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Short Note

Haplotype characterization of a stranded Common Minke Whale calf (*Balaenoptera acutorostrata* Lacépède, 1804): is the Mediterranean Sea a potential calving or nursery ground for the species?

Nicola Maio[1,*], Massimo Giovannotti[2], Vincenzo Caputo Barucchi[2], Agnese Petraccioli[1], Francesco Pollaro[3], Fabio Maria Guarino[1], Andrea Splendiani[2], Roberta De Stasio[1], Gaetano Odierna[1]

[1]*Dipartimento di Biologia, Complesso Universitario di Monte Sant'Angelo, Università degli Studi di Napoli Federico II, Edificio 7, via Cinthia, 26 – 80126 NAPOLI, Italy*
[2]*Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, via Brecce Bianche, 60100 Ancona, Italy*
[3]*Centro Studi Ecosistemi Mediterranei, Via Caracciolo, Pollica (Salerno), frazione Pioppi, 84068, Italy*

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Abstract

The stranding of a suckling calf of Common Minke Whale (*Balaenoptera acutorostrata*) on the coast near Salerno (Campania, Southern Italy) is reported. The molecular analysis of a partial sequence of the mitochondrial DNA control region shows that the animal bore a haplotype identical to haplotype Ba169 considered as typical of individuals from North Atlantic population. Historical data and our results suggest the possibility that the Mediterranean Sea might be a potential calving or nursery ground for this species.

The Common Minke Whale (CMW) is a cosmopolitan species found in all oceans and virtually at all latitudes, from 65° S to 80° N. The species is discontinuously distributed throughout the Northern Hemisphere and is rare in tropical waters. Its migration patterns are poorly known. It occurs in the North Atlantic (with the subspecies *Balaenoptera acutorostrata acutorostrata* Lacépède, 1804 North Atlantic Minke Whale), in the North Pacific (with the subspecies *B. a. scammoni* Deméré, 1986. North Pacific Minke Whale), and in the Southern Hemisphere (with the un-named subspecies Dwarf Minke Whale), but its presence has not been recorded for the Northern Indian Ocean (Mead and Brownell, 2005). Since 2000, the International Whaling Commission Scientific Committee has recognized the Antarctic population of Minke Whale as a good species, *B. bonaerensis* Burmeister, 1867, which is partially sympatric in the Southern hemisphere with the Dwarf Minke Whale. Accordingly, molecular phylogenetic analyses confirm the specific rank of *B. bonaerensis* (Árnason et al., 1993; Pastene et al., 1994; Born et al., 2003). Regarding *B. a. acutorostrata*, Andersen et al. (2003) identified four genetically discrete groups in the North Atlantic: 1) West Greenland, 2) a central group (East Greenland and Jan Mayen), 3) a northeastern group (Svalbard, Barents Sea, Vestfjorden/Lofoten in NW Norway), and 4) North Sea. It should be stressed that the subdivision into four genetically discrete groups in the North Atlantic has been questioned by Bakke et al. (1996) and Anderwald and Evans (2008). Anderwald et al. (2011) in a study with large sample size showed evidence of a mixed assemblage of two putative cryptic populations (Putative Breeding Stocks PBS1 and PBS2) represented in approximately equal proportions in most areas, except the Norwegian North Sea (having a higher proportion of individuals from PBS1) and West Greenland (having a higher proportion of individuals from PBS2). The Common Minke Whale (*Balaenoptera acutorostrata* Lacépède, 1804) (CMW henceforth) is a species listed in the Appendix I of CITES (except for the population of West Greenland listed in Appendix II), and it is considered a "threatened species" in the Annex II of the Barcelona Convention for Protection against Pollution in the Mediterranean Sea. This species is also included in the Appendix II of the Bern Convention on the Conservation of European Wildlife and Natural Habitats, and considered a "Strictly protected fauna species". It is a "species in need of strict protection" in European Union by the Annex IV of the Council Directive 92/43/EEC of May 21th 1992 on the conservation of natural habitats and of wild fauna and flora, known as "Habitats Directive". Finally, it is listed as "Vulnerable" at the European level, and classified as "Least Concern" on the IUCN Red List of Threatned Species (vers.2016–2) (Reilly et al., 2008). In the Mediterranean Sea, according to the Annex 4 of Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS), the CMW is considered as a "visitor species"; "occasional" in the Italian seas and "vagrant", with one

*Corresponding author
Email address: nicomaio@unina.it (Nicola Maio)

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record, in the Black Sea (Notarbartolo di Sciara and Demma, 1997; Reeves and Notarbartolo di Sciara, 2006; Notarbartolo di Sciara and Birkun, 2010; Podestà et al., 2014; Cagnolaro et al., 2015). Genetic tagging with DNA markers is a suitable tool for assessing the genetic composition, migratory movements, site fidelity and genetic effects of overexploitation, already successfully used in other Mediterranean whales (Caputo and Giovannotti, 2009). On April 01, 2010, a young specimen of CMW was found beached lifeless at Santa Teresa beach (Salerno, southern Italy), (Fig. 1a-d). This individual was a very young male of 3.30 m body length (head-to-tail length). The carcass was in good preservation conditions, at the initial stage of decomposition (Code 2, fresh carcass) according to Geraci and Loundsbury (2005). The umbilical scar was still neither completely healed nor completely re-epithelized (Fig. 1d). Even if the external examination showed a rather poor nutritional status, the measurements of blubber (2.0–5.0 cm) were in the standard range and, during necropsy, milk was found within the stomach chambers, while the intestine was completely devoid of contents. This indicates that the whale died shortly after suckling (Harms et al., 2008; Shoham-Frider et al., 2014). Finally, the examination of the carcass revealed that the cranial bone sutures were not completely closed.

Figure 1 – (a) The suckling calf of CMW stranded at Salerno in 2010 (photo by F. Di Nocera). Particular of the head (b), flipper (c) and ventral view: the arrow indicate the umbilical scar (d) (photos by N. Maio).

The possible cause of death may be the lesions observed in the lungs and related to the infection caused by *Klebsiella pneumoniae* (see Kawashima et al., 2004 for disease to Cetacean lungs from this bacterium), as suggested by necroscopic and histopathological analyses performed by the staff of the Diagnostic Provincial Section (DPS) of Salerno of the Experimental Zooprophylactic Institute of southern Italy (EZISI) (Maio et al., 2012). The skeleton was preserved in the Zoological Museum of The University of Naples (Cagnolaro et al., 2014). In order to ascertain the population of origin of the young stranded individual of Salerno we conducted a molecular study, namely the analysis of a partial sequence of the mitochondrial DNA (mtDNA, henceforth) control region. The results of this analysis are here presented together with a historical survey of stranded or incidentally caught *B. acutorostrata* individuals along the coasts of the Mediterranean Sea. The relative records, gathered through bibliographic and museological searches (Cagnolaro et al., 2014), were collected according to Bearzi et al. (2011). Genomic DNA was extracted from a piece of muscle preserved in 70% ethanol using a standard phenol-chloroform protocol (Sambrook et al., 1989). PCR amplification of the first 500 base pairs (bp) at the 5' end of the mtDNA control region was carried out with primers MT4 (Árnason et al., 1993) and P2R (Pastene et al., 2007). The PCR protocol included an initial denaturation step at 95 °C for 5 min followed by 35 cycles of 30 s at 94 °C; 30 s at 50 °C; 30 s at 72 °C and a step of final elongation of 10 min at 72 °C. PCR products were purified with the ExoSAP-IT Kit (Amersham Pharmacia Biotech) and cycle-sequenced using the ABIPrism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequence was then resolved on an ABI310 Genetic Analyser (Applied Biosystems). The nucleotide sequence was confirmed by sequencing PCR products in both directions, using the above primers, and comparing the sequence obtained to similar sequences previously identified by Pastene et al. (2007). To determine the haplotype of the stranded individual analysed, the sequence obtained was aligned with homologous sequences retrieved from GenBank and representing *B. acutorostrata* and *B. bonaerensis* haplotypes described by Pastene et al. (2007). One sequence of *Eschrichtius robustus* and one of *Eubalaena glacialis* were used as outgroups. The alignment was carried out in ClustalW (Larkin et al., 2007) using default parameters. The phylogenetic relationships between *B. acutorostrata* haplotypes were determined using the Neighbor-Joining (NJ) method as implemented in MEGA version 5 (Tamura et al., 2011). NJ analysis was based on Kimura 2-parameters distances (K2P) and the confidence at nodes assessed by 1000 bootstrap replicates. The alignment of 343 bp of the control region of the CMW from Salerno (GenBank accession number, AN, KX079634) with homologous sequences of various individuals from Antarctic, North-East Pacific, North- West Pacific, Sea of Japan, North Atlantic, Brazil, Mediterranean Sea Pastene et al. (2007) revealed that the studied individual bore a haplotype identical to Ba169, considered as a Northern Atlantic haplotype (Pastene et al., 2007, 2010) (Fig. 2). This haplotype is identical to the haplotype N48 borne by a specimen from West Greenland area (Andersen et al., 2003).

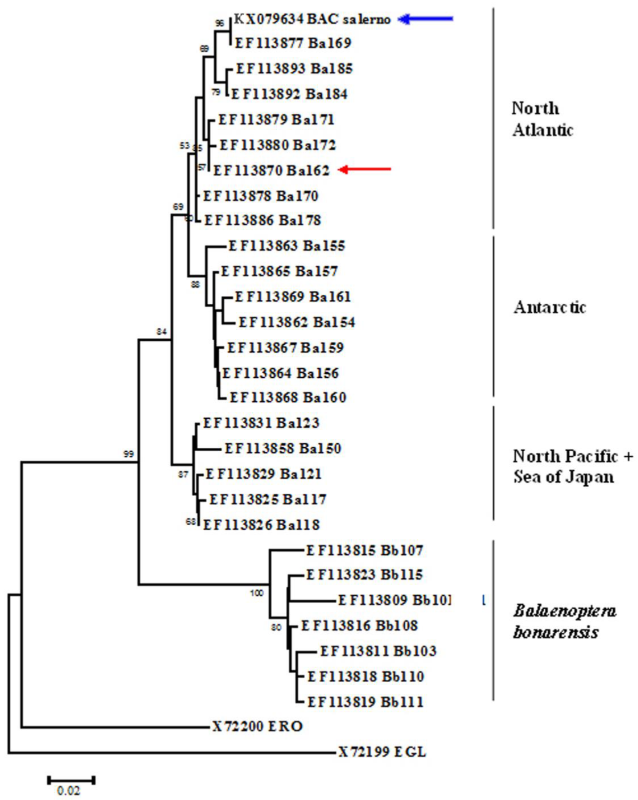

Figure 2 – Neighbor-Joining tree based on mtDNA control region sequences. Blu arrow: Salerno specimen (this work). Red arrow: calf stranded on the Israeli coast in 2000. Ba: *Balaenoptera acutorostrata*; Bb: *Balaenoptera bonaerensis*. For each sequence used, GenBank accession number and haplotype code according to Pastene et al. (2007) are given. ERO: *Eschrichtius robustus*; EGL: *Eubalaena glacialis* (Árnason et al., 1993). Bootstrap values higher than 50% are indicated at nodes.

The observations made on the carcass suggest the animal was a suckling, few months-old calf. The historical survey from 1771 to 2016, revealed 49 records of stranded or caught CMW from the Mediterranean Sea (Cagnolaro et al., 2014) (Fig. 3). Fifteen of the 49 records were represented by young calves, less than four meters long (Fig. 3). Date of capture or stranding, location and size of these 15 specimens are given in Tab. 1.

Thirteen additional records of CMW strandings on the Mediterranean coasts from 1944 to 2012 reported by the Mediterranean

Database on Cetacean Strandings (MEDACES, updated February 2015) were not included in Tab. 1 because not supported by scientific paper or photographic records. Taking into account the period 1993–2007 Kerem et al. (2012), calculated a rate of around four occurrences of the CMW per year in the Mediterranean. This frequency is an order of Magnitude above the "several occurrences in a decade" definition for "visitor" status.

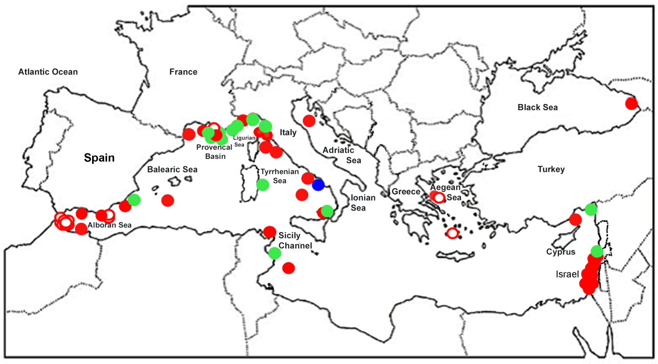

Figure 3 – Known occurrences of CMWs in the Mediterranean Sea. Red circles: historical data of specimens more than four meters long. Green circles: young specimens less than four meters long. Blue circles: suckling calf, this report. Red/white circles: records by MEDACES. (Modified from Notarbartolo di Sciara and Birkun, 2010).

Other two CMW calves, with the same haplotypes (Ba162) but differing from that of the calf here studied, are known in the Mediterranean sea: a calf from Toulon region (France) in 1998 (Macé et al., 1999) and a 3.5 m male suckling calf (with fresh milk traces in the forestomach), stranded 3.5 km off Akko shore on the Israeli coast in May 8, 2000, Ba162 (red arrow in Fig. 2). The latter haplotype was assigned to the Northern Atlantic populations by Pastene et al. (2007) and Kerem et al. (2012) (see also Tab. 1). Furthermore, this haplotype was identical to the haplotypes Bacu001 and N1 possessed by a specimen from North West Atlantic (Rosel and Wilcox, 2014) and a specimen from North Atlantic (Bakke et al., 1996), respectively. Independently from the group of origin, the molecular data for the individuals of CMW occurring in the Mediterranean Sea indicate that they belong to North Atlantic populations. In the Mediterranean Sea, the presence of CMW is currently considered only "occasional" (Cagnolaro et al., 1983, 1993, 2015; Notarbartolo di Sciara and Demma, 1997; Reeves and Notarbartolo di Sciara, 2006; Podestà et al., 2014) and "clearly uncommon" in the Ligurian and Tyrrhenian Seas (Van Waerebeek et al., 1999; Haug et al., 1996). The present finding of a suckling calf together with all the historical data given in Tab. 1, in particular the ones recorded during the last four decades, are of particular interest. In fact, fifteen calves ranging from 300 to 380 cm (between 3 and 5 months old) were recorded from the Mediterranean (12 from central basin). Thirteen of them were recorded in spring (87%), with eight calves observed in April (53%). This fits well with delivery season of this species mainly occurring during winter at low latitude warm waters. In fact, soon after birth, the calves of this species follow their mothers towards high latitude cold waters, to spend their first summer. At birth the calves are 2.6-2.8 m long (up to 3.0 m in Perrin et al., 2009, and will stay with their mothers for about 6 months (sometimes one year or even two years) before being weaned at a length of between 4.5 m and 5.5 m (Stewart and Leatherwood, 1985; Jefferson et al., 1993; Notarbartolo di Sciara and Demma, 1997; Van Waerebeek et al., 1999; Harms et al., 2008; Perrin and Brownell, 2009; Christiansen et al., 2014). However, nursering calves records documented during the last 40 years also suggest that members of this species regularly migrate, in the winter season, from the North Atlantic Ocean to the central Mediterranean Sea (particularly to the Ligurian and Tyrrhenian Seas) where possibly females give birth to their offspring. The finding of a very young calf in the study area is compatible with the hypothesis that some female individuals enter the Mediterranean Sea to deliver (Fraija-Fernández et al., 2015). The possibility that CMWs might deliver their calves in the Mediterranean Sea was also suggested by Van Waerebeek et al. (1999) and Öztürk et al. (2015). Van Waerebeek et al. (1999) and Robineau (2005) did not exclude that a year-round resident population may exist in the Mediterranean. Interestingly, our historical survey shows that juvenile records are mainly concentrated in the Ligurian Provencal basin. All these evidences support the hypothesis that the Mediterranean Sea can also be used as a potential calving or nursery ground by the CMWs. This hypothesis is not in conflict with the possibility that the Mediterranean basin may be also a stopover for mother-calf pairs on the way back further North. On the whole, the assertion that there is no evidence for a stable and viable Mediterranean subpopulation of CMW (Notarbartolo di Sciara and Birkun, 2010) stands and all data confirm and emphasize the importance of this region for this whales. In conclusion, our hypothesis, if confirmed, could have a relevant impact from a conservational point of view. In fact, in the light of the data presented in this study, CMW should be considered as a "vulnerable species" in the Mediterranean in the IUCN Red List. The "vulnerable" status for this species in the Mediterranean would be justified also because of the small number of individuals and their confinement in a partially degraded marine environment, as evidenced by Notarbartolo di Sciara and Birkun (2010).

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Table 1 – Records of nursing or unweaned calves of CMW less than four meter long. F: female; M: male.

Date	Location	Event	Animals (gender, size)	Source and Notes
1878, 18 February	Villefranche-sur-Mer, Nice, France	Captured	1 (3.00 m)	(Reeves and Notarbartolo di Sciara, 2006)
1916, 26 April	Camogli (Province of Genoa, Italy)	Stranded, possibly by caught	1 (3.35 m)	Skull and stuffed skin in Civic Museum of Natural History "G. Doria" of Genoa (Cagnolaro et al., 2014)
1975, May	Mahdia (Tunisia)		1 (about 3 m)	(Reeves and Notarbartolo di Sciara, 2006)
1977, 9 June	Bandol, France	Captured	1 F (3.75 m)	(Reeves and Notarbartolo di Sciara, 2006)
1982, 20 April	St. Raphael (France)		1 F (3.60 m) dead Stillborn	Umbilical cord and placenta still attached (Duguy, 1983; Van Waerebeek et al., 1999; Bompar, 2000; Reeves and Notarbartolo di Sciara, 2006; Notarbartolo di Sciara and Birkun, 2010; Cagnolaro et al., 2015)
1991, 17 May	Turas, Bosa (Province of Nuoro, Italy)	Stranded	1 (3.50 m)	(Reeves and Notarbartolo di Sciara, 2006)
1998, 12 April	Antignano (Province of Livorno, Italy)	Stranded	1 (3.40 m)	Skeleton in Natural History Museum of the Mediterranean of the Livorno Province reported as "newborn" by Roselli et al. (2014)
1998, 24 April	Near Giens peninsula, France	Stranded after caught in a net	1 M (3.40 m)	(Robineau, 2005)
1998, May	Toulon region, France	By caught	1 M (3.65 m)	(Macé et al., 1999; Reeves and Notarbartolo di Sciara, 2006)
2000, 8 May	Akko, Israel	Entangled in gill net	1 M (3.50 m)	(Pastene et al., 2007; Kerem et al., 2012)
2008, 11 August	Anse de Bonnieux (Martigues, France)	Stranded	1 M (3.80 m)	(Dhermain et al., 2009, 2011)
2010, 1 April	Salerno, Italy	Stranded	1 M (3.30 m)	Skeleton in Zoological Museum of the University of Naples Federico II (Maio et al., 2012; Cagnolaro et al., 2014)
2014, 28 April	Santa Pola, Alicante, Spain	Stranded	1 F (3.00 m)	(Fraija-Fernández et al., 2015)
2015, 10 April	Yumurtalik, Turkey	Stranded	1 F (3.55 m)	(Öztürk et al., 2015)
2016, 10 April	Baia S. Antonio, Milazzo (Province of Messina, Italy)	Stranded	1 F (3.27 m)	(Insacco et al., in press)

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