



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



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## ABSTRACT

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 orbitostylic 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 unsolved; 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 (Douadey 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, Sphyrnidae (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

**Abbreviations:** ATP, Adenosine triphosphate; bp, Base pairs; CO, Cytochrome oxidase; Cytb, Cytochrome B; *D-loop*, Control region; mt, Mitochondrial; myr, Million years; ML, Maximum likelihood; ND, Nicotine adenine dehydrogenase; PCR, Polymerase chain reaction; rRNA, Ribosomal RNA; tRNA, Transference RNA.

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**Table 1**  
Elasmobranch species used in this study. Mitochondrial genomes from Pacific (PAC) and Gulf of Mexico (GM) individuals of *Carcharhinus leucas*.

Order/species	Family	mtDNA size	GB ref. #	Reference
<i>Carcharhiniformes</i>				
<i>Carcharhinus leucas</i> (PAC)	Carcharhinidae	16,704	NC023522	Chen et al. (2015b)
<i>Carcharhinus leucas</i> (GM)	Carcharhinidae	16,702	KJ210595	Díaz-Jaimes et al. (2014)
<i>Carcharhinus macroti</i>	Carcharhinidae	16,701	NC024862	Chen et al. (2014a)
<i>Carcharhinus sorrah</i>	Carcharhinidae	16,707	NC023521	Chen et al. (2015c)
<i>Carcharhinus acronotus</i>	Carcharhinidae	16,719	NC024055	Yang et al. (2014a)
<i>Carcharhinus plumbeus</i>	Carcharhinidae	16,706	NC024596	Blower and Ovenden (2014)
<i>Carcharhinus falciformis</i>	Carcharhinidae	16,680	KF801102	Galván-Tirado et al. (2014)
<i>Carcharhinus obscurus</i>	Carcharhinidae	16,706	NC020611	Blower et al. (2013)
<i>Carcharhinus melanopterus</i>	Carcharhinidae	16,706	NC024284	Chen et al. (2014b)
<i>Carcharhinus amblyrhynchoideus</i>	Carcharhinidae	16,705	NC023948	Feutry et al. (2014)
<i>Prionace glauca</i>	Carcharhinidae	16,705	NC022819	Chen et al. (2015d)
<i>Glyphis garricki</i>	Carcharhinidae	16,702	NC023361	Feutry et al. (2015)
<i>Glyphis glyphis</i>	Carcharhinidae	16,701	KF006312	Chen et al. (2014c)
<i>Galeocerdo cuvier cuvier</i>	Carcharhinidae	16,703	NC022193	Chen et al. (2014d)
<i>Scoliodon macrorhynchus</i>	Carcharhinidae	16,693	JQ693102	Chen et al. (2014e)
<i>Sphyrna zygaena</i>	Sphyrnidae	16,731	KM489157	Bolaño-Martínez et al. (2014)
<i>Sphyrna lewini</i>	Sphyrnidae	16,726	NC022679	Chen et al. (2015a)
<i>Sphyrna tiburo</i>	Sphyrnidae	16,723	KM453976	This study
<i>Mustelus griseus</i>	Triakidae	16,754	NC023527	Chen et al. (2014f)
<i>Mustelus manazo</i>	Triakidae	16,707	NC000890	Cao et al. (1998)
<i>Scyliorhinus canicula</i>	Scyliorhinidae	16,697	NC001950	Delabre et al. (1998)
<i>Lamniformes</i>				
<i>Carcharodon carcharias</i>	Lamnidae	16,744	NC022415	Chang et al. (2014a)
<i>Lamna ditropis</i>	Lamnidae	16,699	NC024269	Chang et al. (2014b)
<i>Isurus oxyrinchus</i>	Lamnidae	16,701	NC022691	Chang et al. (2015a)
<i>Isurus paucus</i>	Lamnidae	16,704	NC024101	Chang et al. (2014c)
<i>Cetorhinus maximus</i>	Cetorhinidae	16,670	NC023266	Hester et al. (2013)
<i>Carcharias taurus</i>	Odontaspidae	16,773	NC023520	Chang et al. (2015b)
<i>Alopias pelagicus</i>	Alopiidae	16,692	NC022822	Chen et al. (2015e)
<i>Alopias superciliosus</i>	Alopiidae	16,719	NC021443	Chang et al. (2014d)
<i>Megachasma pelagios</i>	Megachasmidae	16,694	NC021442	Chang et al. (2014e)
<i>Mitsukurina owstoni</i>	Mitsukurinidae	17,743	NC011825	Unpublished
<i>Orectolobiformes</i>				
<i>Orectolobus japonicus</i>	Orectolobidae	16,706	KF111729	Chen et al. (2015f)
<i>Rhincodon typus</i>	Rhincodontidae	16,875	NC023455	Alam et al. (2014)
<i>Chiloscyllium griseum</i>	Hemiscylliidae	16,755	NC017882	Chen et al. (2013)
<i>Chiloscyllium plagiosum</i>	Hemiscylliidae	16,726	NC012570	Unpublished
<i>Chiloscyllium punctatum</i>	Hemiscylliidae	16,703	NC016686	Chen et al. (2014g)
<i>Heterodontiformes</i>				
<i>Heterodontus francisci</i>	Heterodontidae	16,708	NC003137	Arnason et al. (2001)
<i>Heterodontus zebra</i>	Heterodontidae	16,720	NC021615	Chen et al. (2014h)
<i>Squatinaformes</i>				
<i>Squatina formosa</i>	Squatinae	16,690	NC025328	Corrigan et al. (2014)
<i>Squatina japonica</i>	Squatinae	16,689	NC024276	Chai et al. (2014)
<i>Squaliformes</i>				
<i>Squalus acanthias</i>	Squalidae	16,738	NC002012	Rasmussen and Arnason (1999)
<i>Cirrhigaleus australis</i>	Squalidae	16,543	KJ128289	Yang et al. (2014b)
<i>Pristiophoriformes</i>				
<i>Pristiophorus japonicus</i>	Pristiophoridae	18,430	NC024110	Unpublished
<i>Hexanchiformes</i>				
<i>Hexanchus griseus</i>	Hexanchidae	17,405	KF894491	Unpublished
<i>Myliobatiformes</i>				
<i>Gymnura poecilura</i>	Gymnuridae	17,874	NC_024102	Chen et al. (2014i)
<i>Torpediformes</i>				
<i>Narcine entemedor</i>	Narcinidae	17,081	KM386678	Castillo-Paez et al. (2014)
<i>Rajiformes</i>				
<i>Rhinobatos schlegelii</i>	Rhinobatidae	16,780	NC023951	Chen et al. (2014j)
<i>Zearaja chilensis</i>	Rajidae	16,909	KJ913073	Vargas-Caro et al. (2014)
<i>Pristiformes</i>				
<i>Anoxypristis cuspidata</i>	Pristidae	17,243	NC026307	Chen et al. (2015g)
<i>Chimaeriformes</i>				
<i>Callorhynchus milii</i>	Callorhynchidae	16,769	NC014285	Inoue et al. (2010)

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

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, Rhinobatiformes, Myliobatiformes, Torpediniformes and Pristiformes in order to assess the phylogenetic relationships between sharks and rays but also within Galeomorphii.

## 2. Materials and methods

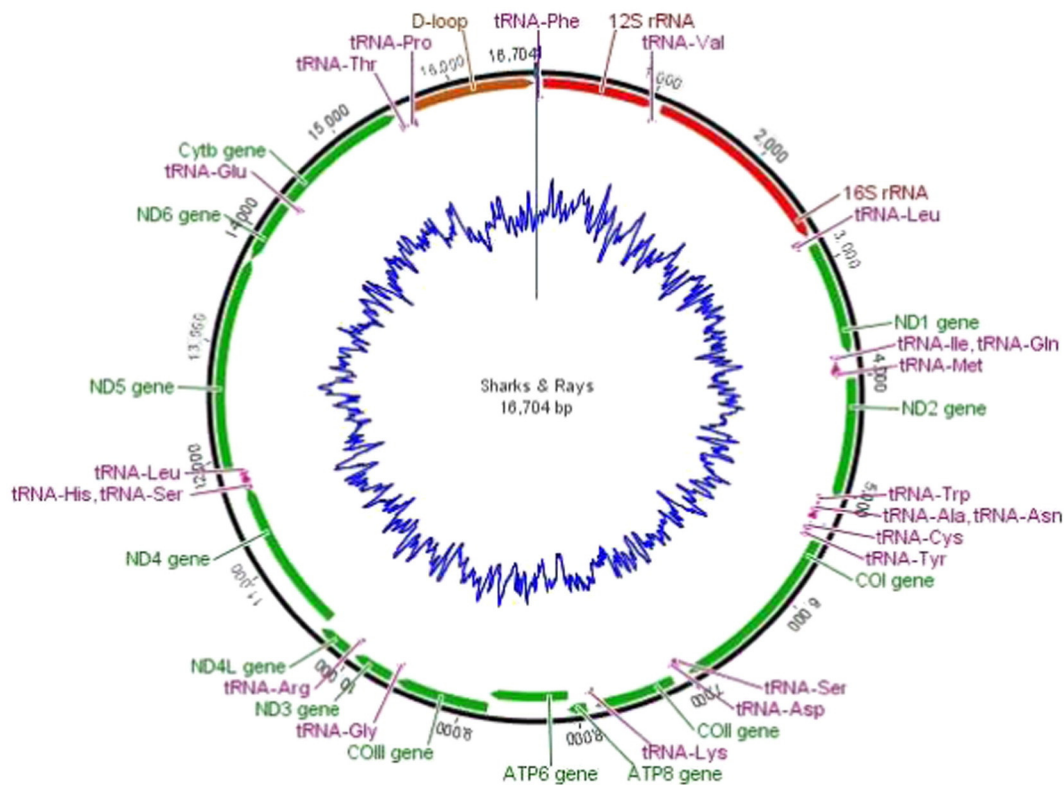
A muscle tissue biopsy of bonnethead was obtained from commercial fishing boats operating in Campeche Mexico, and stored in the Laboratorio de Genética de Organismos Acuáticos at the Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM). The genomic DNA was isolated 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, Torpediniformes and Pristiformes (Batoidea), and including the mtDNA genome of *Callorhynchus 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](http://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).

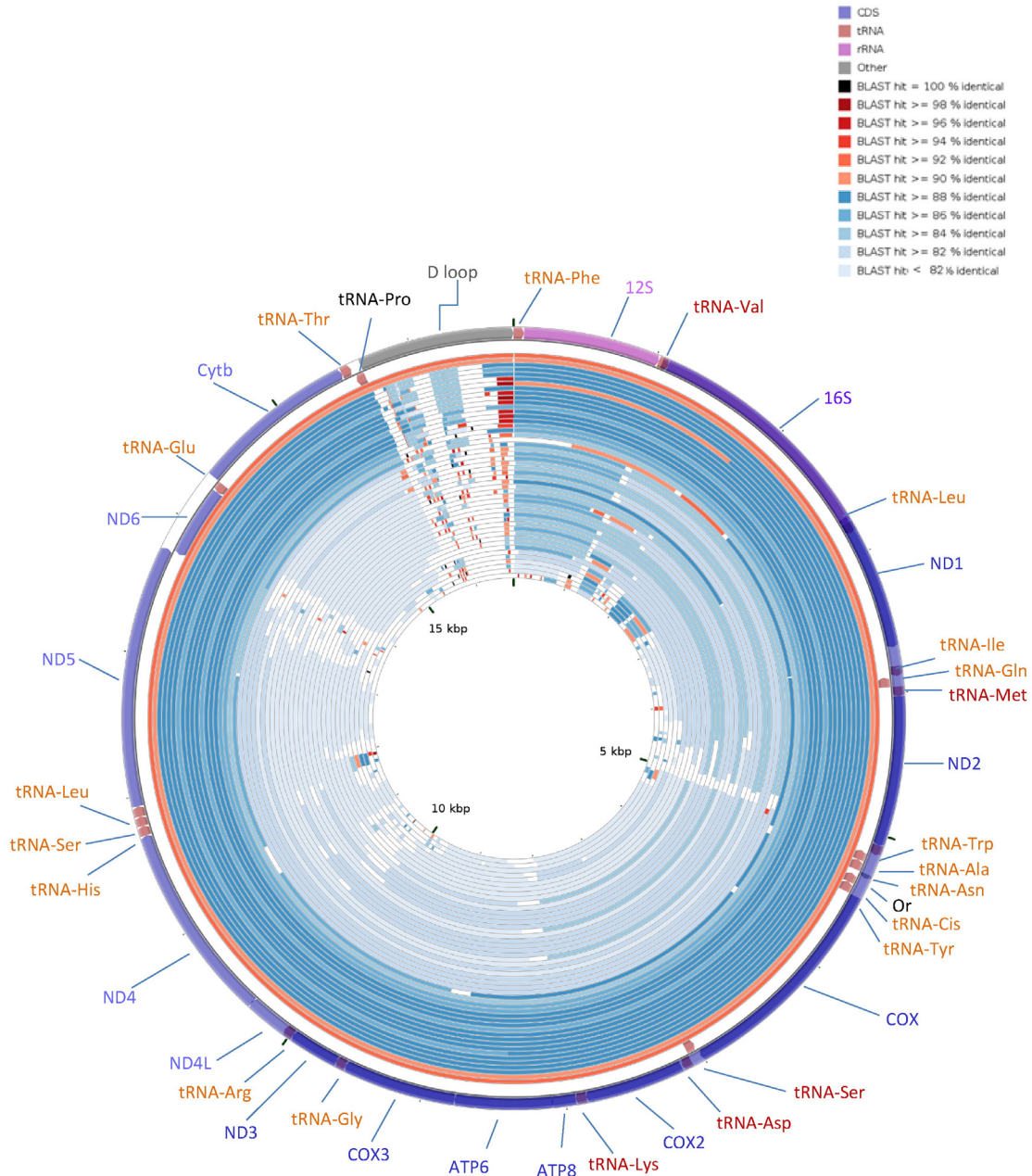
A partitioned Bayesian phylogenetic analysis excluding tRNAs was conducted with parallel version of Mr. Bayes 3.0b4 (Ronquist and Huelsenbeck, 2003) using 20,000 burn-in and 50,000,000 of generations. The unlink option was selected and also the gamma-shaped rate variation option, to allow each partition 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



**Fig. 2.** Graphical representation of the alignment results showing nucleotide identity between *S. tiburo* mitogenome and other 48 shark species, the first two external mitogenomes in red corresponds to *S. lewini* and *S. zygaena* respectively, followed by *Carcharhinus sorrah*, *C. macloiti*, *C. amblyrhynchoides*, *C. falciformis*, *C. plumbeus*, *C. acronotus*, *C. melanopterus*, *C. obscurus*, *Galeocerdo cuvier*, *Prionace glauca*, *Glyphis glyphis*, *G. garriki*, *Mustelus griseus*, *M. manazo*, *Scoliodon macrorhynchus*, *C. leucas*, *Alopias pelagicus*, *Charcharias taurus*, *A. superciliosus*, *Heterodontus francisci*, *Cetorhinus maximus*, *Mitsukurina owstoni*, *Lamna ditropis*, *Orectolobus japonicus*, *Scyliorhinus camicula*, *Chiloscyllium punctatum*, *Heterodontus zebra*, *Isurus paucus*, *Carcharodon carcharias*, *Rhyncodon typus*, *Cirrhigaleus australis*, *Megachasma pelagios*, *Squalus acanthias*, *Chiloscyllium griseum*, *Isurus oxyrinchus*, *Chiloscyllium plagiosum*, *Squatina Formosa*, *S. japónica*, *Pristiophorus japonicus*, *Hexanchus griseus*, *Rhinobatos schlegelii*, *Anoxypristis cuspidata*, *Zearaja chilensis*, *Narcine entemedor*, *Gymnura poecilura*, and *Callorhynchus milii*.

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. zygaena* (16,731; Bolaño-Martínez et al., 2014) but also similar to the reported mtDNA genome of other Carcharhinidae 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 *Cytb* (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 *Pristiophorus japonicus* (Squatiformes) 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 Squatiformes (mean 17,018 ± 792.7), followed by Lamniformes (16,813.9 ± 327.7), Orectolobiformes (16,753 ± 71.3), Heterodontiformes (16,714 ± 8.5) and Carcharhiniformes (16,708.5 ± 15.3). Within the Carcharhiniformes, an important difference in size between the genus *Carcharhinus* (16,703.5 ± 10.2) and *Sphyrna* (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; Poorvliet and Hoarau, 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. zygaena*. However within Carcharhiniformes, representatives of the Sphyrnidae family (genus *Sphyrna* sp.) had a slightly larger mtDNA genome (mean 16,727 ± 4.04) than representatives of the Carcharhinidae family (16,702 ± 8.5) (genus *Carcharhinus*, *Galeocerdo*, *Glyphis*, *Prionace* and *Scoliodon*) as resulted of 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 Carcharhiniformes, the average of the mean number of nucleotide differences among sequences of the representative species (14) of five genera (*Sphyrna*, *Carcharhinus*, *Galeocerdo*, *Glyphis*, and *Scoliodon*), showed informative sites for some portions of the mtDNA genome; specifically the control region showed an average number of nucleotide differences ( $d_{xy}$ ) of 0.194, followed by genes *ND2* ( $d_{xy} = 0.153$ ), *Cytb* ( $d_{xy} = 0.151$ ), and *ND5* ( $d_{xy} = 0.145$ ). 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*, *Cytb* 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* ( $d_{xy} = 0.091$ ), *ND5* ( $d_{xy} = 0.09$ ) and *ND4* ( $d_{xy} = 0.089$ ) whereas the control region displayed among the lower variation ( $d_{xy} = 0.050$ ) similar to that of *COI* ( $d_{xy} = 0.052$ ). Based on analyses of the complete mtDNA genome of the spear-tooth 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 Galeomorphii, Squalomorphii, Squatinomorphii and Rajomorphii 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 (Douady 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))),

**Table 2**  
Comparison between mitogenomes of *Sphyrna tiburo* and *S. lewini*.

<i>Sphyrna tiburo</i>				<i>Sphyrna lewini</i>			
Gene	From (bp)	To (bp)	Size (bp)	Gene	From (bp)	To (bp)	Size (bp)
<i>tRNA<sup>Phe</sup></i>	1	72	72	<i>tRNA<sup>Phe</sup></i>	1	71	70
<i>12S rRNA</i>	73	1025	953	<i>12S rRNA</i>	72	1027	955
<i>tRNA<sup>Val</sup></i>	1026	1097	71	<i>tRNA<sup>Val</sup></i>	1028	1099	71
<i>16S rRNA</i>	1098	2768	1670	<i>16S rRNA</i>	1100	2768	1,668
<i>tRNA<sup>Leu</sup></i>	2769	2843	74	<i>tRNA<sup>Leu</sup></i>	2769	2843	74
<i>ND1</i>	2844	3818	974	<i>ND1</i>	2844	3818	974
<i>tRNA<sup>Ile</sup></i>	3819	3887	68	<i>tRNA<sup>Ile</sup></i>	3819	3887	68
<i>tRNA<sup>Gln</sup></i>	3889	3960	71	<i>tRNA<sup>Gln</sup></i>	3889	3960	71
<i>tRNA<sup>Met</sup></i>	3961	4029	68	<i>tRNA<sup>Met</sup></i>	3961	4029	68
<i>ND2</i>	4030	5074	1044	<i>ND2</i>	4030	5074	1044
<i>tRNA<sup>Trp</sup></i>	5075	5145	70	<i>tRNA<sup>Trp</sup></i>	5075	5145	70
<i>tRNA<sup>Ala</sup></i>	5147	5215	68	<i>tRNA<sup>Ala</sup></i>	5147	5215	68
<i>tRNA<sup>Asn</sup></i>	5216	5288	72	<i>tRNA<sup>Asn</sup></i>	5216	5288	72
<i>tRNA<sup>Cys</sup></i>	5323	5388	65	<i>tRNA<sup>Cys</sup></i>	5324	5390	66
<i>tRNA<sup>Tyr</sup></i>	5390	5459	69	<i>tRNA<sup>Tyr</sup></i>	5392	5461	69
<i>COI</i>	5461	7017	1556	<i>COI</i>	5463	7019	1556
<i>tRNA<sup>Ser</sup></i>	7018	7088	70	<i>tRNA<sup>Ser</sup></i>	7020	7090	70
<i>tRNA<sup>Asp</sup></i>	7092	7161	69	<i>tRNA<sup>Asp</sup></i>	7094	7163	69
<i>COII</i>	7169	7859	690	<i>COII</i>	7171	7861	690
<i>tRNA<sup>Lys</sup></i>	7860	7933	73	<i>tRNA<sup>Lys</sup></i>	7862	7935	73
<i>ATP8</i>	7935	8102	167	<i>ATP8</i>	7937	8104	167
<i>ATP6</i>	8093	8775	682	<i>ATP6</i>	8095	8777	682
<i>COIII</i>	8776	9561	785	<i>COIII</i>	8778	9563	785
<i>tRNA<sup>Gly</sup></i>	9564	9633	69	<i>tRNA<sup>Gly</sup></i>	9566	9635	69
<i>ND3</i>	9634	9982	348	<i>ND3</i>	9636	9984	348
<i>tRNA<sup>Arg</sup></i>	9983	10,052	69	<i>tRNA<sup>Arg</sup></i>	9985	10,054	69
<i>ND4L</i>	10,053	10,349	296	<i>ND4L</i>	10,055	10,351	296
<i>ND4</i>	10,343	11,723	1380	<i>ND4</i>	10,345	11,725	1380
<i>tRNA<sup>His</sup></i>	11,724	11,792	68	<i>tRNA<sup>His</sup></i>	11,726	11,794	68
<i>tRNA<sup>Ser</sup></i>	11,793	11,860	67	<i>tRNA<sup>Ser</sup></i>	11,795	11,861	66
<i>tRNA<sup>Leu</sup></i>	11,861	11,932	71	<i>tRNA<sup>Leu</sup></i>	11,862	11,933	71
<i>ND5</i>	11,933	13,762	1829	<i>ND5</i>	11,934	13,763	1829
<i>ND6</i>	13,758	14,279	521	<i>ND6</i>	13,759	14,280	521
<i>tRNA<sup>Glu</sup></i>	14,278	14,347	69	<i>tRNA<sup>Glu</sup></i>	14,281	14,350	69
<i>Cyt B</i>	14,352	15,496	1144	<i>Cyt B</i>	14,353	15,497	1144
<i>tRNA<sup>Thr</sup></i>	15,497	15,568	71	<i>tRNA<sup>Thr</sup></i>	15,498	15,569	71
<i>tRNA<sup>Pro</sup></i>	15,571	15,639	68	<i>tRNA<sup>Pro</sup></i>	15,572	15,640	68
<i>D-loop</i>	15,640	16,731	1091	<i>D-loop</i>	15,641	16,726	1085

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 Squalimorfes and proposed four superorders (galeomorphs, squalomorphii, squatinimorphii and batoids) was confirmed. As a result, the monophyly for Squalimorphii was confirmed with the inclusion of Squatinimorfes, 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 Sphyrnidae, 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) (Odontaspidae (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

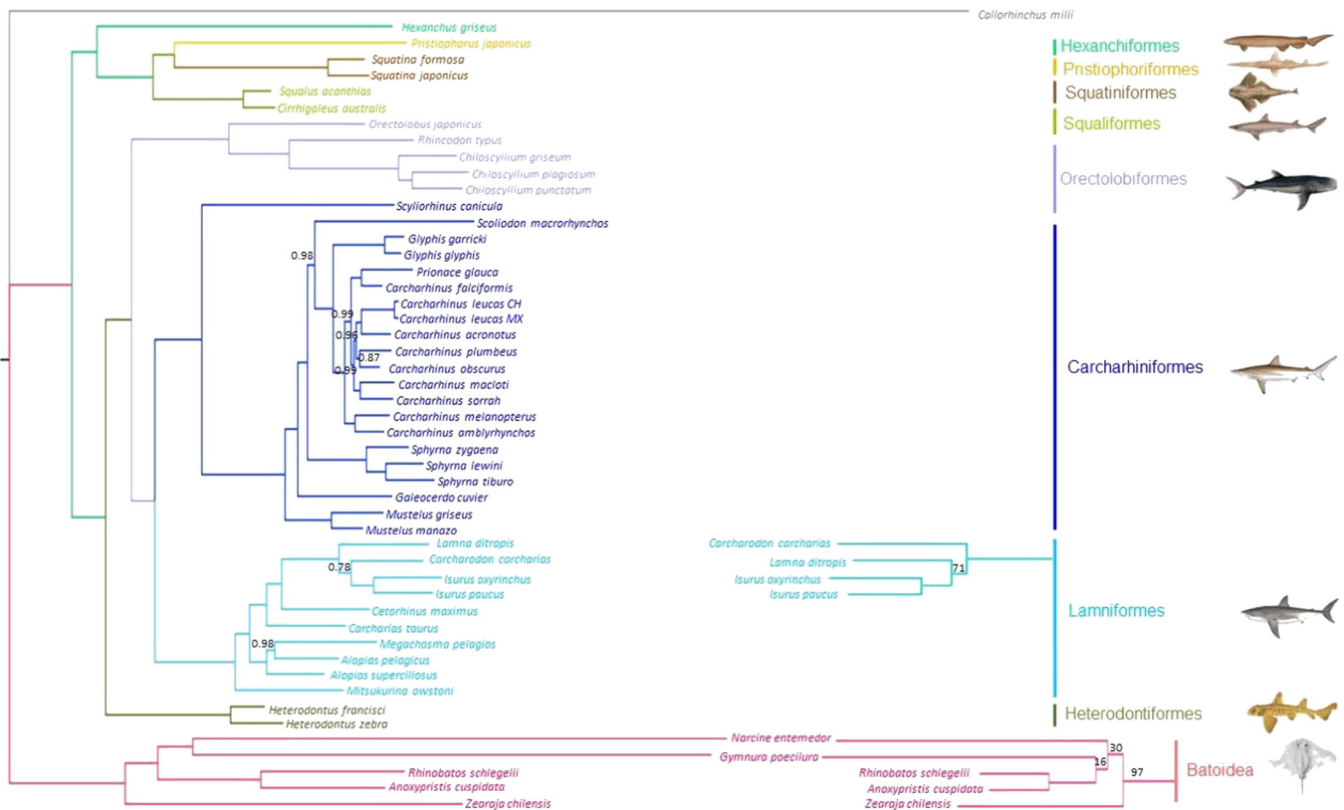


Fig. 3. Left: Bayesian phylogenetic tree using whole mtDNA for sharks and rays showing the posterior probability values for branches (branches without numbers are values equal to 1.0). Right: Clades of the Maximum Likelihood tree which differ from the Bayesian analyses, only bootstrap values below 100%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

directed to identify potentially key habitats as those used as nursery grounds.

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