

An Initial Population Structure and Genetic Diversity Analysis for *Stenella clymene* (Gray, 1850): Evidence of Differentiation Between the North and South Atlantic Ocean

Supplemental Materials

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Supplemental Table

Table S1. Number of sequences (*N*) of *Stenella* species used, its locality, and GenBank accession number for mtDNA regions D-loop, CoI, and Cyt b. AO = Atlantic Ocean, EIO = Eastern Indian Ocean, ETP = East Tropical Pacific, GOM = Gulf of Mexico, IO = Indian Ocean, MS = Mediterranean Sea, NAO = North Atlantic Ocean, NEA = North Eastern Atlantic, NWA = North Western Atlantic, NWP = North Western Pacific, PO = Pacific Ocean, and SAO = South Atlantic Ocean.

Species	Locality	D-loop		CoI		Cyt b		
		<i>N</i>	GenBank	<i>N</i>	GenBank	<i>N</i>	GenBank	
<i>Stenella attenuata</i>	ETP	--	--	7	EU496336-39	EU496353	7	AF084096-97
	GOM				EU557096	NC010205		EF093030
	NWA							EU557096
	NWP							NC012051
	PO							X56294 X92525
<i>Stenella clymene</i>	GOM	20	KX343034	13	KX346580 (SAO11)	24	KX346590 (SAO11)	
	NAO		(SAO01)		KX346581 (SAO09)		KX346591 (SAO09)	
	SAO		KX343035		KX346582 (SAO08)		KX346592 (SAO08)	
			(SAO04)		KX346583 (SAO07)		KX346593 (SAO07)	
			KX343036		KX346584 (SAO06)		KX346594 (SAO06)	
			(SAO06)		KX346585 (SAO05)		KX346595 (SAO05)	
			KX343037		KX346586 (SAO04)		KX346596 (SAO04)	
			(SAO07)		KX346587 (SAO03)		KX346597 (SAO02)	
			KX343038		KX346588 (SAO02)		KX346598 (SAO01)	
			(SAO08)		KX346589 (SAO01)		AF084083 EU517711-12	
			KX343039		EU496346-48		KF691958 KF691985-91	
			(SAO09)				KF691994-95 KF692012-13	
		KX343040						
		(SAO10)						
		KX343041						
		(SAO12)						
		DQ845446-47						
		GQ504137-48						

Species	Locality	D-loop		CoI		Cyt b	
		N	GenBank	N	GenBank	N	GenBank
<i>Stenella coeruleoalba</i>	AO IO	--	--	20	DQ466000-09	48	AF084081-82 DQ466016-18
	GOM				EF090640-44 EU496341-		DQ466020-24 EF090637
	MS NEA				44		EU580088 KF691950-51
	NWA				KF281695		KF691959-66 KF691976
	NWP PO						KF691978 KF691984 KF691992-93 KF691996-2011 KF692014-18
<i>Stenella frontalis</i>	AO	--	--	9	EF090645-46 EU496340	5	AF084089-90
	GOM				EU496349-52 EU496354		EU121092
	NEA				KF281696		EU517713-14
	NWA						
<i>Stenella longirostris</i>	AO EIO	--	--	5	EU496331-35	89	AF084100-03
	ETP IO						EU121093 EU517703
	GOM						EU517715
	NWA PO						KC161126-83 KF691952-57 KF691967-75 KF691977 KF691979-83 X56292-93 X92524

DNA Extraction Protocol for Samples in Formaldehyde (Adapted from Mesquita et al., 2001)

Adapted Lysis Solution – Final volume: 50 ml

Tris-HCl (1M, pH 8.0): 0.5 ml

EDTA (0.5 M, pH 8.0): 1.0 ml

NaCl (1M) 1.0 ml

10% SDS: 8.0 ml

ddH₂O: 39.5 ml

Sample was cut to the minimum possible size with forceps, and the scalpel blade was properly sterilized. Sample was placed in a microcentrifuge tube (1.5 ml), and 200 μ l of lysis solution and 25 μ l of proteinase K (20 mg /ml) were added. The microcentrifuge tube was left in the water bath at 55° C for 4 d; and on each day, 12.5 μ l of proteinase K (250 μ l/mL) was added, and the sample was vortexed slightly. On the last day, the sample was removed from water-bath at 55° C and placed in another bath at 95° C for 10 min, stopping the digestion action of the enzyme. Then, 1 ml of phenol 99% (pH 8.0) was added, and the tube was centrifuged at 4,200 rpm for 20 min. Supernatant was placed in a new microcentrifuge tube (1.5 ml), and 500 μ l of phenol, 480 μ l chloroform,

and 20 μ l of isoamyl alcohol were added. The tube was centrifuged at 4,200 rpm for 20 min, and the supernatant was placed in a new microcentrifuge tube (1.5 ml). Two volumes of cold absolute ethanol and ammonium acetate (7 M) were added in a proportion of 1/10 of the sample volume. Sample was left overnight in the freezer. The next day, the sample was centrifuged at 19,600 rpm for 20 min, and this time the supernatant was discarded. Then, 70 μ l of ethanol (70%) was added, and the tube was centrifuged at 14,000 rpm for 5 min. Again, the supernatant was discarded. The pellet was left to dry at room temperature. When the microcentrifuge tube was completely dry, the DNA was resuspended in 50 μ l TE (1%) and stored at 4° C.