

GENETIC CHARACTERISATION OF *THUNNUS THYNNUS* IN THE MEDITERRANEAN: ANALYSIS AT THE MITOCHONDRIAL LEVEL

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Abstract

Currently two differentiated tuna stocks with spawning areas in the Gulf of Mexico and the Mediterranean Sea are recognised although it is proposed that further population sub-structuring may exist. Samples were collected during 2013-14 (n=80) and the Mitochondrial D-loop region was amplified. Pairwise ϕ_{ST} analysis demonstrated that there was significant differentiation between this study's samples (Grech, Libya-Eastern Mediterranean) and that of the Gulf of Mexico (Atlantic) ($p=0.025$). No evidence of differentiation was found between this sample and the historical samples of the Eastern Mediterranean, Libyan samples ($p=0.0678$, $p=0.0623$ respectively) or the Western Mediterranean ($p=0.0549$). The implications are discussed.

Keywords: Mediterranean Sea, Genetics, South-Eastern Mediterranean

Introduction

The Atlantic Bluefin tuna (*Thunnus thynnus*) is an economically important fish found in the Atlantic Ocean and Mediterranean Sea. Currently two differentiated tuna stocks are recognised. However as the Mediterranean Sea consists of two partly enclosed basins (Western and Eastern) differing in oceanography and abiotic parameters it is proposed that further population sub-structuring may exist. The aim of this study was to determine whether sub-structuring of the mitochondrial DNA from *T. thynnus* (representing the Eastern Mediterranean) could be observed.

Material and Methods

25 frozen muscle samples collected in 2013 from Libyan waters, and stored at -20°C were provided by the Malta Aquacultural Research Station, Marsaxlokk. 55 fresh muscle samples were collected from a tuna pen to the South of Malta in 2014. DNA was extracted from samples, then the Mitochondrial D-loop region was amplified using the primer pairs CSBDH/L15998 and PRO5/12SAR3 [1]. Arlequin version 3.5.1.3 [2] was used to calculate the number of haplotypes (nh), haplotype diversity (h) and nucleotide diversity (π) for both sets of data. Tajima's D (D) [3] and Fu's F [4] were used to test whether the studied *T. thynnus* population was stable.

Results and Discussion

The mitochondrial DNA sequences obtained in this study showed high homology to sequences available in the public domain in GenBank (>99.0%). Pairwise ϕ_{ST} analysis demonstrated that there was significant differentiation between this study's samples (Grech, Libya-Eastern Mediterranean) and that of the Gulf of Mexico (Atlantic) ($p=0.025$) as presented in [5] (Table 1). In contrast no evidence of differentiation was found between this sample and the historical samples of the Eastern Mediterranean [5] and Libyan samples [6] ($p=0.0678$, $p=0.0623$ respectively) (Table 1). When the samples in this study were compared to the historical samples of the Western Mediterranean [5] no difference was found between this study (Grech, Libya-Eastern Mediterranean) and that of the Western Mediterranean (p value of 0.0549). The haplotype diversity (h) of the studied samples using the CSBDH/L15998 and PRO5/12SAR3 primer pairs was 0.985 and 0.945 respectively. The nucleotide diversity (π) was also high at 0.0152 and 0.0149 respectively. These values were comparable to those obtained for *T. thynnus* throughout the Atlantic and Mediterranean regions [1, 5, 7, 8]. These values indicate that the most variation was observed between samples. Tajima's D (D) and Fu's F were both negative at -1.520 and -5.674 ($p<0.001$) for primer set CSBDH/L15998 and -1.296 ($p=0.032$) and -3.859 ($p<0.001$) for primer set PRO5/12SAR3. These indicate an excess of rare nucleotides following a recent population explosion after a population bottleneck and is in accordance with data such as that obtained in [4]. At the mitochondrial level significant large-scale population sub-structuring was reported in this study between the Gulf of Mexico (Atlantic region) and the Eastern Mediterranean region. This is in agreement with the management strategies proposed by ICCAT [9]. No strong evidence was found to support segregation of tuna in the two Mediterranean basins [9]. We recommend extending this study using larger numbers.

Tab. 1. AMOVA pairwise comparison among different *T. thynnus* samples.

	Gulf of Mexico (Carlsson <i>et al.</i> , 2007)	West Med (Carlsson <i>et al.</i> , 2007)	East Med (Carlsson <i>et al.</i> , 2007)	Libya <i>et al.</i> (Vinas <i>et al.</i> , 2001)
West Med (Carlsson <i>et al.</i> , 2007)	0.0129 ($P=0.0139$)	/	/	/
East Med (Carlsson <i>et al.</i> , 2007)	0.0134 ($P=0.1105$)	0.0174 ($P=0.0482$)	/	/
Libya (Vinas <i>et al.</i> , 2001)	0.0201 ($P=0.0639$)	0.0179 ($P=0.0667$)	0.0132 ($P=0.0589$)	/
Study Samples (Libya)	0.0264 ($P=0.0254$)	0.018 ($P=0.0549$)	0.0128 ($P=0.0678$)	0.0327 ($P=0.0623$)

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