

Supplemental figures and tables for:

Worldwide Phylogeography of Rough-Toothed Dolphins (*Steno bredanensis*) Provides Evidence for Subspecies Delimitation

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Marine Mammal Science 38(4): 1371-1397

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TABLE S1 Sample collection information for rough-toothed dolphins. Tissue type refers to how the sample was collected: biopsy was collected from a biopsy dart of a live individual, tissue was collected from a stranded individual, and tooth was collected from a stranded individual or museum specimen. Curator indicates where the sample is housed.

Sample location	Ocean	Number of samples	Tissue type	Curator
East South Africa	Indian	3	Tooth	Bayworld Museum
Oman	Indian	3	Tissue	Oregon State University
Sri Lanka	Indian	1	Biopsy	Southwest Fisheries Science Center and Oregon State University
Marianas	Western Pacific	3	Biopsy	Southwest Fisheries Science Center and Oregon State University
Taiwan	Western Pacific	3	Tissue	Southwest Fisheries Science Center and Oregon State University
Hawaiian Islands	Central Pacific	189	Biopsy, tissue, tooth	Smithsonian, Southwest Fisheries Science Center and Oregon State University
French Polynesia, Samoan Islands	Central Pacific	72	Biopsy	Oregon State University and University of Auckland
New Zealand	Central Pacific	3	Tooth	Oregon State University Museum of New Zealand Te Papa
Eastern Tropical Pacific	Eastern Pacific	56	Biopsy, Tissue	Southwest Fisheries Science Center and Oregon State University
Gorgona Island, West coast of Central America	Eastern Pacific	5	Tooth	Smithsonian
West Coast of U.S.	Eastern Pacific	2	Tissue	Southwest Fisheries Science Center and Oregon State University
East Coast of U.S.	Western Atlantic	30	Tissue, tooth	Smithsonian, Oregon State University, and Portland State University
Caribbean	Western Atlantic	6	Tissue, tooth	Oregon State University Portland State University
Gulf of Mexico	Western Atlantic	2	Tissue	Oregon State University Portland State University
Brazil	Western Atlantic	1	Tissue	Southwest Fisheries Science Center and Oregon State University
Total		379		

TABLE S2 Intron and mtDNA markers used in this study including base pairs (approximate product size optimized for delphinids), primer pairs, location, and references.

Locus	Approximate product size (base pairs)	Primer pair	Location	Primer sequence 5' –Forward Reverse -3'	Reference
Act-1	900	Act3 Act1385	Nuclear	GGTTATCTGATGTATTCC CTTGTGAACTGATTACAGTCC	Palumbi and Baker (1994) Conway (2005)
CHRNA1	360	CHRNA1F CHRNA1R	Nuclear Chromosome 2	GACCATGAAGTCAGACCAGGAG GGAGTATGTGGTCCATCACCAT	Lyons et al. (1997)
CAT	520	CATF CATR	Nuclear Chromosome 11	AAAGACTGACCAGGGCATCA AGGGTAGTCCTTGTGAGGCC	Lyons et al. (1997)
GBA	310	GBAF GBAR	Nuclear Chromosome 1	AAAAGCTTCGGCTACAGCTC TCCCTTCACTTTCTGGA ACTTC	Lyons et al. (1997)
IFN1	340	IFN1F IFN1R	Nuclear Chromosome 9	TTCTCCTGCCTGAAGGACAG GGATCTCATGATTTCTGCTCTGAC	Lyons et al. (1997)
DBY7	200	DBY7F DBY7R	Nuclear Y chromosome	GGTCCAGGAGARGCTTTGAA CAGCCAATTCTCTTGTGGG	Hellborg and Ellegren (2003)
Control Region	800	Dlp1.5 Dlp8	Mitochondrial	TCACCCAAAGCTGRARTTCTA GGAGTACTATGTCCTGTAACCA	Baker et al. (1998) Dalebout et al. (2004)

TABLE S3 Long-range PCR fragments used to sequence the mitogenome identified by fragment number. Origin and Terminal end specify where the primer started and ended specific to *Steno bredanensis* when aligned with the published 16,385 bp *Steno bredanensis* mitogenome sequence from Vilstrup et al. (2011) (GenBank Accession no. JF339982.1). T_A refers to the annealing temperature used in the thermocycle protocol.

Fragment	Primer pair	Primer sequence 5' –Forward Reverse -3'	Fragment length	Origin <i>Steno bredanensis</i>	Terminal end <i>Steno bredanensis</i>	T _A °C	Reference
1	1.4UPF DelNDR	AATCCAGGTCGGTTTCTATCT CAATTGATGAGTAGGCTATAATTTTC	1,952	2,536	4,488	62	2 5
2	Pma6800CO1F DelHDND4R	GAGAAGCMTTYRCATCCAAAC GGGTCAGAGAAGAATGTTAAAG	3,610	6,779	10,389	59	1 1
3	Mys10000ND4LF Mys13000ND5R	CGATCCCACCTAATRTCCGCA GCTCAGGCGTTGGTATAAGA	3,024	9,986	13,011	64	3 3
4A	PmaHS13660F Pma12sRNAR	GCCTYAACCAACCYTAYCTR GTGCTTGATACCWGCTCCTTTT	3,834	12,741	190	62.5	4 1
4B	Mys13000ND5F CytB2	TCTTATACCAACGCCTGAGC CCCTCAGAATGATATTTGTCCTCA	1,624	12,996	15,220	62.5	1 6
5A	EjapM13tpheF 1.4UPR	AAAGCAAGACACTGAAAATGT AGATAGAAACCGACCTGGATT	2,502	55	2,557	60	1 1
5B	Eu1635F DelHD3106R	AGCAGCCATCAATTAAGAAAGC GACRGCTAGGCTTGATATTGC	1,452	1,654	3,106	63	1 1
6	DelND2F Pma6916tSerR	GAAAATTATAGCCTACTCATCAATTG GTTTCGAKTCCTTCCTTTCTT	2,465	4,463	6,928	62.5	5 4
Control region	Dlp1.5 Dlp10	TCACCCAAAGCTGRARTTCTA CCACAGTACTATGTCCGTATT	1,235	15,150	16,385	55	6 7

Note. Primer references are designated in the Reference section as follows: (1) Alexander et al. (2013) study (2) Allan Wilson Center, Massey University, New Zealand, (3) designed by M. Vant, (4) modified from Allan Wilson Center primers, (5) DelND2F, DelND2R Caballero et al. 2015, (6) CytB2 and Dlp1.5 (Dalebout et al. 2004), and (7) Dlp10 (Baker et al 1993).

TABLE S4 Genbank Accession numbers of the mtDNA haplotypes for rough-toothed dolphins in this study. Haplotypes not used in the mitogenome study are labeled N/A. Haplotype numbers assigned here are used in Figure 5.

Haplotype number	Haplotype name	Accession number Control region	Accession number Mitogenome
1	Medit1	MW929453	N/A
2	Medit2	MW929454	N/A
3	EAtl_1	MW929455	N/A
4	EAtl_2	MW929456	N/A
5	EAtl_3	MW929457	N/A
6	EAtl_4	MW929458	N/A
7	WAtl_1	JQ798153	N/A
8	WAtl_2	MW929459	N/A
9	WAtl_3	MW929460	N/A
10	WAtl_4	MW929461	OL461802
11	WAtl_5	EF027007	OL461801
12	WAtl_6	MW929462	OL461805
13	WAtl_7	MW929463	OL461799
14	WAtl_8	MW929464	OL461804
15	WAtl_9	MW929465	OL461800
16	WAtl10	JQ798152	N/A
Same as WAtl_7	WAtl11	Same as WAtl_7	OL461803
17	WAtl12	AY842471	N/A
18	Ind_1	MW929466	OL461797
19	Ind_2	MW929467	N/A
20	Ind_3	MW929468	N/A
21	Ind_4	MW929469	N/A
22	WPac_1	MW929470	OL461796
23	WPac_2	KU745651	OL461798
24	CPac_1	JQ798145	N/A
25	CPac_2	JQ798146	N/A
26	CPac_3	JQ798147	N/A
27	CPac_4	KU745666	N/A
28	CPac_5	JQ798148	OL461808
29	CPac_6	KU745664	N/A
30	CPac_7	KU745654	OL461811
31	CPac_8	MW929471	N/A
32	CPac_9	JQ798149	OL461813
33	CPac10	KU745653	OL461814
34	CPac11	MW929472	N/A
35	CPac12	KU745657	OL461809
36	EPac_1	JQ798154	N/A
37	EPac_2	MW929472	N/A
38	EPac_3	JQ798155	OL461812
39	EPac_4	JQ978150	N/A
40	EPac_5	MW929474	N/A
41	EPac_6	MW929475	OL461807
42	EPac_7	MW929476	N/A
43	EPac_8	MW929477	N/A

44	EPac_9	MW929478	N/A
45	EPac10	MW929479	N/A
46	EPac11	MW929480	N/A
47	EPac12	MW929481	N/A
48	EPac13	MW929482	N/A
49	EPac14	MW929483	OL461810
50	EPac15	MW929484	OL461806
51	EPac16	MW929485	N/A

TABLE S5 Species-specific substitution rates (per site per myr) for the family Delphinidae generated in the multispecies BEAST phylogenetic reconstruction using the protein-coding genes from the mitogenome. The BEAST xml file from Alexander et al. (2013) was used with the addition of a *Steno bredanensis* sequence from this study.

Species	Lower HPD	Higher HPD	Median	Mean
<i>Lagenorhynchus obscurus</i>	0.0041	0.008	0.0059	0.0073
<i>Grampus griseus</i>	0.0052	0.0113	0.0078	0.0105
<i>Stenella attenuata</i>	0.0056	0.0107	0.008	0.0097
<i>Stenella coeruleoalba</i>	0.0063	0.0113	0.008	0.0096
<i>Lagenorhynchus albirostris</i>	0.0064	0.0159	0.0119	0.0156
<i>Cephalorhynchus heavisidii</i>	0.0068	0.0131	0.0097	0.0117
<i>Orcinus orca</i>	0.0088	0.016	0.0122	0.0160
<i>Tursiops truncatus</i>	0.0069	0.0199	0.0123	0.0158
<i>Delphinus delphis</i>	0.0041	0.0115	0.0072	0.0093
<i>Globicephala melas</i>	0.0067	0.0146	0.0101	0.0137
<i>Sousa chinensis</i>	0.0065	0.0103	0.0078	0.0093
<i>Cephalorhynchus commersonii</i>	0.0064	0.0139	0.0091	0.0114
<i>Tursiops aduncus</i>	0.0064	0.014	0.0098	0.0117
<i>Cephalorhynchus eutropia</i>	0.0047	0.0123	0.0081	0.0101
<i>Lagenorhynchus cruciger</i>	0.0039	0.0107	0.0068	0.0083
<i>Delphinus delphis</i>	0.0069	0.0167	0.0104	0.0135
<i>Cephalorhynchus hectori maui</i>	0.0063	0.0145	0.0092	0.0125
<i>Lagenorhynchus australis</i>	0.0039	0.0108	0.0069	0.0085
<i>Steno bredanensis</i>	0.0073	0.0120	0.0098	0.0115

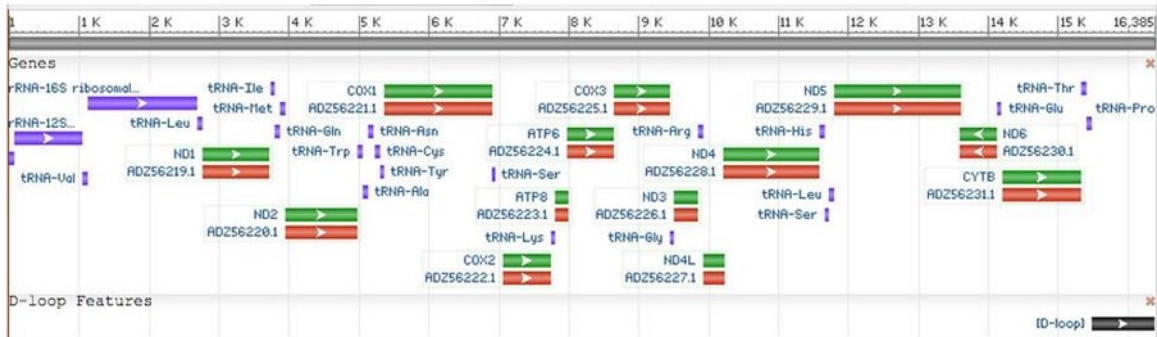


FIGURE S1 The protein-coding genes (green) and the control region (black) for the rough-toothed dolphin (*Steno bredanensis*) sample Sbr11638. The concatenated protein-coding mitogenome data set included all of the protein-coding genes here except ND6 (12 total genes). Reasons for the exclusion of ND6 are discussed in the Methods section.



FIGURE S2 Proximity plots for rough-toothed dolphins (*Steno bredanensis*) in the Atlantic (red) and Pacific/Indian (turquoise) oceans from Random Forest models using the mtDNA CR data set.