

Stingray diversification across the end-Cretaceous extinctions

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Abstract

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The evolution of stingrays (Myliobatiformes) is assessed using a new phylogeny with near-complete genus-level sampling, and additional molecular data. Stingrays diversified into three primary clades: (A) river stingrays, round rays and typical stingrays, (B) butterfly rays and stingarees and (C) eagle and manta rays. The enigmatic sixgill and deepwater rays (*Hexatrygon* and *Plesiobatis*) are not basal stingrays, but are part of the second clade. There is extensive clade-specific variation in molecular evolutionary rates across chondrichthyans: the most appropriate (autocorrelated) divergence dating methods indicate that the extant stingray radiation commenced in the late Cretaceous and continued across the K-Pg boundary. This is highly consistent with the fossil record, and suggests that Cretaceous stingrays, being primarily benthic taxa, were less affected by the K-Pg event than taxa inhabiting the water column. The largest pelagic radiation of stingrays (myliobatids: eagle and manta rays) evolved very shortly after the K-Pg boundary, consistent with rapid ecological expansion into newly-vacated pelagic niches.

Keywords

molecular phylogeny, divergence dating, molecular clock, mass extinctions, Chondrichthyes, Batoidea, Myliobatoidea, cartilaginous fish.

Introduction

Stingrays (Myliobatiformes) are one of the most species-rich (>200) clades of cartilaginous fish (Chondrichthyes), with many economically and medically relevant taxa, and considerable ecological diversity and importance (e.g. marine and freshwater, benthic macropredators and pelagic filter-feeders). Their monophyly is robustly supported by extensive molecular sequence data (e.g. Aschliman et al., 2012a) and numerous evolutionary novelties (such as the caudal sting, and loss of ribs: Carvalho et al., 2004). However, relationships between the major groups (~10 families) of stingrays remain uncertain, in contrast to the rest of the generally well-resolved chondrichthyan tree (Aschliman et al., 2012a). Molecular genetic analyses have not robustly resolved the affinities of the long-branch taxon *Hexatrygon*, while the monophyly of several genera (e.g. *Dasyatis*, *Himantura* sensu stricto) remains relatively untested.

The tempo of stingray diversification also requires further investigation. The earliest well-supported crown myliobatoids occur in the late Cretaceous (~70Ma: Claeson et al., 2010), and the first taxa described from relatively complete fossils are not known until Eocene (Carvalho et al., 2004). In contrast, molecular divergence dating suggests the crown-clade radiated substantially earlier (~87–104 Ma: Aschliman et al., 2012a).

Here, we present a taxonomically and genetically expanded analysis of stingray diversification, with relaxed-clock analyses that account for the substantial clade-specific rate variation. Our enlarged molecular analysis is more congruent with the fossil data, with both sources of evidence suggesting that crown stingrays diversified shortly before the K-Pg bolide impact and were not greatly affected by the resultant extinctions, radiating immediately afterwards into vacated pelagic niches.

Materials and methods

Taxon sampling. Taxon sampling included 97 chondrichthyan species including 48 stingrays (adding 54 new taxa in total to the matrix of Aschliman et al., 2012a). The new matrix includes all stingray genera except one from the Myliobatidae (*Aetomylaeus*) and two from the Pomatotrygonidae (*Paratrygon* and *Plesiotrygon*). We sequenced partial gene fragments of mitochondrial *ND4* (705bp; 51 taxa), nuclear *RAG1* (1418bp; 22 taxa) and one new locus, nuclear *POMC* (800bp; 71 taxa). PCR primer details for each locus are presented in table 1. Voucher information and GenBank accession numbers for all the taxa included in the analysis are available in supplementary table S1.

Table 1. Names and sequences of primers used for PCR and sequencing in this study.

Primer name	Gene	Primer sequence 5' to 3'	Source
ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arevalo et al. 1994
L11424-ND4	ND4	TGACTTCCWAAAGCCCATGTAGA	Inoue et al. 2001
H12293-Leu	tRNA-Leu	TTGCACCAAGAGTTTTTGGTTCCTAAGACC	Inoue et al. 2001
POMC-F	POMC	AGCCATTTCGCTGGAACAA	Todd Reeder ^a
G1009F	POMC	ATCCCAATCTACCCYGGCAA	This study
G1010R	POMC	GACCATCCTTGAYGATGACATTCC	This study
G1030R	POMC	TGRCCATCCTTGAYGATGACAT	This study
G1280F	POMC	AAGCCAGCTTCAGCCYATYGAAGA	This study
G1299F	POMC	GTGGAMAAGAAMMTSGAATCCCAAT	This study
G1300F	POMC	ATGTAYTGATGSCCTGCAAAGTGGA	This study
G1428F	POMC	GAGAMCATCMGGAATTAYGTCATGGG	This study
G1510R	POMC	CCTAAARAGRGTCCARYAGAGGKTTCTGRC	This study
G1550F	POMC	GAGGTGTGTAGCAATGGGCAGAG	This study
G1552R	POMC	ATTCCTGAAGAGGGTGAGCAGTG	This study
Of2fu	RAG1	CTGAGCTGCAGCCAGTATCATAAAATGT	Holcroft 2004
G1027F	RAG1	GTTACCMGGTTATTRTTTCATTYGA	This study
G1028R	RAG1	ATTCATTSCCTTCACTKGCCC	This study
Chon-Rag1-R029	RAG1	AGTGTACAGCCARTGATGYTTCA	Iglésias et al. 2005
G1204R	RAG1	GATTKGTGCGCCAAAYTTCATAGC	This study
G1206F	RAG1	CACRGGGTATGATGARAAGCTGGT	This study

^aPrimer sequence provided by Todd Reeder, San Diego State University, San Diego, CA.

Alignment. The additional *ND4* and *RAG1* sequences were aligned against the alignment blocks provided in Aschliman et al., (2012a). *POMC* sequences were aligned using MAFFT v6.587b (Katoh and Toh 2008) and the alignment refined by eye. The full alignment (with MrBayes partitioning and MCMC commands) is available as supplementary Appendix 1.

Phylogenetic Analyses. The alignment was analysed using MrBayes v3.2.1 (Ronquist et al., 2012), using relaxed-clock (dated) and clock-free (topology only) methods. The optimal partitioning scheme and substitution models were selected using the Bayesian Information Criterion in PartitionFinder (Lanfear et al., 2012). The relaxed-clock dated analyses used internal calibrations similar to calibrations 1-9 in Aschliman et al. (2012a); these were employed as offset exponentials using the same hard minimum and soft 95% maximum.

However, the root age constraint (chondrichthyans: holocephalan-elasmobranch divergence) was substantially reinterpreted. Recent molecular phylogenetic analyses (Inoue et al. 2011, Licht et al. 2012) found crown ages of ~421 and ~413 Ma respectively for crown chondrichthyans, but this could have been influenced by their hard minimum on this

divergence of 410 Ma. The hard minimum was based on Coates and Sequiera (2001), who provisionally accepted the attribution of *Stensioella* to Holocephala and thus to crown Chondrichthyes. However, the phylogenetic affinities of *Stensioella* are highly contentious and it could be a placoderm, i.e. not a holocephalan at all (e.g. Long 2011). Phylogenetic affinities of other putative early holocephalans (e.g. *Melanodus*: Darras et al. 2008) are similarly equivocal. In fact, the oldest uncontroversial chondrichthyan, based on articulated remains, is the same age as *Stensioella* (Miller et al. 2003), and this is a stem rather than a crown chondrichthyan (Davis et al. 2012), and so lies outside the root node in our tree.

A more conservative interpretation of the elasmobranch fossil record indicates that robust fossil evidence for crown chondrichthyans, i.e. the root node of our tree, extends only to 300my: "crown chondrichthyan neurocranial specializations can be traced back to at least the Upper Carboniferous (300 Mya) (e.g., *Iniopera* for euchondrocephalans and *Tristychius* for euselacians). Stem holocephalans can be traced back to at least the Upper Carboniferous, while stem neoselachians can be traced back to either the Late Permian (250 Mya) based on

the putative fossil record of Synchodontiformes, or the Late Carboniferous if *Cooleyella* is a neoselachian” (Pradel et al. 2011, citations omitted). For this reason, we place a wide flat prior on root age (i.e. crown chondrichthyans) of 300–425mya, which encompasses conservative (300) and liberal (410) minimum palaeontological dates, and the molecular estimate (421) based on the latter. In practice, this prior on the root had little effect on the clade of interest (stingrays), because of other more proximal calibrations. Removing this prior did not appreciably change the resultant dates in stingrays or in batoids in general.

MrBayes enforces monophyly of calibrated nodes, but these nodes were all generally obtained with high support in the (topologically unconstrained) clock-free analyses (see below). The TK autocorrelated relaxed clock (Thorne and Kishino 2002) was used, as it was strongly favoured by stepping-stone analyses (Ronquist et al., 2012) over both the uncorrelated relaxed clock (igr) or strict clock (Bayes Factor comparison *sensu* Kass and Raftery 1995). Because saturation of fast-evolving sites can distort divergence times by compressing basal nodes (e.g. Soubrier et al. 2012), the dating analyses were performed with (1) the entire nuclear and mitochondrial data, (2) with mtDNA third codon positions deleted, and (3) with all mtDNA deleted (i.e. nuclear only).

Analyses employed 4 runs (each with 4 chains - 1 cold and 3 heated), with 40 million steps, sampling every 4000, with a burnin of 20% confirmed as adequate (sampled topologies were essentially identical across runs with standard deviation of clade frequencies ~ 0.01 or less; samples for numerical parameters were also essentially identical, with variance between vs within runs approaching unity (Ronquist et al., 2012). The majority-rule consensus tree was obtained from the combined post-burnin samples.

Results and discussion

The dated (fig. 1) and undated (fig. 2) analyses with the nuclear and mitochondrial data (first and second codons) retrieved very similar tree topologies. Support values from the dated analysis are mentioned below; however, all relationships discussed are also found in the undated analysis, and in analyses with all nuclear and mitochondrial data or only nuclear data. These phylogenetic conclusions are thus robust to methods used and to data subsampling.

Relationships between the major clades of chondrichthyans are similar to those found recently based on molecular data (Aschliman et al., 2012a), as expected due to overlapping genes used; many are also highly concordant with morphological evidence (Aschliman et al. 2012b). As with the previous study, monophyly of batoids (fig. 1 clade A), skates, thornbacks+electric rays, and stingrays is supported; guitarfishes form two clades on the stingray stem, with sawfishes nested within one of these clades; and panrays are the sister group to stingrays. Relationships within stingrays (fig 1 clade A), however, are now resolved more robustly: the following relationships have posterior probabilities of 1.0. Stingrays form three primary clades (fig.1), (C) potamotrygonids (river stingrays), urotrygonids (round rays)

and dasyatids (typical stingrays, whiptail rays, etc.), (D) *Hexatrygon* (sixgill stingray), gymnurids (butterfly rays), urolophids (stingarees) and *Plesiobatis* (deepwater stingray), and (E) myliobatids (eagle and manta rays), in agreement with Naylor et al., (2012). Within the myliobatid clade, *Aetobatus* is recovered as sister to all other sampled myliobatids. In contrast, previous work weakly retrieved *Hexatrygon* and *Plesiobatis* as basal stingrays (Aschliman et al., 2012a) and molecular and morphological analyses recovered *Aetobatus* as nested within myliobatids (reviewed in Aschliman, 2014).

The monophyly of several genera is refuted or at least questioned. “*Himantura*” *schmardae* (which often enters freshwater) is again confirmed (pp=1.0) as related to neotropical freshwater stingrays (*vide* Lovejoy, 1996; Aschliman et al., 2012; Naylor et al., 2012), and distant from other dasyatids including other (core) *Himantura*. Core *Himantura* and *Dasyatis* are again also both strongly inferred to be paraphyletic (i.e. grades) (Naylor et al., 2012); each has an apomorphic, monotypic genus (*Urogymnus* and *Pteroplatytrygon* respectively) nested inside with high support (PP>0.95). *Mobula* is also inferred to be paraphyletic with respect to *Manta*, in agreement with recent morphological (Adnet et al., 2012) and molecular studies (Aschliman, 2011; Naylor et al., 2012; Poorvliet et al., 2015) but with lower support.

The branch lengths from the undated analyses of elasmobranchs (fig. 2) suggested extensive rate variation that is phylogenetically autocorrelated (related species tend to share similar rates) and consequently the autocorrelated TK model (Thorne and Kishino, 2002) was a better fit than the uncorrelated IGR model. All the dated analyses using the preferred TK model retrieved similar divergence dates within stingrays (table 2); discussion will focus on the tree from the nuc+mt data excluding mt third codons (fig. 1), but other subsets of the data produced qualitatively the same results. The major clades of batoids diverged 200–140Ma. Stingrays diverged from their sister group (*Zanobatus*, panrays) ~ 147 Ma, but do not diversify until about ~ 76 Ma.

Table 2. Age of various clades of rays based on autocorrelated relaxed clock (TK) analyses of three different subsets of the molecular data: all nuclear and mitochondrial, nuclear and mitochondrial first & second codons only (tree in Fig. 1), and nuclear only. Mean (and 95% highest posterior density interval) divergence dates in Ma are shown. Letters in parentheses are discussed in the text and refer to nodes in Fig 1.

	nuc + all mito	nuc + mito 1&2	nuc only
Batoids (A)	230.9 (146.7-274.8)	215.8 (167.8-268.7)	174 (160-193.6)
Stingrays (B)	74.3 (69.4-80.3)	76.2 (69.5-84.1)	73.2 (67-92.8)
Myliobatids (E)	66.6 (65-69.9)	66.7 (65-70.5)	67 (65-70.5)
Mobulines (F)	30.2 (16.7-43.4)	26.2 (12.5-40.4)	40.2 (27.9-51.5)

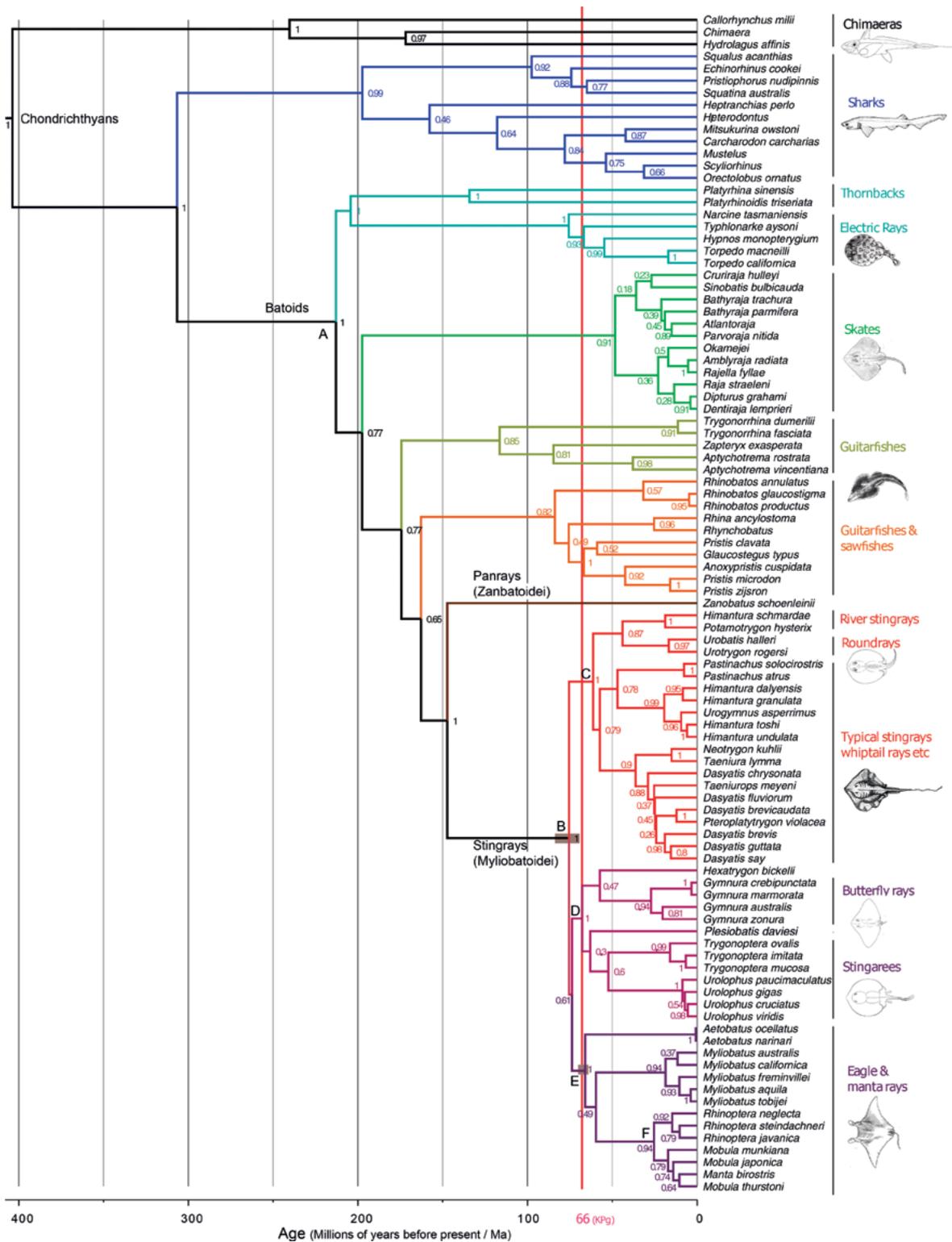


Figure 1. Dated molecular phylogeny of elasmobranchs, with emphasis on stingrays (Myliobatoidei), based on an autocorrelated relaxed clock (TK) analysis of the nuclear and mitochondrial data (excluding third codons). Numbers at nodes are posterior probabilities, node heights are mean estimated ages; the K-Pg extinction (66Ma) is marked with a red line. Stingray clades A-F are discussed in text. Node bars indicate 95% HPDs for stingrays and myliobatids (eagle and manta rays). Images are all public domain and from NOAA except for shark (Tony Ayling), whiptail (Pearson Foresman), stingaree and chimaera (both copyright expired).



Figure 2. Molecular phylogeny of elasmobranchs, with emphasis on stingrays (Myliobatiformes), based on undated (clock-free) analysis of the nuclear and mitochondrial data (excluding third codons). Numbers at nodes are posterior probabilities; branch lengths proportional to inferred divergence (see scale). The rooting could be anywhere along the arrowed branch (tree here is arbitrarily rooted at left end of this branch). For full details of specimen numbers, see table S1.

This long stem lineage leading to a much younger crown radiation is consistent with (a) low diversity throughout the Cretaceous, with diversification only occurring shortly before the K-Pg boundary. However, it could also be generated even with high diversities throughout the Cretaceous, if (b) the end-Cretaceous mass extinctions (~66Ma) extinguished many archaic stingrays, leaving only a few closely-related lineages to cross the Paleogene boundary, or (c) continuously high speciation and extinction rates throughout the Cretaceous generated high taxon turnover (e.g. Crisp and Cook, 2009; Rabosky, 2010). These scenarios can be difficult to test using only molecular phylogenies, and are better tested against the fossil record (Rabosky, 2010), which is most consistent with scenario (a). Throughout most of the Cretaceous, stingrays are neither abundant nor diverse, and taxa robustly assigned to the crown-clade (i.e. using quantitative methods) are first known in the late Cretaceous, when several taxa appear simultaneously (Claeson et al., 2010). There is no major drop in stingray diversity at the K-Pg boundary, with fossils suggesting *Myliobatis* actually survived across the boundary (e.g. Claeson et al., 2010; Guinot et al., 2012). Scenarios (b) and (c), in contrast, entail a very different fossil pattern, predicting the existence of numerous morphologically and taxonomically diverse archaic (stem) stingrays which suffer extinction either at the K-Pg boundary (b) or throughout the Cretaceous (c).

The retrieved dates are broadly consistent with previous work (e.g. Aschliman et al. 2012a), again expected due to overlapping genes and calibrations. There are some notable differences, however. Diversification within skates (Rajiodea) is more recent (~50Ma cf ~80Ma). Also, the late Cretaceous (~76Ma) radiation of crown stingrays (Myliobatiformes) is younger than previously proposed (~90Ma), and more congruent with the oldest well-supported crown stingrays, which appear ~70 mya as part of a late Cretaceous pulse of diversification across elasmobranchs in general (Guinot et al., 2012). In typical sharks this diversification was soon curtailed by the K-Pg extinctions, but rays and skates were less affected (Guinot et al., 2012). The bolide impact more strongly affected surface (rather than benthic) food webs, by curtailing surface productivity and/or initiating surface acidification, though there is evidence for rapid ecosystem recovery (e.g. Alegret et al., 2012). Intriguingly, the inferred age of the largest pelagic radiation of rays (myliobatids: Eagle and Manta Rays) coincides almost exactly with the K-Pg extinctions (fig. 1), consistent with immediate radiation of benthic K-Pg survivors into vacated surface ecospace. Within myliobatids, we estimate that mobulines (*Manta*+*Mobula*) split from rhinopterines only ~26 million years ago (Fig. 1 F) which is in agreement with other studies using similar calibration points (Aschliman et al., 2012a, Poorvliet et al., 2015). This late divergence is not consistent with the placement of the genus *Burnhamia* (Palaeocene onwards) on the mobuline stem (Adnet et al., 2012); these fossils were attributed to the mobuline stem on the basis of one trait related to dental occlusion (Adnet et al., 2012), and the possibility that they fall outside the mobuline-rhinopteryne split needs to be investigated.

Overall, these results suggest that the species richness of modern stingrays is attributable to both a late Cretaceous

pulse followed shortly by survivorship of benthic forms across the K-Pg boundary, which would have enhanced opportunities for immediate subsequent diversification into vacated pelagic habitats.

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Data Accessibility

Table S1 (tissue sources, museum voucher specimens and Genbank numbers) follows the References, and Appendix 1 (alignment in MrBayes format) is available as ESM from the *Memoirs of Museum Victoria* website.

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Table S1. Voucher and GenBank accession number for all taxa included in the analysis. Specimens sequenced as part of our study are indicated. We thank the following people for tissues: Andrew Bentley, University of Kansas; Jonathan Sandoval-Castillo, Macquarie University; Jenny Giles, Tom Kashiwagi & Vera Schluessel, University of Queensland; Samuel Iglésias, *Museum of Natural History* Paris; Eric Lewallen, University of Toronto; Mark McGrouther, Australian Museum; Jenny Ovenden & Stirling Peverell, QDPI; Theodore Pietsch, UWBM; David Vaughan, Two Oceans Aquarium; Terry Walker & Mattias Braccini, MAFRI; William White, CSIRO; Ian Whittington & Leslie Chisholm, South Australian Museum and the myriad of other collectors who have lodged tissues with the Australian Biological Tissue Collection, South Australian Museum. Institutional Abbreviations as per Sabaj Perez (2013).

Taxon	mt genome	RAG1	SCFD2	ND4	POMC	Voucher	this study
Family Anacanthobatidae							
<i>Cruriraja hullei</i>	JN184057	JN184104	JN184146				
<i>Sinobatis bulbicauda</i>	JN184078		JN184147			CSIROH6417-04	
Family Arhynchobatidae							
<i>Atlantoraja castelnaui</i> ^c	JN184055	JN184105				INIDEP-T0406d	
<i>Atlantoraja cyclophora</i> ^c			JN184148			INIDEP-T0474d	
<i>Bathyraja parmifera</i>	JN184056	JN184106	JN184149				
<i>Bathyraja trachura</i>				KT187484	KT187463	KU28471	x
<i>Pavoraja nitida</i>	JN184067	JN184107	JN184150				
Family Dasyatidae							
<i>Dasyatis brevis</i> ^c	JN184058	JN184114	JN184157			BJ_564e	
<i>Dasyatis brevis</i> ^c					KT187416	ABTC89931 ^a	x
<i>Dasyatis brevicaudata</i>		KT187539		KT187487	KT187445	ABTC83883	x
<i>Dasyatis chrysonota</i>				KT187488	KT187446	ABTC98141	x
<i>Dasyatis fluviorum</i>		KT187540		KT187489	KT187447	ABTC79230	x
<i>Dasyatis guttata</i>				KT187490	KT187448	ABTC84485	x
<i>Dasyatis say</i>		KT187541		KT187491	KT187449	KU30237	x
" <i>Himantura schmardae</i> "	JN184062	JN184126	JN184169			ROM66845	
<i>Himantura dalyensis</i>		KT187543		KT187498	KT187451	ABTC85517	x
<i>Himantura granulata</i>				KT187499	KT187452	ABTC85506	x
<i>Himantura toshi</i>		KT187544		KT187500	KT187453	ABTC85510	x
<i>Himantura undulata</i>		KT187545		KT187501	KT187454	AMS I.39533-003	x
<i>Neotrygon kuhlii</i> ^c	JN184065	JN184115	JN184158			BO_424e	
<i>Neotrygon kuhlii</i> ^c					KT187417	ABTC79231	x
<i>Pteroplatytrygon violacea</i>		KT187542		KT187516	KT187450	ABTC84484	x
<i>Pastinachus solocirostris</i>	JN184066	JN184116	JN184159			KA_44e	
<i>Pastinachus atrus</i>		KT187550		KT187512	KT187472	ABTC82862	x
<i>Taeniura lymma</i> ^c	JN184079	JN184117	JN184160			BO_122e	
<i>Taeniura lymma</i> ^c					KT187418	SAMAF9731	x
<i>Taeniurops meyeri</i>				KT187524	KT187460	ABTC103186	x
<i>Urogymnus asperrimus</i> ^c	JN184084	JN184118	JN184161				
<i>Urogymnus asperrimus</i> ^c					KT187419	ABTC84266	x
Family Gymnuridae							
<i>Gymnura crebripunctata</i>	JN184060	JN184119	JN184162			BJ_637e	
<i>Gymnura australis</i>		KT187547		KT187495	KT187456	ABTC85659	x
<i>Gymnura marmorata</i>		KT187548		KT187496	KT187468	KU28376	x
<i>Gymnura zonura</i>				KT187480	KT187461	ABTC103190	x

Taxon	mt genome	RAG1	SCFD2	ND4	POMC	Voucher	this study
Family Hexatrygonidae							
<i>Hexatrygon bickelli</i> ^c	JN184061	JN184120	JN184163			UFTAI-074	
<i>Hexatrygon bickelli</i> ^c					KT187420	MNHP2005-2746	x
Family Mobulidae							
<i>Mobula japonica</i> ^c	JN184063	JN184122	JN184165			BJ_773e	
<i>Mobula japonica</i> ^c					KT187421	ABTC84483	x
<i>Mobula munkiana</i>				KT187505	KT187441	ABTC100704	x
<i>Mobula thurstoni</i>				KT187506	KT187442	ABTC100706	x
Family Myliobatidae							
<i>Aetobatus ocellatus</i>	JN184054	JN184121	JN184164			AU_41e	
<i>Aetobatus narinari</i>				KT187479	KT187462	ABTC85034	x
<i>Myliobatis australis</i> ^c	JN184064	JN184123	JN184166				
<i>Myliobatis australis</i> ^c					KT187422	ABTC82333	x
<i>Myliobatis aquila</i>				KT187507	KT187436	ABTC98143	x
<i>Myliobatis californica</i>		KT187538		KT187508	KT187437	KU28359	x
<i>Myliobatis freminvillei</i>				KT187509	KT187438	KU29698	x
<i>Myliobatis tobijei</i>				KT187510	KT187439	ABTC103184	x
<i>Manta birostris</i> ^c				KT187504	KT187440	ABTC101307	x
<i>Manta alfredi</i>		FJ235624					
Family Narcinidae							
<i>Narcine tasmaniensis</i>	JN171594	JN184094	JN184136				
Family Narkidae							
<i>Typhlonarke aysoni</i>	JN184082	JN184096	JN184138				
Family Plesiobatidae							
<i>Plesiobatis daviesi</i> ^c	JN184070	JN184125	JN184168				
<i>Plesiobatis daviesi</i> ^c					KT187424	MNHP2005-2743	x
Family Platyrhinidae							
<i>Platyrhina sinensis</i> ^c	JN184068	JN184111	JN184154				
<i>Platyrhina sinensis</i> ^c					KT187414	UF159203	x
<i>Platyrhinoidis triseriata</i> ^c	JN184069	JN184112	JN184155				
<i>Platyrhinoidis triseriata</i> ^c					KT187415	ABTC99921	x
Family Potamotrygonidae							
<i>Potamotrygon hystrix</i>	JN184071	JN184127	JN184170			PU_1e	
Family Pristidae							
<i>Pristis clavata</i>	JN184072	JN184097	JN184139			AU_15e	
<i>Pristis microdon</i>		KT187551		KT187514	KT187473	ABTC84466	x
<i>Pristis zijsron</i>				KT187515	KT187475	ABTC84469	x
<i>Anoxypristis cuspidata</i>				KT187481	KT187427	ABTC84463	x

Taxon	mt genome	RAG1	SCFD2	ND4	POMC	Voucher	this study
Family Rajidae							
<i>Amblyraja radiata</i> ^c	NC000893						
<i>Amblyraja radiata</i> ^c		JN184108	JN184151			MCZ159184	
<i>Dipturus grahami</i>				KT187493	KT187465	AMS I.40275-003	x
<i>Dentiraja lemprieri</i>				KT187492	KT187466	ABTC84470	x
<i>Okamejei cf.boesemani</i> ^c		JN184109	JN184152			BO_410e,KA_336e	
<i>Okamejei kenoei</i> ^c	NC007173	JN184110	JN184153				
<i>Rajella fyllae</i>	JN184073						
<i>Raja straeleni</i>				KT187517	KT187476	ABTC98144	x
Family Rhinidae							
<i>Rhina ancylostoma</i> ^c	JN184074	JN184099	JN184141			NT_111e	
<i>Rhina ancylostoma</i> ^c					KT187409	AMS I.40552-001	x
Family Rhinobatidae							
<i>Glaucostegus typus</i> ^c	JN184059	JN184098	JN184140			AU_1e	
<i>Glaucostegus typus</i> ^c					KT187408	ABTC79227	x
<i>Rhinobatos glaucostigma</i> ^c	JN184075	JN184100	JN184142			BJ_733e	
<i>Rhinobatos glaucostigma</i> ^c					KT187410	ABTC100713	x
<i>Rhinobatos annulatus</i>				KT187518	KT187429	ABTC98145	x
<i>Rhinobatos productus</i>				KT187520	KT187428	ABTC83909	x
<i>Trygonorrhina dumerilii</i> ^{b,c}	JN184081	JN184102	JN184144			CSIROH6346-22	
<i>Trygonorrhina dumerilii</i> ^{b,c}					KT187412	ABTC82335	x
<i>Trygonorrhina fasciata</i>				KT187528	KT187459	ABTC89718	x
<i>Zapteryx exasperata</i> ^c	JN184087	JN184103	JN184145			BJ_694e	
<i>Zapteryx exasperata</i> ^c					KT187413	ABTC83911	x
<i>Aptychotrema rostrata</i>				KT187482	KT187457	ABTC84837	x
<i>Aptychotrema vincentiana</i>				KT187483	KT187458	SAMAF9368	x
Family Rhinopteridae							
<i>Rhinoptera steindachneri</i> ^c	JN184076	JN184124	JN184167			BJ_595e	
<i>Rhinoptera steindachneri</i> ^c					KT187423	ABTC100715	x
<i>Rhinoptera javanica</i> ^c				KT187521	KT187443	ABTC103188	x
<i>Rhinoptera bonasus</i> ^c		AY949029					
<i>Rhinoptera neglecta</i>				KT187519	KT187444	ABTC85511	x
Family Rhynchobatidae							
<i>Rhynchobatus djiddensis</i>	JN184077	JN184101	JN184143			AU_75e	
<i>Rhynchobatus laevis</i>					KT187411	AMS I.40490-002	x
Family Torpedinidae							
<i>Torpedo macneilli</i>	JN184080	JN184095	JN184137				
<i>Torpedo californica</i>				KT187523	KT187478	KU29260	x
<i>Hypnos monoptygius</i>		KT187549		KT187503	KT187469	ABTC78372	x

Taxon	mt genome	RAG1	SCFD2	ND4	POMC	Voucher	this study
Family Urolophidae							
<i>Urolophus cruciatus</i> ^c	JN184085	JN184129	JN184172				
<i>Urolophus cruciatus</i> ^c					KT187426	SAMAF9366	x
<i>Urolophus gigas</i>		KT187535		KT187529	KT187433	SAMAF9354	x
<i>Urolophus paucimaculatus</i>		KT187536		KT187530	KT187434	ABTC78373	x
<i>Urolophus viridis</i>		KT187537		KT187531	KT187435	ABTC82287	x
<i>Trygonoptera imitata</i>		KT187533		KT187525	KT187430	ABTC84430	x
<i>Trygonoptera mucosa</i>				KT187526	KT187431	SAMAF9571	x
<i>Trygonoptera ovalis</i>		KT187534		KT187527	KT187432	ABTC82328	x
Family Urotrygonidae							
<i>Urobatis halleri</i> ^c	JN184083	JN184128	JN184171			BJ_554e	
<i>Urobatis halleri</i> ^c					KT187425	ABTC89952	x
<i>Urotrygon rogersi</i>		KT187546		KT187532	KT187455	ABTC89933	x
Family Zanobatidae							
<i>Zanobatus schoenleinii</i>	JN184086	JN184113	JN184156			SE_173e	
OUTGROUP TAXA							
Family Callorhynchidae							
<i>Callorhynchus milii</i> ^c		AAVX01004067					
<i>Callorhynchus milii</i> ^c				KT187485	KT187404	CI145 ^d	x
Family Chimaeridae							
<i>Chimaera monstrosa</i> ^c	NC003136						
<i>Chimaera phantasma</i> ^c		JN184088	JN184130		AB095987		
<i>Hydrolagus affinis</i>				KT187502	KT187403	MCZ162006	x
Family Echinorhinidae							
<i>Echinorhinus cookei</i>				KT187494	KT187467	ABTC100720	x
Family Heterodontidae							
<i>Heterodontus francisci</i> ^c	NC003137						
<i>Heterodontus francisci</i> ^c		JN184089				BJ_540e	
<i>Heterodontus mexicanus</i> ^c			JN184131			BJ_690e	
<i>Heterodontus francisci</i> ^c					KT187405	ABTC101370	x
Family Hexanchidae							
<i>Hepranchias perlo</i>				KT187497	KT187470	ABTC96351	x
Family Lamnidae							
<i>Carcharodon carcharias</i>				KT187486	KT187464	ABTC69275	x
Family Mitsukurinidae							
<i>Mitsukurina owstoni</i> ^c	NC011825						
<i>Mitsukurina owstoni</i> ^c		JN184090	JN184132				

Taxon	mt genome	RAG1	SCFD2	ND4	POMC	Voucher	this study
Family Orectolobidae							
<i>Orectolobus ornatus</i>				KT187511	KT187471	ABTC78364	x
Family Pristiophoridae							
<i>Pristiophorus nudipinnis</i>				KT187513	KT187474	SAMAF9448	x
Family Scyliorhinidae							
<i>Scyliorhinus canicula</i> ^a	NC001950						
<i>Scyliorhinus canicula</i> ^a		JN184092					
<i>Scyliorhinus retifer</i> ^a			JN184134				
Family Squalidae							
<i>Squalus acanthias</i> ^a	NC002012						
<i>Squalus acanthias</i> ^a		JN184093	JN184135			RDM_48e	
<i>Squalus acanthias</i> ^a					KT187407	ABTC84201	x
Family Squatinidae							
<i>Squatina australis</i>				KT187522	KT187477	SAMAF11185	x
Family Triakidae							
<i>Mustelus manazo</i> ^c	NC000890						
<i>Mustelus mustelus</i> ^c		JN184091					
<i>Mustelus lenticulatus</i> ^c			JN184133				
<i>Mustelus antarcticus</i> ^c					KT187406	SAMAF11156	x

Museum abbreviations for newly added taxa follow Sabaj Perez (2013). See Aschliman *et al.* (2012a) for details of other specimens.

Classifications as in Aschliman *et al.* (2012a), except Australian taxa according to Last and Stevens (2009).

^a ABTC refers to the Australian Biological Tissue Collection of the South Australian Museum

^b *Trygonorrhina fasciata* in Aschliman *et al.* (2012a)

^c Composite taxa

^d Christopher Izzo, University of Adelaide

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