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# CLASSIFICATION OF SPECIES WITHIN THE GENUS *HEXANCHUS* AND *HEPTRANCHIAS* THROUGH MTDNA SEQUENCES.

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## Abstract

In this study, the mtDNA region from the 12S rRNA gene to the 16S rRNA gene has been sequenced from a number of Hexanchidae specimens (*Hexanchus griseus*, *Hexanchus nakamurai* and *Heptranchias perlo*). A total of 150 polymorphic nucleotide positions have been identified, with the interspecific divergence being 5.46%, while the intraspecific divergence between the identified haplotypes ranged between 0.24% and 0.95%.

**Keywords:** *Elasmobranchii*, *South-Central Mediterranean*

## Introduction

The Family Hexanchidae (Order: Hexanchiformes), has only four extant species [1], making this Family one of the smallest shark groups, with its members being described through morphological characters [1,2]. Nonetheless in other shark Families, mtDNA sequences have been readily used as genetic markers to identify or confirm very similar species [3,4].

## Methodology

Tissue samples were collected from *Hexanchus griseus* (Central Mediterranean n=6; North-East Atlantic n=4; South-West Pacific n=5; and North-East Pacific n=4); *Hexanchus nakamurai* (North-West Pacific n=4) and *Heptranchias perlo* (Central Mediterranean n=4; North-East Atlantic n=1; and North-West Pacific n=2). DNA was extracted using proteinase K digestion followed by standard phenol-chloroform extraction method. Amplification was carried out using 12SA (AAACTGGGATTAGATACCCACTAT) and 16SA (ATGTTTTGATAAACAGGCG) primers [5] (95°C for 4min followed by 30 cycles of 30sec at 95°C, 30sec at 55°C and 1min at 72°C, with a final extension of 10min at 72°C). PCR products were sequenced in both directions using ABI3730xl. Additionally, two homologous sequences [3], one of *Hexanchus vitulus* (a synonym to *Hexanchus nakamurai*) and one of *Heptranchias perlo* were also added to the analyses. Sequences were then aligned together using Geneious R6 and the shortest homologous sequence was used for analyses (1404bp). Intraspecific and interspecific variation were estimated using Arlequin v3 [6], while a Maximum Likelihood phylogram was constructed using the best fit mutation model through MEGA v5 [7].

## Results and Discussion

150 polymorphic nucleotide positions were recorded, forming 11 haplotypes. The intraspecific variation ranged between 0.24% and 0.95%, with the highest genetic differences being noted between the most distant sampling locations (Fig 1). Mediterranean specimens of *Hexanchus griseus* and *Heptranchias perlo* shared haplotypes with specimens from the North-East Atlantic Ocean.

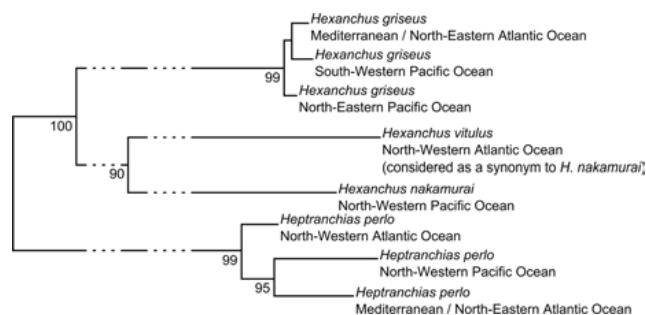


Fig. 1. A schematic phylogram of the three Hexanchidae species analysed in this study. The numbers near nodes represent bootstrap values.

The interspecific genetic divergence was around 5.46%. Clear genetic divergence was noted between the genus *Heptranchias* and the genus *Hexanchus*, with the latter being monophyletic. The genetic divergence within the species *Hexanchus nakamurai*, a senior synonym to *Hexanchus vitulus* [2], was quite high. In fact *Hexanchus nakamurai* specimens collected from the North Western Pacific

Ocean exhibited a 4.4% divergence from that originating from the North Western Atlantic Ocean (Fig 1). Similar observations were noted in another study [4], using a different gene, where again high intraspecific divergence was recorded between *Hexanchus nakamurai* of Indo-Pacific origin and that of an Atlantic origin. Thus it can be concluded that there is a possibility of a third species within the genus *Hexanchus*, that is *Hexanchus nakamurai* might be split into a species within the Atlantic Ocean and another one in the Indo-Pacific Ocean. Given that *Hexanchus nakamurai* has been recorded in the Mediterranean Sea, then its identification would be best if supported by phylogenetic analyses to better understand the evolutionary connections of this poorly known species

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