Complete mitochondrial DNA genome of bonnethead shark, *Sphyrna tiburo*, and phylogenetic relationships among main superorders of modern elasmobranchs

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Elasmobranchs are one of the most diverse groups in the marine realm represented by 18 orders, 55 families and about 1200 species reported, but also one of the most vulnerable to exploitation and to climate change. Phylogenetic relationships among main orders have been controversial since the emergence of the Hypnosqualean hypothesis by Shirai (1992) that considered batoids as a sister group of sharks. The use of the complete mitochondrial DNA (mtDNA) may shed light to further validate this hypothesis by increasing the number of informative characters. We report the mtDNA genome of the bonnethead shark *Sphyrna tiburo*, and compare it with mitogenomes of other 48 species to assess phylogenetic relationships. The mtDNA genome of *S. tiburo* is quite similar in size to that of congeneric species but also similar to the reported mtDNA genome of other Carcharhinidae species. Like most vertebrate mitochondrial genomes, it contained 13 protein coding genes, two rRNA genes and 22 tRNA genes and the control region of 1086 bp (D-loop). The Bayesian analysis of the 49 mitogenomes supported the view that sharks and batoids are separate groups.

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1. Introduction

Sharks are one of the oldest groups in nature with a diversification dated to have occurred 460–300 million years (myr) ago (Heinicke et al., 2009). As a consequence, sharks are one of the most diverse taxa in the marine realm, playing an important role in the ecosystems due to their position as top- or mid-level predators. This highlights the importance of diversity and the value of evolutionary studies regarding sharks since many species are exploited by humans around the world (Dulvy et al., 2014). Phylogenetic relationships at several levels ranging from superorders to families, or even genera within families, are still controversial. Although it has been widely accepted that modern sharks (Neoselachia) are monophyletic, the relationships among the four main superordinal groups (Galeomorphii, Squalomorphii, Squatinomorphii and Rajomorphii), and the arrangement of orders within these groups remain unsolved. As an example, whereas Bigelow and Schroeder (1948) suggested that batoids are a separate group from sharks, more recent morphological evidence provided by Shirai (1992) placed batoids as a group derived from sharks, which is known as the “hypnosqualean” hypothesis. Nevertheless, although most molecular studies suggest rejection of the hypnosqualean hypothesis, these studies are based on single nuclear or mitochondrial DNA (mtDNA) genes or a set of sequences ranging from 2.4 to 5.8 kb (Duoady et al., 2003; Winchell et al., 2004; Naylor et al., 2005). Likewise, within orders some morphological studies have placed Squalomorphs and Squatinimorphs as the orbitostylig group, based on the sharing of a potential synapomorphy: a projection from the upper-jaw cartilage inside of the ocular orbit (Maisey, 1980).

Similarly, the systematic position of orders within Galeomorphii is unresolved: whereas morphological studies with no exception place Lamniformes as sister order of Carcharhiniformes (Compagno, 1973; Carvalho, 1996), some molecular studies places Orectolobiformes as the sister group of Carcharhiniformes (Vélez-Suazo and Agnarsson, 2011). However, other studies confirm Lamniformes as the sister group of Carcharhiniformes (Duoady et al., 2003; Naylor et al., 2012). Furthermore, within Carcharhiniformes there are some unsolved relationships as there are some families probably paraphyletic such as the hammerhead sharks, Sphyridae (Lim et al., 2010).

Many molecular phylogenies up to date are based on the use of individual genes. However, with the advent of Next Generation Sequencing (NGS) protocols, databases for species' complete mtDNA...
genomes have increased notably and the analyses of mitogenomes are providing new insights on phylogenetic reconstruction (Qin et al., 2015). The bonnethead shark *Sphyrna tiburo*, is seasonally distributed within estuarine, coastal, and continental shelf waters in the western Atlantic from North Carolina, U.S. to southern Brazil, the Gulf of

| Order/species                  | Family               | mtDNA size | GB ref. #       | Reference                  |
|-------------------------------|----------------------|------------|----------------|---------------------------|
| **Carcharhiniformes**         |                      |            |                |                           |
| Carcharhinus leucas (PAC)     | Carcharhinidae       | 16,704     | NC023522       | Chen et al. (2015b)       |
| Carcharhinus leucas (GM)      | Carcharhinidae       | 16,702     | KJ20595        | Díaz-Jaimes et al. (2014) |
| Carcharhinus macloti           | Carcharhinidae       | 16,701     | NC024862       | Chen et al. (2014a)       |
| Carcharhinus sorrah            | Carcharhinidae       | 16,707     | NC023521       | Chen et al. (2015c)       |
| Carcharhinus acronotus         | Carcharhinidae       | 16,719     | NC024055       | Yang et al. (2014a)       |
| Carcharhinus plumbeus          | Carcharhinidae       | 16,706     | NC024596       | Blower and Ovenden (2014) |
| Carcharhinus falciformis       | Carcharhinidae       | 16,680     | KF01102        | Galván-Tirado et al. (2014)|
| Carcharhinus obscurus          | Carcharhinidae       | 16,706     | NC020611       | Blower et al. (2013)      |
| Carcharhinus melanopterus      | Carcharhinidae       | 16,706     | NC023948       | Chen et al. (2014b)       |
| *Prionace glauca*              | Carcharhinidae       | 16,705     | NC022819       | Feutry et al. (2014)      |
| **Scyliorhinidae**            |                      |            |                |                           |
| **Carcharodon dorado**         | Lamnidae             | 16,744     | NC022415       | Chang et al. (2014a)      |
| **Lamna ditropis**             | Lamnidae             | 16,699     | NC024269       | Chang et al. (2014b)      |
| **Isurus oxyrinchus**          | Lamnidae             | 16,701     | NC022691       | Chang et al. (2015a)      |
| **Isurus paucus**              | Lamnidae             | 16,704     | NC024101       | Chang et al. (2014c)      |
| **Carcharias taurus**          | Odontaspidae         | 16,773     | NC023202       | Chang et al. (2015b)      |
| **Alopias pelagicus**          | Alopiidae            | 16,692     | NC022822       | Chen et al. (2015e)       |
| **Alopias superciliosus**      | Alopiidae            | 16,719     | NC021443       | Chang et al. (2014d)      |
| **Megachasma pelagios**        | Megachasmidae        | 16,694     | NC021442       | Chang et al. (2014e)      |
| **Mitsukurina owstoni**        | Mitsukurinidae       | 17,743     | NC011825       | Unpublished               |
| **Orectolobiformes**           |                      |            |                |                           |
| Orectolobus japonicas         | Orectolobidae        | 16,706     | KF111729       | Chen et al. (2015f)       |
| **Blyynodon typus**            | Rhincodontidae       | 16,875     | NC023455       | Alam et al. (2014)        |
| **Chiloscyllium giseum**       | Hemicryptidae        | 16,755     | NC017862       | Chen et al. (2013)        |
| **Chiloscyllium plagiosum**    | Hemicryptidae        | 16,726     | NC012570       | Unpublished               |
| **Chiloscyllium punctatum**    | Hemicryptidae        | 16,703     | NC016686       | Chen et al. (2014g)       |
| **Heterodontiformes**          |                      |            |                |                           |
| **Heterodontus francisci**     | Heterodontidae       | 16,708     | NC003137       | Arnason et al. (2001)     |
| **Heterodontus zebra**         | Heterodontidae       | 16,720     | NC021615       | Chen et al. (2014h)       |
| **Squatinaformes**             |                      |            |                |                           |
| **Squatina formosa**           | Squatinidae          | 16,690     | NC025328       | Corrigan et al. (2014)    |
| **Squatina japonica**          | Squatinidae          | 16,689     | NC024276       | Chai et al. (2014)        |
| **Squaliformes**               |                      |            |                |                           |
| Squallus acanthias             | Squalidae            | 16,738     | NC002012       | Rasmussen and Arnason (1999)|
| Cirrhigaleus australis         | Squalidae            | 16,543     | KJ128289       | Yang et al. (2014b)       |
| **Pristiphoriformes**          |                      |            |                |                           |
| Pristiphorus japonicus         | Pristiophoridae      | 18,430     | NC024110       | Unpublished               |
| **Hexanchiformes**             |                      |            |                |                           |
| Hexanchus griseus              | Hexanchidae          | 17,405     | KF894491       | Unpublished               |
| **Myliobatiformes**            |                      |            |                |                           |
| Gymnura poecilura              | Gymnuridae           | 17,874     | NC024102       | Chen et al. (2014)        |
| **Torpediformes**              |                      |            |                |                           |
| Narcine entemoder              | Narcinidae           | 17,081     | KM366678       | Castillo-Paez et al. (2014)|
| **Rajiformes**                 |                      |            |                |                           |
| Rhinobatos schlegeli           | Rhinobatidae         | 16,780     | NC023951       | Chen et al. (2014j)       |
| Zearaja chilenis               | Rajidae              | 16,909     | KJ913073       | Vargas-Caro et al. (2014) |
| **Pristiformes**               |                      |            |                |                           |
| Anoxypristis cuspidata         | Pristiidae           | 17,243     | NC026307       | Chen et al. (2015b)       |
| **Chimaeriformes**             |                      |            |                |                           |
| Callorhinus milii              | Callorhinidae        | 16,769     | NC014285       | Inoue et al. (2010)       |
Mexico and the Caribbean, including the eastern Pacific from southern California, USA to Ecuador (Compagno, 1984). Some studies based on acoustic and conventional tagging in estuarine waters of the Gulf of Mexico coast of Florida have suggested that S. tiburo is a long-term resident within a specific estuary, with low dispersal among different estuaries (Heupel et al., 2006; Bethea and Grace, 2013). The proclivity of individuals to remain or return for extended periods to areas where they were born is one of the main criteria for philopatry (Feldheim et al., 2014). These nursery areas are critical for protection of neonates and young juveniles and for subsequent recruitment into the adult population. Assessing genetic differences between populations is constrained by the use of single/individual genes because of the low genetic variation that characterizes mtDNA in elasmobranchs. The use of longer sequences or whole mtDNA genomes will increase the number of informative characters and thus our capability for defining phylogeographic patterns or philopatric signals in this species.

In this study we report the complete mitochondrial genome of S. tiburo using a protocol based on next generation sequencing and compared the resultant mitogenome with mtDNA genome sequences of other 48 shark and ray species including representatives from the orders Carcharhiniformes, Lamniformes, Orectolobiformes, Heterodontiformes, Pristiophoriformes, Rajiformes, Myliobatiformes, Torpediniformes and Pristiformes in order to assess the phylogenetic relationships between sharks and rays but also within Galeomorphii.

2. Materials and methods

A muscle tissue biopsy of bonnethead was obtained from commercial fishing boats operating in Campeche Mexico, and stored in the Laboratorio de Genética de Organismos Acuáticos at the Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM). The genomic DNA was isolated using Wizard Genomics DNA Purification Kit (Promega®).

For the library preparation the DNA was sheared by sonication with Bioruptor® and the KAPA BIOSYSTEMS® library preparation protocol with slight modifications was followed. In brief, fragmented DNA was ligated to Illumina universal TruSeq adapters containing eight custom nucleotide indexes (Faircloth and Glenn, 2012). Fragments were size selected in a ~250–450 bp range and enriched through PCR, purified and normalized. A library for sequencing in Illumina MiSeq v3 600 cycle kit was prepared to produce paired-end 300 nucleotide reads at the Genomics Facility from the University of Georgia (UGA).

The total reads were quality filtered, assembled and annotated in Geneious® 7.1.5 using as reference the mtDNA genome of Sphyrna lewini (accession NC022679). We report the first complete sequence of the mitochondrial genome of bonnethead S. tiburo, obtained by NGS methods.

Our laboratory has assembled the complete mitogenome of other shark species as Sphyrna zygaena (KM489157), Carcharhinus leucas (KJ210595), Carcharhinus falciformis (KF801102) and Carcharodon carcharias (KJ934896). We used these mitogenomes and others available in GenBank (Table 1), to perform phylogenetic analyses comparing the orders of the subclasses Elasmobranchii; Carcharhiniformes, Lamniformes, Orectolobiformes and Heterodontiformes (Galeomorphii), Hexanchiformes, Squaliformes, Pristiophoriformes and Squatiniformes (Squalimorphii), Myliobatiformes, Rajiformes, Torpediformes and Pristiformes (Batoidea), and including the mtDNA genome of Callorhinchus milii (Chimaeriformes) as external group. A total of 49 mitogenomes were analyzed.

The sequences of the complete mitogenomes were aligned using the MUSCLE application available at Geneious® 7.1.5 with 8 iterations. From the alignment we obtained the positions of each gene, tRNA, rRNA, and control region. We evaluated the appropriate model of substitution in JModelTest obtaining the GTR+I+G as the most probable model. We obtained a graph of the consensus sequence (Fig. 1), as well as the graphical representation of the sequence alignment using Geneious version 7.1 created by Biomatters available from http://

Fig. 1. Gene organization map of the consensus sequence from the alignment of multiple shark and ray species. The protein-coding genes, tRNAs, rRNAs and non-coding regions are shown in different colors. The blue ring in the middle shows GC contents.
www.geneious.com. We also made a graphical comparison of the *S. tiburo* mitogenome with other shark mitogenomes available in GenBank (Table 1) through a BLAST using the CGView Comparison Tool (CCT) (Grant et al., 2012) (Fig. 2).

A partitioned Bayesian phylogenetic analysis excluding tRNAs was conducted with parallel version of Mr. Bayes 3.0b4 (Ronquist and Huelsenbeck, 2003) using 20,000 burn-in and 50,000,000 of generations. The unlink option was selected and also the gamma-shaped rate variation option, to allow each partition to run with its own set of parameters. Likewise a tree inference using a maximum likelihood (ML) algorithm in the partitioned data excluding tRNAs, was also made using the software RAxML-HPC v. 8 (Stamatakis, 2014) with the GTRCAT model, and 100 bootstrap replicates. We used an individual representative of Chimaeriformes (*C. milii*) as an external group. In order to identify those genes containing the higher number of variable sites useful to address divergence at the inter-generic level within Carcharhiniformes as well as the inter-specific level within the Carcharhinidae family, the mean number of differences at the nucleotide level for individual mtDNA genes was estimated.

### 3. Results and discussion

**3.1 Genome structure and genetic variation**

In this study we report the complete mitochondrial genome sequence of the bonnethead shark *S. tiburo* (GenBank accession number...
able genes were

tionships at the same level (Vélez-Zuazo and Agnarsson, 2011) and although genes representative species (14) of the mean number of nucleotide differences among sequences of the KM453976) of a specimen collected from Campeche, Gulf of Mexico. A total of 2,402,505 X2 paired reads were obtained, which after filtered and assembled resulted in the complete genome sequence containing 16,723 nucleotides. The S. tiburo mitogenome is quite similar in size to that of the congeneric species, S. lewini (16,726 bp; Table 2) (Chen et al., 2015a) and S. zygaea (16,731; Bolaño-Martínez et al., 2014) but also similar to the reported mtDNA genome of other Charcharhinidae species (range 16,680–16,754; Table 1). Like most vertebrate mitochondrial genomes, it contained 13 protein coding genes, two rRNA genes and 22 tRNA genes and the control region of 1086 bp (D-loop) (Table 2). All genes are arranged in a similar fashion as most of vertebrate mitogenomes (Fig. 1) and for most of them the starting codon (ATG) was identified with the exception of the CO subunit I (COI) gene which had GTG as starting codon. For most genes the stop codon (TAA) was identified except for some genes whereas incomplete codons were contained for ND2, ND3, ND4, ND6 (T-), and Cyb (TA-).

3.2 Genome length and gene divergence across the compared shark species

In general although all shark mitogenomes exhibited high similarities in size among species (Fig. 2), larger mitogenomes were observed for species from the most basal lineages, with the Japanese sawshark Pristis japonicus (Squaliformes) having the largest mtDNA genome (18,430 bp) followed by longtail butterfly ray Gymnura poecilura (17,874 bp) (Myliobatiformes) and the goblin shark Mitsukurina owstoni (17,743 bp) (Lamniformes). Among orders, the mtDNA genome was larger in the Squaliformes (mean 17,018 ± 792.7), followed by Lamniformes (16,813.9 ± 327.7), Orectolobiformes (16,753 ± 71.3), Heterodontiformes (16,714 ± 8.5) and Charcharhiniformes (16,708.5 ± 15.3). Within the Charcharhiniformes, an important difference in size between the genus Carcharhinus (16,703.5 ± 10.2) and Sphyra (16,726.7 ± 4.04) was observed. The main differences in mtDNA genome size correspond to the high content of tandem repeats characterizing the control region in elasmobranchs (Castro et al., 2007; Poirviet and Hoaaru, 2013) which has been reported also for teleost fishes (Stärner et al., 2004; Chen et al., 2004).

S. tiburo had a similar size for the mtDNA genome as its congeneric species, S. lewini and S. zygaea. However within Charcharhiniformes, representatives of the Sphyridae family (genus Sphyra spp.) had a slightly larger mtDNA genome (mean 16,727 ± 4.04) than representatives of the Charcharhinidae family (16,702 ± 8.5) (genus Carcharhinus, Galeocerdo, Glyphis, Priacanthus and Scyllodon) as resulted from a short insertion of 44 bp in the control region.

The alignment of the 48 representative sharks and rays species of the main elasmobranch orders (Fig. 2) allowed the identification of several informative mtDNA regions at different levels of phylogenetic analyses (e.g. ranging from the inter–generic level to the inter-specific level).

At the inter-generic level within Charcharhiniformes, the average of the mean number of nucleotide differences among sequences of the representative species (14) of five genera (Sphyra, Carcharhinus, Galeocerdo, Glyphis, and Scyllodon), showed informative sites for some portions of the mtDNA genome; specifically the control region showed an average number of nucleotide differences (d\textsubscript{xy}) of 0.194, followed by genes ND2 (d\textsubscript{xy} = 0.153), Cyb (d\textsubscript{xy} = 0.151), and ND5 (d\textsubscript{xy} = 0.145). Although the control region showed a higher number of differences, it was characterized by several large portions of gaps among genera. In turn, ND2 has been used widely to assess phylogenetic relationships at the family level for elasmobranchs (Naylor et al., 2005), although genes ND4, Cyb and COI have been also used to evaluate relationships at the same level (Vélez-Zuazo and Agnarsson, 2011 and references therein).

At the inter-specific level within genus Carcharhinus, the most variable genes were ND2 (d\textsubscript{xy} = 0.091), ND5 (d\textsubscript{xy} = 0.09) and ND4 (d\textsubscript{xy} = 0.089) whereas the control region displayed among the lower variation (d\textsubscript{xy} = 0.050) similar to that of COI (d\textsubscript{xy} = 0.052). Based on analyses of the complete mtDNA genome of the speartooth shark Glyphis glyphis, of individuals from several river drainages of Australia (Feutry et al., 2014), the mtDNA genes ND5, ND2 and 12S, were identified also as informative at the intra-specific level (between populations) whereas the control region showed a lower amount of informative sites and was not informative for population differentiation. Similar results were reported for the zebra shark, Stegostoma fasciatum where the ND4 was the most informative gene at the intra-specific level as compared with the mtDNA control region (Dudgeon et al., 2009). Due to its faster mutational rate, the usefulness of the ND2 gene to address genetic divergence/phylogenetic questions at inter- and intra-specific level has been emphasized by Naylor et al. (2005, 2012), using a wide number of elasmobranch species.

3.3 Phylogenetic relationships

The mitogenomes of 48 shark and ray species representing the Galeomorphi, Squalomorphi, Squatinomorphi and Rajomorphi elasmobranch superorders were compared using C. milii (Chimaeriformes) as external group (Fig. 3). In general, by using the whole mtDNA genome the Bayesian and ML tree phylogenies were consistent with most molecular studies using individual mtDNA and/or nuclear genes (Douday et al., 2003; Winchell et al., 2004; Naylor et al., 2005; Vélez-Zuazo and Agnarsson, 2011), but differ from studies based on morphological data in supporting the main hypotheses. For example both, Bayesian and ML tree topologies were coincident on placing batoids (Rajidae (Pristiformes (Torpediformes, Myliobatiformes))),

| Gene | From (bp) | To (bp) | Size (bp) |
|------|-----------|---------|-----------|
| tRNA\textsuperscript{Cyb} | 1 | 72 | 72 |
| tRNA\textsuperscript{COI} | 77 | 1025 | 948 |
| tRNA\textsuperscript{ND2} | 1026 | 1097 | 71 |
| tRNA\textsuperscript{ND3} | 1098 | 2768 | 1670 |
| tRNA\textsuperscript{ND4} | 2769 | 2843 | 74 |
| tRNA\textsuperscript{ND5} | 3819 | 3887 | 68 |
| tRNA\textsuperscript{tRNASer} | 3889 | 3960 | 71 |
| tRNA\textsuperscript{tRNAAsp} | 3961 | 4009 | 28 |
| tRNA\textsuperscript{tRNAAsn} | 4030 | 4074 | 44 |
| tRNA\textsuperscript{tRNATr} | 5126 | 5282 | 76 |
| tRNA\textsuperscript{tRNATrp} | 5323 | 5388 | 65 |
| tRNA\textsuperscript{tRNATyr} | 5390 | 5459 | 69 |
| COI | 5461 | 7017 | 1556 |
| COI\textsuperscript{COI} | 7018 | 7088 | 70 |
| COI\textsuperscript{ND2} | 7092 | 7161 | 69 |
| COI\textsuperscript{ND3} | 7169 | 7859 | 690 |
| COI\textsuperscript{ND4} | 7860 | 7933 | 73 |
| COI\textsuperscript{ND5} | 8093 | 8775 | 682 |
| COI\textsuperscript{ND6} | 8776 | 9561 | 785 |
| tRNA\textsuperscript{tRNASer} | 9564 | 9633 | 69 |
| tRNA\textsuperscript{tRNAAsp} | 9634 | 9982 | 348 |
| tRNA\textsuperscript{tRNATrp} | 9883 | 10052 | 69 |
| tRNA\textsuperscript{tRNATrp} | 10053 | 10349 | 296 |
| tRNA\textsuperscript{tRNATyr} | 10343 | 11230 | 887 |
| tRNA\textsuperscript{tRNATyr} | 11274 | 11792 | 68 |
| tRNA\textsuperscript{tRNATyr} | 11793 | 11860 | 67 |
| tRNA\textsuperscript{tRNAAsp} | 11861 | 11932 | 71 |
| tRNA\textsuperscript{tRNAAsp} | 11933 | 12762 | 1829 |
| tRNA\textsuperscript{tRNAAsp} | 12758 | 14279 | 521 |
| tRNA\textsuperscript{tRNAAsp} | 14278 | 14347 | 69 |
| tRNA\textsuperscript{tRNAAsp} | 14352 | 15496 | 1144 |
| tRNA\textsuperscript{tRNAAsp} | 15497 | 15568 | 71 |
| tRNA\textsuperscript{tRNAAsp} | 15571 | 15639 | 68 |
| D-loop | 15640 | 16731 | 1091 |

Table 2

Comparison between mitogenomes of Sphyra tiburo and S. lewini.
as sister group of sharks, rejecting the Hypnosqualea hypothesis of Shirai (1992) which suggested that Batoids are derived from sharks (see Douady et al., 2003 and references therein). The mitogenome evidence supported the previous hypothesis based on morphological data separating Batoids from sharks (Bigelow and Schroeder, 1948, 1953) and is also consistent with most of the molecular evidence showed by Douady et al. (2003), Winchell et al. (2004) and Naylor et al. (2005) based on the analysis of 2.4–5.8 kbp including mtDNA and nuclear (Rag gene) data. Likewise, the monophyly of modern sharks or “Neoselachian” but with some differences in the arrangement of the 4 monophyletic superorders proposed by Compagno (1977) was clearly identified. The monophyly for three elasmobranch superorders as suggested by Maisey (1984) that organized neoselachians into three groups, the first based on the orbitostylic jaw suspension (Hexanchiformes, Squaliformes, Pristiophoriformes and Squatiniformes), the galeomorphs (Heterodontiformes, Orectolobiformes, Lamniformes and Carcharhiniformes) and batoids (skates and rays) and differs from the point of view of Compagno (1977) who placed Squatiniformes as a separated group of Squalimorphs and proposed four superorders (galeomorphs, squalomorphs, squatinimorphs and batoids) was confirmed. As a result, the monophyly for Squalomorphii was confirmed with the inclusion of Squatinimorphs, supporting the group with the orbitostylic jaw suspension (Hexanchiformes (Squaliformes (Squatiniformes, Pristiophoriformes))) according to the proposal of Maisey (1984) (Fig. 3).

Finally, within Galeomorphii, mtDNA genome sequences supported the association ((Lamniformes, Carcharhiniformes) Orectolobiformes) with Heterodontiformes in a basal position as suggested by de Carvalho (1996) and Shirai (1996) based on morphology and is also compatible with the molecular studies of Naylor et al. (2005) and Heincke et al. (2009) based on sequences of either the mtDNA and/or nuclear DNA, but differs from the views of Douady et al. (2003), Winchell et al. (2004), Human et al. (2006), Mallatt and Winchell (2007) and Vélez-Zuazo and Agnarsson (2011) who based on sequences of mtDNA and/or nuclear genes considered Lamniformes and Orectolobiformes as a sister group. Similarly, the mtDNA genome supported a sister relationship between Squatiniformes and Pristiophoriformes with Squaliformes being basal and Hexanchiformes as paraphyletic which is consistent with most of the molecular studies (Douady et al., 2003; Naylor et al., 2005; Mallatt and Winchell, 2007; Human et al., 2006; Vélez-Zuazo and Agnarsson 2011) but differs from the morphological evidence of Compagno (1973) and de Carvalho (1996) that found Pristiophoriformes nested as sister group with Squaliformes and Batoidea respectively.

At the family level, it was not possible to confirm the monophyly for Carcharhinidae as the tiger shark Galeocerdo cuvier appeared as paraphyletic and Sphyridae, which was monophyletic, as sister taxa of Carcharhinidae. This arrangement was reported before by Vélez-Zuazo and Agnarsson (2011), and Naylor et al. (2012) based on sequences of several mtDNA genes. Finally, the monophyly for Lamnidae was confirmed with families ordered as follows; (Mitsukurinidae (Alopiidae, Megachasmidae) (Odontaspididae (Cetorhinidae (Lamnidae))).

### 3.4 Conclusions

- The mtDNA genome for Sphyrna tiburo was 16,723 bp, similar in size to that of other Sphyrnid sharks which were slightly longer than those of Carcharhinid sharks, containing similar number and arrangement of genes as most vertebrate mtDNAs.
- The Bayesian and ML trees were similar to most of phylogenies based on molecular data and also to some other phylogenies based on morphological data confirming monophyly of Neoselachian and batoidea as sister group of sharks.
- The ND2 gene was informative at several levels from the inter-generic to intra-specific, as suggested before. This information will be valuable to develop molecular markers to perform population genetic analyses.

![Fig. 3](image-url)

**Fig. 3.** Left: Bayesian phylogenetic tree using whole mtDNA for sharks and rays showing the posterior probability values for branches (branches without numbers are values equal to 1.0). Right: Clades of the Maximum Likelihood tree which differ from the Bayesian analyses, only bootstrap values below 100% are shown.
directed to identify potentially key habitats as those used as nursery grounds.

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