VNTRS IN THE MTDNA CONTROL REGION OF THE BLUNTNOSE SIXGILL SHARK, *HEXANCHUS GRISEUS*.

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Abstract
In most shark species the mtDNA control region is approximately 1070bp. However, genetic analyses of *Hexanchus griseus* specimens collected from different locations, have led to the identification of a long mtDNA control region, ranging between 1570bp and 1752bp. This exceptionally long region contains a number of 45bp repeats which lead to the formation of VNTRs that are not evenly distributed globally but rather by geographical locations.

Keywords: Elasmobranchii, Deep waters, Conservation, Genetics, Mediterranean Sea

The mtDNA control region is the main non-coding sequence of the mtDNA which resides between tRNA-Pro and tRNA-Phe genes. The expected length of the control region in most shark species is around 1070bp, nonetheless in species such as *Rhincodon typus* [1], *Carcharodon carcharias* [2] and Hexanchiformes [3,4], this region is even longer.

To study the complete control region of *Hexanchus griseus*, 146 specimens of the species were collected from different geographical locations (see acknowledgments for list of locations), with the majority being of Mediterranean origin (n=126). The sequence between the 3' of cytochrome b gene to the 5' of 12S rRNA gene has been amplified and sequenced in 2012 (Accession numbers: KF894454-90), using both forward and reverse primers, to ensure the inclusion of the complete control region in the analyses. The sequences obtained were aligned to the mtDNA control region of other shark species to confirm neighbouring genes through homology.

Although different specimens exhibited different lengths for this locus, no length heteroplasmy was noted in the *H. griseus* specimens studied, as only one PCR product was noted per individual. A search for the repeated motif has yielded a 45bp sequence that was imperfectly repeated up to eleven times within domain 1 of the control region (Figure 1). The imperfect repetition of the motif has yielded 14 motif sequences which differed from each other solely through transitions. Analysis of each motif sequence has shown that when single stranded most of them, especially the most common ones, can fold on themselves to form highly stable secondary structures leading to the conclusion that such sequences are probably the result of repeated strand slippage during DNA replication through stem-loop formation [5].

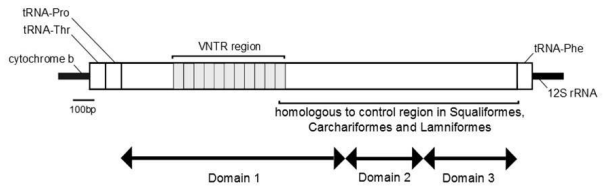

Fig. 1. The mtDNA control region and neighbouring genes for *H. griseus*, with Mediterranean specimens exhibiting a maximum of 11 repeats in the VNTR region.

The specimens collected from the Mediterranean Sea exhibited the longest VNTR region containing 9 (4.76%), 10 (91.27%) and 11 (3.97%) copies of the repeat. No significant difference was detected between the number of repeats recorded from the eastern, central and western Mediterranean specimens. Specimens from the Eastern Atlantic Ocean (n=11) exhibited 10 repeats, while those from the Pacific Ocean (n=9) exhibited 7 repeats. When the specimens from the Pacific Ocean were analysed against those from the Atlantic Ocean, a significant difference ($P < 0.001$) in the number of repeats was noted. This indicates that the global distribution of the number of repeats is linked to specific geographical regions.

The patterns observed with the VNTRs together with analyses of point mutations strengthen the fact that even though *H. griseus* is widely distributed throughout all oceans, its population is subdivided into genetically distinct units. Thus highlighting the need to consider each group separately when designing managment plans for the conservation of this species.

Acknowledgments:
We would like to thank all the Maltese fishermen for supporting the project, and a number of international entities who aided in tissue collection, including: Mr. F. Burns, Marine Scotland Science, Scotland; Dr. M. Freitas, Director of the Marine Biology Station of Funchal and the curator of the Museum of Natural History of Funchal, Madeira; Mr. J. Gamatham and Mr. P. Kainge, The National Marine Information and Research Centre, Ministry of Fisheries and Marine Resources, Republic of Namibia; Dr. J. Guallart and Nuria Bufort and captain and crew of the fishing ship "Pausep", Valencia, Spain; Dr. H-C, Ho, National Museum of Marine Biology and Aquarium, Taiwan; Dr. H. Kabasakal, Ichthyological Research Society, Istanbul, Turkey; Dr. P. Lymberakis, Natural History Museum of Crete, Crete, Greece; Dr. K. Maslenikov, The University of Washington Fish Collection, Washington, USA; Dr. A. Miliou, Archipelagos, Institute of Marine and Environmental Research of the Aegean Sea, Greece; Dr. A. Stewart, New Zealand Foundation for Research Science and Technology through Te Papa Biosystematics of NZ EEZ fishes subcontract within NIWA's Marine Biodiversity and Biosecurity OBI program (contract C01X0502); Prof. F. Tinti and Dr. A. Velona, Dept. Experimental and Evolutionary Biology, Faculty of Sciences, University of Bologna, Italy; Dr. H.J. Walker, Marine Vertebrates Collection, Scripps Institute of Oceanography, University of California, USA; Dr. N. Ziani, AILERONS, France.

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