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Mitogenomics of electric rays: evolutionary considerations within Torpediniformes (Batoidea; Chondrichthyes)

JUAN DIEGO GAITÁN-ESPITIA[1,2], JAIBER J. SOLANO-IGUARAN[1,3], DANIELA TEJADA-MARTINEZ[1,4] and JULIAN F. QUINTERO-GALVIS[1,3]*

[1]*Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Casilla 567, Valdivia, Chile*
[2]*CSIRO Oceans & Atmosphere, GPO Box 1538, Hobart 7001, TAS, Australia*
[3]*Programa de Magister en Ciencias mención Genética, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile*
[4]*Programa de Doctorado en Ciencias mención Ecología y Evolución, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile*

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Torpediniformes (electric rays) is a relatively diverse group of benthic coastal elasmobranchs found in all shallow tropical to temperate waters around the world. Despite its ecological and evolutionary importance, the interrelationships within this lineage of cartilaginous fishes and its phylogenetic position within Batoidea remain controversial. In this study, we report the first complete sequences of two tropical electric rays, *Narcine bancroftii* and *Narcine brasiliensis*, using a combination of 454 and Sanger sequencing technologies. These species are a common bycatch of artisanal fishery communities on the north-east Caribbean coast of Colombia and are considered Critically Endangered according to the International Union for Conservation of Nature classification system. Overall, the two newly sequenced mitogenomes exhibit similarities in size, transcriptional orientation, gene order, and nucleotide composition in comparison to other batoids. Based on the concatenated alignment of protein-coding genes, our phylogenetic analyses support the hypothesis that electric rays are closely related to thornback rays (Platyrhinidae), forming a clade in a sister position to a group containing the remaining three batoid orders. Within Torpediniformes, our results reject the nonmonophyletic hypothesis of the genus *Narcine* reported in previous morphological and molecular studies.

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ADDITIONAL KEYWORDS: elasmobranches – electric rays – mitogenome – phylogenetic relationships.

INTRODUCTION

Batoidea (skates, stingrays, and their allies) represents one of the most species-rich groups of extant chondrichthyans with about 630 species spread amongst up to 23 families and four orders (Fowler, 2005; Naylor *et al.*, 2012). This cartilaginous fish lineage is recognized as a monophyletic group sister to all living sharks (Douady *et al.*, 2003; Aschliman *et al.*, 2012a). Nonetheless, the phylogenetic relationships within Batoidea remain controversial amongst morphologists and molecular biologists, with the most contentious issues concerning the phylogenetic position of the Torpediniformes (Rocco, 2013). From a morphological point of view, Torpediniformes share no known synapomorphies with any other particular taxon within batoids and are considered the sister group of the other batoid taxa (McEachran & Aschliman, 2004; Claeson, 2014). Similar observations have been made in other studies using ribosomal genes (16S and 18S) and on the karyological structure of

*Corresponding author. E-mail: juadiegaitan@gmail.com

batoids finding that the unique genomic organization, the large quantities of highly repeated DNA, and the characteristic distribution of heterochromatin of electric rays place the Torpediniformes distant from the other batoid species (Rocco *et al.*, 2007; Rocco, 2013). By contrast, molecular studies using partial mitochondrial genomes (mitogenomes) and nuclear genes have suggested that Rajiformes is sister to all other batoids, implicating a disc-like ancestral form, rather than a shark-like form, as previously thought (Aschliman *et al.*, 2012a; Naylor *et al.*, 2012; Franklin, Palmer & Dyke, 2014). In these studies, the electric rays were recovered in a close relationship with the Platyrhinidae (Aschliman *et al.*, 2012a; Naylor *et al.*, 2012), which is a group of 'guitarfishes'. Other authors using mitochondrial and molecular markers have found different results, recovering a clade with Pristiformes and Rhinobatiformes as the ancestral batoid group and Torpediniformes in a sister relationship to the Myliobatiformes (Pavan-Kumar *et al.*, 2014). These different hypotheses regarding the evolutionary relationships amongst batoids (Fig. 1) warrant further exploration with additional markers and taxon sampling.

Mitochondrial DNA (mtDNA) is widely used in a range of comparative studies as a tool to infer species relationships (Gaitán-Espitia, Nespolo & Opazo, 2013; Cea, Gaitán-Espitia & Cárdenas, 2015; Dilly, Gaitán-Espitia & Hofmann, 2015; Gaitán-Espitia & Hofmann, 2016). Characteristics of this molecular resource such as the lack of introns, unambiguous orthology, lack of recombination, broadly uniform rate of molecular evolution, and phylogenetic signal at diverse taxonomic ranks have led many to consider it as a reliable resource for investigations of molecular divergence and deep evolutionary relationships (Boore & Brown, 1998; Bernt *et al.*, 2013a; Botero-Castro *et al.*, 2013; Perseke *et al.*, 2013). Nevertheless, mtDNA can be uninformative if only small portions of the mitogenome are used (Galtier *et al.*, 2009; Poortvliet *et al.*, 2015). This is particularly true in cartilaginous fishes because of the slow rates of mutation exhibited by this group (Martin, Naylor & Palumbi, 1992; Martin, 1995). In these cases, greater resolution can be achieved by using complete mitogenome sequences (Alam *et al.*, 2014; Gillett *et al.*, 2014; Poortvliet *et al.*, 2015), particularly of groups with minimally sequenced genomes such as Torpediniformes. Although a great number of entire mitochondrial genomes have been reported in the Organelle Genome Resources of the National Center for Biotechnology Information for Batoidea (~37), most of these genomes are for species in the orders Myliobatiformes and Rajiformes with 19 and 11, respectively, followed by the Pristiformes/Rhiniformes group, which contains six. To date, the Torpediniformes are represented only by the complete mitogenome of the giant electric ray *Narcine*

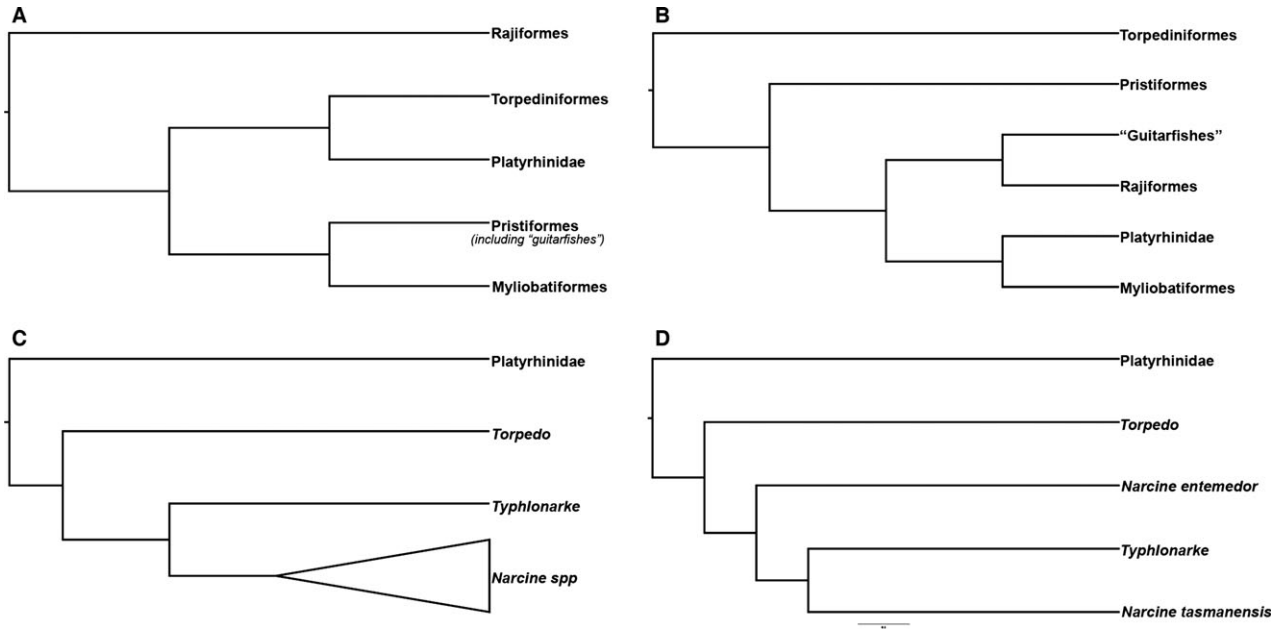

Figure 1. Phylogenetic hypotheses regarding the evolutionary relationships amongst batoid fishes. Trees were pruned and modified to better reflect the different levels of comparison and the taxa included in the present study. Reconstructions based on (A) partial mitochondrial and nuclear genes (Aschliman *et al.*, 2012a) and (B) morphological characters (McEachran & Aschliman, 2004) of major groups. Competing hypotheses within Torpediniformes depicting (C) the monophyly (Claeson, 2014) and (D) the paraphyly (Naylor *et al.*, 2012) of the genus *Narcine*.

entemedor (Castillo-Páez, del Río-Portilla & Rocha-Olivares, 2014), and three partially sequenced genomes (*Narcine tasmaniensis*, *Typhlonarke aysoni*, and *Torpedo macneilli*) (Table 1). This relatively diverse group of batoids is unquestionably monophyletic, being easily distinguished from other batoids by presenting well-developed pectoral electric organs derived from branchial musculature, smooth skin devoid of dermal denticles or spines, and a highly modified posteriorly arched shoulder girdle, amongst other characters (de Carvalho, Séret & Compagno, 2002). Within Torpediniformes, 12 extant genera gathered in four subfamilies (Torpedininae, Hypninae, Narcininae, and Narkidae) are recognized (Aschliman, Claeson & McEachran, 2012b), but the inter-relationships amongst them remain unresolved (Fig. 1C, D) based on the available molecular (Aschliman *et al.*, 2012a; Naylor *et al.*, 2012) and morphological data (Aschliman *et al.*, 2012b; Claeson, 2014).

In this study we report the first complete sequences of two tropical electric rays, *Narcine bancroftii* and *Narcine brasiliensis* using a combination of 454 and Sanger sequencing technologies. These species are a common bycatch of artisanal fishery communities on the north-east Caribbean coast of Colombia (Gaitán-Espitia & López, 2008; Ramírez-Hernandez *et al.*, 2011), and are considered Critically Endangered according to the International Union for Conservation of Nature classification system (de Carvalho *et al.,* 2007; Rosa & Furtado, 2007). We characterized the mitogenomic architectures of both species and compared them with other available electric ray mitogenomes. Furthermore, phylogenetic analyses using the protein-coding genes of mitochondrial genomes were conducted with representative species of the other batoid orders to investigate evolutionary relationships and taxonomic groupings.

MATERIAL AND METHODS

Sample and DNA isolation

Fresh liver samples were obtained from five *N. bancroftii* and five *N. brasiliensis* males caught as bycatch by the artisanal fishery community of Don Diego (11°18′N, 73°43′W) and Ahuyama (11°53′N, 72°17′W) on the north-east Caribbean coast of Colombia. Species were identified following the keys and descriptions of McEachran & Carvalho (2002). Intact mitochondria were then removed from approximately

Table 1. List of species used in this study

Order	Family	Species	GenBank accession no.
Torpediniformes	Narcinidae	*Narcine brasiliensis*	KT119410
		Narcine bancroftii	KT119411
		Narcine entemedor	KM386678
		Narcine tasmaniensis	JN171594
		Typhlonarke aysoni	JN184082
	Torpedinidae	*Torpedo macneilli*	JN184080
Myliobatiformes	Dasyatidae	*Dasyatis akajei*	KC526959
	Gymnuridae	*Gymnura poecilura*	KJ617038
	Hexatrygonidae	*Hexatrygon bickelli*	JN184061
	Mobulinae	*Mobula japonica*	JX392983
	Myliobatidae	*Aetobatus flagellum*	KF482070
	Plesiobatidae	*Plesiobatis daviesi*	AY597334
	Potamotrygonidae	*Potamotrygon motoro*	KF709642
	Urolophidae	*Urobatis halleri*	JN184083
Pristiformes	Pristidae	*Pristis clavata*	KF381507
	Rhinobatinae	*Rhinobatos hynnicephalus*	KF534708
	Anoxypristis	*Anoxypristis cuspidata*	KP233202
	Zanobatidae	*Zanobatus schoenleinii*	JN184086
	Rhinidae	*Rhina ancylostoma*	JN184074
	Rhynchobatidae	*Rhynchobatus djiddensis*	JN184077
Rajiformes	Anacanthobatidae	*Sinobatis bulbicauda*	JN184078
	Arhynchobatidae	*Pavoraja nitida*	KJ741403
	Platyrhinidae	*Platyrhina sinensis*	JN184068
	Rajidae	*Raja rhina*	KC914434
	Atlantoraja	*Atlantoraja castelnaui*	KM507724
Squalidae	Squalidae	*Squalus acanthias*	Y18134
Chimaeriformes	Chimaeridae	*Chimaera monstrosa*	AJ310140

120 mg tissue of each specimen using a Mitochondrial Isolation Kit (Thermo Scientific). The isolated mitochondrial pellet was used for the mtDNA extraction by means of a Mitochondrial DNA Isolation kit (Bio-Vision, Mountain View, CA). Samples of each species were pooled for sequencing.

Sequencing, assembly, and mitogenome annotation

Libraries for both electric ray species were sequenced using a combination of 454 (Roche Genome Sequencer GS FLX Titanium) and Sanger sequencing technologies on ABI 3730XL sequencers by Eurofins MWG Operon (Huntsville, USA). DNA samples were nebulized, individually bar-coded to perform emulsion-based clonal amplification and sequenced to approximately 20-fold coverage. As pyrosequencing technology produces characteristic sequencing errors, mostly imprecise signals for longer homopolymer runs (Luo *et al.*, 2012), we developed a stringent quality control procedure, using only reads with high quality scores (i.e. over 40 Q-score). In addition, we used the condensation tool found in the NextGENe software v. 2.3.3 (Softgenetics, State College, PA, USA) to correct for homopolymer errors and other base call errors produced by the pyrosequencing process. The 454 reads were trimmed of adapters, filtered based on quality values (cut-off: base-caller error probability $P_{error} = 0.01$; modified-Mott trimming algorithm), and assembled using the software GENEIOUS PRO 8.1 (Drummond *et al.*, 2010).

Protein-coding genes (PCGs), ribosomal RNA (rRNA) genes, transfer RNA (tRNA) genes, and non-coding regions of mtDNA sequences were predicted and annotated using MitoAnnotator, a web-based tool developed specifically for fish mitochondrial genome annotation (Iwasaki *et al.*, 2013). The limits of PCGs and rRNAs were manually adjusted based on the location of adjacent genes and the presence of start and stop codons (Table 1). tRNA genes were identified by their cloverleaf structure and the anticodon in the intergenic regions using ARWEN v. 1.2 (Laslett & Canbäck, 2008) and tRNAscan-SE v. 1.21 (Schattner, Brooks & Lowe, 2005), following the generalized vertebrate mitochondrial tRNA settings. The sequences obtained in this work have been deposited in GenBank under the accession numbers KT119410 (*N. brasiliensis*) and KT119411 (*N. bancroftii*).

Alignments and phylogenetic reconstructions

Nucleotide sequences of the PCGs of the batoid species used in this study (Table 1) were translated into amino acid sequences using the vertebrate mitochondrial genetic code, and aligned separately using the MAFFT platform of the TranslatorX multiple sequence alignment program (Abascal, Zardoya & Telford, 2010). Alignments were performed using the L-INS-i option (accurate for alignment of ≤ 200 sequences) and default settings. The alignments were back-translated into the corresponding nucleotide sequences. This alignment procedure helped avoid the destruction of codons and displacement of nucleotides and aimed to obtain a reliably homologous region (Abascal *et al.*, 2010; Gaitán-Espitia *et al.*, 2013). Ambiguously aligned sites were removed using GBLOCKS v. 0.19b implemented in TranslatorX (Abascal *et al.*, 2010) with default settings. Nucleotide sequences for individual PCG alignments were concatenated before the phylogenetic analysis.

A best partition scheme (BPS) analysis for the concatenated alignment was conducted with the program PartitionFinder (Lanfear *et al.*, 2012), as described in Cea *et al.* (2015). A total of 33 data blocks [11 PCGs excluding the *apocytochrome b* (*Cytb*) and *reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunit 6* (*Nad6*) genes] were defined, following the criteria of one data block for each codon position in each gene. The BPS included six subsets (Supporting Information Table S1) with the models of molecular evolution used for both Bayesian inference (BI) and maximum likelihood (ML) analyses. ML inference was performed with RAXML v. 7.2.6 (Stamatakis, 2006), using the graphical interface RaxML-GUI (Silvestro & Michalak, 2011) using the GTR + gamma model and the rapid bootstrap option with 1000 replicates. In addition, a BI Markov chain Monte Carlo analysis was conducted using MrBayes v. 3.2 (Ronquist & Huelsenbeck, 2003). The rate parameter was allowed to vary. Parameter estimation was 'unlinked' for the shape of the gamma distribution used to model rate variation among sites, the substitution matrix, the proportion of invariable sites, and the estimation of state frequencies. Six Markov chains were used, with each chain started from a random tree. The 'temperature' parameter was set to a default value of 0.2. Two simultaneous runs of 10 000 000 generations were conducted, and trees were sampled every 1000 generations. To establish whether the Markov chains had reached a steady state, we plotted the −ln likelihood scores of sampled trees against generation time using TRACER v. 1.5 (Rambaut & Drummond, 2009). Trees inferred prior to stationarity (i.e. lack of improvement in the likelihood score) were discarded as burn-in (first 10% of the sampled trees), and the remaining trees were used to construct a 50% majority-rule consensus tree.

RESULTS AND DISCUSSION

Mitogenome architecture of electric rays

The complete mitogenomes of the tropical electric rays *N. brasiliensis* and *N. bancroftii* encode the typical metazoan mtDNA genes, including cytochrome oxidase subunits (*Cox1*, *Cox2*, and *Cox3*), *Cytb*, reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunits (*Nad1*, *Nad2*, *Nad3*, *Nad4*, *Nad4L*, *Nad5*, and *Nad6*), the Adenosine Triphosphate synthase subunits (*Atp6–Atp8*), the small and large ribosomal RNAs (*12S* and *16S* rRNAs), and a full set of tRNAs (Table 2, Fig. 2). All 22 tRNAs were within the size range of 68 to 75 bp, and each of them folded into a typical cloverleaf secondary structure as predicted by ARWEN and tRNAscan-SE. With the exception of the *Cox1* gene, which starts with a GTG codon, all other PCGs have the usual ATG start codon (Table 2), whereas the most frequent stop codon is TAA (Table 2). The size (16 997 and 16 971 bp for *N. brasiliensis* and *N. bancroftii*, respectively) and the architecture (Table 2, Fig. 2) of these two newly sequenced mitogenomes are consistent with the genomic features previously reported in other batoids (Inoue *et al.*, 2010; Castillo-Páez *et al.*, 2014; Chen *et al.*, 2014). In fact, transcriptional orientation, gene order/size, and nucleotide composition are highly conserved features amongst electric rays, skates, 'guitarfishes', stingrays, and their allies (Table S2). It is likely that the lack of gene rearrangements in the mitogenome of these cartilaginous fishes is maintained by functional (Blier *et al.*, 2006), molecular (Blier, Dufresne & Burton, 2001), and/or phylogenetic constraints (Bernt *et al.*, 2013b).

Nevertheless, some differences were observed in the size of the largest noncoding region, located between the *tRNA-Pro* and *tRNA-Phe* genes (Table 2, Fig. 2), which contains the putative origin for mitochondrial DNA replication (POR) in Batoidea (Castillo-Páez *et al.*, 2014; Chen *et al.*, 2014). In Torpediniformes, Rajiformes, Pristiformes, and 'guitarfishes', the POR ranges between 1060 and 1328 bp, whereas in Myliobatiformes the POR region exhibits the largest sizes, ranging between 1907 and 4490 bp (Poortvliet & Hoarau, 2013; Yang *et al.*, 2013; Austin *et al.*, 2014; Zhang *et al.*, 2015). These differences are probably explained by insertions and/or tandem-duplication events in the POR region (Ray & Densmore, 2002; Gaitán-Espitia *et al.*, 2013) after the split of the stingrays from the rest of the batoids approximately 160 Mya (Aschliman *et al.*, 2012a).

Phylogenetic reconstruction

Batoidean evolutionary relationships have been examined in many morphological and molecular studies that have helped to disentangle the higher phylogenetic framework of living batoid fishes. Nevertheless, the phylogenetic relationships within this group remain controversial due in great part to the phylogenetic position of the Torpediniformes (Rocco, 2013) and the unresolved inter-relationships within electric rays (Aschliman *et al.*, 2012a; Naylor *et al.*, 2012; Claeson, 2014). Our phylogenetic reconstruction using both BI and ML analyses produced identical topologies with similar branch lengths. These analyses support the monophyly of each of the four Batoidea orders, with high posterior probabilities and bootstrap values (Fig. 3). Importantly, our phylogenetic reconstruction supports the hypothesis that electric rays are sister to thornback rays (Platyrhinidae; Aschliman *et al.*, 2012a; Naylor *et al.*, 2012), which were previously considered to belong to the 'guitarfishes' by Compagno (1999) and to Myliobatiformes by McEachran & Aschliman (2004) and Nelson (2006). In our study, the Torpediniformes + Platyrhinidae clade was found in a sister relationship to a group containing the Myliobatiformes + Pristiformes (including 'guitarfishes') and the Rajiformes (Fig. 3). The position of electric rays in our phylogeny is congruent with previous morphological (McEachran & Aschliman, 2004; Schaefer & Summers, 2005; Aschliman *et al.*, 2012b; Claeson, 2014) and molecular studies (Rocco *et al.*, 2007; Rocco, 2013). Other important differences between our results and those presented in previous publications are related to the phylogenetic position of skates, 'sawfishes', and 'guitarfishes'. For instance, phylogenetic reconstructions using partial mitochondrial and nuclear genes have recovered Rajiformes (Aschliman *et al.*, 2012a; Naylor *et al.*, 2012) or Pristiformes + Rhinobatiformes (Pavan-Kumar *et al.*, 2014) as the most derived batoid group with moderate to weak BI and ML supports. Within the last group, our results indicate that the family Zanobatidae is the most external taxon of the clade (Fig. 3), which departs from the previously proposed incorporation of this group into the Myliobatiformes clade (McEachran & Aschliman, 2004; Nelson, 2006; Aschliman *et al.*, 2012a). It is possible that the discrepancies discussed above can be explained by the different methodologies implemented (e.g. Aschliman *et al.*, 2012a) and in particular by the distinct datasets analyzed (morphological, nuclear, mitochondrial and cytogenetic). In this study, in addition to assessing the variation at a mitogenomic scale, we used a methodology that concatenates PCG sequences and sets the best model of evolution for each codon position within each of the PCGs. This method has been described as an accurate strategy with which to account for variable evolutionary histories of different loci in mitochondrial phylogenomic analyses (Leavitt *et al.*, 2013), allowing in

Table 2. Mitochondrial genome content and general features of the *Narcine brasiliensis* and *Narcine bancroftii*

		Narcine brasiliensis						*Narcine bancroftii*					
Name	Direction	Length (bp)	Min	Max	Start codon	Stop codon	AT%	Length (bp)	Min	Max	Start codon	Stop codon	AT%
tRNA-Phe	Forward	70	1	70				70	1	70			
12S rRNA	Forward	972	75	1046				966	76	1.041			
tRNA-Val	Forward	70	1048	1117				70	1042	1.111			
16S rRNA	Forward	1686	1118	2803				1683	1112	2.794			
tRNA-Leu	Forward	75	2804	2878				75	2796	2.87			
NAD1 gene	Forward	975	2879	3853	ATG	ACA	62.9	975	2871	3.845	ATG	ACA	62.2
tRNA-Ile	Forward	70	3855	3924				70	3847	3.916			
tRNA-Gln	Reverse	70	3923	3992				70	3915	3984			
tRNA-Met	Forward	69	3996	4064				69	399	4058			
NAD2 gene	Forward	1044	4066	5109	ATG	CTA	64.2	1	4059	5102	ATG	CTA	64.2
tRNA-Trp	Forward	69	5112	5180				70	5102	5171			
tRNA-Ala	Reverse	69	5183	5251				69	5174	5242			
tRNA-Asn	Reverse	72	5254	5325				72	5245	5316			
tRNA-Cys	Reverse	68	5359	5427				68	5352	5419			
tRNA-Tyr	Reverse	69	5432	5501				69	5424	5492			
COX1 gene	Forward	1560	5502	7061	GTG	TAT	63.1	1560	5494	7053	GTG	TAT	62.9
tRNA-Ser	Reverse	72	7064	7135				73	7056	7128			
tRNA-Asp	Forward	69	7136	7204				69	7129	7197			
COX2 gene	Forward	693	7209	7901	ATG	TCT	62.6	693	7202	7894	ATG	TCT	62.3
tRNA-Lys	Forward	75	7903	7977				75	7896	797			
ATP8 gene	Forward	168	7979	8147	ATG	TAA	68.2	168	7973	814	ATG	TAA	68.5
ATP6 gene	Forward	684	8138	8821	ATG	TAA	69.9	687	8131	8817	ATG	TAA	69.3
COX3 gene	Forward	786	8822	9607	ATG	TCA	58.5	786	8818	9603	ATG	TCA	57.6
tRNA-Gly	Forward	72	9609	9680				72	9605	9676			
NAD3 gene	Forward	351	9681	10,031	ATG	GAA	65.2	351	9677	1003	ATG	GAA	64.7
tRNA-Arg	Forward	71	10,031	10,101				71	10,027	10,097			
NAD4L gene	Forward	297	10,102	10,398	ATG	TGT	64	297	10,098	10,394	ATG	TGC	64.6
NAD4 gene	Forward	1383	10,392	11,774	ATG	TTT	67	1383	10,388	11,770	ATG	TTT	66.8
tRNA-His	Forward	69	11,775	11,843				69	11,771	11,839			
tRNA-Ser	Forward	68	11,844	11,911				69	11,840	11,908			
tRNA-Leu	Forward	73	11,913	11,985				73	11,908	11,980			
NAD5 gene	Forward	1824	11,986	13,809	ATG	CGA	64.7	1818	11,981	13,798	ATG	CGA	64.7
NAD6 gene	Reverse	516	13,797	14,312	ATG	GTA	66.9	516	13,786	14,301	ATG	GTA	64.7
tRNA-Glu	Reverse	69	14,315	14,383				69	14,302	14,370			
CYTB gene	Forward	1140	14,387	15,526	ATG	CTA	61.5	1140	14,374	15,513	ATG	CTA	61.4
tRNA-Thr	Forward	73	15,529	15,601				74	15,516	15,589			
tRNA-Pro	Reverse	69	15,606	15,674				69	15,595	15,663			

ATP, Adenosine triphosphate synthase subunit; COX, cytochrome oxidase subunit; CYTB, apocytochrome b; NAD, reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunit; rRNA, ribosomal RNA; tRNA, transfer RNA. Min and Max refer to initial and final position in the mitogenome.

our case a better resolution of the phylogenetic relationships amongst orders of Batoidea.

Within Torpediniformes, the tropical electric rays *N. brasiliensis* and *N. bancroftii* are sister to each other, sharing a most recent common ancestor with the giant electric ray *N. entemedor* and the Australian numbfish *N. tasmaniensis* (Fig. 3). In our analyses, this group was in a close relationship with the blind electric ray *Ty. aysoni* (Fig. 3). These two lineages share some morphological features such as disc shape, the presence of stout jaws, and strong labial cartilages (Nelson, 2006), and their divergence time is estimated to be approximately 64 Mya, at the beginning of the Cenozoic (Aschliman *et al.*, 2012a). In conjunction with these results, our BI and ML analyses suggest that the short-tail electric ray *To. macneilli* branched off early in the monophyletic electric ray lineage (Fig. 3), with the estimated divergence time for the split between *Torpedo* and the rest of the numbfishes dated at the end of the Mesozoic, approximately 73 Mya (Aschliman *et al.*, 2012a). Based on these findings, our results reject the nonmonophyletic hypothesis of the genus *Narcine* reported in other studies using the *Nad2* gene

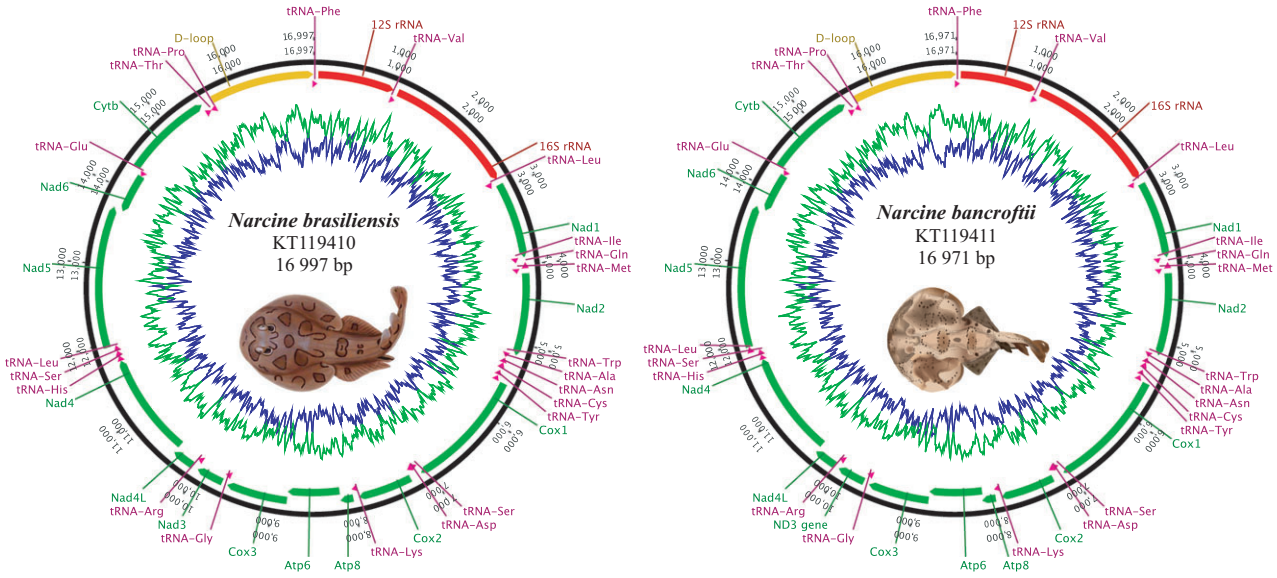

Figure 2. Schematic representation of the mitochondrial genome architecture, AT (blue) and CG (green) content of the tropical electric rays *Narcine brasiliensis* and *Narcine bancroftii*. Abbreviations: Atp, Adenosine Triphosphate synthase subunit; Cox, cytochrome oxidase subunit; Cytb, apocytochrome b; Nad, reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunit; rRNA, ribosomal RNA; tRNA, transfer RNA.

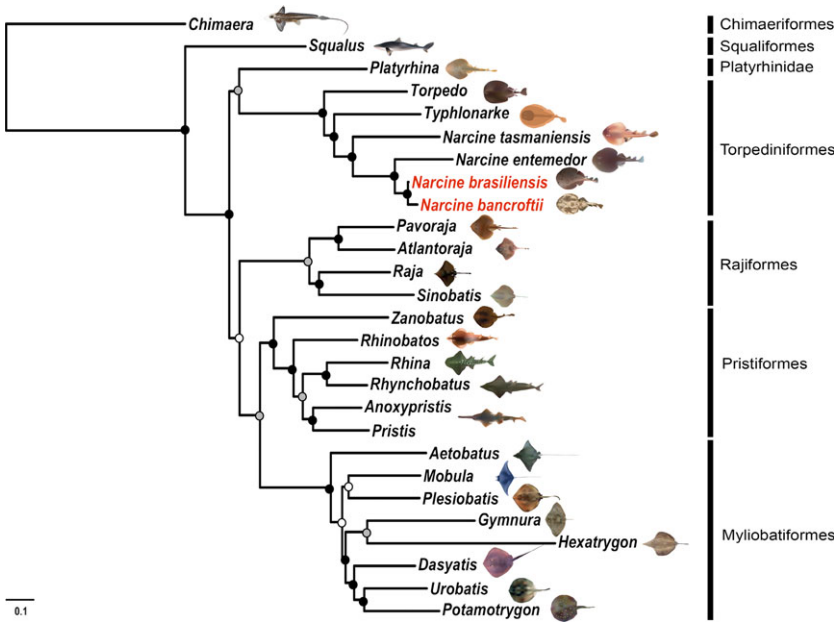

Figure 3. Maximum likelihood (ML) tree of concatenated protein-coding genes describing phylogenetic relationships amongst batoids**.** The ML bootstrap and Bayesian posterior probability values for each node are indicated (black circles: bootstrap value ≥ 90% and posterior probability of 1; grey circles: bootstrap value < 90% and posterior probability of 1; white circles: bootstrap value < 90% and posterior probability < 1). The scale bar represents the number of nucleotide substitutions per site.

(Naylor *et al.*, 2012), and resolve one of the complex cases of generic nonmonophyly within electric rays. However, further studies including additional data sets (both mtDNA and nuclear DNA) and more representative species of the subfamilies Torpedininae, Hypninae, Narcininae, and Narkidae, are required in

order to obtain a more accurate resolution of the phylogenetic relationships within Torpediniformes.

CONCLUSION

The sequenced mitogenomes of the tropical electric rays *N. brasiliensis* and *N. bancroftii* exhibit similarities in size (16 997 and 16 971 bp, respectively), transcriptional orientation, gene order, and nucleotide composition in comparison to other electric rays, skates, 'guitarfishes', stingrays, and their allies. With the incorporation of these two newly sequenced mitogenomes, our molecular phylogenetic reconstruction of the order Torpediniformes is the most complete to date, including six mitogenomes in total. Based on the concatenated alignment of PCGs, our phylogenetic analyses support the hypothesis that electric rays are closely related to thornback rays (Platyrhinidae), forming a clade in a sister position to the remaining three batoid orders. Within Torpediniformes, our results reject the non-monophyletic hypothesis of the genus *Narcine* reported in previous morphological and molecular studies. Further mitogenomic exploration of interfamily relationships within Torpediniformes will require increased taxon sampling of poorly represented groups such as Torpedininae, Hypninae, and Narkidae.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the supporting information tab for this article:

Table S1. Best partition scheme and best-fit models of molecular evolution for the mitochondrial protein-coding genes alignment.

Table S2. Structural characteristics of the mitochondrial genomes of the elasmobranches species used in this study.