

OCEANIC GIANTS IN THE MEDITERRANEAN: FIRST MITOCHONDRIAL ANALYSIS OF LEATHERBACK TURTLES (*DERMOCHELYS CORIACEA*) IN THE ADRIATIC AND TYRRHENIAN SEAS

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The leatherback turtle *Dermochelys coriacea* (Vandelli, 1761) is an occasional trophic visitor of the Mediterranean basin. So far, very few individuals have been genetically analysed from this sea and none from Italy. We sequenced a mitochondrial DNA fragment of three specimens of *D. coriacea* found in recent years along the North-Adriatic and Tyrrhenian shores. They were all females approaching adult stage. Causes of death were attributable to the main threats for sea turtles in Mediterranean waters, all related to human activities (collisions with boats, entanglement in fishing nets and plastic debris ingestion). Two different mitochondrial haplotypes were observed, with the two North-Adriatic turtles sharing the same one. Compared to known *Dermochelys* sequences and previous genetic characterization of rookeries, these results suggest that the most probable origin of at least two of the three leatherbacks was the western Atlantic.

Key words: leatherback turtle, mtDNA, origin, central Mediterranean, human threats

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Sedmopruga usminjača *Dermochelys coriacea* (Vandelli, 1761) povremeni je posjetitelj Mediterana. Dosad je genetički analizirano vrlo malo tih jedinki, pritom nijedna iz Italije. Sekvencionirali smo fragment mtDNA tri primjerka *D. coriacea* pronađenih u posljednje vrijeme na obalama sjevernog Jadrana i Tirenskog mora. Sve su bile subadultne ženke. Uzorci smrti pripisani su glavnim prijetnjama morskim kornjačama u Mediteranu, a sve su povezane s ljudskom aktivnošću (sudari s plovilima, zapetljavanje

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u ribarske mreže i gutanje plastičnog otpada). Zabilježena su dva mitohondrijalna haplotipa, pri čemu su dvije sjevernojadranske kornjače imale isti haplotip. U usporedbi s poznatim *Dermochelys* sekvencama i ranijim genetskim istraživanjima gnijezdišta, ovi rezultati sugeriraju da je najvjerojatnije porijeklo bar dviju od tri usminjača bio zapadni Atlantik.

Ključne riječi: sedmopruga usminjača, mtDNA, porijeklo, centralni Mediteran, humane ugroze

INTRODUCTION

The leatherback turtle *Dermochelys coriacea* (Vandelli, 1761) is distributed worldwide at tropical and subtropical latitudes. It is reported in Mediterranean and Italian waters occasionally during feeding migrations (CASALE *et al.*, 2003), but no nesting within the basin has ever been confirmed by direct observations. The species is globally listed as Vulnerable in the IUCN Red List of Threatened Species (A2b,d: www.iucnredlist.org), although threats and trends of abundance vary considerably in different populations. In order to assess population structure (DUTTON *et al.*, 2013) and to allow the identification of source rookeries for stranded or incidentally caught animals (PROSDOCIMI *et al.*, 2014; RODEN *et al.*, 2017; VARGAS *et al.*, 2019), western and eastern Atlantic colonies were genetically characterised for a variable fragment of the mitochondrial DNA (mtDNA). Very few individuals found in the Mediterranean have been previously genotyped (VELLA & VELLA, 2016; RODEN *et al.*, 2017), and none from Italian waters, which are a central crossroad in migration routes within the basin and an important foraging area for Mediterranean and Atlantic sea turtles (GAROFALO *et al.*, 2013a; 2013b). To trace the origin of three individuals from Italy [two found along the North-Adriatic coast (NOVARINI *et al.*, 2010, 2017) and one bycaught in a fishing net in the Tyrrhenian waters off Calabria, Fig.1], we sequenced mtDNA control region fragment, the same marker analysed previously for the nesting colonies in the western and eastern Atlantic (DUTTON *et al.*, 2013).

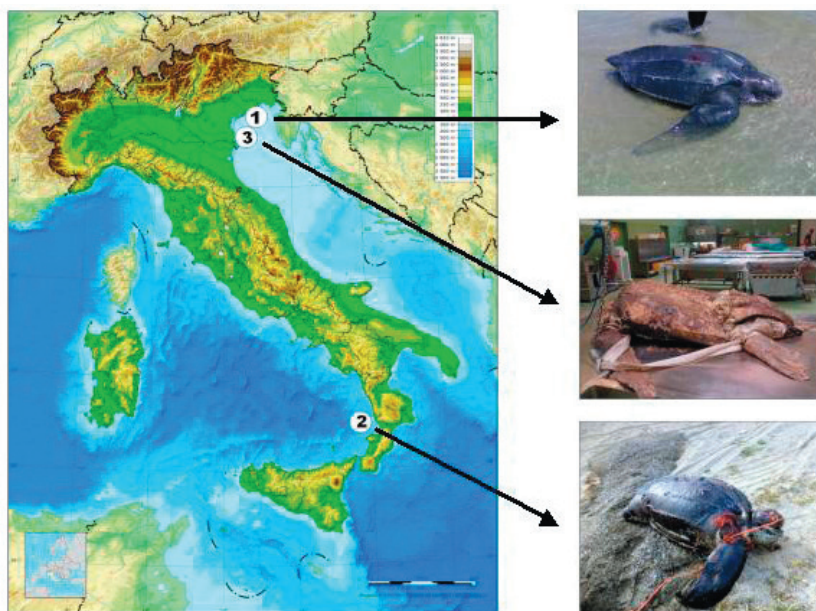


Fig. 1. Recovery sites of the turtles analysed. Photo credits: 1) C. Rigato; 2) M.A.R.E. Calabria; 3) UniPD.

MATERIALS AND METHODS

Information about the three leatherback turtles is summarized in Tab. 1. Size and sex of the animals, as well as causes of death, were determined during necropsy, although the 2014 specimen had missing gonads due to the bad state of the carapace and was therefore sexed from external morphology (NOVARINI *et al.*, 2017). Muscle samples (25 mg) were collected and frozen. DNA was extracted with the QIAamp Mini Kit (QIAGEN). A mtDNA control region fragment of 763 bp encompassing the tRNA^{Pro} and the D-loop was amplified and sequenced using the primer pair LCM15382/H950G (ABREU-GROBOIS *et al.*, 2006), following the protocols as in (MARCHIORI *et al.*, 2017). These primers are used to amplify the same DNA segment of different sea turtle species: *Caretta caretta*, *Chelonia mydas* and *D. coriacea*. Sequences were analysed using the DNA Sequencing Analysis Software version 5.1 (Applied Biosystems). The multiple alignment program included in the package Vector NTI version 9.1 (Invitrogen) was used to align the sequences. Our sequences were deposited and compared with those available from the same species in GenBank (www.ncbi.nlm.nih.gov). Haplotypes were classified according to the nomenclature used in DUTTON *et al.* (2013).

Tab. 1. Field data, necropsic results and mtDNA haplotypes for the turtles.

ID	Date of recovery	Site of recovery	Cause of death	CCL (cm)	Sex	mtDNA haplotype	GenBank Acc. N°
1	02.06.2009	Lido di Venezia (VE)	Bacterial gastroenteritis	138	F	Dc3.1	MK674798
2	11.10.2013	Lamezia Terme (CZ)	Asphyxia in fishing nets	143	F	Dc1.1	MK674797
3	03.10.2014	Chioggia (VE)	Boat strike	147	F	Dc3.1	MK674798

RESULTS

The sequenced leatherback turtles (Tab. 1) were all females, at threshold sizes (STEWART *et al.*, 2007) between subadult and adult stages (mean CCL: 142.7 ± 4.5 cm). All causes of death appeared to be related to the main threats for sea turtles in the Mediterranean waters: collisions with boats, entanglement in fishing nets and plastic debris ingestion, which in sample 1 may have caused severe bacterial gastroenteritis (POPPI *et al.*, 2012). Two different haplotypes were found: Dc1.1 and Dc3.1 (sequences deposited in GenBank, accession numbers: MK674797 and MK674798, respectively). The latter haplotype (Dc3.1) was shared by the two North-Adriatic turtles.

DISCUSSION AND CONCLUSIONS

DUTTON *et al.* (2013) described seven and nine distinct Management Units (MUs) for *Dermochelys coriacea* based on mtDNA and nuclear microsatellite data, respectively, from known leatherback nesting colonies of the western and eastern Atlantic. Ten haplotypes were found, of which Dc1.1 was the most common and was present in all rookeries at frequencies ranging from 39% (Brazil) to 94% (Florida). The second most common haplotype in the same colonies was Dc3.1, which was found in all western Atlantic (Caribbean) populations and in Gabon (Africa) at frequencies ranging from

3% (U.S. Virgin Islands) to 61% (Brazil). Haplotypes Dc1.1 and Dc3.1, separated by five mutations, are central in the two-star-shaped phylogroup network of D-loop haplotypes recovered by DUTTON *et al.* (2013). While haplotype Dc1.1 is frequent in both eastern and western Atlantic colonies (so it is not a good “source-tag”), haplotype Dc3.1 is widespread in western Atlantic colonies and rare in the eastern ones. The presence of Dc3.1 in two of our three turtles may thus be indicative of their western Atlantic origin, even though they cannot be ascribed to a specific colony.

Further analysis using nDNA markers (microsatellites) would allow more precise assignment of leatherbacks to the nesting population of origin (STEWART *et al.*, 2016; RODEN *et al.*, 2017), while more extensive sampling is needed to better assess patterns of connectivity between Mediterranean foraging areas and Atlantic nesting populations. Our three female *D. coriacea* were large sub-adults, and they all appear to have died as a result of human activities off the Italian coasts. Therefore, anthropogenic impacts in the central Mediterranean area may negatively affect not only the Mediterranean populations of loggerhead and green turtles (GAROFALO *et al.*, 2013a; 2013b), but also Atlantic leatherbacks, just before recruitment to their reproductive populations.

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SUMMARY

Oceanic giants in the Mediterranean: first mitochondrial analysis of leatherback turtles (*Dermochelys coriacea*) in the Adriatic and Tyrrhenian seas

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the same haplotype. The two haplotypes cannot be ascribed to a single colony, due to their common presence in most of the Atlantic rookeries previously characterized. However, the leatherbacks found in North-Adriatic waters could probably have originated from the western Atlantic, judging from the evaluation of the frequencies of their haplotype in the possible source rookeries.