Online ISSN: 0718-1957 RBMO
Revista de Biología Marina y Oceanografía
Vol. 57, N° especial: 132-144, 2022
<https://doi.org/10.22370/rbmo.2022.57.Especial.3339>

ARTICLE

Combining morphological and molecular information to infer phylogenetic relationships of lamniform sharks

Combinando información morfológica y molecular para inferir las relaciones filogenéticas de los tiburones lamniformes

Sebastián Cona[1*], Claudio Cornejo[2], Sebastián Hernández[3,4] and Christian M. Ibáñez[1]

[1]Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andres Bello, Avenida República 440, Santiago, Chile
[2]Programa de Doctorado en Sistemática y Biodiversidad, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario S/N, Concepción, Chile
[3]Biomolecular Laboratory, Center for International Programs, Universidad Veritas, Calle 31, Avenida 20, Zapote, San José, Costa Rica
[4]Sala de Colecciones Biológicas, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile
*Corresponding author: conasebastian6@gmail.com

Resumen.- Los tiburones del orden Lamniformes se restringen a 15 especies existentes, agrupadas dentro de 10 géneros y 8 familias. Estas especies están caracterizadas por tener dos aletas dorsales sin espinas y una válvula intestinal de forma de anillo. Sus relaciones filogenéticas no son congruentes entre diferentes métodos y enfoques, como el uso de datos morfológicos o moleculares. El presente estudio evalúa las relaciones filogenéticas de las especies del orden Lamniformes mediante reconstrucciones filogenéticas basadas en datos morfológicos y moleculares, utilizando ambos sets de datos simultáneamente mediante inferencia Bayesiana. El árbol consenso de la reconstrucción Bayesiana morfológica muestra que Lamnidae y Alopiidae son monfiléticos, mientras que Odontaspididae es polifilético. Se identificaron ocho sinapomorfías morfológicas para Alopiidae, seis en Lamnidae y una para Odontaspididae. En el árbol consenso de la reconstrucción Bayesiana molecular Lamnidae y Odontaspididae es monofilético, mientras Alopiidae es polifilético. En el árbol consenso de la reconstrucción Bayesiana de datos combinados (morfología y ADN), Lamnidae, Alopiidae y Odontaspididae son monofiléticos. Los resultados obtenidos sugieren que, al usar caracteres combinados dentro de un análisis Bayesiano filogenético, las probabilidades posteriores aumentan, y es de gran ayuda para la sistemática en el orden Lamniformes. Debido a la presencia de grupos no-monofiléticos, familias monotípicas y el fuerte apoyo a la división de los lamniformes en dos clados, se necesita una revisión urgente de la clasificación de estas especies de tiburones.

Palabras clave: Elasmobranquios, sistemática, morfología, filogenia, monofilia

Abstract.- Sharks of the order Lamniformes are restricted to 15 extant species grouped into 10 genera and 8 families. These species are characterized by two spine-less dorsal fins and a ring-shaped intestinal valve. Their phylogenetic relationships are not congruent among different methods and approaches, such as the use of morphological or molecular data. The present study evaluates the phylogenetic relationships of species of the order Lamniformes by means of phylogenetic reconstructions through Bayesian inference based on morphological and molecular data and using both datasets combined. The consensus tree of the morphological Bayesian reconstruction shows that Lamnidae and Alopiidae are monophyletic, while Odontaspididae is polyphyletic. Eight synapomorphies are detected in Alopiidae, six in Lamnidae, and one for Odontaspididae. In the Bayesian molecular reconstruction consensus tree, Lamnidae and Odontaspididae are monophyletic, and Alopiidae is polyphyletic. In the consensus tree of the Bayesian reconstruction of combined data, Lamnidae, Alopiidae and, Odontaspididae are monophyletic. The results obtained suggest that posterior probabilities increase when using combined characters in a Bayesian phylogenetic analysis, which is greatly advantageous for systematics of the order Lamniformes. Due to the presence of non-monophyletic groups, monotypic families, and the strong support for the division of lamniforms into two clades, a crucial review for the classification of species is needed.

Key words: Elasmobranchs, systematics, morphology, phylogeny, monophyly

INTRODUCTION

Extant sharks of the order Lamniformes (Chondrichthyes: Elasmobranchii) comprise a group of 15 species catalogued into 10 genera and 8 families (Stone & Shimada 2019). This order comprises a monophyletic group (Compagno 1973, 1977) according to synapomorphic traits that include an elongated ring-type intestinal valve, and the absence of nictitating membrane (Compagno 1990, 2002; Shimada 2005, Nelson 2006, Williams 2015, Stone & Shimada 2019). Lamniform or mackerel sharks are medium- to large-sized species [*i.e.*, up to 9.8 m long in *Cetorhinus maximus* (Gunnerus, 1765), most acting as top predators in pelagic ecosystems (Cortés 1999, Compagno 2002, López *et al.* 2009)]. Lamniform sharks have circumglobal distribution, mainly at mid-to-low latitudes (temperate and tropical seas), while some species reach cold boreal and subantarctic waters (Compagno 2002, Schnetz *et al.* 2016). These sharks are economically important due to their commercial exploitation around the world, either as target species or as bycatch in other commercial fisheries (Compagno 1984a, 2001; Camhi *et al.* 1998, Acuña *et al*. 2002, López *et al.* 2009, Fischer *et al.* 2012). Given their economic and ecological relevance, research focused on the relationships of lamniform sharks began with Jordan (1898), which was later organized by Bigelow & Schroeder (1958).

Lamniforms constitute a phylogenetic enigma since both morphological and molecular data have resulted in different tree topologies. Compagno (1973 & 1977) were the first phylogenetic studies with elasmobranchs, which were conducted comparing the condrocranium morphology. Later, Compagno (1990) considered morphological comparisons of the whole shark body. Since the description of *Megachasma pelagios* Taylor, Compagno & Struhsaker, 1983, collected in a research vessel in Hawaii, the relationship of *M. pelagios* within the order Lamniformes was a matter of debate among different authors (Compagno 1973, 1977, 1990; Taylor *et al.* 1983, Maisey 1985, Long & Waggoner 1996). This controversy was raised by the feeding strategy (planktivorous) and internal anatomy (jaw, teeth, intestinal valve) of this species (Compagno & Struhsaker 1983, Compagno 1984a, 1990), as *Cetorhinus maximus* Gunnerus, 1765, from a different family, is also planktivorous feeder and shares similar teeth morphology (homoplasy) with *M. pelagios*. Therefore, these taxa should be assessed and compared with other sharks of the order Lamniformes. First, Compagno (1973, 1977) grouped *M. pelagios* into its own family, Megachasmidae, based on several phenetic differences compared with all other lamniform sharks, suggesting the family as a primitive sister-group of the rest of lamniforms. Other studies proposed that *C. maximus* and *M. pelagios* formed a monophyletic group (Cetorhinidae) considering the mandibular suspension associated with their specialized feeding apparatus (Maisey 1985) or similar teeth morphology (Long & Waggoner 1996) (Fig. 1A, C). Conversely, another cladistic study concluded that *Cetorhinus* and *Megachasma* are not sister groups, suggesting that these species evolved their filter feeding strategy independently (Compagno 1990, Shimada *et al*. 2009) (Fig. 1B). Moreover, Shirai

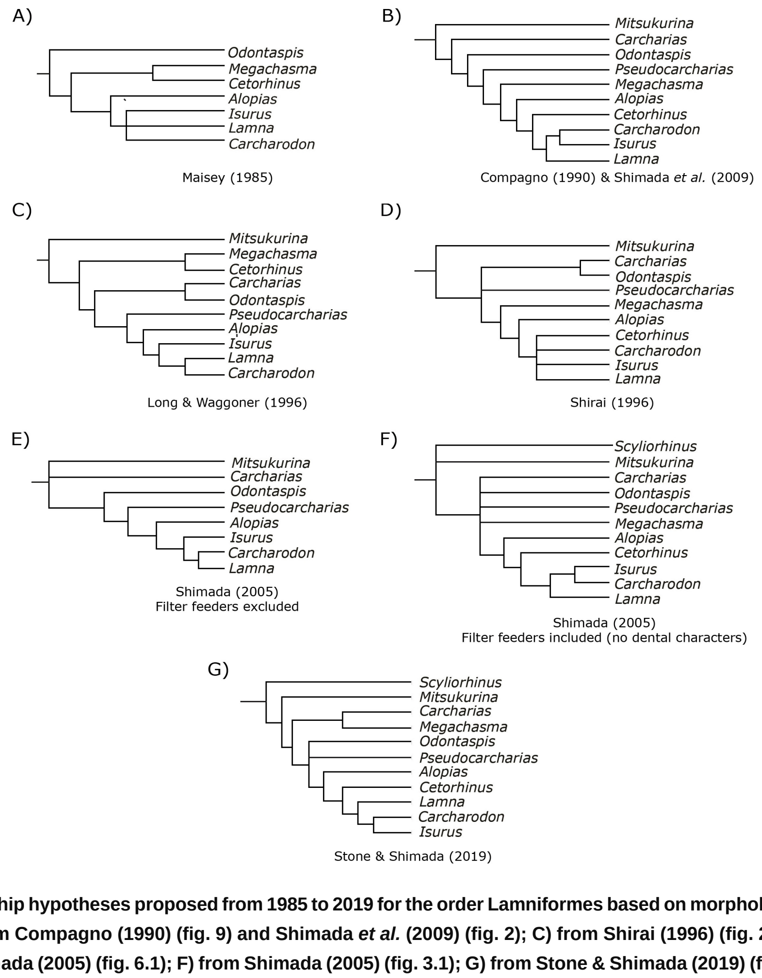

Figure 1. Simplified relationship hypotheses proposed from 1985 to 2019 for the order Lamniformes based on morphological character data. A) from Maisey (1985) (fig. 2); B) from Compagno (1990) (fig. 9) and Shimada *et al.* (2009) (fig. 2); C) from Shirai (1996) (fig. 2); D) from Long & Waggoner (1996) (fig. 1A); E) from Shimada (2005) (fig. 6.1); F) from Shimada (2005) (fig. 3.1); G) from Stone & Shimada (2019) (fig. 8B). Cladograms with less than 2 non-monotypic families were excluded / Hipótesis de relaciones del orden Lamniformes propuestas desde el año 1985 hasta el 2019, basadas en caracteres morfológicos. A) obtenida de Maisey (1985) (fig. 2); B) obtenida de Compagno (1990) (fig. 9) y Shimada *et al.* (2009) (fig. 2); C) obtenida de Shirai (1996) (fig. 2); D) obtenida de Long & Waggoner (1996) (fig. 1A); E) obtenida de Shimada (2005) (fig. 6.1); F) obtenida de Shimada (2005) (fig. 3.1); G) obtenida de Stone & Shimada (2019) (fig. 8B). Cladogramas con menos de 2 familias no monotípicas fueron excluidos

(1996) proposed the phylogenetic relationships of major Neoselachian sharks (Fig. 1D) using many morphological characters (*e.g.*, skeletal, fins, axial skeleton). This study by Shirai (1996) presented *Mitsukurina owstoni* Jordan, 1898 as sister species to the rest of lamniform sharks, and the families Odontaspididae and Megachasmidae as sister group to Alopiidae, Lamnidae and Cetorhinidae. Later, Shimada (2005), also based on morphology [*i.e.*, anatomy and teeth morphology, suggested the monophyly of Alopiidae and Lamnidae (Fig. 1E, F)]. Other research explored evolutionary relationships according to the mineralization pattern of teeth in Alopiidae and Lamnidae, supported by phylogenetic reconstructions that considered molecular data (Naylor *et al.* 2012, Schnetz *et al.* 2016). Recently, Stone & Shimada (2019) resurrected the family Carchariidae (Fig. 1G) for the genus *Carcharias* Rafinesque, 1810 due to the continuous polyphyly obtained for the family Odontaspididae in previous phylogenetic studies and the lack of morphological data available for *Odontaspis noronhai* (Maul, 1955).

The molecular phylogenetic approaches for lamniform sharks have mostly considered mitochondrial genes, suggesting that the phylogeny of this group of sharks is composed of two main clades (Fig. 2). For lamniform sharks, molecular phylogenetic studies started with the use of allozymes, reconstructing the relationships within Alopiidae

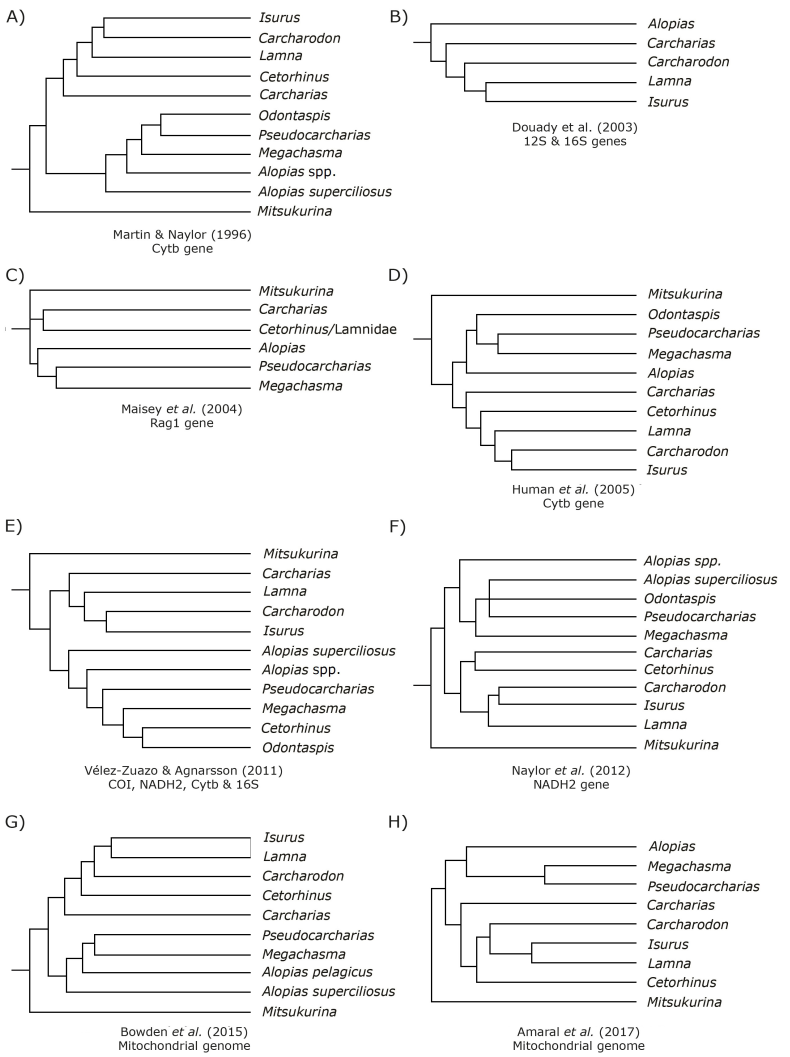

Figure 2. Simplified relationship hypotheses posed from 1996 to 2018 for the order Lamniformes based on molecular characters of nuclear or mitochondrial gene data. A) from Martin & Naylor (1996) (fig. 5); B) from Douady *et al.* (2003) (fig. 1); C) from Maisey *et al.* (2004) (fig. 5A); D) from Human *et al.* (2005) (fig. 2); E) from Vélez-Zuazo & Agnarsson (2011) (fig. 4); F) from Naylor *et al.* (2012) (fig. 2.2); G) from Bowden *et al.* (2015) (fig. 1); H) from Amaral *et al.* (2018) (fig. 5). Cladograms with less than 2 non-monotypic families were excluded / Hipótesis de relaciones del orden Lamniformes simplificadas, propuestas desde el año 1996 hasta el 2018, basadas en caracteres moleculares tomados de genes nucleares o mitocondriales. A) obtenida de Martin & Naylor (1996) (fig. 5); B) obtenida de Douady *et al.* (2003) (fig. 1); C) obtenida de Maisey *et al.* (2004) (fig. 5A); D) obtenida de Human *et al.* (2005) (fig. 2); E) obtenida Vélez-Zuazo & Agnarsson (2011) (fig. 4); F) obtenida de Naylor *et al.* (2012) (fig. 2.2); G) obtenida de Bowden *et al.* (2015) (fig. 1); H) obtenida de Amaral *et al.* (2018) (fig. 5); Cladogramas con menos de 2 familias no monotípicas fueron excluídos

(Eitner 1995). Later, research using one (*i.e.*, Cytb, NADH2 or RAG1, Martin & Naylor 1997, Maisey *et al.* 2004, Human *et al.* 2006, Naylor *et al.* 2012) or two molecular markers (12S and 16S, Douady *et al.* 2003) found different phylogenetic relationships (Fig. 2). Among all studies, Vélez-Zuazo & Agnarsson (2011) resolved the major relationships within Selachimorpha using four mitochondrial genes (COI, NADH2, Cytb, 16S) and one nuclear gene (RAG1) (Fig. 2E), supporting the monophyly of Lamniformes. Similarly, Bowden *et al.* (2016) and Amaral *et al.* (2018) (Fig. 2G, H) reconstructed the phylogeny of lamniform sharks using the available mitochondrial genomes.

In summary, as in any phylogenetic reconstruction, different topologies can be obtained according to the type of data –morphological (Fig. 1) and/or molecular (Fig. 2)– and the methods (most using Parsimony and likelihood) employed to perform analyses. Hence, the aim of this study was to provide phylogenetic relationships of lamniform sharks through Bayesian inference combining morphological and molecular characters as an alternative to enhance the tree topology in the order Lamniformes (*i.e.*, Alopiidae, Lamnidae and Odontaspididae).

Materials and methods

Phylogenetic reconstruction

To elucidate the evolutionary relationships of lamniform sharks (Table 1), three phylogenetic analyzes based on morphological, molecular, and combined data were performed. In all phylogenetic analyses, the trees were rooted using *Scyliorhinus canicula* (Linnaeus, 1758) as outgroup.

Morphological phylogeny

The morphological data matrix was generated selecting 42 non-ordered morphological characters published by Shimada (2005) and Stone & Shimada (2019), in addition to 25 binary morphological characters based on descriptions of external anatomy obtained from Compagno (1984a, b; 2001). The multi-state characters from Shimada (2005) and Stone & Shimada (2019) were transformed by simplifying multistate characters into binary data (*i.e.*, absent or present, lower or higher, small or large, and equal or different). All characters were added to a single data matrix, resulting in 67 binary characters to perform the phylogenetic Bayesian analysis (Suppl. Material, Appendix 1).

Table 1. GenBank access codes of mitochondrial sequences of each species used in the molecular phylogenetic analysis in this study / Código de acceso GenBank de las secuencias mitocondriales usadas en el análisis filogenético para cada especie en este estudio

Family	Species	Cytb	NADH2	COI	12S
Outgroup					
Scyliorhinidae	*Scyliorhinus canicula*	Y16067	JQ518686	Y16067	Y16067
Lamniformes					
Alopiidae	*Alopias superciliosus*	KC757415	KC757415	KC757415	KC757415
	Alopias vulpinus	MF374733	MF374734	MF374735	MF374736
	Alopias pelagicus	KF020876	KF412639	KF412639	KF020876
Cetorhinidae	*Cetorhinus maximus*	NC023266	JQ518731	FJ519307	NC023266
Lamnidae	*Carcharodon carcharias*	L08031	JQ518732	DQ108328	KX389266
	Isurus oxyrinchus	L08036	NC022691	KJ146036	NC022691
	Isurus paucus	L08037	NC024101	KF899543	NC024101
	Lamna ditropis	LDU91438	JQ518735	KF918878	NC024269
	Lamna nasus	L08038	JQ518990	KJ146041	NC033911
Megachasmidae	*Megachasma pelagios*	U91440	JQ518736	EU398905	NC021442
Mitsukurinidae	*Mitsukurina owstoni*	EU528660	JQ519120	JX124812	EU528659
Carchariidae	*Carcharias taurus*	U91447	JQ518737	FJ519764	KT337317
Odontaspididae	*Odontaspis ferox*	U91445	JQ518738	GU130673	MT702386
	Odontaspis noronhai	-	JQ518739	KF899559	-
Pseudocarchariidae	*Pseudocarcharias kamoharai*	NC026216	NC026216	NC026216	NC026216

The Bayesian phylogenetic reconstruction was conducted in BayesPhylogenies ver.1.1 software (Pagel *et al.* 2004), using the morphological model of irreversible time (M2P model), previously selected based on the Bayes Factor calculated in Tracer ver.1.6 (Rambaut *et al.* 2013). M2P model allows the rates of gain and loss of traits to differ along trees. By means of Markov Chain Monte Carlo (MCMC), four chains were run, each using 40,000,000 iterations. Likelihood convergence and Effective Sample Size (ESS) were evaluated in Tracer, and 10% of MCMC was discarded as burn-in to build the 50% cut-off majority consensus tree.

To map morphological synapomorphies along trees, a Maximum Parsimony (MP) phylogenetic reconstruction was performed in T.N.T ver.1.5 software (Goloboff & Catalano 2016), using the Wagner parsimony and 10,000 bootstrap replicates.

Molecular phylogeny

Cytochrome b (CYTB), NADH dehydrogenase subunit 2 (NADH2), Cytochrome C oxidase I (COI), and small subunit: SSU ribosomal RNA (12S) mitochondrial genes downloaded from GenBank were used, which were available either as partial gene samples or as complete mitochondrial genomes (Table 1) (NCBI 2021)[1]. Saturation of each gene was evaluated using the substitution saturation index (Iss) and critical substitution saturation index (Iss.c) test introduced by Xia *et al.* (2003) implemented in DAMBE ver.7.2 software (Xia 2018). Sequences of each gene were aligned with MUSCLE algorithm (Edgar 2009) implemented in MEGA X software (Kumar *et al.* 2018). All gene matrices were combined in a partitioned data matrix in Mesquite ver.3.5 software (Maddison & Maddison 2018). The best substitution model was estimated for each mitochondrial nucleotide sequence in jModelTest ver.2.1.1 software (Darriba *et al.* 2012), using the Bayesian Information Criterion (BIC) and the corrected Akaike Information Criterion (AICc) (Akaike 1998, Bhat & Kumar 2010). The Bayesian phylogenetic reconstruction was performed in BayesPhylogenies v.1.1 software (Pagel *et al.* 2004) with four independent MCMC using 40,000,000 iterations.

Combined character phylogeny

Morphological (binary characters) and molecular (mitochondrial genes) data were used to build a concatenated matrix with the adjustments for each analysis mentioned above. The concatenated matrix was employed to perform a Bayesian analysis of combined data using the best substitution model for each dataset.

To identify congruence between each data set (morphological, molecular, and combined), Bayesian reconstructed trees were evaluated using APE package implemented in R ver.4.02 (Paradis & Schliep 2019, R Core Team 2020).

Results

Morphological phylogeny

From the 67 morphological characters evaluated in this study, 44 (65.67%) were recognized as informative according to the Bayesian analysis. Two major clades (Fig. 3A) were retrieved from the phylogenetic reconstruction, where *Cetorhinus maximus* was positioned as sister group to the rest of lamniform sharks with a high Posterior Probability (PP= 1.0). Clade 1 was composed by the families Alopiidae, Megachasmidae, Mitsukurinidae, Odontaspididae, and Pseudocarchariidae. Megachasmidae was positioned as sister group to the rest of the families within Clade 1. Alopiidae was positioned as sister group to the Pseudocarchariidae, Odontaspididae, Mitsukurinidae and Carchariidae. Odontaspididae was polyphyletic, with *Odontaspis ferox* (Risso, 1810) positioned as sister species of *O. noronhai*, *M. owstoni* and *Carcharias taurus* Rafinesque, 1810. Clade 2 was composed solely of the Lamnidae, with two subclades, one of them corresponding to *Lamna* Cuvier, 1816, and the other grouping *Carcharodon carcharias* (Linnaeus, 1758) and *Isurus* Rafinesque, 1810 as sister groups. The parsimony phylogenetic analysis revealed eight morphological synapomorphies for Alopiidae and six synapomorphies for Lamnidae (Table 2).

[1]NCBI. 2021. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda. <https://www.ncbi.nlm.nih.gov>

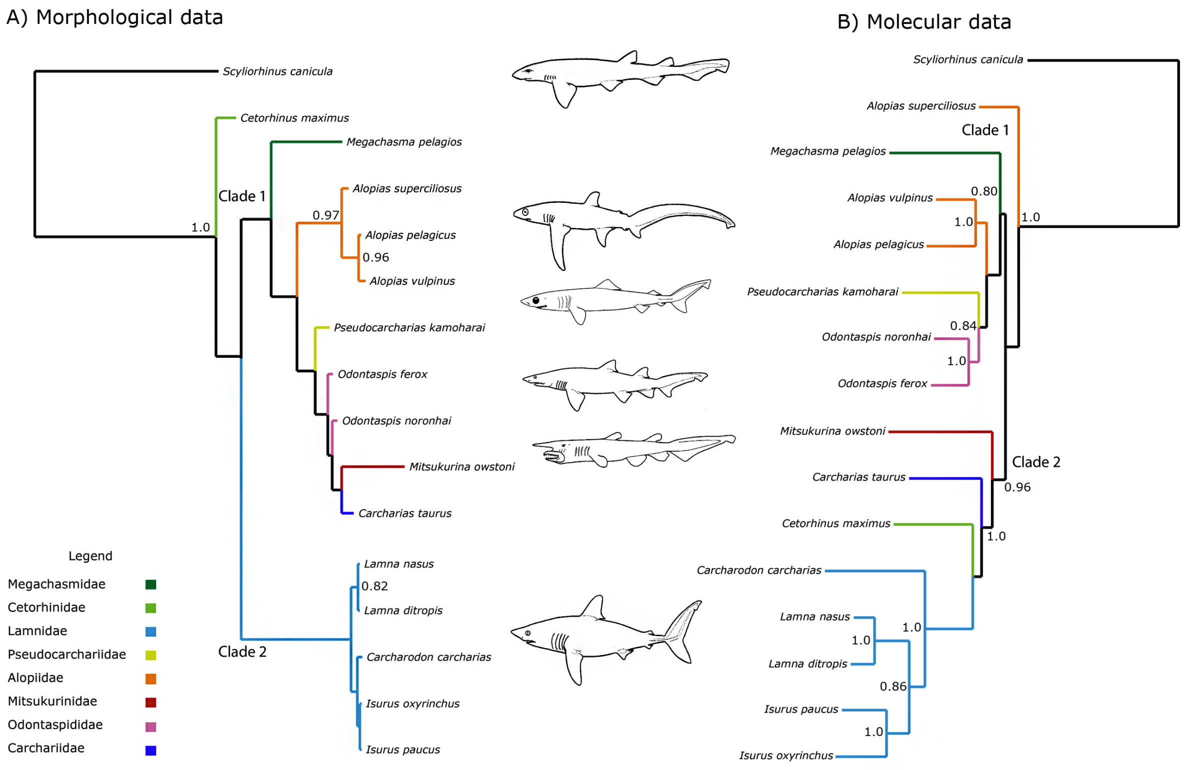

Figure 3. Mirrored reconstructed Bayesian trees of: A) morphological data and B) molecular data. Numbers at the nodes are estimated posterior probabilities (probabilities > 0.7 are shown). Colors of tree branches indicate the family of each species (see legend) / Reconstrucción de los árboles filogenéticos Bayesianos de: A) datos morfológicos y B) datos moleculares. Los números de los nodos corresponden a la probabilidad posterior (solo > 0,7 son presentados). Los colores de las ramas de los árboles indican las familias para cada especie (ver leyenda)

Table 2. List of synapomorphies identified in the families Alopiidae, Lamnidae and Odontaspididae according to the morphological dataset / Lista de sinapomorfías identificadas en las familias Alopiidae, Lamnidae y Odontaspididae del set de datos morfológicos

Family	Synapomorphies
Alopiidae	(1) Deep notch on dorsal side of palatoquadrate immediately lateral to upper dental bulla, (2) Large orbital diameter compared to cranial length behind nasal capsules; (3) Convex overall outline of posterior edge of cranium when viewed dorsoventrally, (4) Over 200 total vertebrae, (5) An approximately equal length of upper caudal fin lobe compared to precaudal body length; (6) Elongated caudal fin; (7) Small to moderate mouth; (8) Small to moderate branchial slits.
Lamnidae	(1) Mesial process of palatoquadrate present; (2) Ventral level of nasal capsules depressed below level of basal plate; (3) Cranial width at preorbital processes compared to that at nasal capsules equal or narrower; (4) Cranial width at preorbital processes compared to cranial length behind level of preorbital processes much lesser (“long cranial roof); (5) Large stapedial foramina of cranium; (6) Short or moderate pelvic fin.
Odontaspididae	(1) Lateral rostral cartilages form part of anterior fontanelle of cranium.

Molecular phylogeny

Xia´s test did not find saturation in both the coding (NADH2: Iss= 0.4673 < Iss.c= 0.776, P < 0.01; COI: Iss= 0.4721 < Iss.c 0.736, P = 0.0094; Cytb: Iss= 0.454 < Iss.c= 0.7711, P < 0.01) and non-coding gene (12S: Iss= 0.461 < Iss.c 0.7622, P = 0.025). The best substitution model was the same for all genes (Cytb: Generalized time-reversible model (GTR+G+I), NADH2: GTR+G+I and 12S: GTR+G+I) except for COI: Hasegawa, Kishino and Yano model (HKY+G) (Hasegawa *et al*. 1985). The molecular Bayesian analysis recognized 1,258 informative characters (33%) out of 3,813. Two major clades were retrieved from the phylogenetic reconstruction (Fig. 3B). Clade 1 was composed of Alopiidae, Pseudocarchariidae, Megachasmidae, and Odontaspididae. Clade 2 was represented by Mitsukurinidae, Cetorhinidae, Lamnidae and Carchariidae. Alopiidae was polyphyletic (PP= 1.0), with *Alopias superciliosus* (Lowe, 1841) as sister group to the rest of the families in Clade 1. Lamnidae was monophyletic, with *C. carcharias* as the sister group of *Lamna* and *Isurus* (PP= 1.0). Odontaspididae was monophyletic (PP= 0.83). Both Bayesian analyses denoted the low congruence between morphological and molecular phylogenetic reconstructions (Fig. 3).

Combined character phylogeny

The likelihood values of combined data were similar to values obtained from molecular data, and both were higher than values from morphological data (Table 3). The Bayesian analysis of combined data recognized 1,288 informative characters out of 3,880 (33.2%). Two main clades were retrieved from the phylogenetic reconstruction (Fig. 4). Clade 1 was composed of monophyletic Alopiidae (PP= 0.85), as sister group to *M. pelagios*, *Pseudocarcharias kamoharai* (Matsubara, 1936) and *O. ferox*. Clade 2 was represented by *M. owstoni*, *C. taurus*, *C. maximus* and Lamnidae. Within clade 2, *M. owstoni* was positioned as sister group to the rest of the families (PP= 0.88), while Lamnidae was monophyletic and sister group to *C. maximus* (PP= 1). Lamnidae was composed of two clades, presenting a topology similar to the morphological phylogenetic reconstruction, with *Lamna* as sister group (PP= 1.0) to *Carcharodon* Smith, 1838 and *Isurus*, the latter two being sister groups (PP= 0.92). The family Odontaspididae resulted polyphyletic, with *O. ferox* in Clade 1 (PP= 0.71) and *C. taurus* in Clade 2 (PP= 0.89).

Congruence between morphological and molecular trees yielded 39% across nodes, while the molecular and combined data yielded 85% of congruence across nodes.

Table 3. Descriptive statistics of each Bayesian analysis. HPD95 (Highest posterior density 95% Interval), ESS (effective sample size) / Estadísticas descriptivas de cada análisis Bayesiano. HPD95 (Intervalo de 95% de mayor densidad posterior), ESS (Tamaño de muestra efectivo)

Parameters	Morphological data	Molecular data	Combined data
Mean	-435.13	-21,217.68	-21,762.17
Median	-434.70	-21,217.34	-21,762.00
HPD95	-446.41 – -424.62	-21,229.17 – - 21,206.77	-21,775.43 – -21,749.09
ESS	18,380.47	5,278.10	91.92

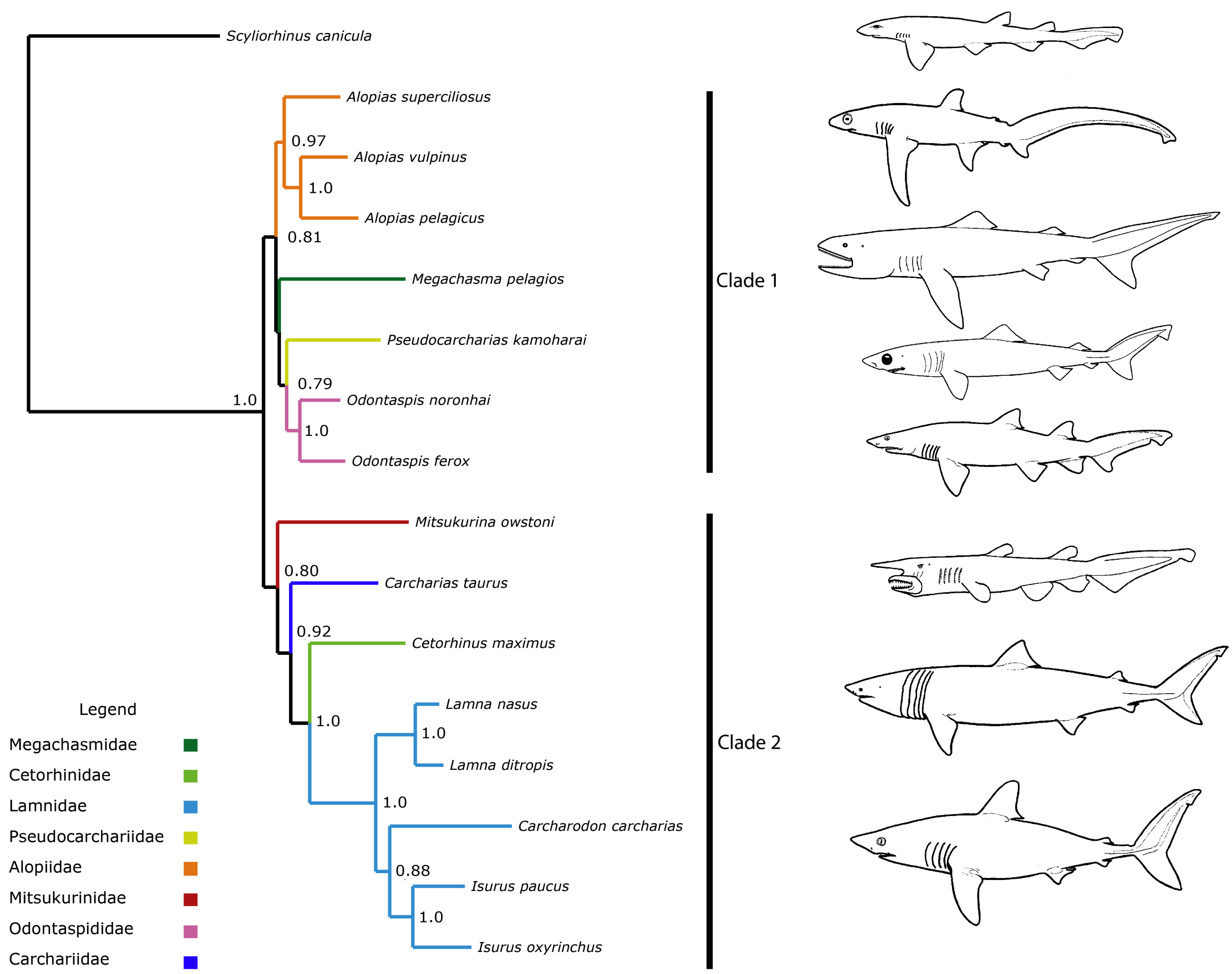

Figure 4. Combined data Bayesian phylogenetic consensus tree of extant Lamniformes based on morphological and molecular data. Numbers at the nodes are estimated posterior probabilities (probabilities > 0.7 are shown). Colors of the tree branches indicate the family of each species (see legend) / Árbol filogenético consenso de data combinada Bayesiano de los Lamniformes existentes basado en datos morfológicos y moleculares. Los números en los nodos representan la probabilidad posterior estimada (solo > 0,7 son presentados). Los colores de las ramas del árbol indican la familia de cada especie (ver leyenda)

Discussion

Despite the amount of phylogenetic research concerning the order Lamniformes (Figs. 1 and 2), this is the first study that combines morphological and molecular data to reconstruct a phylogeny using Bayesian analysis and considering all extant lamniform species. Based on the topology and posterior probabilities of morphological, molecular, and combined phylogenetic reconstructions, the combined data matrix provided the highest support (Fig. 4).

Morphological phylogeny

Given the large percentage (65.67%) of informative characters in this study, it is suggested that the number of morphological data is sufficient to support the results obtained. Shimada (2005) and other authors (Compagno 1990, Long & Waggoner 1996, Bemis *et al.* 2015) employed characters that presented low homology, mainly obtained from dental morphology and from the fossil record (Hubbel 1996, Naylor *et al.* 1997, Shimada 2005, Flammensbeck *et al.* 2018). However, it is worth noting that dental morphology only provides homoplasy due to convergent evolution, given that the teeth morphology of filter feeding sharks is problematic when used as a set of homologous characters, causing a subjective detection of homologies, which are not comparable to previous works (Maisey 1985, Long & Waggoner 1996, Yabumoto *et al.* 1997, Shimada 2005, 2007; Bemis *et al.* 2015, Schnetz *et al.* 2016, Stone & Shimada 2019). Therefore, dental characters were not used in this study, but instead focused on internal and external anatomy, and for this reason, our phylogenetic hypothesis (Fig. 3A) contrasts with morphology-based phylogenies that include dental characters. In the phylogenetic hypothesis posed by Long & Waggoner (1996) (Fig. 1C), Mitsukurinidae is a sister group to the rest of lamniforms, Megachasmidae and Cetorhinidae are sister groups, Odontaspididae is monophyletic, and Alopiidae is a sister group to Lamnidae. In Shimada (2005) (Fig. 1F), Mitsukurinidae is sister group to the rest of lamniform sharks, Odontaspididae, Pseudocarchariidae and Megachasmidae show polytomy and are sister to Alopiidae, Cetorhinidae and Lamnidae. Both studies lack a consistent topology in their phylogenetic hypotheses, which suggests that the use of dental characters is not a good approach. The phylogenetic hypothesis of Stone & Shimada (2019, fig. 8A, B, C) heavily contrasts with our result (Fig. 3A) despite using the same 42 (out of 44) morphological characters. Clearly the use of phylogenetic methods that do not incorporate the uncertainty of relationships between species (*i.e.*, Parsimony) results in an unreliable phylogenetic hypothesis, with possible erroneous groupings as sister groups due to the phenomenon of long-branch attraction (Bergsten 2005, Yang & Rannala 2012).

The synapomorphies obtained in this study (Table 2) reinforce the hypothesis that Alopiidae and Lamnidae are monophyletic. Stone & Shimada (2019) resurrected Carchariidae for *C. taurus* given that Odontaspididae was not monophyletic when *C. taurus* and *O. ferox* were included in past phylogenetic reconstructions (Compagno 1990, 2002; Naylor *et al.* 1997, 2012; Shimada 2005, Bowden *et al.* 2016, Stone & Shimada 2019), hence, Odontaspididae is suggested to be monophyletic. These synapomorphies only partially agree with the diagnostic characters described by Compagno (1984a, 2002), since the main synapomorphies detected here correspond to skeletal morphology, which is generally ignored in the diagnostic descriptions conducted by this author.

Molecular phylogeny

Previous molecular phylogenetic studies (Fig. 2) show that Lamniformes is represented by two main clades –with *M. owstoni* as sister group the rest of lamniforms–, one clade containing the families Lamnidae and Cetorhinidae and the other integrated by Alopiidae and the rest of the families. The family Alopiidae is paraphyletic in our consensus tree, which is a common result in other molecular studies (Fig. 2A, F, G) (Martin & Naylor 1997, Naylor *et al.* 1997, Vélez-Zuazo & Agnarsson 2011, Naylor *et al.* 2012), with *A. superciliosus* being the species that determines the paraphyletic arrangement. Similarly, the polyphyly of Odontaspididae, with *C. taurus* and *O. ferox* separated between the main clades, was also detected in previous molecular studies (Fig. 2A, D, E, F), where all hypotheses lacked the simultaneous use of the three species that comprise the family (Naylor *et al.* 1997, Human *et al.* 2006, Vélez-Zuazo & Agnarsson 2011, Naylor *et al.* 2012). In addition, the phylogenetic hypotheses of recent studies that compare whole mitochondrial genomes (Bowden *et al.* 2015, Amaral *et al.* 2017) are not consistent as these do not include species such as *Alopias vulpinus* (Bonnaterre, 1788) or *Odontaspis* spp.

Phylogenetic congruence

The congruence analysis yielded a low percentage (39%) between the morphological and molecular phylogenetic trees, which suggests that there is low congruence between both types of characters in the evolutionary lineages of lamniform sharks. Despite the low congruence between trees, the family Lamnidae (Fig. 3, clade 1A, B) is monophyletic in both analyses. Low congruence between different datasets is common in several phylogenetic studies (*e.g.*, Patterson *et al.* 1993, Brower *et al.* 1996, Farías *et al.* 2000, López-Fernández *et al.* 2005, Cachera & Le Loc'h 2017, Cornejo *et al.* 2018). The lack or low congruence between phylogenetic trees is expected given that mitochondrial genes code for metabolic processes rather than morphological features (Taanman 1999, Hickman *et al.* 2008, Hara *et al.* 2018). The morphological consensus tree showed two main lineages of lamniform sharks that could be separated based on their distinct feeding behaviors, since feeding habits are a major determinant of shark morphology (*i.e.*, jaw suspension, elongated caudal fin, size and morphology of fins and gills) and lamniforms consume a large number of preys (Maisey 1980, 1984, 1985; Cortés 1999, Helfman *et al.* 2009). Another aspect that can be inferred in morphological lineages is the oceanic distribution, as clade 2, except for Alopiidae, Megachasmidae, and Pseudocarchariidae, is composed of species with a relatively small oceanic distribution associated with the coastal zone (Compagno 2002). While the rest of lamniforms have a wide oceanic distribution, not always associated with the continental shelf, which could require different body morphology to sustain long swimming distances (Compagno 2002).

Combined data phylogeny

Our results combining both datasets (morphological and molecular) (Fig. 4) recovered the best phylogenetic hypothesis (highest PP values of each node) and an increased congruence with the molecular tree (85%), where the family Alopiidae is a monophyletic group, in contrast with the paraphyly in the molecular-based tree (Fig. 3B). In this study, the monophyly of the family Lamnidae agrees with previous research, although the phylogenetic position of *Carcharodon* differs by being either a sister taxon of *Lamna* (Martin 1995, Long & Waggoner 1996, Shimada 2005) or *Isurus* (Compagno 1990, Dulvy & Reynolds 1997, Human *et al*. 2006, Vélez-Zuazo & Agnarsson 2011, Naylor *et al*. 2012). Regarding to *C. taurus*, this study present evidence of both morphological and molecular data that supports that this species should not be grouped within Odontaspididae, as Stone & Shimada (2019) proposed. Compagno (1984) included *Carcharias* and *Odontaspis* Agassiz, 1838 in the same family according to paleontological records based on teeth morphology (Glikman 1964, 1967; Herman 1977), which, as mentioned earlier, can constitute a problem to sustain the classification of species within families.

The fact that lamniform sharks showed two main clades, a polyphyletic family and several monotypic families suggests that these sharks are in need for crucial taxonomic revision and should be divided into two superfamilies. The morphological and molecular data supporting these two clades indicate that the classification problems were rooted in a poor taxonomy, and hence, complete morphological data should be employed to assess these systematic issues within the family Odontaspididae instead of focusing exclusively on novel molecular datasets (Maisey 1980, 1984, 1985; Shirai 1992, Carvalho & Maisey 1996, Ebach *et al*. 2006). The results presented here reinforce the use of combined morphological and molecular data, as morphology has regained importance in phylogenetics (Giribet 2015).

In conclusion, this study considerably enhanced the phylogeny of lamniform sharks by combining 45 morphological characters and a molecular database of 1,242 characters, which constitutes the best supporting evidence of the monophyly of the families Alopiidae and Lamnidae and the best phylogenic reconstruction for this group of sharks. Although to continue enhancing the phylogeny of Lamniformes, it is imperative to incorporate more molecular markers (both mitochondrial and nuclear).

Acknowledgments

We appreciate the comments of several colleagues who suggested improvements during the development of this study, among them, Cristián Canales. C.F. Cornejo was supported by CONICYT- Beca Doctorado Nacional (21191261) and EDPG LPR-161 Project from Dirección de Postgrado de la Universidad de Concepción, Chile.

Literature cited

Acuña E, JC Villarroel & R Grau. 2002. Fauna íctica asociada a la pesquería de pez espada (*Xiphias gladius* Linnaeus). Gayana 66(2): 263-267.

Amaral CR, F Pereira, D Silva, A Amorim & EF de Carvalho. 2018. The mitogenomic phylogeny of the Elasmobranchii (Chondrichthyes). Mitochondrial DNA Part A 29(6): 867-878.

Akaike H. 1998. Information theory and an extension of the maximum likelihood principle. In: Parzen E, K Tanabe & G Kitagawa (eds). Selected papers of hirotugu Akaike. Springer Series in Statistics (Perspectives in Statistics), pp. 199-213. Springer, New York.

Bemis WE, JK Moyer & ML Riccio. 2015. Homology of lateral cusplets in the teeth of lamnid sharks (Lamniformes: Lamnidae). Copeia 103(4): 961-972.

Bergsten J. 2005. A review of long-branch attraction. Cladistics 21(2): 163-193.

Bhat HS & N Kumar. 2010. On the derivation of the Bayesian Information Criterion, 4 pp. School of Natural Sciences, University of California, Merced.

Bigelow HB & WC Schroeder. 1958. A large white shark, *Carcharodon carcharias*, taken in Massachusetts Bay. Copeia 1958(1): 54-55.

Bowden DL, C Vargas-Caro, JR Ovenden, MB Bennett & C Bustamante. 2016. The phylogenomic position of the grey nurse shark *Carcharias taurus* Rafinesque, 1810 (Lamniformes, Odontaspididae) inferred from the mitochondrial genome. Mitochondrial DNA Part A, 27(6): 4328-4330.

Brower AVZ, R DeSalle & A Vogler. 1996. Gene trees, species trees, and systematics: a cladistic perspective. Annual Review of Ecology and Systematics 27(1): 423-450.

Cachera M & F Le Loc'h. 2017. Assessing the relationships between phylogenetic and functional singularities in sharks (Chondrichthyes). Ecology and Evolution 7(16): 6292-6303.

Camhi M, S Fowler, J Musick, A Bräutigam & S Fordham. 1998. Sharks and their relatives ecology and conservation. Occasional Paper, IUCN Species Survival Commission 20: 1-39.

Carvalho MD & JG Maisey. 1996. Phylogenetic relationships of the Late Jurassic shark *Protospinax* Woodward 1919 (Chondrichthyes: Elasmobranchii). In: Arratia G & G Viohl (eds). Mesozoic fishes: Systematics and paleoecology, pp. 9-46. Verlag Dr Friedrich Pfeil, Munich.

Compagno LJV. 1973. Interrelationships of living elasmobranchs. Zoological Journal of the Linnean Society 53: 15-61.

Compagno LJV. 1977. Phyletic relationships of living sharks and rays. American Zoologist 7(2): 303-322.

Compagno LJV. 1984a. Order Lamniformes. In: Compagno LJV (ed). FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1 - Hexanchiformes to Lamniformes. FAO Fisheries Synopsis 125(4/1): 212-249.

Compagno LJV. 1984b. Family Scyliorhinidae. In: Compagno LJV (ed). FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1 - Hexanchiformes to Lamniformes. FAO Fisheries Synopsis 125(4/2): 253-368.

Compagno LJV. 1990. Relationships of the megamouth shark, *Megachasma pelagios* (Lamniformes: Megachasmidae), with comments on its feeding habits. National Oceanic and Atmospheric Administration Technical Report, National Marine Fisheries Service 90: 357-379.

Compagno LJV. 2002. Order Lamniformes. In: Compagno LJV (ed). Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Vol. 2. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Catalogue for Fishery Purposes 1(2): 51-121.

Cornejo C, CM Ibáñez & CE Hernández. 2018. Evaluación sistemática del género *Helcogrammoides* (Blenniformes, Trypteriigidae): Pequeños peces con grandes problemas. Revista de Biología Marina y Oceanografía 53(S1): 15-24.

Cortés E. 1999. Standardized diet compositions and trophic levels of sharks. ICES Journal of Marine Science 56(5): 707-717.

Douady CJ, M Dosay, MS Shivji & MJ Stanhope. 2003. Molecular phylogenetic evidence refuting the hypothesis of Batoidea (rays and skates) as derived sharks. Molecular Phylogenetics and Evolution 26(2): 215-221.

Dulvy NK & JD Reynolds. 1997. Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. Proceedings of the Royal Society of London, Biological Sciences 264(1386): 1309-1315.

Ebach MC, DM Williams & JJ Morrone. 2006. Paraphyly is bad taxonomy. Taxon 55(4): 831-832.

Eitner BJ. 1995. Systematics of the genus *Alopias* (Lamniformes: Alopiidae) with evidence for the existence of an unrecognized species. Copeia 1995(3): 562-571.

Farías IP, G Ortí & A Meyer. 2000. Total evidence: molecules, morphology, and the phylogenetics of cichlid fishes. Journal of Experimental Zoology 288(1): 76-92.

Fischer J, K Erikstein, B D'Offay, S Guggisberg & M Barone. 2012. Review of the Implementation of the International Plan of Action for the Conservation and Management of Sharks. FAO Fisheries and Aquaculture Circular 1076: 1-120.

Flammensbeck CK, J Pollerspöck, F Schedel, NJ Matzke & N Straube. 2018. Of teeth and trees: A fossil tip-dating approach to infer divergence times of extinct and extant squaliform sharks. Zoologica Scripta 47(5): 539-557.

Giribet G. 2015. Morphology should not be forgotten in the era of genomics–a phylogenetic perspective. Zoologischer Anzeiger - A Journal of Comparative Zoology 256: 96-103.

Glikman LS. 1964. Sharks of the Paleogene and their stratigraphic significance, 229 pp. Doklady Akademii Nauk Soyuza Sovetskikh Sotsialisticheskikh Respublik, Moscow. [In Russian]

Glikman LS. 1967. Subclass Elasmobranchii (sharks). In: Obruched DV (ed). Osnovy Paleontologii. Beschelyustnye, Ryby [Fundamentals of Palaeontology. Agnathans, Fishes], pp. 196-237. Nauka, Moscow, [Israel Program for Scientific Translations] [Translated from the Russian]

Goloboff P & S Catalano. 2016. TNT version 1.5, including a full implementation of phylogenetics morphometrics. Cladistics 32(3): 221-238.

Hara Y, K Yamaguchi, K Onimaru, M Kadota, M Koyanagi, S Keeley, K Tatsumi, K Tanaka, F Motone, Y Kageyama, R Nozu, N Adachi, O Nishimura, R Nakagawa, C Tanegashima, I Kiyatake, R Matsumoto, K Murakumo, K Nishida, A Terakita, S Kuratani, K Sato, S Hyodo & S Kuraku. 2018. Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates. Nature Ecology & Evolution 2(11): 1761-1771.

Hasegawa M, H Kishino & TA Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22(2): 160-174.

Herman J. 1977. Les Sélaciens des terrains néocrétacés et paléocènes de Belgique et des contrées limitrophes. Elements d'une biostratigraphie intercontinentale. Memoirs of the Geological Survey of Belgium 15: 1-450.

Hickman CP, SR Larry & A Larson. 2008. Organic evolution. In: Hickman CP Jr, LS Roberts, SL Keen, A Larson, H I'Anson & DJ Eisenhour (eds). Integrated principles of zoology, pp. 104-136. WCB/McGraw-Hill, Boston.

Hubbel G. 1996. Using tooth structure to determine the evolutionary history of the white shark. In: Klimley PA & DG Ainley (eds). Great white sharks. The biology of *Carcharodon carcharias*, pp. 9-18. Academic Press, San Diego.

Human BA, EP Owen, LJ Compagno & EH Harley. 2006. Testing morphologically based phylogenetic theories within the cartilaginous fishes with molecular data, with special reference to the catshark family (Chondrichthyes; Scyliorhinidae) and the interrelationships within them. Molecular Phylogenetics and Evolution 39(2): 384-391.

Jordan DS. 1898. Description of a species of fish (*Mitsukurina owstoni*) from Japan: The type of a distinct family of lamnoid sharks. Proceedings of the California Academy of Sciences (Series 3) Zoology 1(6): 199-204.

Kumar S, G Stecher, M Li, C Knyaz & K Tamura. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549.

Long DJ & BM Waggoner. 1996. Evolutionary relationships of the white shark: A phylogeny of lamniform sharks based on dental morphology. In: Klimley PA & DG Ainley (eds). Great white sharks. The biology of *Carcharodon carcharias*, pp. 33-47. Academic Press, San Diego.

López S, R Meléndez & P Barría. 2009. Alimentación del tiburón marrajo *Isurus oxyrinchus* Rafinesque, 1810 (Lamniformes: Lamnidae) en el Pacífico suroriental. Revista de Biología Marina y Oceanografía 44(2): 439-451.

López-Fernández H, RL Honeycutt, ML Stiassny & KO Winemiller. 2005. Morphology, molecules, and character congruence in the phylogeny of South American geophagine cichlids (Perciformes, Labroidei). Zoologica Scripta 34(6): 627-651.

Maddison WP & DR Maddison. 2018. Mesquite: a modular system for evolutionary analysis. Version 3.51 <http://www.mesquiteproject.org>

Maisey JG. 1980. An evaluation of jaw suspension in sharks. American Museum Novitates 2706: 1-17.

Maisey JG. 1984. Chondrichthyan phylogeny: a look at the evidence. Journal of Vertebrate Paleontology 4(3): 359-371.

Maisey JG. 1985. Relationships of the megamouth shark, *Megachasma*. Copeia 1985(1): 228-231.

Maisey JG, GJ Naylor & DJ Ward. 2004. Mesozoic elasmobranchs, neoselachian phylogeny and the rise of modern elasmobranch diversity. In: Arratia G & A Tintori (eds). Mesozoic fishes 3. Systematics, paleoenvironments and biodiversity, pp. 17-56. Verlag Dr. Friedrich Pfeil, München.

Martin AP. 1995. Mitochondrial DNA sequence evolution in sharks: rates, patterns, and phylogenetic inferences. Molecular Biology and Evolution 12(6): 1114-1123.

Martin AP & GJP Naylor. 1997. Independent origins of filter feeding in megamouth and basking sharks (Order Lamniformes) inferred from phylogenetic analysis of cytochrome b gene sequences. In: Yano K, JF Morrisey, Y Yabumoto & K Nakaya (eds). Biology of the megamouth shark, pp. 39-50. Tokai University Press, Tokyo.

Naylor GJ, AP Martin, EG Mattison & WM Brown. 1997. Interrelationships of lamniform sharks: testing phylogenetic hypotheses with sequence data. In: Kocher TD & CA Stepien (eds). Molecular systematics of fishes, pp. 199-218. Academic Press, San Diego.

Naylor GJ, JN Caira, K Jensen, KA Rosana, N Straube & C Lakner. 2012. Elasmobranch phylogeny: a mitochondrial estimate based on 595 species. In: Carrier JC, JA Musick & MR Heithaus (eds). Biology of sharks and their relatives, pp. 31-56. CRC Press, Boca Raton.

Nelson JS. 2006. Order Lamniformes. In: Nelson JS (ed). Fishes of the world 4: 57-59. John Wiley & Sons, Edmonton.

Pagel M, A Meade & D Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. Systematic Biology 53(5): 673-684.

Paradis E & K Schliep. 2019. APE 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35(3): 526-528.

Patterson C, DM Williams & CJ Humphries. 1993. Congruence between molecular and morphological phylogenies. Annual Review of Ecology and Systematics 24(1): 153-188.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>

Rambaut A, M Suchard, D Xie & A Drummond. 2013. Tracer v1.6, <http://tree.bio.ed.ac.uk/software/tracer/>

Schnetz L, C Pfaff & J Kriwet. 2016. Tooth development and histology patterns in lamniform sharks (Elasmobranchii, Lamniformes) revisited. Journal of Morphology 277(12): 1584-1598.

Shimada K. 2005. Phylogeny of lamniform sharks (Chondrichthyes: Elasmobranchii) and the contribution of dental characters to lamniform systematics. Paleontological Research 9(1): 55-72.

Shimada K. 2007. Mesozoic origin for megamouth shark (Lamniformes: Megachasmidae). Journal of Vertebrate Paleontology 27(2): 512-516.

Shimada K, CK Rigsby & SH Kim. 2009. Labial cartilages in the smalltooth sandtiger shark, *Odontaspis ferox* (Lamniformes: Odontaspididae) and their significance to the phylogeny of lamniform sharks. The Anatomical Record 292(6): 813-817.

Shirai S. 1992. Phylogenetic relationships of the angel sharks, with comments on elasmobranch phylogeny (Chondrichthyes, Squatinidae). Copeia 1992(2): 505-518.

Shirai S. 1996. Phylogenetic interrelationships of neoselachians (Chondrichthyes, Euselachii). In: Stiassny MLJ, LR Parenti & GD Johnson (eds). Interrelationships of fishes, pp. 9-34. Academic Press, San Diego/London.

Stone NR & K Shimada. 2019. Skeletal anatomy of the bigeye sand tiger shark, *Odontaspis noronhai* (Lamniformes: Odontaspididae), and its implications for lamniform phylogeny, taxonomy, and conservation biology. Copeia 107(4): 632-652.

Taanman JW. 1999. The mitochondrial genome: structure, transcription, translation and replication. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1410(2): 103-123.

Taylor LR, LJV Compagno & PJ Struhsaker. 1983. Megamouth, a new species, genus and family of lamnoid shark (*Megachasma pelagios*, family Megachasmidae) from the Hawaiian Islands. Proceedings of California Academy of Science 43: 87-110.

Vélez-Zuazo X & I Agnarsson. 2011. Shark tales: a molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). Molecular Phylogenetics and Evolution 58(2): 207-217.

Williams T. 2015. Sharks, batoids and chimaeras of the North Atlantic. FAO Species Catalogue for Fisheries Purposes No. 7 / North Atlantic Sharks Relevant to Fisheries Management: A Pocket Guide, 523 pp. FAO, Rome.

Xia X. 2018. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. Molecular Biology and Evolution 35: 1550-1552.

Xia X, Z Xie, M Salemi, L Chen & Y Wang. 2003. An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26: 1-7.

Yabumoto Y, M Goto & T Uyeno. 1997. Dentition of a female megamouth, *Megachasma pelagios*, collected from Hakata Bay, Japan. In: Yano K, JF Morrissey, Y Yabumoto & K Nakaya (eds). Biology of megamouth shark, pp. 63-75. Tokai University Press, Tokyo.

Yang Z & B Rannala. 2012. Molecular phylogenetics: principles and practice. Nature Reviews Genetics 13(5): 303-314.

Editor: Francisco Concha

Received 28 June 2019

Accepted 02 November 2021

Supplementary material

Appendix 1. List of characters used in the morphological Bayesian analysis. Characters 1-42 extracted from Shimada (2005) and Stone & Shimada (2019); Characters 43-67 obtained from the descriptions by Compagno (1984a, b; 2002). See references section for more information / Lista de caracteres usados en el análisis Bayesiano morfológico. Los caracteres 1-42 fueron extraídos de Shimada (2005) y Stone & Shimada (2019); Los caracteres 43-67 fueron generados de las descripciones de Compagno (1984a, b; 2002). Para más información, revisar los artículos citados

Number	Character	0	1
1	Dental bullae	absent	present
2	"Orbital process" of palatoquadrate	present	absent
3	Mesial process of palatoquadrate	absent	present
4	Notch on dorsal side of palatoquadrate immediately lateral to upper dental bulla	absent	deep
5	Rostral node of cranium	absent	present
6	Rostral appendices of cranium	absent	present
7	Medial rostral cartilage of cranium	narrow	broad
8	Rostral length anterior to nasal capsule compared to total cranial length	short (proportion < 0.2)	long (proportion ≥ 0.2)
9	Separation between base of lateral rostral cartilages and nasal capsules	absent	present
10	Lateral rostral cartilages form part of anterior fontanelle of cranium	no	yes
11	Length of nasal capsules compared to cranial length behind rostrum	long (proportion ≥ 0.30)	short (proportion < 0.30)
12	Ventral level of nasal capsules	elevated above or approximately equal to, level of basal plate	depressed below level of basal plate
13	Interruption of subethmoid fossa between right and left nasal capsules	absent	present
14	Cranial width at preorbital processes compared to that at nasal capsules	equal or narrower	much wider
15	Cranial width at postorbital processes compared to that at preorbital processes	approximately equal or narrower	much wider
16	Orbital diameter compared to cranial length behind nasal capsules	large (proportion ≥ 0.55)	small (proportion < 0.55)
17	Dorsal extent of cranial roof	approximately equal level to dorsal edge of orbit	arched far above dorsal edge of orbit
18	Cranial height (excluding rostral cartilages and nasal capsules) compared to cranial length behind nasal capsules	low (proportion < 0.60)	high (proportion > 0.60)
19	Cranial width at preorbital processes compared to cranial length behind level of preorbital processes	approximately equal or greater ("short cranial roof")	much lesser ("long cranial roof")
20	Overall outline of posterior edge of cranium when viewed dorsoventrally	convex	straight
21	Prominent lateral wing of suborbital shelf cranium	absent	present
22	Stapedial foramina of cranium	small or moderate	large
23	Secondary calcification of vertebrae with endochordal radii radiating from notochordal sheath	absent	present
24	Total vertebral count	≤ 200	> 200
25	Nictitating lower eyelid	present	absent
26	Labial furrows	present	absent
27	Intestinal vale type	spiral	ring
28	Number of turns of valvular intestine	≤ 32	> 32
29	"Nuchal groove" on each side of head above gills	absent	present
30	Precaudal pit at origin of upper caudal lobe	absent	present

Number	Character	0	1
31	Precaudal keel	absent	present
32	Secondary caudal keel	absent	present
33	Pectoral fin origin	under or anterior to fourth gill opening	behind fourth gill opening
34	Pectoral fin radials	aplesodic	plesodic
35	First dorsal fin radials	aplesodic	semiplesodic
36	Position of first dorsal fin	directly above or posterior to level of pelvic fins	anterior to level of pelvic fins
37	Height of second dorsal fin compared to first dorsal fin	approximately equal or larger	much smaller
38	Size of pelvic fins compared to that of first dorsal fin	approximately equal or larger	much smaller
39	Height of anal fin compared to that of first dorsal fin	approximately equal or larger	much smaller
40	Length of upper caudal fin lobe compared to precaudal body length	much shorter	approximately equal
41	Length of lower caudal fin lobe compared to that of upper caudal fin lobe	much shorter	approximately equal
42	Total number of tooth rows on each jaw	≤ 40	> 40
43	Maximum total length (cm)	< 500 cm	> 500 cm
44	Eye position	lateral	dorsolateral
45	Eye size	small or moderate	large
46	Complete preorbital walls	absent	present
47	Ventral caudal lobe	absent	present
48	Precaudal pits	absent	present
49	Anal fin shape	rounded	angular
50	Elongated caudal fin	absent	present
51	Snout length	elongated	short or moderately elongated
52	Mouth position	subterminal	terminal
53	Teeth development	developed	rudimentary or little developed
54	Mouth size	small or moderate	large
55	Branchial slits	small or moderate	large
56	Gill rakers	absent	present
57	Gill openings well extended over the dorsal part of the head	not extended	extended
58	Number of teeth rows	< 120	>120
59	Pectoral fin length	short	long
60	Pelvic fin length	short or moderate	long
61	Small teeth	absent	present
62	Large teeth	absent	present
63	Vertebral calcification	strong	reduced
64	Dermal denticles size	small	large
65	Dermal denticles texture	soft	rough
66	Pectoral fins width	narrow	wide
67	Lunate caudal fin	absent	present