

What is the Relationship within the Family Lamnidae?

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Abstract: Uncertainties remain about the relationship within the family Lamnidae, which includes the white shark, 2 makos, the porbeagle, and salmon shark. Several alternative hypotheses have been suggested related to the Lamnidae phylogeny. The aim of this study was to find an accurate answer to the family Lamnidae interrelationship. To that extend, the mitochondrial cytochrome b gene was studied in all the Lamnidae species and 2 outgroups from the families Alopiidae and Odontaspidae. The monophyly of 2 makos were obtained from both DNA and amino acid (AA) parsimony analysis. Based on our analysis, first the white shark joins this group, which is a sister taxon to the genus *Isurus* (makos), as reported by Naylor. In the AA parsimony analysis, the 2 makos and porbeagle-white shark branches come together as the monophyletic family *Isurus*, but not *Lamna*. These results agree with Compagno's suggestion and Martin's findings.

Key Words: Lamnidae, phylogenetic, relationship

Lamnidae Familyasi İçindeki İlişki

Özet: Beyaz köpek balığı, 2 mako, porbeagle ve Salmon türü köpek balıklarını kapsayan Lamnidae familyası içerisindeki ilişki çok net değildir. Bu familyanın kendi içerisindeki sınıflandırmasına yönelik pekçok farklı hipotezler üretilmiştir. Bu çalışma, Lamnidae familyası içindeki ilişkilere yönelik yeterli bir cevap bulabilmek amacı ile tüm Lamnidae türlerini ve Alopiidae ve Odontaspidae ailesinden 2 harici örnek kullanılarak mitokondrial cyt b geni ile yapılmıştır. Hem DNA hemde AA prsimony analizlerinde 2 mako türü monofiletik grub olarak elde edilmiştir. Bu gruba Naylor'ın sonucuna uygun olarak, 1 analizde makoların bağlı olduğu *Isurus* cinsinin kardeş cinsi olan beyaz köpek balığı katılırken, AA analizi sonucunda porbeagle-beyaz köpek balığı ile 2 mako cinsi bir arada grubu oluşturmaktadır. *Isuruslar* bu analizde monofiletik grub halinde iken *Lamna* cinsi monofiletik değildir. Bu sonuç Compagno'nun önerisi ve Martin'in buluşu ile aynıdır.

Anahtar Sözcükler: Lamnidae, genetic sınıflandırma, ilişki

Introduction

Evolutionary relationships within and between different orders of sharks have been discussed by taxonomists for a long time. In the order Lamniformes (mackerel sharks) there are 7 families: Lamnidae (mackerel sharks), Alopiidae (thresher shark), Odontaspidae (sand tiger shark), Cetorhinidae (basking shark), Megachasmidae (megamouth sharks), Mitsukurinidae (goblin sharks), and Pseudocarchariidae (crocodile shark) (1).

The Lamnidae is a very small family and includes only 5 species, including the well-known predators the white shark, 2 makos, the porbeagle, and salmon shark (2). Mostly, they have a very large body size and are known

as fast swimming sharks; sometimes called the lamnid, isurids, or mackerel sharks. Their bodies have adapted to high-speed swimming, for which they have a conical snout, very large gills for more efficient gas exchange, a streamlined fusiform body, a very small second dorsal fin, and a strong lunate tail. Some of these sharks can maintain a body temperature 7-10 °C higher than the water temperature by exchanging heat through the circulatory system, which keeps heat inside the body (3). They are mostly seen in coastal waters, in cool to tropical seas (1).

Many researchers have tried to better understand the elasmobranch phylogeny and the relationship between the different orders. First, Compagno (4,5) presented a

new classification model in which he grouped the elasmobranch orders into 4 superorders: Galeomorphii, Squatinimorphii, Squalomorphii, and Batoidea. He assigned Carcharhiniformes, Lamniformes, and Orectolobiformes into the superorder Galeomorphii. He consistently defended the monophyly of each galeomorph order, particularly his suggestion about the monophyly of the Lamniformes (4), based on dentition and many other anatomical formations. Lamnoid sharks are generally considered closely related to carcharhinoid sharks because of their similarities in fin positions and tooth morphology. However, in spite of these similarities, Compagno defended Lamniformes as monophyletic. In addition, in 1988, 12 orders of recent elasmobranchs, which were considered monophyletic, were divided into 2 major monophyletic groups by Compagno; the Galeomorphii and Squalea. Once again, Lamniformes were placed in Galeomorphii. When he explained the lamnoid assemblage, Compagno proposed 5 families (Mitsukurinidae, Odontospididae, Pseudocarcharinidae, Megachasmidae, and Alopidae) and 3 subfamilies (Alopiinae, Cetorhininae, and Lamninae, which includes Carcharodon, Isurus, and Lamna) inside this order. Some researchers supported this; others did not (6). Several similar studies have been performed on this subject, and this recent elasmobranch classification has been widely accepted, with the differences of Lamninae being a subfamily instead of family in the current classification.

The first appearance of each lineage can be found in the known fossil records. One group of sharks in the order Lamniformes are very well preserved in the fossil records. From these records, which provide information about rates and patterns of molecular evolution, independent phylogenetic hypotheses can be made. Molecular data (DNA or amino acid sequences) also provide extensive information about the relationships. Agreement on the origination and the time of divergence between the molecular data and fossil record can be used in the proposition of the best hypothesis.

There are still uncertainties about the relationship within the family Lamnidae and alternative hypotheses have been suggested. For example, one hypothesis proposed by Compagno indicates that Isurus (mako sharks) and Carcharodon are sister taxa, and Lamna (porbeagle and salmon sharks) is the most ancestral genus of the family, based on a survey of morphological characteristics (7).

In an alternative hypothesis, Long and Waggoner proposed that Carcharodon and Lamna are sister taxa, based on analysis of tooth characters (8,9).

A third alternative hypothesis was proposed by Maisey, based on similar morphological characters. This proposition included 14 species of Lamniformes. The author suggested that the families Lamnidae and Alopidae are monophyletic and the sister groups, Cetorhinus and Megachasma, join this group first (10,11).

In addition, fossil records clearly show that 2 makos are sister taxa (12).

Based on Compagno's conclusion of these 2 hypotheses, Carcharodon and Isurus have been identified as sister taxa, while, as alternatively suggested, the fact that Carcharodon and Lamna have been identified as sister taxa cannot be ignored. One possible reason could be that in the distant past all 3 genera separated from each other within a short period time. This possibility is supported by the estimations of sequence divergence, which are very similar to each other (*Isurus-Carcharodon* = 7.1%, *Isurus-Lamna* = 8.4%, and *Carcharodon-Lamna* = 6.2%) (9).

In addition, Martin tried to elucidate the relationship within the family Lamnidae by parsimony phylogenetic analysis of the mitochondrial cytochrome b gene. The results strongly suggested (100%) that the family Lamnidae is monophyletic. The monophyly of the 2 species of Isurus, and the monophyly of Isurus and Carcharodon were supported with reasonably strong values (85%-97% and 78%-88%, respectively) in addition to the finding that as a sister taxa Lamna was the most ancestral extant genus of this family (corroborated by Compagno (7)) (9). These findings, indicating that the family Lamnidae is a monophyletic group, were supported using recombinant activation gene (Rag-1) phylogenetic analysis by Martin (13) and with the mitochondrial NADH 2 and cytochrome b genes by Naylor et al. (2).

In another study, by Martin et al. (14), the SSR locus (Loc6) was amplified using primers Loc6F 5'-ATT GTT TCG TGG CCT AGG TG-3' and Loc6R 5'-AGC CAC ATC GAT AAT CCC AG-3' from Lamniform sharks, and several different length deletions were obtained in the region flanking the repeat motif, of which one was present in all species of the Lamnidae.

The order Lamniformes is commonly considered the sister group of Carcharhiniformes, but recent studies

based on molecular data have led to the proposal of an alternative hypothesis in which Lamniformes and Orectolobiformes (carpet sharks) are assigned as the sister groups of Carcharhiniformes (15). Similarly, Winchell et al. proposed a close relationship among lamniforms, carcharhiniforms, and orectolobiforms based on 12S and 16S rRNA, tRNA valine, and mitochondrial cytochrome b gene molecular analysis (16).

A molecular phylogenetic study of the mitochondrial 12S-16S locus and tRNA valine by Douady et al. also supported the Lamniform-Carcharhiniform association (17).

Phylogenetic distribution of different modes of reproduction also put the lamniforms, carcharhiniforms, and orectolobiforms shark orders together (18).

These previous studies report diverse results about the relationship among the family Lamnidae. There seems to be uncertainty about which species is the sister taxon to the *Isurus* (makos). In this study, to find an accurate answer to the family lamnidae interrelationship the mitochondrial cytochrome b gene of 2 makos (*Isurus paucus* and *Isurus oxyrinchus*), the porbeagle (*Lamna nasus*), salmon shark (*Lamna ditropis*), and white shark (*Carcharodon carcharias*), in addition to 2 outgroups, the thresher shark (*Alopias vulpinus*) from the family Alopidae, and the smalltooth sandtiger shark (*Odontaspis ferox*) from the family Odontaspidae, were analyzed to gain a better understanding of the relationship among the family Lamnidae.

Materials and Methods

DNA sources

Five 5 species of the family Lamnidae and 2 species from families Alopidae and Odontaspidae, as an outgroup, were used (Table).

Phylogenetic Analysis

An approximately 1100-bp mitochondrial cytochrome b gene nucleotide sequence was obtained from GenBank and aligned. The best possible alignment was used in a tree construction. The sequence was loaded into the Eyeball sequence editor (20). The sequence alignments were formed using the MEGALIGN program within the DNASTAR package and adjustments were made visually. GenBank accession numbers were as follows: *Alopias vulpinus* cytochrome b result: U91442; *Isurus oxyrinchus* cytochrome b result: L08036; *Isurus paucus* cytochrome b result: L08037; *Carcharodon carcharias* cytochrome b result: L08031; *Lamna nasus* cytochrome b result: L08038; *Odontaspis ferox* cytochrome b result: U91445; *Lamna ditropis* cytochrome b result: U91438 (2).

The data were analyzed using maximum parsimony (MP) methods within the PHYLIP v.3.5c program (21). The analyses reliability was later tested by bootstrapping (22) with 1000 replications of the data. Similarly, transversion parsimony (TP) was applied to data that included only transversion signals by manually converting it into purines and pyrimidines using MP methods within the PHYLIP v.3.5c program (21). The reliability was tested by bootstrapping (22) with 1000 replications of the data.

Results

The analysis of the molecular data obtained from the mitochondrial cytochrome b gene nucleotide and amino acid (AA) analysis was carried out. The mitochondrial cytochrome b gene nucleotide sequence was obtained from GenBank (see materials and methods section for accession numbers). The sequence alignment of the 1145 bp nucleotide of the mitochondrial cytochrome b gene was taken from GenBank and used in the DNA and AA analysis. *Alopias vulpinus* (thresher) was included in the

Table. List of species in this study (19).

Species name	Family name	Common name
<i>Isurus paucus</i>	Lamnidae	longfin mako
<i>Isurus oxyrinchus</i>	Lamnidae	shortfin mako
<i>Lamna nasus</i>	Lamnidae	porbeagle
<i>Lamna ditropis</i>	Lamnidae	salmon shark
<i>Carcharodon carcharias</i>	Lamnidae	white shark
<i>Alopias vulpinus</i>	Alopidae	thresher shark
<i>Odontaspis ferox</i>	Odontaspidae	smalltooth sandtiger shark

data as an outgroup. We obtained similar results from the 2 analyses. In the first result, 2 makos (shortfin and longfin) were assigned together, followed by the joining first of *Carcharodon carcharias* (white shark), followed by *Lamna nasus* (porbeagle)-*Lamna ditropis* grouping, which was obtained from DNA parsimony analysis. In the AA parsimony analysis the 2 makos were assigned together and the *Carcharodon carcharias* (white shark)-*Lamna nasus* (porbeagle) grouping joined it. In this analysis *Lamna ditropis* joins later. This result was somewhat similar to Long and Waggoner's (8) proposition based on the analysis of dentition characters (9) (Figures 1 and 2).

Discussion

It was clearly seen that the monophyly of the 2 makos (*I. paucus* and *I. oxyrinchus*) were obtained from both analyses. To this group, *Carcharodon* (white shark), which is a sister taxon to the genus *Isurus* as in Naylor's (2) findings, joins first. Following this, *L. nasus* joins, which is followed by the *L. ditropis* monophyletic group, the most ancestral genus of the family Lamnidae, according to the mitochondrial cytochrome b gene nucleotide MP analysis. Interestingly, AA parsimony analysis gave a unique result in which *I. paucus*-*I. oxyrinchus* (2 makos) and *L. nasus*-*C. carcharias* branch together. In addition, *Isurus* was determined to be a monophyletic group, but *Lamna* was not. This result

confirms Compagno's (7) suggestion and Martin's (9) findings.

The phylogenetic hypothesis of the Lamnidae in relation to geological time (in millions of years ago), based on the molecular data, indicates that the 2 species of *Isurus* separated from a common ancestor 34-48 million years ago, *Isurus* and *Carcharodon* split 43-60 million years ago (during the Paleocene or early Eocene), and *Lamna* and the others separated 46-65 million years ago. (9). This indication shows that *Lamna* separated from the *Isurus* and *Carcharodon* groups, and agrees with the nucleotide analysis data.

It is known that mutation rates are very important for gene selection. Mitochondrial genes, which evolve more slowly in fish, especially sharks, as compared to mammals, despite the fact that nucleotide changes occur more quickly than nuclear genes, represent more reliable results.

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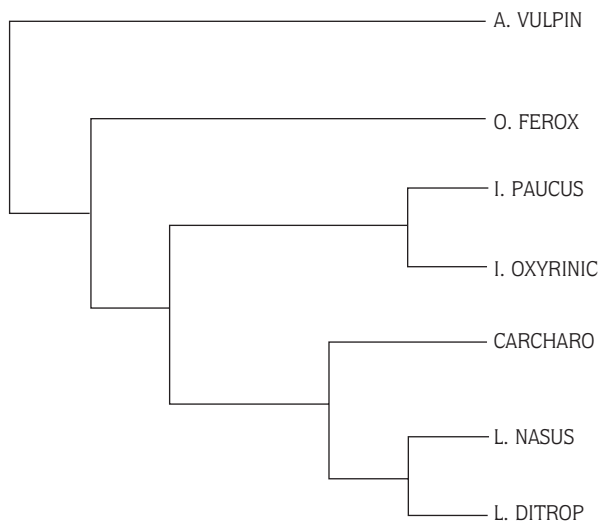


Figure 1. Majority consensus bootstrap tree of the MP analysis of the mitochondrial cytochrome b nucleotide sequences of the Lamnidae species.

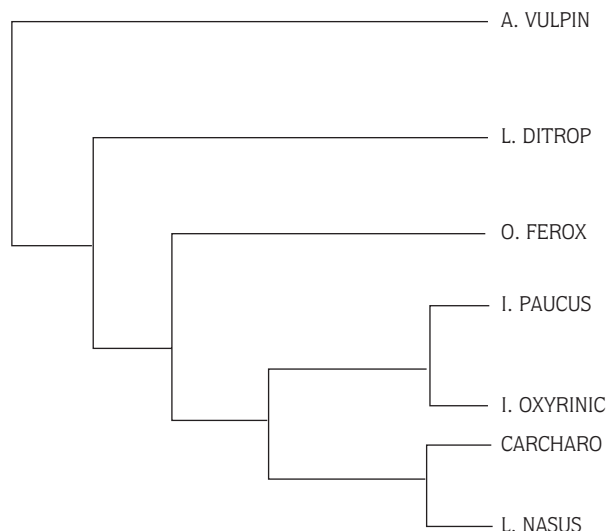


Figure 2. Majority consensus bootstrap tree of the AA parsimony analysis of the mitochondrial cytochrome b nucleotide sequences of the Lamnidae species.

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