian Sea: a further reason. *Biol. Mar. Medit.*, 7: 117-27.
- Panigada S., Notarbartolo di Sciara G., Zanardelli Panigada M., Airoldi S., Borsani J. F., Jahoda M. 2005. Fin whales (*Balaenoptera physalus*) summering in the Ligurian Sea: distribution, encounter rate, mean group size and relation to physiographic variables. J. Cetacean Res. Manage., 7: 137-145.
- Sambrook J., Fritsch E.F. and Maniatis T. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, New York.
- Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. and Higgins D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, 56: 604-614.
- Zanardelli M., Notarbartolo di Sciara G. and Jahoda M. 1992. Photoidentification and behavioural observations of fin whales summering in the Ligurian Sea. *European Res. Cetaceans*, 6: 86–89.