

# Supplementary material of Molecular Taxonomy and Diversification of Atlantic Skates (Chondrichthyes, Rajiformes): Adding more Pieces to the Puzzle of Their Evolutionary History

**Table S1.** Complete list of skate species occurring in the North East (NEA), Central East (CEA), South East (SEA) and South West (SWA) Atlantic Ocean. References related to the barcode data and the last global assessment by IUCN are included. LC: least concern, NT: near threatened, VU: vulnerable, EN: endangered, CR: critically endangered, NE: not evaluated. Grey highlights species and geographical areas covered by this study.

Scientific Name (Author/s, Year)	Common Name	NEA	CEA	SEA	SWA	Barcoded in the area	Barcoded in other areas	References	Global IUCN Last assessed
<i>Amblyraja doellojuradoi</i> (Pozzi, 1935)	Southern thorny skate				x	yes	no	[1]	LC 2019
<i>Amblyraja frerichsi</i> <sup>1</sup> (Krefft, 1968)	Thickbody skate				x	no	no		DD 2004
<i>Amblyraja georgiana</i> <sup>1</sup> (Norman, 1938)	Antarctic starry skate				x	yes	no	Unpublished	DD 2019
<i>Amblyraja hyperborea</i> (Collett, 1879)	Arctic skate	x		x		yes	yes	[2,3]	LC 2016
<i>Amblyraja jenseni</i> <sup>1</sup> (Bigelow & Schroeder, 1950)	Shorttail skate	x				yes	no	[2]	LC 2019
<i>Amblyraja radiata</i> (Donovan, 1808)	Starry ray	x				yes	yes	[2–5]	VU 2019
<i>Amblyraja taaf</i> <sup>1</sup> (Meisner 1987)	Whiteleg skate				x	no	no	[8]	DD 2020
<i>Atlantoraja castelnaui</i> (Miranda Ribeiro, 1907)	Spotback skate				x	yes	no	[1,6,7]	CR 2019
<i>Atlantoraja cyclophora</i> (Regan, 1903)	Eyespot skate				x	yes	no	[1,6]	EN 2019
<i>Atlantoraja platana</i> (Günther, 1880)	La Plata skate				x	yes	no	[1,6]	EN 2019
<i>Bathyrāja albomaculata</i> (Norman, 1937)	White-dotted skate				x	yes	no	[1]	VU 2019
<i>Bathyrāja brachyurops</i> (Fowler, 1910)	Broadnose skate				x	yes	no	[1,4]	NT 2019
<i>Bathyrāja cousseauae</i> Díaz de Astarloa & Mabrugaña, 2004	Joined-fins skate				x	yes	no	[1]	LC 2019
<i>Bathyrāja griseocauda</i> (Norman, 1937)	Graytail skate				x	yes	no	[1]	EN 2019
<i>Bathyrāja hesperaficana</i> Stehmann, 1995	West African skate		x			no	no		DD 2004
<i>Bathyrāja macloviana</i> (Norman, 1937)	Patagonian skate				x	yes	no	[1]	NT 2019
<i>Bathyrāja magellanica</i> (Philippi, 1902)	Magellan skate				x	yes	no	[1]	LC 2019
<i>Bathyrāja meridionalis</i> Stehmann, 1987	Dark-belly skate				x	yes	no	[8]	LC 2019
<i>Bathyrāja multispinis</i> (Norman, 1937)	Multispine skate				x	yes	no	[1,4]	NT 2019
<i>Bathyrāja pallida</i> (Forster, 1967)	Pale ray	x				yes*	no		LC 2019

<i>Bathyraja papilionifera</i> Stehmann, 1985	Butterfly skate			x	yes	no	[1]	LC	2019
<i>Bathyraja richardsoni</i> (Garrick, 1961)	Richardson's ray	x			this study	yes	[8,9]	LC	2015
<i>Bathyraja scaphiops</i> (Norman, 1937)	Cuphead skate			x	yes	yes	[1]	LC	2019
<i>Bathyraja schroederi</i> (Krefft, 1968)	Whitemouth skate			x	no	no		LC	2019
<i>Bathyraja smithii</i> (Müller & Henle, 1841)	African softnose skate			x	yes	no	Unpublished	LC	2019
<i>Bathyraja spinicauda</i> (Jensen, 1914)	Spinytail skate	x			yes	no	[2,3]	NT	2019
<i>Cruriraja durbanensis</i> (von Bonde & Swart, 1923)	Smoothnose legskate			x	no	no		DD	2019
<i>Cruriraja hulleyi</i> Aschliman, Ebert & Compagno, 2010	Roughnose legskate			x	yes	no	Unpublished	LC	2019
<i>Dactylobatus clarkii</i> (Bigelow & Schroeder, 1958)	Hookskate			x	no	no		LC	2019
<i>Dipturus argentinensis</i> Díaz de Astarloa, Mabragaña, Hanner & Figueroa, 2008	Argentine Skate			x	yes	no	[1]	DD	2019
<i>Dipturus batis</i> (Linnaeus, 1758)	Blue skate	x			yes	no	[5,10,11]	CR	2006
<i>Dipturus doutrei</i> (Cadenat, 1960)	Violet skate		x	x	no	no		DD	2009
<i>Dipturus intermedius</i> (Parnell, 1837)	Flapper skate	x			yes	no	[3]	NE	-
<i>Dipturus lamillai</i> Concha, Caira, Ebert & Pomper, 2019	Warrah Skate			x	no	no		LC	2020
<i>Dipturus leptocaudus</i> (Krefft & Stehmann, 1975)	Thintail skate			x	no	no		VU	2019
<i>Dipturus mennii</i> Gomes & Paragó, 2001	South Brazilian skate			x	no	no		CR	2019
<i>Dipturus nidarosiensis</i> (Storm, 1881)	Norwegian skate	x	x	x	yes	yes	[3,4,10,12–15]	NT	2015
<i>Dipturus oxyrinchus</i> (Linnaeus, 1758)	Longnosed skate	x	x		yes	yes	[3,4,10,11,13,16–19]	NT	2014
<i>Dipturus pullopunctatus</i> (Smith, 1964)	Slime skate			x	yes	no	[4,20]	LC	2020
<i>Dipturus springeri</i> (Wallace, 1967)	Roughbelly skate			x	yes	no	[4,20]	LC	2019
<i>Dipturus teevani</i> (Bigelow & Schroeder, 1951)	Prickly brown ray			x	no	no		LC	2019
<i>Dipturus trachydermus</i> (Bigelow & Schroeder, 1950)	Roughskin Skate			x	no	no		EN	2019
<i>Gurgesiella dorsalis</i> McEachran & Compagno, 1980	Onefin skate			x	no	no		VU	2019
<i>Leucoraja circularis</i> (Couch, 1838)	Sandy ray	x	x		yes	yes	[3,11,13,16,19]	EN	2014
<i>Leucoraja compagno</i> (Stehmann, 1995)	Tigertail skate			x	no	no		DD	2019
<i>Leucoraja fullonica</i> (Linnaeus, 1758)	Shagreen ray	x			yes	yes	[3]	VU	2014
<i>Leucoraja leucosticta</i> (Stehmann, 1971)	Whitedappled skate			x	no	no		DD	2018
<i>Leucoraja naevus</i> (Müller & Henle, 1841)	Cuckoo ray	x			yes	no	[4,5,11,13,16,19]	LC	2014
<i>Leucoraja wallacei</i> (Hulley, 1970)	Yellowspotted skate			x	yes	no	[20]	VU	2020

<i>Malacoraja krefftii</i> (Stehmann, 1978)	Krefft's ray	x				no	yes	Unpublished	LC	2014
<i>Malacoraja obscura</i> Carvalho, Gomes & Gadig, 2005	Brazilian Soft Skate				x	no	no		LC	2019
<i>Malacoraja spinacidermis</i> (Barnard, 1923)	Soft skate	x	x	x		no	yes	[2]	LC	2020
<i>Neoraja africana</i> (Stehmann & Séret, 1983)	West African pygmy skate		x			no	no		DD	2006
<i>Neoraja caerulea</i> (Stehmann, 1976)	Blue ray	x				yes	no	[12]	LC	2014
<i>Neoraja iberica</i> Stehmann, Séret, Costa & Baro, 2008	Iberian pygmy skate	x				yes	no	[12,16]	LC	2014
<i>Neoraja stehmanni</i> (Hulley, 1972)	South African Dwarf Skate				x	no	no		LC	2019
<i>Psammobatis bergi</i> Marini, 1932	Blotched sandskate				x	yes	no	Unpublished	LC	2019
<i>Psammobatis extenta</i> (Garman, 1913)	Zipper sandskate				x	yes	no	Unpublished	LC	2019
<i>Psammobatis lentiginose</i> McEachran, 1983	Freckled sandskate				x	yes	no	[1]	LC	2019
<i>Psammobatis normani</i> McEachran, 1983	Shortfin sandskate				x	yes	no	[1]	LC	2019
<i>Psammobatis parvacauda</i> McEachran, 1983	Smalltail sandskate				x	no	no		DD	2007
<i>Psammobatis rudis</i> Günther, 1870	Smallthorn sandskate				x	yes	no	[1]	LC	2019
<i>Psammobatis rutrum</i> Jordan, 1891	Spade sandskate				x	this study	no		LC	2019
<i>Raja asterias</i> Delaroche, 1809	Mediterranean starry ray	x				yes	yes	[13,19]	NT	2014
<i>Raja brachyura</i> Lafont, 1871	Blonde ray	x	x			yes	yes	[3,5,11,13,16,17,19]	NT	2008
<i>Raja clavata</i> Linnaeus, 1758	Thornback ray	x	x	x		yes	yes	[3–5,11,13,16–19,21]	NT	2005
<i>Raja herwigii</i> Krefft, 1965	Cape Verde skate		x			yes*	no	Unpublished	DD	2007
<i>Raja maderensis</i> Lowe, 1838	Madeira skate		x			yes	no	[16,22]	VU	2014
<i>Raja mauritaniensis</i> <sup>2</sup> Capapé, 1977	African ray		x			no	no		NE	-
<i>Raja microocellata</i> Montagu, 1818	Small-eyed ray	x	x			yes	no	[5,16]	NT	2006
<i>Raja miraletus</i> Linnaeus, 1758	Brown ray	x	x			yes	yes	[4,11,13,16–19,21]	LC	2019
<i>Raja montagui</i> Fowler, 1910	Spotted ray	x	x			yes	yes	[3–5,11,13,16,19]	LC	2007
<i>Raja ocellifera</i> Regan, 1906	Twineye skate				x	yes	no	Unpublished	EN	2020
<i>Raja parva</i> Last & Séret, 2016	African brown skate		x	x		yes**	no	[23]	NE	-
<i>Raja straeleni</i> Poll, 1951	Biscuit skate		x	x		yes	no	[20]	DD	2009
<i>Raja undulata</i> Lacepède, 1802	Undulate ray	x	x			yes	yes	[13,16]	EN	2003

<i>Rajella barnardi</i> (Norman, 1935)	Bigthorn skate	x	x	yes	no	Unpublished	LC	2004	
<i>Rajella bathyphila</i> (Holt & Byrne, 1908)	Deep-water ray	x	x	this study	yes	[2]	LC	2019	
<i>Rajella bigelowi</i> (Stehmann, 1978)	Bigelow's ray	x	x	yes	yes	Unpublished	LC	2019	
<i>Rajella caudaspinosa</i> (von Bonde & Swart, 1923)	Munchskin skate		x	this study	no		LC	2019	
<i>Rajella dissimilis</i> (Hulley, 1970)	Ghost skate		x	yes	no	[4,20]	LC	2004	
<i>Rajella fyllae</i> (Lütken, 1887)	Round ray	x		yes	yes	[2,3]	LC	2019	
<i>Rajella kukujevi</i> (Dolganov, 1985)	Mid-Atlantic skate	x		yes	no	[12]	LC	2014	
<i>Rajella leoparda</i> (von Bonde & Swart, 1923)	Leopard skate		x	yes	no	Unpublished	LC	2018	
<i>Rajella lintea</i> (Fries, 1838)	Sailray	x		yes	yes	[3]	LC	2019	
<i>Rajella ravidula</i> (Hulley, 1970)	Smoothback skate		x	no	no		LC	2019	
<i>Rajella sadowskii</i> (Kreff & Stehmann, 1974)	Brazilian skate			x	no	no	DD	2004	
<i>Rioraja agassizii</i> (Müller & Henle, 1841)	Rio skate			x	yes	no	[1,7]	VU	2019
<i>Rostroraja alba</i> (Lacepède, 1803)	White skate	x	x	x	yes	yes	[13,16,18–20]	EN	2006
<i>Sympterygia acuta</i> Garman, 1877	Bignose fanskate			x	yes	no	[1]	CR	2019
<i>Sympterygia bonapartii</i> Müller & Henle, 1841	Smallnose fanskate			x	yes	no	[1]	NT	2019
<i>Zearaja brevicaudata</i> (Marini, 1933)				x	yes	no	[27]	NE	-

<sup>1</sup> These species were considered possibly conspecific with *Amblyraja hyperborea* by Weighman [28] and treated as junior synonym of *A. hyperborea* by Last et al. [29].

<sup>2</sup> *Raja mauritaniensis* was proposed as a replacement name for the junior homonym *Raja Africana* by White & Fricke [30]. The species had previously been treated as a questionably valid species by Weighman [28] and Last et al. [29].

\*Sequences deposited in BOLD but not publicly available.

\*\*Sequences not deposited in BOLD or GenBank.

**Table S2.** Primer sequences in 5'-3' direction, final concentration ( $C_i$ , in mM) and annealing temperature ( $T_a$ , in °C) utilised in this study for PCR amplification of the two partial mitochondrial fragments Cytochrome Oxidase subunit I (COI) and Nycotinamide dehydrogenase subunit 2 (NADH2). Grey boxes mark the M13-sequencing primer.

Region	Primers 5'-3'	$C_i$ mM	$T_a$ °C	Reference
COI	FishF2: TCGACTAATCATAAAGATATCGGCAC	0.5	54	[24]
	FishR2: ACTTCAGGGTGACCGAAGAATCAGAA			
	VF2_t1: TGTAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC	0.2	52	[25]
	Fish F2_t1: TGTAACGACGGCCAGTTCGACTAATCATAAAGATATCGGCAC			
	FR1d_t1: CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA			
	Fish R2_t1: CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA			
	FF2d: TTCTCCACCAACCACAARGAYATYGG	0.2	52	[25]
	FR1d: CACCTCAGGGTGTCCGAARAAYCARAA			
NADH2	NADH2 Skates FW2: CTACTAATGAACCCCTCGT	0.2	55	[21]
	NADH2 Skates RV: GCTTTGAAGGCTTTTGGT			
	ND2Met47: TTTTGGGCCCATACC	0.5	54	[26]
	ND2Trp18: GCTTTGAAGGCTTTTGGT			

**Table S3.** Cases of specimen misidentification. Percentage of similarity (through BOLD Identification Engine) and sampling area of the individuals are presented; the number of misidentified individuals in relation to the total number assessed is provided in parenthesis in the Morphological ID and Barcode ID column.

Morphological ID	Barcode ID	BOLD ID (% Similarity)	Major ocean area, Location	E-Voucher morphological evaluation
<i>Amblyraja radiata</i> (1/20)	<i>Rajella fyllae</i> (1/14)	99.69-100	NEA Iceland	<i>R. fyllae</i>
<i>Atlantoraja castelnaui</i> (1/19)	<i>Atlantoraja cyclophora</i> (1/25)	99.85-100	SWA Brazil	<i>A. cyclophora</i>
<i>Atlantoraja cyclophora</i> (1/26)	<i>Atlantoraja platana</i> (1/26)	100	SWA Brazil	<i>A. platana</i>
<i>Cruriraja</i> sp. (3/4)	<i>Rajella barnardi</i> (3/13)	99.5	SEA Angola	No available picture
<i>Cruriraja</i> sp. (1/4)	<i>Rajella leoparda</i> (1/22)	99.66	SEA Angola	Juvenile
<i>Cruriraja hulleyi</i> (1/22)	<i>Raja straeleni</i> (1/20)	100	SEA South Africa	Juvenile
<i>Dipturus pullopunctatus</i> (2/10)	<i>Rostroraja alba</i> (2/14)	100	SEA South Africa	No available picture
<i>Leucoraja fullonica</i> (1/3)	<i>Dipturus batis</i> (1/10)	100	NEA Iceland	No available picture
<i>Leucoraja wallacei</i> (1/17)	<i>Raja straeleni</i> (1/20)	100	SEA South Africa	Juvenile
<i>Psammobatis extenta</i> (1/1)	<i>Psammobatis rutrum</i> (1/10)	100	SWA Brazil	<i>P. rutrum</i>
<i>Raja</i> sp. (7/7)	<i>Raja herwigi</i> – <i>Raja clavata</i> (7/7)	98.77-98.44	SEA Angola	<i>Raja</i> sp.
<i>Rajella caudaspinosa</i> (1/8)	<i>Cruriraja hulleyi</i> (1/21)	100	SEA South Africa	No available picture
<i>Rajella caudaspinosa</i> (1/8)	<i>Rajella barnardi</i> (1/13)	99.5	SEA South Africa	<i>R. barnardi</i>
<i>Rajella caudaspinosa</i> (2/8)	<i>Rajella leoparda</i> (2/22)	99.66	SEA South Africa	<i>R. leoparda</i>
<i>Rajella dissimilis</i> (1/7)	<i>Raja straeleni</i> (1/20)	100	SEA South Africa	Juvenile
<i>Rajella leoparda</i> (1/28)	<i>Cruriraja hulleyi</i> (1/21)	100	SEA South Africa	No available picture
<i>Rajella leoparda</i> (1/28)	<i>Raja straeleni</i> (1/20)	100	SEA South Africa	Juvenile
<i>Rajella leoparda</i> (4/28)	<i>Rajella dissimilis</i> (4/6)	100	SEA South Africa	<i>R. dissimilis</i>
<i>Rioraja agassizii</i> (2/12)	<i>Atlantoraja platana</i> (2/26)	100	SWA Brazil	<i>A. platana</i>
<i>Rostroraja alba</i> (1/16)	<i>Cruriraja hulleyi</i> (1/21)	100	SEA South Africa	No available picture
<i>Rostroraja alba</i> (2/16)	<i>Raja straeleni</i> (2/20)	99.33-99.5	SEA South Africa	No available picture

**Table S4.** Sequence diversity for Cytochrome Oxidase subunit I for Atlantic skates included in the present study. For each taxon, the following fields are presented: number of individuals (N), maximum intraspecific genetic distance (Max Intra-Sp), distance to nearest neighbour (Distance to NN), number of haplotypes (nH), haplotype diversity (Hd), nucleotide diversity ( $\pi$ ), sampling locations, Barcode Index Number (BIN) obtained from BOLD (blue text refers to Unique BIN, red text refers to Discordant BIN, black text refers to Concordant BIN), and abbreviation ID used in phylogenetic analyses. IUCN Categories are also indicated: least concern (LC), near threatened (NT), vulnerable (VU), endangered (EN), critically endangered (CR), not evaluated (NE).

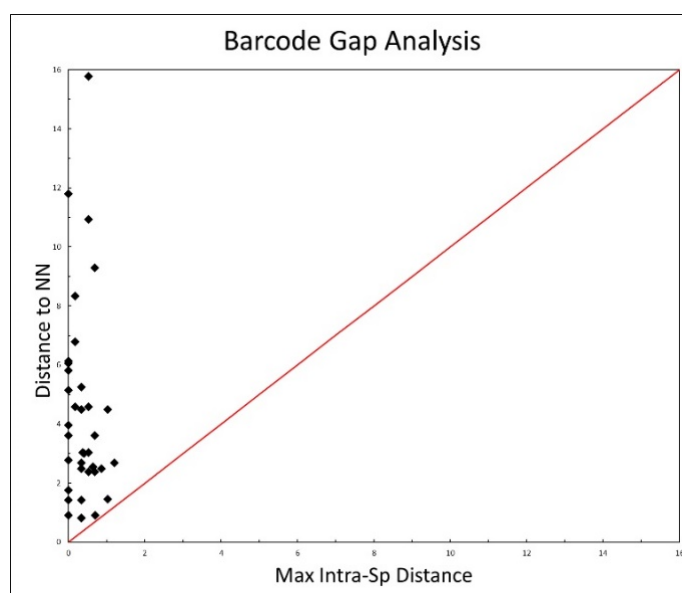
Species	N	Max Intra-Sp	Distance to NN	Geographical areas	IUCN category	BIN	Elasmo-Atl/ Total number in BOLD	ID
ARHYNCHOBATIDAE								
<i>Atlantoraja castelnaui</i>	18	0.17	8.33	SWA Brazil	CR	BOLD:AAB4961	18/30	Atcas1
<i>Atlantoraja cyclophora</i>	25	1.03	4.49	SWA Brazil	EN	BOLD:AAB1882	25/35	Atcyc1
<i>Atlantoraja platana</i>	26	0.34	4.49	SWA Brazil	EN	BOLD:AAB1884	26/29	Atpla1
<i>Bathyraja richardsoni</i>	6	0.37	3.04	NEA Mid-Atlantic Ridge	LC	BOLD:AAA8067	6/10	Bric1
<i>Bathyraja spinicauda</i>	10	0.52	3.04	NEA Iceland	NT		10/225	Bspi1
<i>Psammobatis rutrum</i>	10	0.52	10.94	SWA Brazil	LC	BOLD:AAE0763	10/10	Prut1
<i>Rioraja agassizii</i>	10	0.69	9.3	SWA Brazil	VU	BOLD:AAB1883	10/29	Raga1
GURGESIELLIDAE								
<i>Cruriraja huleyi</i>	21	0.52	15.78	SEA South Africa	LC	BOLD:AAB7055	21/30	Chul1
RAJIDAE								
<i>Amblyraja hyperborea</i>	13	0.34	2.69	NEA Iceland	LC	BOLD:ABZ5141	13/207	Ahyp1
<i>Amblyraja radiata</i>	19	1.20	2.69	NEA Iceland	VU	BOLD:AAA4500	19/259	Arad1
<i>Dipturus pullopunctatus</i>	9	0.00	6.14	SEA South Africa	LC	BOLD:AAD3653	9/38	Dpul1
<i>Dipturus batis</i>	10	0.69	3.61	NEA Iceland; Western English Channel	CR	BOLD:AAC0752	10/33	Dbat1
<i>Dipturus intermedius</i>	5	0.00	3.61	NEA Western English Channel	NE	BOLD:AAA8675	5/41	Dint1
<i>Dipturus nidarosiensis</i>	1	N/A	5.81	CEA Morocco	NT	BOLD:AAE1030	1/14	Dnid1
<i>Leucoraja fullonica</i>	2	0.00	6.06	NEA Celtic Sea	VU	BOLD:AAE3437	2/8	Lful1
<i>Leucoraja naevus</i>	2	0.52	2.39	NEA Celtic Sea	LC	BOLD:AAB0817	2/70	Lnae1
<i>Leucoraja wallacei</i>	16	0.69	2.39	SEA South Africa	VU	BOLD:ABZ1536	16/23	Lwal1
<i>Raja brachyura</i>	2	0.52	4.59	NEA Celtic Sea; Portugal	NT	BOLD:AAA4358	2/59	Rbra1
<i>Raja clavata</i>	14	0.70	0.91	NEA Celtic Sea; Irish Sea; North Sea; Norwegian Sea	NT	BOLD:ACF2419	16/143	Rcla1
<i>Raja maderensis</i>	2	0.00	0.91	CEA Madeira	VU			Rmad1

<i>Raja microocellata</i>	4	0.17	4.59	NEA Bristol Channel, Bay of Biscay	NT	BOLD:AAE7627	4/7	Rmic1
<i>Raja cf. miraletus Angola 1</i>	13	0.64	2.55	SEA Angola	LC	<a href="#">BOLD:ABZ6837</a>	13/13	Rmir10
<i>Raja cf. miraletus Angola 2</i>	4	0.00	3	SEA Angola		<a href="#">BOLD:AAA4475</a>	4/4	Rmir5
<i>Raja cf. miraletus Senegal</i>	4	0.41	5.15	CEA Cape Verde		BOLD:AAZ1588	4/5	Rmir1
<i>Raja ocellifera</i>	13	0.64	2.55	SEA South Africa	EN	BOLD:AAA4359	13/20	Rmir32
<i>Raja montagui</i>	63	0.34	5.26	NEA Celtic Sea; Irish Sea	LC	BOLD:AAA4356	63/110	Rmon1
<i>Raja sp.</i>	7	0.00	1.76	SEA Angola		<a href="#">BOLD:AAA4361</a>	7/7	Rsp1
<i>Raja straeleni</i>	20	1.03	1.46	SEA South Africa	DD	BOLD:ACF2420	20/26	Rstr1
<i>Raja undulata</i>	2	0.17	6.78	NEA Bay of Biscay; Western English Channel	EN	BOLD:AAL0858	2/12	Rund1
<i>Rajella barnardi</i>	13	0.34	0.82	SEA Angola; South Africa	LC	<b>BOLD:AAA4360</b>	35/37	Rlbar1
<i>Rajella leoparda</i>	22	0.34	0.82	SEA Angola; South Africa	LC			Rlleo1
<i>Rajella bathyphila</i>	3	0.00	2.78	NEA Iceland	LC	BOLD:ABZ5259	3/20	Rlbat1
<i>Rajella caudaspinosa</i>	4	0.34	2.49	SEA South Africa	LC	<a href="#">BOLD:ADK9017</a>	4/4	Rlcau1
<i>Rajella dissimilis</i>	6	0.34	1.43	SEA South Africa	LC	BOLD:ACF1708	6/9	Rldis1
<i>Rajella fyllae</i>	14	0.86	2.49	NEA Iceland	LC	BOLD:AAA4502	14/79	Rlfyl1
<i>Rajella kukujevi</i>	4	0	1.43	NEA mid-Atlantic Ridge; Bay of Biscay; Ireland; Scotland	LC	BOLD:ACF1707	4/10	Rlkuk1
<i>Rajella lintea</i>	1	0	3.96	NEA Iceland	LC	BOLD:AAD0011	1/17	Rllin
<i>Rostroraja alba</i>	14	0	11.8	SEA South Africa	EN	BOLD:AAB8691	14/26	Ralb1



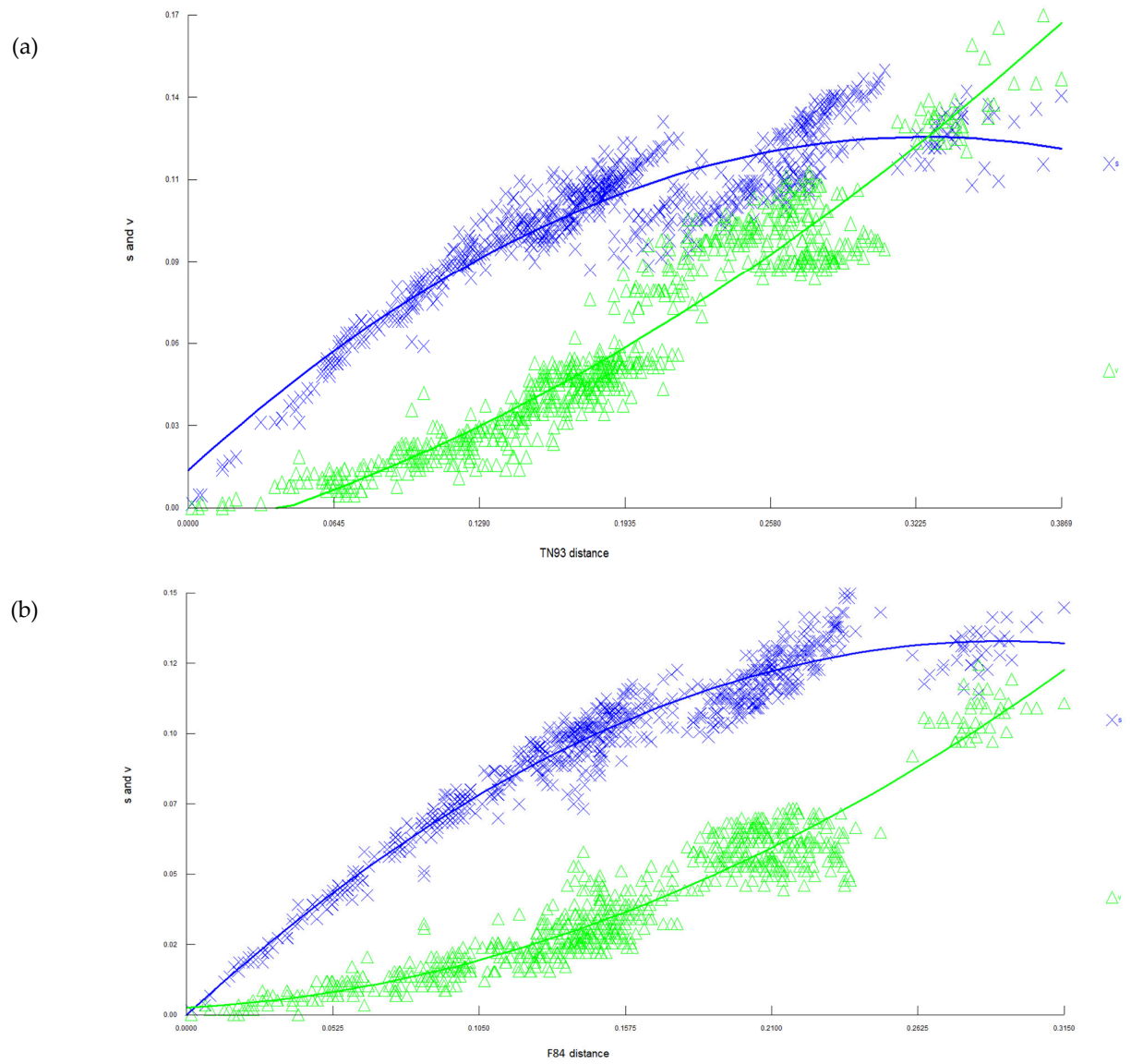
**Table S5.** Substitution saturation test measuring whether the observed index of substitution saturation (Iss) is significantly lower than critical Iss value (Iss.c), with different numbers of operational taxonomic units (Num.OTU) for COI and NADH2.

Marker	Num.OTU	Iss	Iss.c	T	DF	P
NADH2	4	0.315	0.806	21.246	443	0.000
	8	0.316	0.767	18.828	443	0.000
	16	0.317	0.746	18.013	443	0.000
	32	0.324	0.720	16.730	443	0.000
COI	4	0.387	0.802	13.818	255	0.000
	8	0.376	0.761	12.926	255	0.000
	16	0.378	0.737	12.622	255	0.000
	32	0.384	0.712	11.907	255	0.000

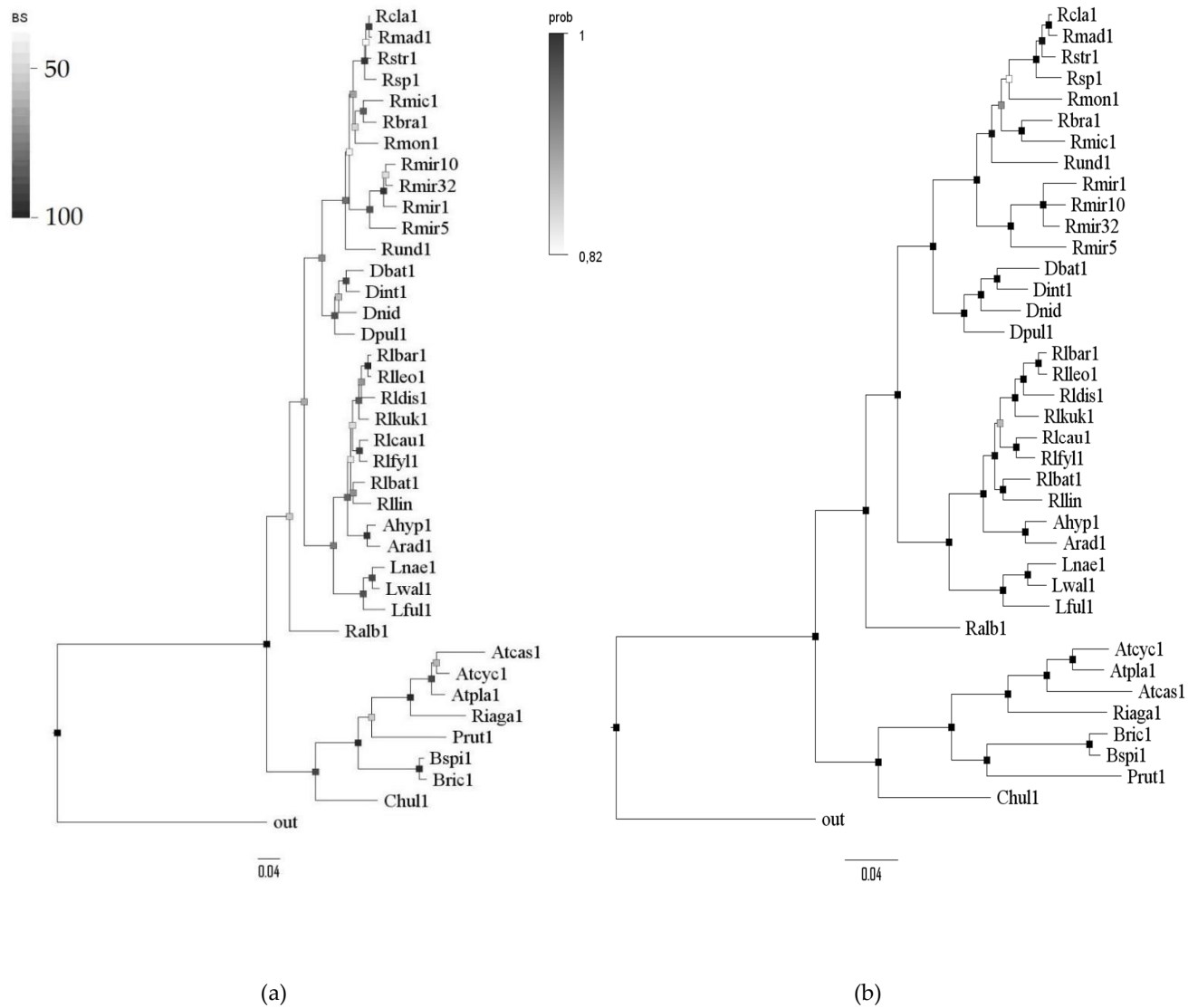


**Figure S1.** Maximum intraspecific distance plotted against the minimum inter-specific (Nearest Neighbour) distances (p-distance values) for the COI barcode sequences of the 38 MOTUs. The 1:1 equivalence (straight line) is indicated.





**Figure S3.** Plot of the number of transitions (s, blue cross) and transversions (v, green triangle) vs. genetic distance. The curves show the trends of variance of transitions and transversions with increasing genetic distance. a) NADH2; b) COI.



**Figure S4.** Maximum-likelihood (a) and Bayesian (b) phylogenetic trees based on concatenated NADH2 and COI datasets. Statistical measures of Maximum-likelihood bootstrap (BS) and Bayesian posterior probability (prob) support are shown at each node. A distance scale bar is given. Names at tips are consistent with the column “ID” in Table S4. The outgroup (out) is represented by homologous sequence of *Squalus acanthias* (GenBank code: NC\_002012).

## References

1. Mabrugaña, E.; Astarloa, J.M.D. de; Hanner, R.; Zhang, J.; Castro, M.G. DNA Barcoding Identifies Argentine Fishes from Marine and Brackish Waters. *PLOS ONE* **2011**, *6*, e28655, doi:10.1371/journal.pone.0028655.
2. Coulson, M.W.; Denti, D.; Guelpen, L.V.; Miri, C.; Kenchington, E.; Bentzen, P. DNA Barcoding of Canada's Skates. *Mol. Ecol. Resour.* **2011**, *11*, 968–978, doi:https://doi.org/10.1111/j.1755-0998.2011.03034.x.
3. Lynghammar, A.; Christiansen, J.S.; Griffiths, A.M.; Fevolden, S.-E.; Hop, H.; Bakken, T. DNA Barcoding of the Northern North-east Atlantic Skates (Chondrichthyes, Rajiformes), with Remarks on the Widely Distributed Starry Ray. *Zool. Scr.* **2014**, *43*, 485–495, doi:https://doi.org/10.1111/zsc.12064.
4. Lago, F.C.; Vieites, J.M.; Espiñeira, M. Development of a FINS- Based Method for the Identification of Skates Species of Commercial Interest. *Food Control* **2012**, *24*, 38–43, doi:10.1016/j.foodcont.2011.08.034.
5. Kneibelsberger, T.; Landi, M.; Neumann, H.; Kloppmann, M.; Sell, A.F.; Campbell, P.D.; Laakmann, S.; Raupach, M.J.; Carvalho, G.R.; Costa, F.O. A Reliable DNA Barcode Reference Library for the Identification of the North European Shelf Fish Fauna. *Mol. Ecol. Resour.* **2014**, *14*, 1060–1071, doi:https://doi.org/10.1111/1755-0998.12238.
6. Ribeiro, A. de O.; Caires, R.A.; Mariguela, T.C.; Pereira, L.H.G.; Hanner, R.; Oliveira, C. DNA Barcodes Identify Marine Fishes of São Paulo State, Brazil. *Mol. Ecol. Resour.* **2012**, *12*, 1012–1020, doi:https://doi.org/10.1111/1755-0998.12007.
7. Ferrette, B.L. da S.; Domingues, R.R.; Rotundo, M.M.; Miranda, M.P.; Bunholi, I.V.; De Biasi, J.B.; Oliveira, C.; Foresti, F.; Mendonça, F.F. DNA Barcode Reveals the Bycatch of Endangered Batoids Species in the Southwest Atlantic: Implications for Sustainable Fisheries Management and Conservation Efforts. *Genes* **2019**, *10*, 304, doi:10.3390/genes10040304.
8. Smith, P.J.; Steinke, D.; Mcveagh, S.M.; Stewart, A.L.; Struthers, C.D.; Roberts, C.D. Molecular Analysis of Southern Ocean Skates (*Bathyrhaja*) Reveals a New Species of Antarctic Skate. *J. Fish Biol.* **2008**, *73*, 1170–1182, doi:10.1111/j.1095-8649.2008.01957.x.
9. Spies, I.B.; Gaichas, S.; Stevenson, D.E.; Orr, J.W.; Canino, M.F. DNA-Based Identification of Alaska Skates (*Amblyraja*, *Bathyrhaja* and *Raja*: Rajidae) Using Cytochrome c Oxidase Subunit I (CoI) Variation. *J. Fish Biol.* **2006**, *69*, 283–292, doi:https://doi.org/10.1111/j.1095-8649.2006.01286.x.
10. Cannas, R.; Follesa, M.C.; Cabiddu, S.; Porcu, C.; Salvadori, S.; Iglésias, S.P.; Deiana, A.M.; Cau, A. Molecular and Morphological Evidence of the Occurrence of the Norwegian Skate *Dipturus Nidarosensis* (Storm, 1881) in the Mediterranean Sea. *Mar. Biol. Res.* **2010**, *6*, 341–350, doi:10.1080/17451000903428496.
11. Costa, F.O.; Landi, M.; Martins, R.; Costa, M.H.; Costa, M.E.; Carneiro, M.; Alves, M.J.; Steinke, D.; Carvalho, G.R. A Ranking System for Reference Libraries of DNA Barcodes: Application to Marine Fish Species from Portugal. *PLOS ONE* **2012**, *7*, e35858, doi:10.1371/journal.pone.0035858.
12. Rodríguez-Cabello, C.; Pérez, M.; Sánchez, F. New Records of Chondrichthyans Species Caught in the Cantabrian Sea (Southern Bay of Biscay). *J. Mar. Biol. Assoc. U. K.* **2013**, *93*, 1929–1939, doi:10.1017/S0025315413000271.
13. Cariani, A.; Messinetti, S.; Ferrari, A.; Arculeo, M.; Bonello, J.J.; Bonnici, L.; Cannas, R.; Carbonara, P.; Cau, A.; Charilaou, C.; et al. Improving the Conservation of Mediterranean Chondrichthyans: The ELASMOMED DNA Barcode Reference Library. *PLOS ONE* **2017**, *12*, e0170244, doi:10.1371/journal.pone.0170244.
14. Ramírez-Amaro, S.; Ordines, F.; Puerto, M.Á.; García, C.; Ramon, C.; Terrasa, B.; Massutí, E. New Morphological and Molecular Evidence Confirm the Presence of the Norwegian Skate *Dipturus Nidarosensis* (Storm, 1881) in the Mediterranean Sea and Extend Its Distribution to the Western Basin. *Mediterr. Mar. Sci.* **2017**, *18*, 251–259, doi:10.12681/mms.1950.
15. Carbonara, P.; Cannas, R.; Donnaloia, M.; Melis, R.; Porcu, C.; Spedicato, M.T.; Zupa, W.; Follesa, M.C. On the Presence of *Dipturus Nidarosensis* (Storm, 1881) in the Central Mediterranean Area. *PeerJ* **2019**, *7*, doi:10.7717/peerj.7009.
16. Serra-Pereira, B.; Moura, T.; Griffiths, A.M.; Gordo, L.S.; Figueiredo, I. Molecular Barcoding of Skates (Chondrichthyes: Rajidae) from the Southern Northeast Atlantic. *Zool. Scr.* **2011**, *40*, 76–84, doi:https://doi.org/10.1111/j.1463-6409.2010.00461.x.
17. Landi, M.; Dimech, M.; Arculeo, M.; Biondo, G.; Martins, R.; Carneiro, M.; Carvalho, G.R.; Brutto, S.L.; Costa, F.O. DNA Barcoding for Species Assignment: The Case of Mediterranean Marine Fishes. *PLOS ONE* **2014**, *9*, e106135, doi:10.1371/journal.pone.0106135.
18. Vella, A.; Vella, N.; Schembri, S. A Molecular Approach towards Taxonomic Identification of Elasmobranch Species from Maltese Fisheries Landings. *Mar. Genomics* **2017**, *36*, 17–23, doi:10.1016/j.margen.2017.08.008.
19. Ramírez-Amaro, S.; Ordines, F.; Picornell, A.; Castro, J.A.; Ramon, C.; Massutí, E.; Terrasa, B. The Evolutionary History of Mediterranean Batoidea (Chondrichthyes: Neoselachii). *Zool. Scr.* **2018**, *47*, 686–698, doi:https://doi.org/10.1111/zsc.12315.
20. Steinke, D.; Connell, A.D.; Hebert, P.D.N. Linking Adults and Immatures of South African Marine Fishes. *Genome* **2016**, doi:10.1139/gen-2015-0212.
21. Ferrari, A.; Tinti, F.; Maresca, V.B.; Velonà, A.; Cannas, R.; Thasitis, I.; Costa, F.O.; Follesa, M.C.; Golani, D.; Hemida, F.; et al. Natural History and Molecular Evolution of Demersal Mediterranean Sharks and Skates Inferred by Comparative Phylogeographic and Demographic Analyses. *PeerJ* **2018**, *6*, e5560, doi:10.7717/peerj.5560.
22. Ball, R.E.; Serra-Pereira, B.; Ellis, J.; Genner, M.J.; Iglésias, S.; Johnson, A.F.; Jones, C.S.; Leslie, R.; Lewis, J.; Mariani, S.; et al. Resolving Taxonomic Uncertainty in Vulnerable Elasmobranchs: Are the Madeira Skate (*Raja Maderensis*) and the Thornback Ray (*Raja Clavata*) Distinct Species? *Conserv. Genet.* **2016**, *17*, 565–576, doi:10.1007/s10592-015-0806-1.
23. Last; Séret A New Eastern Central Atlantic Skate *Raja Parva* Sp. Nov. (Rajoidei: Rajidae) Belonging to the *Raja Miraletus* Species Complex. *Zootaxa* **2016**, *4147*, 477–489, doi:10.11646/zootaxa.4147.4.8.
24. Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R.; Hebert, P.D.N. DNA Barcoding Australia's Fish Species. *Philos. Trans. R. Soc. B Biol. Sci.* **2005**, *360*, 1847–1857, doi:10.1098/rstb.2005.1716.

25. Ivanova, N.V.; Zemlak, T.S.; Hanner, R.H.; Hebert, P.D.N. Universal Primer Cocktails for Fish DNA Barcoding. *Mol. Ecol. Notes* **2007**, *7*, 544–548, doi:<https://doi.org/10.1111/j.1471-8286.2007.01748.x>.
26. Sandoval-Castillo, J.; Rocha-Olivares, A. Deep Mitochondrial Divergence in Baja California Populations of an Aquilopelagic Elasmobranch: The Golden Cownose Ray. *J. Hered.* **2011**, *102*, 269–274, doi:[10.1093/jhered/esr004](https://doi.org/10.1093/jhered/esr004).
27. Gabbanelli, V.; Díaz de Astarloa, J.M.; Gonzalez-Castro, M.; Vazquez, D.M.; Mabragaña, E. Almost a Century of Oblivion: Integrative Taxonomy Allows the Resurrection of the Longnose Skate *Zearaja Brevicaudata* (Marini, 1933) (Rajiformes; Rajidae). *C. R. Biol.* **2018**, *341*, 454–470, doi:[10.1016/j.crvi.2018.10.002](https://doi.org/10.1016/j.crvi.2018.10.002).
28. Weigmann, S. Annotated Checklist of the Living Sharks, Batoids and Chimaeras (Chondrichthyes) of the World, with a Focus on Biogeographical Diversity. *J. Fish Biol.* **2016**, *88*, 837–1037, doi:<https://doi.org/10.1111/jfb.12874>.
29. Last, P.; Weigmann, S.; Yang, L. Changes to the nomenclature of the skates (Chondrichthyes: Rajiformes). In: Last, P.R. & Yearsley, G.K. (Eds.) *Rays of the World: Supplementary information*, CSIRO Australian National Fish Collection: Canberra, Australia, 2016, CSIRO Special Publication: 11–34.
30. White, W.T.; Fricke, R. *Raja Mauritanensis*: A replacement name for *Raja Africana* Capapé, 1977 (Rajiformes: Rajidae), a junior homonym of *Raja Africana* Bloch & Schneider, 1801 (Myliobatiformes: Dasyatidae). *Zootaxa* **2021**, *4970*, 399–400, doi:<https://doi.org/10.11646/zootaxa.4970.2.12>.