THE FIRST GENETIC ANALYSES OF THE LEATHERBACK TURTLE, *DERMOCHELYS CORIACEA* FROM A STRANDING IN CENTRAL MEDITERRANEAN.

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Abstract

On the 16th of July 2015, a dead Leatherback turtle, *Dermochelys coriacea*, stranded in Cirkewwa, Malta and was collected by the Armed Forces of Malta. This is the first stranded record of this species for the Maltese islands. As part of ongoing conservation field research on marine turtles around the Maltese islands, the presence of this species in the central Mediterranean has been monitored too for the past 20 years and has been recorded only three times. The specimen found dead and stranded was tissue sampled for genetic analyses at three mtDNA loci and three nuclear DNA loci. This allowed for a first time genetic study to compare the characters of this species in the Mediterranean with any other genetic sequence data already available for this species from other regions.

Keywords: Turtles, Conservation, Genetics, South-Central Mediterranean

The Leatherback turtle, *Dermochelys coriacea* (Vandelli, 1761), is the most widely distributed sea turtle species in the world, with its population being subdivided into 7 regional management units (RMUs). In 2009, this species has been evaluated as Vulnerable by IUCN, with some of its RMUs being rated at a higher risk of extinction [1]. Mediterranean records for this species are limited to rare sporadic observations of isolated individuals and with no records of nesting sites in the region. Any specimens of this species in the area are reported as migrants from the Atlantic Sea [2,3].

As part of ongoing conservation field research on marine turtles around the Maltese islands, the presence of this species in the central Mediterranean has been monitored as well for the past 20 years and has been recorded few times. On the 16th of July 2015, a dead specimen of *D. coriacea* weighing approximately 200kg and with a carapace of about 2m long from head to tail was collected by the Armed Forces of Malta. From a muscle tissue sample, it was possible to extract genomic DNA which was analysed for the mtDNA control region (CR); 12S rRNA gene (12S); cytochrome c oxidase I gene (COI); brain-derived neurotrophic factor gene (BDNF); oocyte maturation factor Mos (Cmos); and RNA fingerprint protein 35 gene intron 1 (R35), using their respective amplification protocol [4-6]. Each PCR product was sequenced and deposited in GenBank accession numbers KU883271-6 respectively. The sequences were compared to genetic data available in genetic sequence databases.

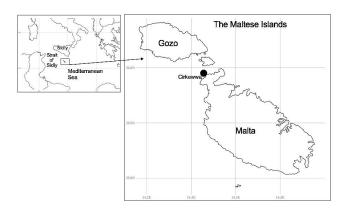


Fig. 1. Map showing location from where the Leatherback turtle, *Dermochelys coriacea*, was collected.

Specific genetic data for this species on most of the studied loci is very limited, and all the sequences obtained during this study matched the few reported sequences at 100% identity. The R35 gene obtained in this study had a 1bp difference (99.9% identity match) a homologous sequence obtained from an Atlantic specimen but completely matched a sequence from a Pacific specimen. However here it has to be noted that genetic data for only two specimens of *D. coriacea* is currently available for this gene and thus the genetic variation of each

RMU at this gene is unknown.

The CR also matched other sequences obtained during other studies. The CR haplotype placed the locally collected specimen within the commonest haplotype found in the Atlantic Sea [7,8], further supporting the hypothesis that Mediterranean Leatherback turtles are of Atlantic origin.

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References

1 - Wallace BP., Tiwari M. and Girondot M., 2013. *Dermochelys coriacea*. The IUCN Red List of Threatened Species 2013: (http://www.iucnredlist.org/). Accessed 4th February 2016.

2 - Casale, P. and Margaritoulis D., (Eds.) 2010. Sea turtles in the Mediterranean: Distribution, threats and conservation priorities. 2010. Gland, Switzerland: IUCN. 294 pp.

3 - Casale P., Nicolosi P., Freggi D., Turchetto M. and Argano R., 2003. Leatherback turtles (*Dermochelys coriacea*) in Italy and in the Mediterranean Basin. Herpetological Journal 13:135-139.

4 - Abreu-Grobois FA., Horrocks J., Formia A., Browne D. and Beggs J., 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analysis. p. 179. In: Proceedings of the 26th Annual Symposium on Sea Turtle Biology and Conservation, Crete, 3rd-8th April 2006. Inter- national Sea Turtle Society, Athens, Greece.

5 - Naro-Maciel E., Reid B., Fitzsimmons NN., Le M., DeSalle R. and Amato G., 2009. DNA barcodes for globally threatened marine turtles: a registry approach to documenting biodiversity. Molecular Ecology Resources, 10:252-263.

6 - Naro-Maciel E., Le M., FitzSimmons NN., and Amato G., 2008. Evolutionary relationships of marine turtles: a molecular phylogeny based on nuclear and mitochondrial genes. Molecular Phylogenetics and Evolution, 49: 659-662.

7 - Molfetti E, Vilaca ST., Georges JY., Plot V., Delcroix E., Le Scao R., Lavergne A., Barrioz S., dos Santos FR. and de Thoisy B., 2013. Recent Demographic History and Present Fine-Scale Structure in the Northwest Atlantic Leatherback (*Dermochelys coriacea*) Turtle Population. Plos One, 8: e58061.

8 - Dutton PH., Roden SE., Stewart KR., LaCasella E., Tiwari M., Formia A., Thome' JC., Livingstone SR., Eckert S., Chacon-Chaverri D., Rivalan P. and Allman P. 2013. Population stock structure of leatherback turtles (*Dermochelys coriacea*) in the Atlantic revealed using mtDNA and microsatellite markers. Conservation Genetics 14:625-636.