GENETIC BARCODING OF ELASMOBRANCHES IN MALTA (CENTRAL MEDITERRANEAN)

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Abstract
The correct identification of species constitutes the first step in accurate fisheries data collection and sustainable management. DNA barcoding of a standardized sequence of the COI gene has proven to be a powerful tool in assisting conventional taxonomic methods in species identification, especially when considering species from taxa that are difficult to identify down to the species level. This paper presents work on a total of 77 elasmobranch specimens collected during commercial fishing activities between 2012 and 2013 within the 25 nautical mile Fisheries Management Zone around the Maltese Islands.

Keywords: Elasmobranchii, Genetics, Fisheries, Conservation, South-Central Mediterranean

Introduction
The Mediterranean Sea hosts around 75 species of elasmobranches. On a regional scale, 31 are within threatened categories, including 14 Critically Endangered, 9 Endangered and 8 Vulnerable, with some of them being in a worse conservation status when compared to their global conservation status [1].

Materials and Methods
Seventy-seven elasmobranch specimens were sampled to assess the efficacy of genetic barcoding as an identification tool across a selection of species landed in Malta during study period 2012-2013. The shark species analysed comprised: *Centrophorus granulosus; Heptranchias perlo; Hexanchus griseus; Mustelus asterias; Mustelus mustelus; Mustelus punctulatus; Prionace glauca; Scyliorhinus canicula; Scyliorhinus stellaris; and Squalus blainville,* and the ray species comprised*: Dasyatis centroura; Dasyatis pastinaca; Dasyatis tortonesei; Dipturus oxyrinchus; Leucoraja circularis; Raja clavata; Raja polystigma;* and *Raja radula*. As *R. clavata* specimens exhibited polychromatism (variety of colour patterns on their dorsal surface) and differences in the number of thorns, specimens of each form noted were collected to analyse these forms genetically. In this study, five of the seven different colour patterns known [2] were analysed, including uniform, spotted, ocellated, reticulated and marbled specimens. All specimens were anatomically identified using identification keys and diagnostic features [3,4]. Genomic DNA was extracted using the standard proteinase K, phenol-chloroform extraction method and a partial sequence of the COI gene was amplified and sequenced in both directions using universal fish primers [5].

Results
Different primer pairs were used to amplify different species, therefore a 610 bp sequence homologous to all taxa was used for the genetic analyses. All sequences obtained were run via Blastn to genetically confirm the species identity with already available genetic barcodes for each species. All sequences matched with >99% identity to already available sequences, confirming the species' identity. A total of 245 bp positions (40.2%) exhibited genetic differences. Using the K2P model [6], mean genetic divergence within species was found to be 0.22%; while within genus the genetic divergence ranged between 4.23% (between *D. pastinaca; D. tortonesei*) to 9.86% (*S. canicula; S. stellaris*). Genetic analyses of *R. clavata* have shown that there were no genetic differences between the different forms analysed, and only two haplotypes were identified, with the least common haplotype appearing only in one specimen. Moreover, this analysis presents the first two definite records of *D. tortonesei* in Maltese fisheries landings. This species differed from its morphologically similar congener D. pastinaca by a K2P divergence of 4.23%, that is well beyond the intraspecific variation threshold [5], further confirming that *D. pastinaca* and *D. tortonesei* are two separate species rather than two synonyms [7].

This study was the first to use DNA barcoding of the COI gene in Malta to analyse the polychromatism noted in *R. clavata* and to genetically confirm the presence of various elasmobranch species including, *H. griseus, P. glauca, D. centroura, D. pastinaca, D. tortonesei, L. circularis, R. polystigma* and *R. radula* within the Maltese Fisheries Management Zone. The latter two species being of higher conservation value since they occur only in the Mediterranean Sea. Results show that DNA barcoding clarified morphological identifications, even when polychromatism was noted. It was also possible to confirm the identity of different species especially within the genus *Mustellus*, *Dasyatis* and *Raja* as these genera contain a number of morphologically similar species. These results illustrate the importance of using more accurate reliable tools to enhance taxonomic identification of species facilitating species-specific monitoring and management for sustainable fisheries and conservation.

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