Locus	Primer sequence	Repeat motif	<i>T</i> <sub>a</sub> (℃)	Size range (bp)	N <sub>a</sub>	$H_O$	$H_E$	п	PI	PIsib	Pooled after amplification for loading	Reference
SCA22	F: GTT TGA GGA GAA GAC ATA C R: CCC TGA CCA CAG	(CT) <sub>7</sub> TTCT(CA) <sub>36</sub>	55	130–146	Null allele	-	-	395	-	-	1a	1
SCO11	F: ACC GCC TCT GTC TGT TTC TC R: AAG TCA CTC GGA GGA GTC CA	(CTAT)6CTAA	55	171–227	9	0.8405	0.6158	395	0.2107	0.4927	1b	2
SCA17	F: TCC TGA GAC CTT GAG TTC R: ATT CAT TTC CAG AGC ATC	(CA) <sub>18</sub>	55	184–192	26	0.8379	0.7961	395	0.0656	0.3682	2a	1
SCA37	F: TGT GTC CTA TTT CTA TTG R: ACA TTC TAC GGA GTC TTC	(CA) <sub>22</sub>	55	227–231	13	0.8329	0.7932	395	0.0655	0.3686	2b	1
SCO28	F: AAA CCA TTC CAT TTT GAG GTA A R: CCC TAG TAT AAG AAC ATG GGA AGA	(GATA) <sub>5</sub>	55	134–146	6	0.7038	0.4881	395	0.3575	0.5917	3a	2
SCA9	F: GTC TTC TTC ATC GGC TGT R: CTG AAA AGA GGG CTA AGG	(CA) <sub>23</sub>	55	192–222	11	0.8784	0.7694	395	0.0875	0.3860	3b	1
SCA27	F: TGC CAG GAA AAT AAG GAG R: GCG TGG AGA GGG TAT ATG	(CA) <sub>21</sub>	55	184–194	17	0.9949	0.7631	395	0.0855	0.3886	4a	1

 Table S1 Summary of genetic variation based on 16 microsatellite loci in the Heaviside's dolphin

Locus	Primer sequence	Repeat motif	<i>T<sub>a</sub></i> (℃)	Size range	Na	$H_O$	$H_E$	п	PI	PIsib	Pooled after amplification	Reference
				(bp)							for loading	
SCA39	F: TGA GAT GCT TCT TAC	$(CA)_{20}$	55	209–215	11	0.9594	0.7169	395	0.1221	0.4206	4b	1
	СТА											
	R: TAT TAC CIT ATG											
	GGC TTG				10	0.0404	0.04.40	<b>a</b> a <b>r</b>		a <b>a</b> 4a 4	_	2
EV14	F: TAA ACA TCA AAG	$(GT)_n$	55	127–151	13	0.8481	0.8143	395	0.0507	0.3494	5a	3
	CAG ACC CC											
	R: CCA GAG CCA AGG											
<b>T</b> . 11	TCA AGA G				10	0.0505	0.0070	205	0.0500	0.0500	~1	
Ttrll	F: CTTTCA ACC TGG CCT	$(CA)_{21}$	55	193–223	10	0.9595	0.8070	395	0.0539	0.3532	56	4
	R: GIT IGG CCA CIA											
$T_{tric}$		$(\mathbf{C}\mathbf{A})$	<b>5 5</b>	02 151	16	0.9622	0.9410	205	0.0201	0 22 4 4	6.	4
1005	F: CAUCITACA GCC	$(CA)_{34}$	55	83-151	10	0.8033	0.8419	393	0.0381	0.3344	oa	4
	$ \begin{array}{c} AAA \ I \ OA \ OA \ OA \ OA \ OA \ OA \$											
	CTG AGT CAT CA											
EV27	$E_{\rm ACC}$ TTC ATT TCC	$(\mathbf{AC})$	55	176 106	0	0 8320	0 6508	305	0 1505	0 1163	66	3
EVSI		$(AC)_n$	55	1/0-180	9	0.6529	0.0398	393	0.1505	0.4405	00	5
	P. TAG TAG AGC CGT											
	GAT AAA GTG C											
SC \ 54	E GTC AGG AGG TTG	$(\mathbf{C}\mathbf{A})_{\mathbf{C}\mathbf{A}}$	55	107 201	3	0 7386	0 5183	30/	0 3010	0 5646	7.	1
SCAJ4	GGA GTA	$(CA)_{20}$	55	197-201	5	0.7580	0.5165	394	0.3019	0.3040	7 a	1
	$\mathbf{R}$											
	GAA AAT CA											
Dde66	$F^{\cdot}$ AAC ATT GCC AGT	$(GT)_{10}$	55	346_362	8	0 9721	0 6479	394	0 1813	0 4692	7h	5
Ducoo	GCC TTA GAA	(01)]9	55	540-502	0	0.9721	0.0179	571	0.1015	0.1072	10	5
	R <sup>·</sup> GTG GAA CAG ACG											
	CGC ATA T											
Dde09	F: GAA GAT TTT ACC	$(CTAT)_{10}$	55	221-245	1	-	-	10	-	-	8a	5
2 4007	CTG CCT GTC	(01111)10	00	221 215	-			10			04	C
	R: GAT CTG TGC TCC											
	TTA GGG AAA											

Locus	Primer sequence	Repeat motif	$T_a$ (°C)	Size range (bp)	Na	H <sub>O</sub>	$H_E$	п	PI	PIsib	Pooled after amplification for loading	Reference
Dde059	F: TAC ACA GCT TAC TTA CCT TAC CAA R: GTC CCT TTG AGC AGA GTT CTA	(GATA) <sub>n</sub>	55	384-432	1	-	-	10	-	-	8b	5
Mean SD					11.69 5.736	$0.8664 \\ 0.0875$	0.7102 0.1154		0.1362	0.4257		

 $T_a$  stands for annealing temperature, bp for allele sizes, and  $N_a$  represents the number of alleles examined within each putative population where observed ( $H_o$ ) and expected ( $H_E$ ) heterozygosities were estimated; *n* indicates the number of individuals used in calculations; dash (-) indicates loci which were not polymorphic, *PI* stands for Probability of Identity per locus, and *PIsibs* indicates Probability of Identity for genetic similarity among siblings per locus. Locus SCA22 did not amplify and loci Dde09 and Dde059 were monomorphic.

References:

- 1. Chen L, Yang G (2008). A set of polymorphic dinucleotide and tetranucleotide microsatellite markers for the Indo-Pacific humpback dolphin (*Sousa chinensis*) and cross-amplification in other cetacean species. *Conservation Genetics* **10**, 697–700.
- 2. Mirimin L, Coughlan J, Rogan E, Cross TF (2006). Tetranucleotide microsatellite loci from the striped dolphin (*Stenella coeruleoalba* Meyen, 1833). *Molecular Ecology Notes* **6**, 493–5.
- 3. Valsecchi E, Amos W (1996). Microsatellite markers for the study of cetacean populations. *Molecular Ecology* 5, 151–6.
- 4. Rosel P, Forgetta V, Dewar K (2005). Isolation and characteriszation of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). *Molecular Ecology Notes* **5**, 830–3.
- 5. Coughlan J, Mirimin L, Dillane E, Rogan E, Cross TF (2006). Isolation and characterization of novel microsatellite loci for the short-beaked common dolphin (*Delphinus delphis*) and cross-amplification in other cetacean species. *Molecular Ecology Notes* **6**, 490–2.

Location	Haplotype diversity ( <i>h</i> )	Nucleotide diversity ( $\pi$ )
Table Bay	$0.6106 \pm 0.0669$	$0.002106 \pm 0.001515$
St. Helena Bay	$0.7749\ \pm 0.0554$	$0.006995 \pm 0.004011$
Lambert's Bay	$0.7631\ \pm 0.0435$	$0.006938 \pm 0.003896$
Hondeklipbaai	$0.8807\ \pm 0.0246$	$0.005766 \pm 0.003369$
Port Nolloth	$0.8920\ \pm 0.0186$	$0.005671 \pm 0.003278$
Luderitz	$0.7136\ \pm 0.0443$	$0.002797 \pm 0.001861$
Walvis Bay	$0.8727\ \pm 0.0272$	$0.005797 \pm 0.003354$
Overall	$0.7868\ \pm 0.0401$	$0.005152 \pm 0.003040$

**Table S2** Genetic variability estimates in mtDNA control region sequences excluding singletons (n = 27) for haplotype diversity (h) and nucleotide diversity ( $\pi$ )

		Research sites (putative populations)										
Locus		Table Bay	St. Helena	Lambert's	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay				
		( <i>n</i> = 54)	Bay $(n = 55)$	Bay $(n = 63)$	( <i>n</i> = 40)	( <i>n</i> = 66)	(n = 62)	( <i>n</i> = 55)				
SCO11	$N_a$	5	5	6	4	4	8	5				
	$H_O$	0.6111	0.8000	0.9365	0.9500	0.8636	0.8871	0.8363				
	$H_E$	0.5152	0.5466	0.6600	0.6642	0.6051	0.6656	0.5950				
	HWE	0.8757	1.0000	1.0000	1.0000	1.0000	0.9965	0.9954				
SCA17	$N_a$	11	10	13	13	14	17	17				
	$H_O$	0.7407	0.6181	0.9365	0.9750	0.9090	0.9032	0.7818				
	$H_E$	0.7464	0.6944	0.8005	0.8265	0.7800	0.8535	0.7749				
	HWE	0.2871	0.0074	1.0000	1.0000	0.9982	0.8190	0.0970				
SCA37	$N_a$	10	7	9	8	9	10	10				
	$H_O$	0.5555 <sup>§*</sup>	0.6363	0.9682	1.0000	0.8939	0.8709	0.9090				
	$H_E$	0.7371	0.7031	0.8213	0.8209	0.8030	0.7651	0.8367				
	HWE	0.0000	0.0798	1.0000	1.0000	0.9958	0.8652	0.7487				
SCO28	$N_a$	3	2	2	2	4	4	3				
	$H_O$	0.5925	0.4909	0.8095	0.7750	0.7272	0.8387	0.6727				
	$H_E$	0.4823	0.4379	0.4958	0.4807	0.4959	0.5222	0.4909				
	HWE	0.6754	0.7426	1.0000	1.0000	1.0000	0.0154	0.9347				
SCA9	$N_a$	5	6	6	8	9	7	7				
	$H_O$	0.7037	0.8363	0.9047	0.9750	0.9545	0.9193	0.8546				
	$H_E$	0.6955	0.7122	0.7439	0.7911	0.8009	0.7870	0.7701				
	HWE	0.2139	0.9903	1.0000	1.0000	0.9991	0.6943	0.8827				
SCA27	$N_a$	11	12	8	8	10	8	9				
	$H_O$	1.0000	0.9818	1.0000	1.0000	0.9848	1.0000	1.0000				
	$H_E$	0.7895	0.8038	0.7147	0.7614	0.7626	0.7348	0.7473				
	HWE	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000				

**Table S3** Summary of genetic variation based on 13 microsatellite loci in the Heaviside's dolphin

				Research	n sites (putative po	pulations)		
Locus		Table Bay	St. Helena	Lambert's	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
		( <i>n</i> = 54)	Bay $(n = 55)$	Bay $(n = 63)$	( <i>n</i> = 40)	( <i>n</i> = 66)	( <i>n</i> = 62)	( <i>n</i> = 55)
SCA39	$N_a$	5	9	7	7	8	7	7
	$H_O$	0.8703	0.9090	1.0000	1.0000	0.9697	0.9839	0.9818
	$H_E$	0.5443	0.7497	0.6794	0.7092	0.7575	0.7322	0.7785
	HWE	1.0000	0.1317	1.0000	1.0000	0.9995	1.0000	0.9997
EV14	$N_a$	10	9	11	8	11	10	8
	$H_O$	$0.6851^{*}$	$0.6181^{*}$	1.0000	0.9750	0.9242	0.8387	0.8909
	$H_E$	0.8043	0.7668	0.8397	0.8307	0.8109	0.8146	0.7807
	HWE	0.0221	0.0192	1.0000	0.9596	0.9961	0.3241	0.9555
Ttr11	$N_a$	5	5	8	4	9	8	8
	$H_O$	0.8888	0.9636	0.9841	1.0000	0.9393	0.9677	0.9818
	$H_E$	0.7613	0.7578	0.8133	0.6535	0.7317	0.8436	0.8634
	HWE	0.9507	0.9999	1.0000	1.0000	1.0000	0.9892	0.9858
Ttr63	$N_a$	10	10	10	10	12	11	13
	$H_O$	0.5925 <sup>§*</sup>	$0.7272^*$	1.0000	0.9750	0.9849	0.8548	0.8909
	$H_E$	0.8200	0.8517	0.7845	0.8098	0.7573	0.8631	0.8734
	HWE	0.0019	0.0199	1.0000	1.0000	1.0000	0.0354	0.0869
EV37	$N_a$	4	4	5	4	6	5	8
	$H_O$	0.6666	0.5636	1.0000	1.0000	0.9090	0.8709	0.8181
	$H_E$	0.5637	0.4946	0.6646	0.7237	0.6981	0.6448	0.7269
	HWE	0.0111	0.9224	1.0000	1.0000	0.9997	0.9928	0.4015
SCA54	$N_a$	3	3	2	3	3	3	3
	$H_O$	0.4074	0.2363	0.9365	0.9750	0.7576	0.8709	1.0000
	$H_E$	0.3456	0.2163	0.5019	0.5503	0.5259	0.5642	0.6198
	HWE	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

		Research sites (putative populations)									
Locus		Table Bay	St. Helena	Lambert's	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay			
		( <i>n</i> = 54)	Bay $(n = 55)$	Bay $(n = 63)$	( <i>n</i> = 40)	(n = 66)	(n = 62)	( <i>n</i> = 55)			
Dde66	$N_a$	4	4	5	5	7	5	3			
	$H_O$	0.9814	0.9818	1.0000	1.0000	0.8636	1.0000	1.0000			
	$H_E$	0.6941	0.6333	0.6318	0.6095	0.6174	0.6746	0.6059			
	HWE	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000			
Average (SD)	$N_a$	6.615 (3.203)	6.615 (3.124)	7.077 (3.252)	6.462 (3.126)	8.154 (3.288)	7.923 (3.639)	7.769 (4.045)			
	$H_O$	0.715 (0.176)	0.720 (0.222)	0.959 (0.056)	0.969 (0.060)	0.898 (0.080)	0.908 (0.060)	0.894 (0.101)			
	$H_E$	0.654 (0.148)	0.644 (0.177)	0.704 (0.114)	0.710 (0.113)	0.703 (0.107)	0.728 (0.109)	0.728 (0.116)			

 $N_a$  indicates the number of alleles examined per each sampling site where observed ( $H_o$ ) and expected ( $H_E$ ) heterozygosities were estimated, HWE stands for Hardy-Weinberg equilibrium; n indicates the number of individuals used in calculations, § denotes loci out of HWE ( $\leq 0.05$ ) and \* indicates evidence for null allele. Locus SCA22 did not amplify and loci Dde09 and Dde059 were monomorphic

Table S4 Sex-biased dispersal results for males and females with respect to  $F_{IS}$ ,  $F_{ST}$ , Ho, Hs, mean assignment and variance assignment

	N	F <sub>IS</sub>	$F_{ST}$	Но	Hs	Mean assignment	Variance of mean assignment
Males	173	-0.2619	0.0277	0.8692	0.6888	0.3567	12.0338
Females	222	-0.2347	0.0219	0.8638	0.6996	-0.2780	15.1182
Overall	395	-0.2468	0.0248	0.8662	0.6947	0.0000	0.0000
<i>P</i> -value		0.9440	0.8870	0.2860	0.9260	0.9640	0.9000

	Table Bay	St. Helena Bay	Lambert's Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay	Total
TBH1	27	4		4	6		1	42
TBH2	2							2
TBH3	1	3		2	4	4	2	16
TBH4	9	2						11
TBH7	1							1
TBH8	6	12	6	7	6	16		53
TBH11	2	2						4
TBH18	1							1
TBH35	1							1
TBH38	1							1
TBH40	1							1
TBH54	1							1
TBH55	1							1
SHB1		2		1	7	27	5	42
SHB8		1						1
SHB14		1						1
SHB15		16	27					43
SHB16		10	5	4	4	2		25
SHB20		1	11	1	3			16
SHB32		1						1
LBH10			5	7	7	4		23
LBH21			3					3
LBH24			2	6	15		6	29
LBH27			1					1
LBH34			3				1	4
НКВ3				1	6		2	9
HKB11				1				1

**Table S5** Haplotype frequencies per sampling location of 51 haplotypes identified in biopsy samples of Heaviside's dolphins obtained at seven locations off the southwest African coast

	Table Bay	St. Helena Bay	Lambert's Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay	Total
HKB19				2	3			5
HKB30				1				1
HKB34				1				1
HKB35				1				1
HKB37				1				1
PNH5					1			1
PNH17					2			2
PNH33					1			1
PNH65					1			1
CH2LDZ						4		4
CH5LDZ						1		1
CH7LDZ						1	7	8
CH19LDZ						1		1
CH45LDZ						1	1	2
CH54LDZ						1		1
CH2WB							14	14
CH4WB							1	1
CH9WB							1	1
CH16WB							4	4
CH18WB							1	1
CH26WB							1	1
CH29WB							5	5
CH33WB							1	1
CH49WB							2	2
TOTAL	54	55	63	40	66	62	55	395

Sampling location (sample size)	Inferred population cluster				
Sampling location (sample size) —	1	2			
Table Bay (54)	0.680	0.320			
St. Helena Bay (55)	0.698	0.302			
Lambert's Bay (63)	0.434	0.566			
Hondeklipbaai (40)	0.362	0.638			
Port Nolloth (66)	0.458	0.542			
Luderitz (62)	0.425	0.575			
Walvis Bay (55)	0.424	0.576			

**Table S6** Proportion of Heaviside's dolphin individuals from each of the seven sampling locations assigned to each of the two clusters inferred fromSTRUCTURE analysis



**Figure S1** Median-joining network of mtDNA control region haplotypes without singletons for Heaviside's dolphins found off the southwest coast of southern Africa.



Figure S2 Average posterior probability (ln *K*) for each of the seven clusters (i.e., *K* 1 to 7) from 15 independent runs.



**Figure S3** Log-likelihood values (Ln Pr(X/K)) from 15 independent runs, where the ad hoc statistic delta  $K(\Delta K)$  shows the most probable number of genetic clusters (*K*).



**Figure S4** Bayesian assignment probabilities where K = 6 clusters were inferred from STRUCTURE analysis. Two sampling sites, Hondeklipbaai and Port Nolloth, are grouped together as in the AMOVA results using mtDNA control region data. Each individual is represented by a thin vertical line, which is portioned into *K* coloured segments that represent the individual's estimated membership fractions in *K* clusters. Black lines separate individuals of different populations. The six putative populations are labelled below the figure.