Patterns of geographic variation between mitochondrial and nuclear markers in Heaviside's (Benguela) dolphins (*Cephalorhynchus heavisidii*)

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Abstract

The Heaviside's (or Benguela) dolphin (*Cephalorhynchus heavisidii*) is endemic to the west coast of southern Africa. The present study investigated the population genetic structure across a large portion of the species distribution using mitochondrial control region and nuclear (microsatellite) markers. A total of 395 biopsy skin samples were analyzed; they were collected from free-ranging Heaviside's dolphins in 7 locations along 1650 km of coast between Table Bay, South Africa and Walvis Bay, Namibia. Both genetic markers rejected the hypothesis of 1 homogenous population but revealed contrasting results in the genetic structuring of putative populations. Mitochondrial DNA suggested either 2 populations or a fine-scale division with 6 (sub) populations, while microsatellite markers were indicative of 2 widespread populations with measurable gene flow between them. Neutrality tests and mismatch distribution of the mitochondrial sequences indicated a departure from mutation–drift equilibrium due to a population expansion at the 2 extremes of the geographic range, but not towards the middle of the distribution. These results highlight the importance of evaluating multiple genetic markers to gain reliable insights into population processes and structure.

Key words: Heaviside's (Benguela) dolphins, microsatellite loci, mitochondrial DNA, population genetic structure, southern Africa

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Introduction

Understanding population structure is important for the conservation of species' genetic diversity (Avise *et al.* 1995, Frankham *et al.* 2010). At a larger scale, oceanographic features such as surface salinity, temperature and primary productivity can influence, directly or indirectly, genetic patterns of cetaceans (Bilgmann *et al.* 2007; Fontaine *et al.* 2007). Among semi-pelagic and coastal delphinids, there are known cases of var-

ied degrees of intra-specific genetic differentiation between neighboring populations (e.g. Andrews *et al*. 2006, 2010; Möller *et al.* 2007; Hollatz *et al.* 2011), and factors influencing such genetic differentiation in coastal areas include habitat type and behavioural specializations, which can, in turn, be influenced by physical coastal features of estuaries and embayments (Möller *et al.* 2007; Gowans *et al*. 2008). This may result in significant genetic structure even over small geographic scales (Hoelzel *et al.* 1998; Natoli *et al.* 2008; Pimper *et al.* 2010; Hamner *et al.* 2012; Pérez-Alvarez *et al.* 2015).

The genus *Cephalorhynchus* comprises 4 species, of which the Heaviside's dolphin, known also as the Benguela dolphin [*Cephalorhynchus heavisidii* (Gray, 1828)] remains one of the least known, despite its coastal occurrence (Best 2007). This species is endemic to coastal waters of southwestern Africa; from the Cape of Good Hope, South Africa (18°28′E, 34°21′S) in the south to southern Angola (provinces Namibe and Benguela) in the north. This represents approximately 2500 km, although the northern limit of the distribution remains uncertain (Best 2007; Fig. 1). These dolphins prefer inshore habitats, usually waters <100 m deep, although occasionally can also be seen over deeper waters and up to 80 km offshore (Best & Abernethy 1994). They are associated with the cold Benguela Current and prey primarily on juvenile hake (*Merluccius capensis* Castelnau, 1861) and kingklip [*Genypterus capensis* (Smith, 1847); Sekiguchi *et al.* 1992]. The International Union for the Conservation of Nature (IUCN) lists this species as Data Deficient (Reeves *et al.* 2013).

In the Western Cape, South Africa, early observations indicated relatively high numbers and year-round presence of these dolphins in inshore waters (Rice & Saayman 1984), but it was not possible to determine whether these high local densities represented philopatric groups or if they were shifting aggregations of different individuals (Best 1988). More recent estimates suggest that there are approximately 6000 individuals that use the southernmost portion of the species' range, a 300-km stretch of coast between Cape Town (18°25′E, 33°54′S) and Lambert's Bay (18°30′E, 32°08′S), with several locations thought to have greater aggregations (e.g. Table Bay, St. Helena Bay and Lambert's Bay; Elwen *et al.* 2009). Although preliminary findings suggest highly dynamic groups and low site fidelity (Behrmann 2011), the population demographic parameters and structure remain unknown.

Only one genetic study has been conducted on Heaviside's dolphins to date, which did not detect unambiguous population genetic structure (Jansen van Vuuren *et al.* 2002), although the geographically limited range of sampling and the use of a single mitochondrial marker could have influenced the outcome. To expand upon that initial study, we investigated the spatial genetic structure of Heaviside's dolphins with expanded geographic coverage across a large portion of the species' range, using multiple genetic markers with different modes of inheritance and mutation rates. Similar studies performed

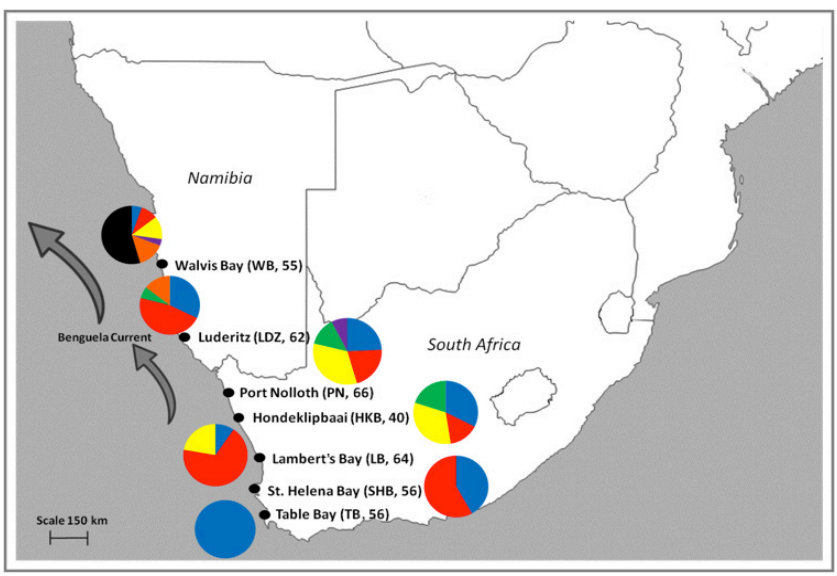

Figure 1 Locations (sampling sites) off the coast of Namibia and the west coast of South Africa where skin biopsies of Heaviside's dolphins were collected between 2009 and 2012, with sample sizes indicated in parentheses. Pie charts represent haplotype frequencies for the mtDNA control region in each sampling location analyzed (TB = blue, SHB = Red, LB = Yellow, HKB = Green, PN = Purple, LDZ Orange, WB = Black).

elsewhere on other species of the genus *Cephalorhynchus*, including Hector's dolphin [*Cephalorhynchus hectori* (P.-J. van Bénéden, 1881); Hamner *et al.* 2012], Commerson's dolphin [*Cephalorhynchus commersonii* (Lacépède, 1804); Pimper *et al.* 2010] and the Chilean dolphin [*Cephalorhynchus eutropia* (Gray, 1846); Pérez-Alvarez *et al.* 2015], have led us to hypothesize that population genetic structure is likely, and we anticipated that our mtDNA sequence data would concur with the nuclear microsatellite markers. We also investigated dispersal parameters, such as sex-specific dispersal (*AIc*, *vAIc*) and gene flow (F_{ST}), that distinguish between an equilibrium model (ancient population separation with ongoing gene flow) and a nonequilibrium model (no gene flow, but remnant shared variation as a result of a recent population split) of population divergence, as measures of genetic differentiation are expected to be higher in the philopatric sex than in the dispersing sex. In this paper, we present current baseline data on the population genetic structure of Heaviside's dolphins and trust that this work may instigate further in-depth studies of this little-known species.

Materials and methods

Sample collection

Biopsy skin samples from 399 free-ranging Heaviside's dolphins were collected during boat surveys at 7 locations along 1650 km of the west coast of South Africa: Table Bay (TB, 18°25′E, 33°54′S), St. Helena Bay (SHB, 18°02′E, 32°75′S), Lambert's Bay (LB, 18°30′E, 32°08′S), Hondeklipbaai (HKB, 17°26′E, 30°31′S), Port Nolloth (PN, 16°86′E, 29°25′S) and Luderitz, Namibia (LDZ, 15°15′E, 26°64′S) and Walvis Bay, Namibia (WB, 14°50′E, 22°95′S) during 4 years between 2009 and 2012 (Fig. 1). A modified pole spear (Hawaiian sling) was used with a stopper and a small stainless steel biopsy tip, as in similar studies elsewhere (e.g. Andrews *et al*. 2006, 2010). This field protocol assured quality genetic samples with generally a minimal negative impact on the animals involved (IWC 1991; Aguilar & Borrell 1994; Barrett-Lennard *et al.* 1996). Total genomic DNA was extracted using a non-hazardous and economical salt extraction protocol (Aljanabi & Martinez 1997). DNA concentrations were determined via Nanodrop spectrophotometer (Thermo Fischer Scientific, Massachusetts, United States).

Sex identification

Because the sex of individuals could not be determined in the field, a polymerase chain reaction (PCR)-based method was used, which allowed the ensuing analysis to be partitioned according to sex. The ZFX and SRY genes (Rosel 2003) were amplified for each individual. A 25-µL mixture was used, made of 1× buffer (10 mM Tris HCl [pH 8.], 50 mM KCl), 1.5 mM $MgCl_2$, 150 µM dNTPs, 0.3 µM of primers ZFX0582F, ZFX0923R, PMSRYF and 0.06 µM of TtSRYR, and 1.5 units of thermostable DNA polymerase (Southern Cross Biotechnology). Positive controls of known sexes and a negative control were used in each PCR reaction. The PCR profile consisted of 35 cycles of 92 °C for 30 s followed by 94 °C for 30 s, 51 °C for 45 s, 72 °C for 45 s, with a final extension of 72 °C for 30 s. The entire 25-µL volume of PCR product was used to determine the fragment patterns on a 2.5–3.0% agarose gel containing Gold View nucleic acid stain (SBS Genetech, Beijing, China) for electrophoresis and visualized by ultraviolet light. Samples produced either 1 band identifying females or 2 bands identifying males.

Sequencing and genotyping

A 580-bp fragment of the mitochondrial DNA control region was amplified at the 5′ end using primers L15926 and H00034 from Rosel *et al.* (1994) in a 25-µL reaction volume containing 2 µL of 20–100 ng/µL genomic DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 150 µM dNTPs, 0.3 µM of each primer, and 2.5 units of SuperTherm *Taq* polymerase (Southern Cross Biotechnology, Cape Town, South Africa). The PCR profile consisted of 1 min at 95 °C followed by 35 cycles of 1.5 min at 94 °C, 2 min at 48 °C and 2–3 min at 72 °C. The final extension included an additional 3 min at 72 °C to ensure complete extension of the PCR products. An aliquot of the PCR product was run on a 1% agarose gel containing ethidium bromide for electrophoresis and visualized by ultraviolet light. PCR products were not purified before sequencing because amplification was successful; the correct size fragment was amplified and no primer dimers were formed. Cycle sequencing was carried out in the forward direction only by Macrogen (Korea) on an Automatic Sequencer 3730xl. Sequences were aligned and edited using BioEdit (Hall 1999) and saved as nexus files.

Sixteen microsatellite loci were attempted, but 3 loci, SCA22 (null allele) and Dde09 and Dde059, were monomorphic or failed to amplify after testing 10 samples each and were removed. Samples were genotyped at 13 microsatellite loci (Gopal *et al.* 2012; Table S1), which included: SCA9, SCA17, SCA27, SCA37,

SCA39 and SCA54 derived from *Sousa chinensis* (Chen & Yang 2008), SCO11 and SCO28 from *Stenella coeruleoalba* (Mirimin *et al.* 2006), Ttr11 and Ttr63 from *Tursiops truncatus* (Rosel *et al.* 2005), Dde66 from *Delphinus delphis* (Coughlan *et al*. 2006), and EV14, EV37 from Valsecchi and Amos (1996). Amplification was carried out in 10-μL reaction volumes; each reaction contained 20–100ng/μL DNA with the following reagent *F* (formal) concentrations taken from Mirimin *et al.* (2006): 1X Green GoTaq Reaction Buffer (Promega, Wisconsin, USA) supplemented with 0.5 mM $MgCl_2$, 1 μM of each primer, 250 μM dNTPs and 0.5 U of GoTaq DNA polymerase (Promega). The thermal profile for all loci consisted of a denaturation step at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. Each locus was amplified individually and PCR products for each loci were run separately on a 2% agarose gel containing ethidium bromide visualized by ultraviolet light for the separation and analysis of the targeted DNA fragment based on their size and charge. Two loci were pooled together and genotyped one at a time at the Central Analytical Facility in Stellenbosch University, with internal size standard (ROX350; Table S1). Electrophoresis was performed on an ABI3130xl using a 50-cm capillary array and POP7 (all supplied by Applied Biosystems). Microsatellite profiles were screened and checked using the software Peak Scanner V. 1.0 (Applied Biosystems) with peak positions recorded manually and problematic profiles re-genotyped/re-analyzed in the sequencer machine.

Genetic diversity

There were 3 datasets used for all analyses, except where specified: females only, males only, and all samples combined. Standard measures of genetic diversity were estimated for the mtDNA data: haplotype diversity (h) and nucleotide diversity (π) using Arlequin 2.0 (Schneider *et al.* 2000). The number of variable sites were counted using the program MEGA (Tamura *et al.* 2007; Kumar *et al.* 2008). Model Test 3.7 (Posada & Crandall 1998) was run in PAUP 4.0b10 (Swofford 2002) to identify the model of evolution that best fit each of the 3 datasets.

Summary statistics for microsatellite data (allele frequencies, observed (H_O) and expected (H_E) heterozygosity) were examined for each sampling site, using Arlequin 2.0 (Schneider *et al.* 2000). Evidence for the presence of null alleles, scoring errors and allele drop-outs was examined across all 13 loci for each sampling site using MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). To check for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium, the heterozygote deficiency test on GENEPOP on the Web (http://genepop.curtin.edu.au/) was carried out for each of the 13 microsatellite loci in each population, with the number of batches set at 100 and dememorization number and iterations per batch set at 1000. For tests of HWE and linkage disequilibrium, the sequential Bonferroni correction was applied to correct probability values for multiple comparisons (Rice 1989). Sample pairs with matching multilocus genotypes were tested for the presence of duplicate samples and also used to estimate the average probability of identity per locus (*PI*) as well as *PIsibs* (Probability of Identity for genetic similarity among siblings) in GenAlEx v. 6.3 (Peakall & Smouse 2006) and the list of sample pairs matching at all loci were removed before analyses were conducted. Four duplicated samples (2 from TB, 1 from SHB and 1 from LB) were found to have exactly the same sex, mtDNA control region sequence and microsatellite profiles across all loci, suggesting they were re-sampled individuals; therefore, they were removed from both mtDNA and microsatellite datasets, resulting in a final total of 395 individuals.

Dispersal patterns

Sex-biased dispersal was examined using the program GenAlEx v. 6.3 (Peakall & Smouse 2006), where for each individual a log-likelihood assignment index correction (*AIc*) value was calculated. A difference in the frequency distribution of *AIc* values among males and females indicates sex-biased dispersal, which was assessed using the Mann–Whitney *U*-test in GenAlEx. *AIc* values average zero for each population, while negative values characterize individuals with a higher probability of being immigrants. For comparison, FSTAT (Goudet 1995) was also used to estimate and compare F_{IS}, F_{ST}, H_O, H_S, mean assignment and the variance of mean assignment.

Population structure

Mitochondrial DNA data

To examine the level of genetic population structure among the localities using the mitochondrial control region data, an analysis of molecular variance (AMOVA: F_{ST} and Φ_{ST}) was performed in Arlequin 2.0 (Schneider *et al.* 2000) and *P*-values were obtained using 10 000 permutations on all 3 datasets.

Relationships among mtDNA haplotypes were investigated using the default parameters of median-joining

networks in Network 4.6 (Bandelt *et al.* 1999). Mantel's test was used to detect correlation between a matrix of pairwise genetic distances and geographic distances (Smouse *et al.* 1986; Mantel for Windows 1.11 [Calvalcanti 2000]). To obtain confidence for the Mantel test, 10 000 randomizations were performed and the axes regressions were performed by the 2-D model, using a linearized fixation index: $(F_{ST}/(1 - F_{ST}))$ plotted against the logarithm of distance (Rousset & Raymond 1997). The geographic distance was estimated using ArcGIS to measure the linear distances between the centers of each sampling site.

A Bayesian clustering approach based on a spatial model in Geneland 4.0.3 was used on the mitochondrial control region data to infer the number of populations and their spatial extent (Guillot *et al.* 2005a,b, 2008; Guillot 2008) in the program R v. 2.13.1 (R Development Core Team 2011). The model uses both haplotypes and spatial coordinates of sampled individuals to cluster them into populations (Guillot *et al.* 2008). For this analysis, an allele frequency correlated model was used, with 100 000 Markov Chain Monte Carlo (MCMC) iterations and thinning of 100, maximum number of nuclei in the Poisson–Voronoi tesselation fixed to 790, and the spatial coordinates considered as true coordinates, with 15 independent runs, with the number of populations set to $1 \leq K \leq 7$ to determine convergence. For comparison, a spatial analysis of molecular variance (SAMOVA) was performed to further examine the population structure (Dupanloup *et al.* 2002).

Microsatellite data

For the microsatellite data, an AMOVA was run in Arlequin 2.0 to examine population structure among sampling locations. The AMOVA was run using F_{ST} and also R_{ST}, which is preferred for microsatellite data because a step-wise mutation model is assumed (Slatkin 1995). The difference between those 2 models is that F_{ST} takes allele frequencies into account, whereas R_{ST} takes into account both allele frequencies and genetic distance.

Population structure was also examined under a spatial model using the Geneland 4.0.3 package in program R v. 2.13.1 (R Development Core Team 2011) and convergence was evaluated by running the same parameters multiple times until the outcome of the multiple runs looked similar. For comparison, the Bayesian clustering method implemented in STRUCTURE 2.3.1 (Pritchard *et al.* 2000) was used to test the assignment of individual samples to genetic clusters. This method does not take into account spatial data but applies the MCMC method to evaluate the likelihood of different subgroups and estimates the most probable number of putative populations (K) that best explains the pattern of genetic variability. The analysis was run using the admixture and correlated allele frequency model with a burn-in length and length of simulation set at 100 000 iterations, respectively. The STRUCTURE analysis was initially run with $K = 1$ to 7, 10 and 15 populations, respectively, to determine which setting would be most suitable for determining genetic clusters in our data. In the end, the STRUCTURE analysis was run with $K = 1$ to 10, and to check for convergence of the Markov chain parameters, 15 replicate runs for each K were performed with the number of populations set to $1 \leq K \leq 10$.

To detect the true number of clusters (K) in the dataset, ΔK was calculated (Evanno *et al.* 2005) from the rate of change in the log probability of data between successive K values, using the program R v. 2.13.1 (R Development Core Team 2011). STRUCTURE assumes a model in which there are K populations, each of which is characterized by a set of allele frequencies at each locus and individuals in the sample are assigned (probabilistically) to populations, or jointly to 2 or more populations if their genotypes indicate that they are admixed (Pritchard *et al.* 2009) as depicted by our dataset. According to Pritchard *et al.* (2009), estimating the number of populations using STRUCTURE should be treated with caution for 2 reasons: (i) it is computationally difficult to obtain accurate estimates of $\Pr(X/K)$ and this method merely provides an *ad hoc* approximation; and (ii) the biological interpretation of K may not be straightforward (Pritchard *et al.* 2009). We used ΔK (from Evanno *et al.* 2005) as an indicator of the signal strength detected by STRUCTURE and the peak modal value was used, as ΔK is more robust than STRUCTURE $\mathrm{Ln}(K)$ in detecting population structure (Evanno *et al.* 2005).

Population demographics

Tajima's D (Tajima 1989) and Fu's F_S test (Fu 1997) were used on the mitochondrial control region data to examine departures from neutrality at each sampling site, which may indicate population bottlenecks or expansions. The examination of deviation from neutrality by both tests was based on 1000 coalescent simulations with consideration of the recombination rate implemented in Arlequin 2.0 (Schneider *et al.* 2000). Expectations of these statistics are nearly zero in a constant size population, whereas significant negative values may point to a recent expansion in population size, and significant positive values may indicate population fragmenta-

tion or recent population bottlenecks (Tajima 1989; Fu 1997). The possibility of demographic change was also investigated with mismatch distributions using the mitochondrial control region data, by comparing the distribution of pairwise differences among the haplotypes for all individuals, with the expected distribution under a model of demographic expansion (e.g. stationary or expanding populations) using Arlequin (Harpending *et al.* 1998; Schneider *et al.* 2000).

To test for evidence of a genetic bottleneck, the heterozygote excess method (Luikart *et al.* 1998) was implemented within the program BOTTLENECK version 1.2.02 (Piry *et al.* 1999). Populations that have undergone bottlenecks exhibit a correlation reduction of the allele number and heterozygosity at polymorphic loci (Piry *et al.* 1999). The 2 phase model (TPM) comprised 95% single step mutations and 5% multiple step mutations for which the variance for mutation size was set to 12 as suggested by Piry *et al*. (1999). Altogether, 10 000 simulations were run. To determine if the number of loci exhibiting heterozygosity excess was significant, the 1-tailed Wilcoxon signed rank test for heterozygote excess was applied. To determine time of expansion ($\tau = 2t\mu$), the following mutation rate was used: 2.3×10^{-3} (Nabholz *et al.* 2008a), where t = time and μ = mutation rate.

Results

Genetic diversity

A 580-bp fragment of the mitochondrial control region was successfully amplified from 395 skin biopsies of Heaviside's dolphins (Table 1). There were 94 transitions, 20 transversions and 0 indels, and the transition:transversion ratio estimate was 4.7. There were 19 parsimony informative and 49 variable sites detected, which defined 51 different haplotypes (GenBank Numbers KX260272-KX260322). The overall mtDNA haplotype (h = 0.9298, SE = 0.005) and nucleotide diversities (π = 0.0065, SE = 0.004) differed by 2 orders of magnitude for the combined dataset (Table 1). Estimates of haplotype diversity were lowest in TB at the southern extreme of the species' range, with the highest haplotype diversity found in HKB. All regions had private haplotypes ranging from just 1 in LB to 8 in both TB and WB. Although nucleotide diversity was also lowest in TB, the values were comparable to those from the other sites. The h and π values were similar for both sexes at all locations (Table 1).

Table 1 Genetic diversity estimates of mtDNA control region sequences including mean haplotype diversity (h) and nucleotide diversity (π) of female (F) and male (M) Heaviside's dolphins in 7 sampling locations off the southwest coast of Africa

					All samples		Females		Males	
Location	n	M	F		h (95% CI)	π (95% CI)	h (95% CI)	π (95% CI)	h (95% CI)	π (95% CI)
Table Bay (TB)	54	23	31		0.7177 (0.6600–0.7754)	0.0030 (0.0010–0.0050)	0.7828 (0.7212–0.8444)	0.0033 (0.0011–0.0055)	0.6126 (0.5079–0.7173)	0.0026 (0.0008–0.0044)
St. Helena Bay (SHB)	55	28	27		0.8364 (0.8082–0.8646)	0.0075 (0.0033–0.00117)	0.8547 (0.0397–0.8944)	0.0070 (0.0030–0.011)	0.8360 (0.7909–0.8811)	0.0081 (0.0036–0.0126)
Lambert's Bay (LB)	63	31	32		0.7706 (0.7278–0.8134)	0.0070 (0.0031–0.0109)	0.7782 (0.7294–0.827)	0.0073 (0.0032–0.0114)	0.7527 (0.6770–0.8284)	0.0067 (0.0028–0.0106)
Hondeklipbaai (HKB)	40	20	20		0.9090 (0.8872–0.9308)	0.0065 (0.0028–0.0102)	0.8526 (0.7919–0.9133)	0.0052 (0.0020–0.0084)	0.8579 (0.7956–0.9202)	0.0065 (0.0027–0.0103)
Port Nolloth (PN)	66	25	41		0.9016 (0.8839–0.9193)	0.0058 (0.0024–0.0092)	0.8939 (0.8689–0.9189)	0.0058 (0.0024–0.0092)	0.9300 (0.9016–0.9584)	0.0060 (0.0025–0.0095)
Luderitz (LDZ)	62	23	39		0.7409 (0.6981–0.7837)	0.0036 (0.0013–0.0059)	0.7328 (0.6806–0.785)	0.0034 (0.0012–0.0056)	0.7708 (0.6995–0.8421)	0.0042 (0.0016–0.0068)
Walvis Bay (WB)	55	22	33		0.8949 (0.8707–0.9191)	0.0058 (0.0024–0.0092)	0.8669 (0.8172–0.9166)	0.0058 (0.0024–0.0092)	0.9328 (0.9058–0.9598)	0.0057 (0.0023–0.0091)
Overall	395	172	223		0.9298 (0.9253–0.9343)	0.0065 (0.0029–0.0101)	0.9285 (0.9222–0.9348)	0.0062 (0.0027–0.0097)	0.9310 (0.9237–0.9383)	0.0068 (0.0030–0.0106)

n represents the overall sample size per location (males and females combined).

There were 27 unique haplotypes that had single site changes, but all these had electropherograms that yielded strong signals, distinct peaks and little to no noise so these mutations were considered informative. However, to ensure that these single site changes could not impact the analysis in any way, haplotype and nucleotide diversities were estimated and the median-joining network was also constructed without these single site mutations (Table S2, Fig. S1). Minor discrepancies were found and because these differences did not affect any of our further interpretations, all 27 unique haplotypes were kept in all the analyses that follow.

For the microsatellite data, high levels of polymorphism (2–17 alleles/13 loci) were found across 13 loci. No evidence was found for large allele dropouts among the 13 loci, although 1 locus (SCA22) showed evidence of null alleles and was, therefore, removed from the dataset. Data from most of the sampling sites, except for Table Bay (SCA37, Ttr63 and Ev14) and St. Helena Bay (Ttr 63 and Ev14), showed no evidence for null alleles or scoring error due to stuttering. One sample obtained from Walvis Bay (CH53WB) could not be amplified at 2 loci (SCA54 and Dde66) and was also removed from the dataset. The average gene diversity for expected heterozygosity estimates over loci, calculated in Arlequin, for the 12 remaining microsatellite loci ranged from 0.6437 (± 0.3325) in St. Helena Bay to 0.7281 (± 0.3725) in Luderitz for the sexes-combined dataset (Table 2). Similar diversity estimates were found when males and females were analyzed separately (Table 2). The number of alleles per locus ranged from 2 to 17 (Table S3), and observed heterozygosity ranged from 0.236 to 1.00, which depicts varying levels of polymorphism found at all loci (Table S1). The probability of identity (*PI*) per locus (mean *PI* = 0.1362) was smaller than the *PIsibs* (mean *PIsibs* = 0.4257), which takes into account the genetic similarity among siblings (Table S1). Following Bonferroni correction ($P = 0.05/13$, Rice 1989), all 13 loci conformed to Hardy–Weinberg expectations, and did not show evidence of linkage disequilibrium. Similarly, there was no indication of linkage disequilibrium when examined by location.

Dispersal patterns

In total, samples from 173 males and 222 females were analyzed. AMOVA done on the mtDNA data for separate sexes revealed significant differences overall for F_{ST} and Φ_{ST}, rejecting the null hypothesis of panmixia F_{ST} (females: 0.133, $P < 0.001$; males: 0.146, $P < 0.001$; Appendix Ia) and Φ_{ST} (females: 0.160, $P < 0.001$; males: 0.165, $P < 0.001$; Appendix Ib). Microsatellite data revealed no indication of significant sex-biased dispersal for F_{ST} values (F_{ST} for males = 0.028, $P = 0.000$, females = 0.022, $P = 0.000$; Appendix II). Assignment index correction (*AIc*) tests were not significant for both the mean (males *mAIc* = 0.150, females *mAIc* = −0.117) and variance values (males *vAIc* = 0.150, females *vAIc* = 0.140, Fig. 2) despite the low negative value of *mAIc* for females. The Mann–Whitney *U*-test also revealed no indication of "sex bias" in dispersal between either sex ($Z = 1.578$, $P = 0.114$). All parameters analyzed using the FSTAT program also did not show any significant difference between males and females (Table S4). Because no statistical indication of sex-biased dispersal differences were found between the sexes, all following analyses were performed on a combined sex dataset to increase statistical power.

Table 2 Microsatellite average genetic diversity over loci (expected heterozygosity) estimates and standard errors (±) for Heaviside's dolphins sampled off the coast of Namibia and west coast of South Africa in 2009–2012

Location	*n*	M	F	Gene diversity		
				All samples	Females	Males
Table Bay	54	23	31	0.6538 (0.3164 ± 0.9912)	0.6650 (0.3198 ± 1.0102)	0.6389 (0.3043 ± 0.9735)
St. Helena Bay	55	28	27	0.6437 (0.3112 ± 0.9762)	0.6546 (0.3135 ± 1.6473)	0.6356 (0.3039 ± 0.9673)
Lambert's Bay	63	31	32	0.7040 (0.3431 ± 1.0649)	0.7097 (0.3432 ± 1.0762)	0.7036 (0.3398 ± 1.0674)
Hondeklipbaai	40	20	20	0.7101 (0.3446 ± 1.0756)	0.7150 (0.3423 ± 1.0877)	0.7144 (0.3420 ± 1.0868)
Port Nolloth	66	25	41	0.7036 (0.3430 ± 1.0642)	0.7046 (0.3418 ± 1.0674)	0.7069 (0.3401 ± 1.0737)
Luderitz	62	23	39	0.7281 (0.3556 ± 1.1006)	0.7307 (0.3553 ± 1.1061)	0.7246 (0.3481 ± 1.1011)
Walvis Bay	55	22	33	0.7246 (0.3535 ± 1.0957)	0.7287 (0.3530 ± 1.1044)	0.7246 (0.3485 ± 1.1007)

Total number of female (F) and male (M) samples per sampling site and the overall total number of individuals sampled (*n*) are shown.

Population structure

This study used 2 different approaches for each gene marker to test for population genetic structure. For the mtDNA data, SAMOVA suggested 2 putative populations (TB *vs* SHB, LB, HKB, PN, LDZ, WB) while Geneland indicated 6 putative populations (TB *vs* SHB *vs* LB *vs* HKB, PN *vs* LDZ *vs* WB). For the microsatellite data, testing for population structure was conducted using Geneland and STRUCTURE. Geneland gave inconclusive results (not shown); however, STRUCTURE suggested a southern (TB and SHB) and a northern population (LB, HKB, PN, LDZ and WB). Details for all the analyses conducted for each genetic marker are given below.

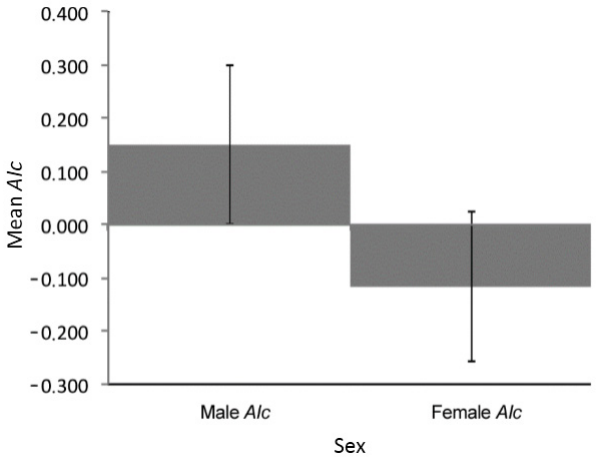

Figure 2 Average corrected assignment index (*AIc*) for male and female Heaviside's dolphins sampled at 7 locations off the Namibian and west South African coast between 2009 and 2012. Grey shading indicates the mean *AIc* value and standard errors are indicated by the black bars.

Mitochondrial DNA data

The substitution model that best fit the data was Hasegawa–Kishino–Yano (HKY, Hasegawa *et al.* 1985), with a gamma correction value of 0.94 for both sexes combined. The results of AMOVA for the combined sexes indicated a significant amount of overall genetic differentiation among the Heaviside's dolphins for both F_{ST} (0.134, $P < 0.0001$; Table 3a) and Φ_{ST} (0.156, $P < 0.0001$; Table 3b). Among a total of 21, 16 Φ_{ST} pairwise comparisons were significantly different before Bonferroni correction, with values ranging from 0.038 to 0.409. F_{ST} pairwise comparisons performed on the combined-sexes dataset were significantly different between all sites except for HKB to PN (Table 3a).

Table 3 Pairwise *F*-statistics for Heaviside's dolphins from 7 sampling sites off the coast of Namibia and west coast of South Africa: (a) F_{ST} for microsatellites (above diagonal) and mtDNA control region (below diagonal); and (b) R_{ST} for microsatellites (above diagonal) and Φ_{ST} for mtDNA control region (below diagonal). Significance of *P*-values: *≤0.05, **≤0.001, ***≤0.0001

	N	Table Bay	St. Helena Bay	Lambert's Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
a								
All Samples		54	55	63	40	66	62	55
Table Bay	54	—	0.01626***	0.03484***	0.04871***	0.03300***	0.02618***	0.03769***
St. Helena Bay	55	0.16525***	—	0.03601***	0.04463***	0.02598***	0.02769***	0.03602***
Lambert's Bay	63	0.24733***	0.04017**	—	0.00957***	0.00902***	0.00907***	0.01635***
Hondeklipbaai	40	0.12769***	0.06459***	0.12081***	—	0.01734***	0.01107***	0.01782***
Port Nolloth	66	0.14002***	0.08924***	0.13147***	−0.00038	—	0.01229***	0.01363***
Luderitz	62	0.24804***	0.14192***	0.21903***	0.11232***	0.10467***	—	0.01021***
Walvis Bay	55	0.18555***	0.12858***	0.16443***	0.07677***	0.06269***	0.14504***	—
b								
All Samples		54	55	63	40	66	62	55
Table Bay	54	—	0.00467	0.03043***	0.03937***	0.04439***	0.02365**	0.01597**
St. Helena Bay	55	0.27704***	—	0.02025*	0.02453***	0.02437***	0.01732*	0.01360*
Lambert's Bay	63	0.40879***	0.03787*	—	0.01661*	0.02236***	0.00714	0.01457**
Hondeklipbaai	40	0.29759***	0.01836	0.10119***	—	0.01097	0.01761	−0.00278
Port Nolloth	66	0.25522***	0.01926	0.08927***	0.00147	—	0.03836***	0.01897***
Luderitz	62	0.30517***	0.12434***	0.26535***	0.10312***	0.12682***	—	0.01083*
Walvis Bay	55	0.36091***	0.04205*	0.07171***	0.02978	0.01751	0.22718***	—

The SAMOVA for the combined-sexes dataset suggested that the 7 sampling sites were best partitioned into 2 populations (K = 2, F_{CT} = 0.197): the northern population, consisting of 6 of the sampling sites (SHB, LB, HKB, PN, LDZ and WB), and the southern population, represented by only 1 site (TB). The Bayesian clustering method based on the correlated spatial model in Geneland suggested that there are at least 6 populations, with each sampling site as a distinct population except for HKB and PN (Fig. 3), which concurs with the F_{ST} pairwise comparisons. The Mantel test analysis across the entire sampling area based on mitochondrial DNA control data suggested no correlation between genetic diversity and linear geographic distance for all samples (r = 0.34, P = 0.88; Appendix III).

The median-joining network shows the relationships among 51 unique haplotypes (GenBank accession numbers KX260272 – KX260322) across all samples (Fig. 4). Five haplotypes (TBH1, TBH8, SHB1, SHB15 and LBH24) were by far the most frequent, occurring in 43 (11%), 56 (14%), 42 (11%), 43 (11%) and 24 (7%) of the samples, respectively. Although these haplotypes were found across all regions, they occurred in strikingly different frequencies depending on locality (Fig. 1, Table S5). Aside from the differing frequencies of the common haplotypes, the network did not show any other obvious pattern relating to population structure (Fig. 4).

Microsatellite data

Genetic differentiation of the microsatellite data using AMOVA among all 7 sampling sites was estimated using pairwise F_{ST} and R_{ST}. The results obtained for both estimators (F_{ST} = 0.024, P = 0.000; R_{ST} = 0.020, P = 0.000) differed with respect to the relative levels of specific pairwise population differentiation comparisons, with statistically significant levels of genetic variation found only for F_{ST} across all localities, while R_{ST} produced fewer significant differences (Table 3).

There was no substantial population structure as assessed with the correlated model in Geneland (results not shown). In contrast, analysis with STRUCTURE using the admixture model provided weak support for 2 distinct populations for all samples (southern: TB and SHB, and northern: LB, HKB, PN, LDZ and WB; Fig. 5). This was further confirmed by examining the number of clusters (K) in the dataset, using ΔK (Evanno *et al.* 2005; Table S6, Figs S2-S4). Based on the bar plot gen-

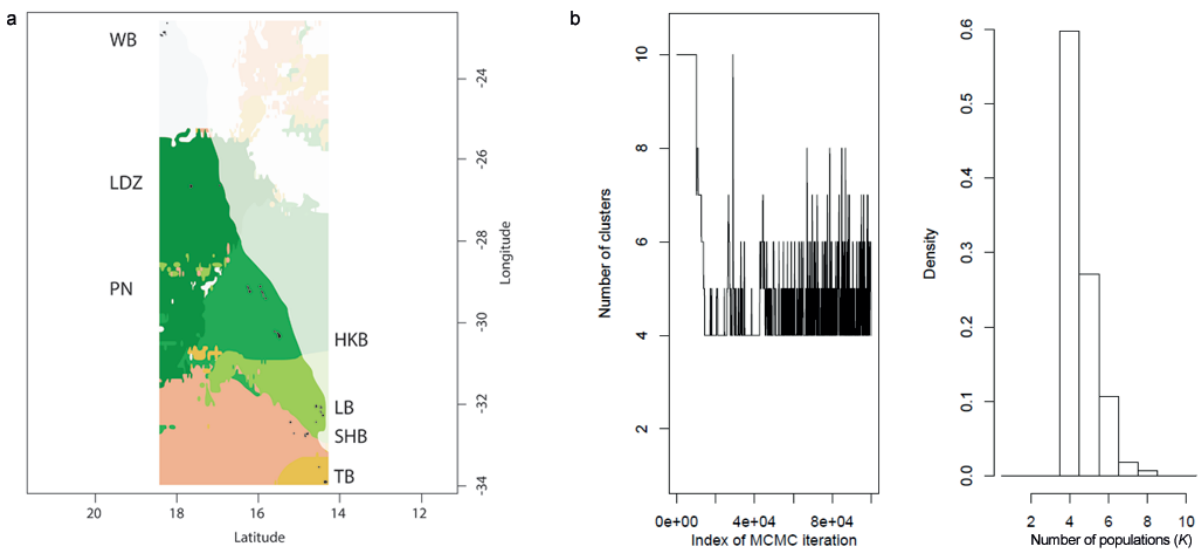

Figure 3 (a) Six populations of Heaviside's dolphins off the southwest coast of southern Africa defined by Bayesian clustering method based on mitochondrial data and the correlated spatial model in Geneland. Darker and lighter colors indicate the posterior probability distribution with each color belonging to a different population, and lighter colored areas showing the highest posterior probabilities of clusters. Black dots represent the relative positions of the sampled individuals. (b) The number of populations simulated from the posterior distribution.

erated with STRUCTURE, the inferred proportion of individuals assigned to each of the 2 clusters suggests that the dolphins sampled in TB and SHB represent 1 cluster, while all those sampled in the other 5 locations (LB, HKB, PN, LDZ and WB) form another cluster (average membership q-value to cluster two = 0.58). The propor-

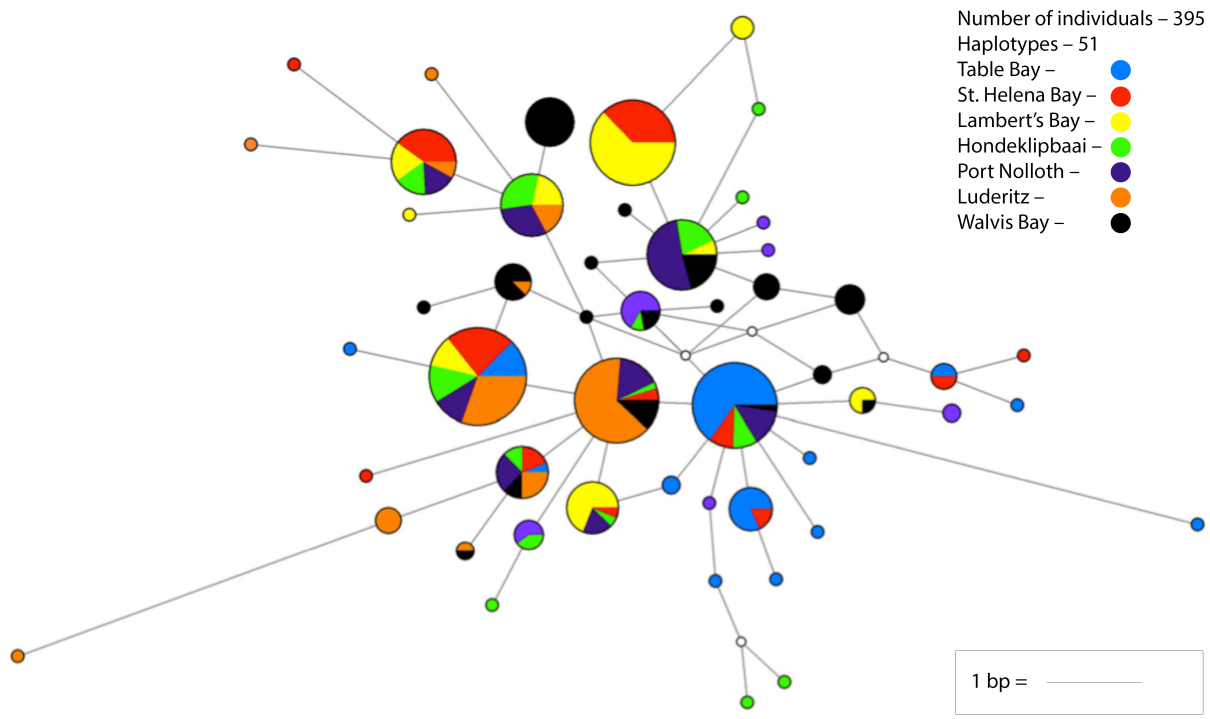

Figure 4 Median-joining network of mtDNA control region haplotypes for Heaviside's dolphins found off the southwest coast of southern Africa. The size of the circles is proportional to the frequency in which each haplotype occurs, and the length of the branches is proportional to the number of base changes between haplotypes. The shortest branches indicate 1 base change. Haplotypes are color coded according to the frequency in which they occur at each sample site. White circles represent missing haplotypes.

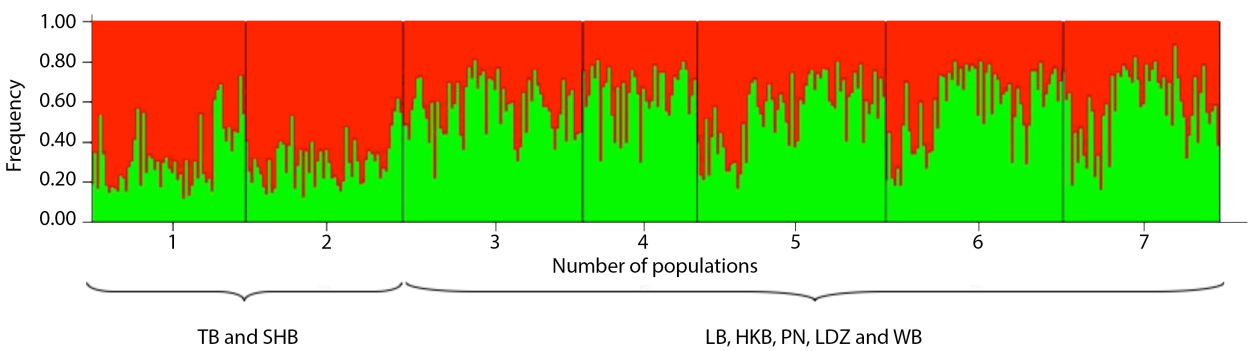

Figure 5 Bayesian assignment probabilities for Heaviside's dolphins off the southwest coast of Africa inferred using the program STRUCTURE. Each vertical line across the x-axis corresponds to a single individual and shading represents the proportional membership coefficient (y-axis) of that individual to each of 2 clusters.

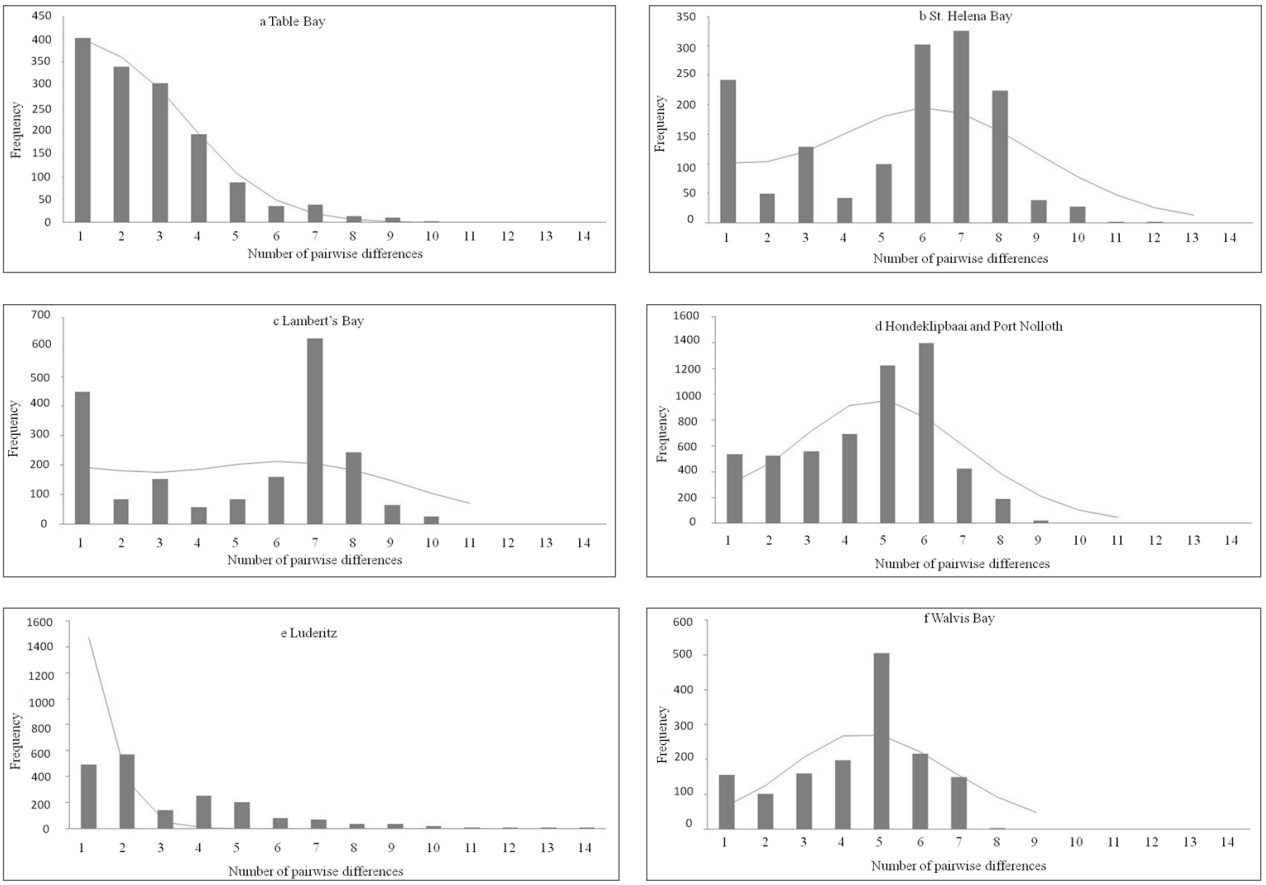

Figure 6 Observed (bars) and expected (line) mismatch distributions for putative populations of Heaviside's dolphins off the Namibian and west South African coast, defined by the mtDNA control region: (a) Table Bay, (b) St. Helana Bay, (c) Lambert's Bay, (d) Hondeklipbaai and Port Nolloth, (e) Luderitz and (f) Walvis Bay.

tion of individuals from HKB was high enough to distinguish it from TB and SHB (Table S6, Figs S2-S4). An additional analysis was run whereby each sampling site was considered a population with Hondeklipbaai and Port Nolloth as 1 population. The bar plot generated by STRUCTURE inferred 6 clusters as defined by mtDNA AMOVA and Geneland (Fig. S4). The Mantel test analysis across the entire sampling area based on microsatellite data suggested no correlation between genetic diversity and linear geographic distance for all samples (r = 0.17, P = 0.75; Appendix IIIb).

Population demographics

The mismatch distribution using the mtDNA control region for the 6 populations defined by Geneland showed 2 distinct patterns (Fig. 6). Mismatch distributions for SHB and LB represented by a multimodal distribution suggest that these populations may be in equilibrium. The mismatch distributions of the remaining populations, TB, WB and HKB/PN, suggest a recent expansion, likely after the last glacial maxima, given the unimodal distribution (mean *Tau* value = 3.59). Fu's F_S test for TB was significant, which suggests that the population is out of mutation–drift equilibrium and further supports a recent demographic shift (Table Bay: F_S = −5.397; P = 0.008, Tajima's D = −2.036; P = 0.005), whereas Fu's F_S test was not significant for Walvis Bay (F_S = −4.749; P = 0.04). This was not the case for HKB/PN, which had non-significant values for both estimates (F_S = −3.541; P = 0.138, Tajima's D = −0.075; P = 0.546). The remaining populations did not show significant departures from equilibrium, which is consistent with the multimodal nature of the mismatch distribution.

In BOTTLENECK analysis performed on the north and south populations as suggested by STRUCTURE, the Wilcoxon signed rank tests under mutational 2 phase model (TPM) produced non-significant results for the southern population (Wilcoxon test, $P = 0.6576$) but significant results for the northern population (Wilcoxon test, $P = 0.0002$), which indicates that this population may have undergone a recent bottleneck. BOTTLENECK analysis performed on the Table Bay population delivered a non-significant outcome (Wilcoxon test, $P = 0.9836$), confirming the mismatch distribution results of a recent expansion. The time of expansion of the Table Bay population was estimated at approximately 52 608 years ago using the mismatch distribution *Tau* value (*Tau* value = 2.42), whereas the time of expansion of the northern (*Tau* value = 5.07) and southern populations (*Tau* value = 4.28) were calculated at approximately 110 118 and 93 028 years ago, respectively.

Discussion

Application of two commonly used genetic markers, the mitochondrial DNA control region and microsatellites, allowed an assessment of genetic diversity, dispersal patterns and population genetic structure of the Heaviside's dolphin across a large portion of the species' range. Assignment index correction (*AIc*) tests suggested lack of sex-biased dispersal. The assignment index method calculates the log-likelihood of each individual's genetic assignment to its population, corrected by the mean log-likelihood of assignment for the population. Positive values are associated with residents, while negative values indicate rare genotypes that are more likely to be associated with immigrants. Sex-biased dispersal was assessed by comparing mean and frequency distribution of assignment bias (*AIc*) for males versus females, without specifying sampling location or putative population of samples. If dispersal was sex-biased, the dispersing sex would have displayed lower *AIc* than the philopatric sex (Goudet *et al.* 2002). In our data, the assignment index correction displayed a different mean and range between sex groups (Fig. 2), but the pattern did not pass the test for statistical significance. Consequently, although some differences between male and female patterns of dispersal cannot be ruled out (with gene flow possibly stronger in males, as known for most marine mammals), the difference indicated by our data was minor and failed to be detected by all the statistical tests performed in our study.

In contrast to the only previous genetic study of this species, which used only mitochondrial DNA and a small sample size (Jansen van Vuuren *et al.* 2002), our findings suggest a measurable genetic structure among the Heaviside's dolphins off the southwest coast of southern Africa. Using SAMOVA, the mitochondrial DNA control region indicated 2 genetically differentiated populations (TB *vs* SHB, LB, HKB, PN, LDZ, WB) among the 7 sampling sites, while analyses with Geneland indicated 6 putative populations (TB *vs* SHB *vs* LB *vs* HKB, PN *vs* LDZ *vs* WB), suggesting further sub-structure within each of these groups. The microsatellite data, based on analyses using STRUCTURE, provided a similar and not mutually exclusive signal, when compared to mtDNA Geneland, clustering the dolphins into a northern population (LB, HKB, PN, LDZ and WB) and a southern population that included the animals from Table Bay and St. Helena Bay. Overall, there appear to be 2 primary populations (northern and southern), but within each there is some limited structure suggesting that gene flow is not entirely ubiquitous between sites.

Genetic variability of populations is shaped by historical events (bottlenecks, range expansion and zone of admixture), ecological and demographic factors (size and age of populations, and social structure) as well as environmental features that influence gene flow. Consequently, present patterns of genetic structuring are often reflective of population structure evolution (Pimper *et al.* 2010; Cipriano *et al.* 2011; Coscarella *et al.* 2011). In the case of Heaviside's dolphins, population genetic structure was detectable through clustering analyses (Geneland and STRUCTURE). However, when AMOVA was conducted, none of the F_{ST} values were high for either marker. Furthermore, F_{ST} values between northern and southern populations were not appreciably higher than those for dolphins sampled at different sites within the 2 putative populations, suggesting that the population structure for the northern and southern putative population is weak (Table 3). Within both northern and southern populations, low genetic differentiation among samples obtained at different sites (i.e. bays) resulted in a high degree of admixture (Fig. 5) indicating that there is gene flow along the coast. Consequently, there appears to be a dynamic meta-population system with a varied degree of connectivity across a greater geographic scale and increased insularity at the peripheries of the current extent of our sampling effort, especially the southern periphery of the species' range.

Despite the grouping of the genetic material from Table Bay with that from St. Helena Bay for the microsatellites, the Table Bay population stands out as distinct for the mitochondrial marker. Furthermore, mitochon-

drial haplotype and nucleotide diversity values were much lower in the dolphins from Table Bay than all the other sites, possibly because this represents the extreme southern limit of the species' range. If the species has only recently expanded into Table Bay, a leading edge effect would be expected where diversity is low, as found in this study. Moreover, the star-shape pattern of the haplotype network for Table Bay is indicative of a population expansion, which is supported by the mismatch distribution and neutrality tests. Thus, the results suggest a founder event precipitated by a southward expansion into Table Bay.

Interesting to note is that the Agulhas Current retroflection has been shown to be unstable, and at irregular intervals it forms eddies called Agulhas "rings" or "loops" that move off into the South Atlantic (Shannon *et al.* 1989). These Agulhas eddies extend through the entire water column in the Cape Basin (offshore from Table Bay), where they split, join or disperse with other eddies (Boebel *et al.* 2003). The conversion between cold and warm water may influence the distribution and abundance of cetaceans (Cockcroft *et al.* 1990); and the edge region of eddies represents the area where nutrients and phytoplankton upwelled in the eddy's center converge, known to represent a fruitful foraging ground for species such as tuna, seabirds and cetaceans (Seki *et al.* 2001; Davis *et al*. 2002; Bakun 2006; Yen *et al*. 2006; Woodworth *et al*. 2012). Such an oceanographic feature at the southern outer limit of the Heaviside's dolphin range could be a factor that has contributed to the population expanding south into a region where greater abundance of food may be facilitated by the Agulhas eddies or where food resources are brought by the eddies closer inshore.

Studies of Hector's dolphin suggest that population isolation may be a result of ecological preferences and strong phylopatry, as indicated by striking genetic differences between Hector's dolphin populations over short distances (Hamner *et al.* 2012). In contrast, Heaviside's dolphins appear to be quite different, with little genetic structure and no sex-biased dispersal, suggesting that this species is less philopatric than its congener. Alternatively, gene flow may take place when Heaviside's dolphins from different inshore locations meet while foraging offshore. Heaviside's dolphins exhibit a diel inshore–offshore movement pattern, with animals travelling to shallow inshore waters in the early morning for resting and socializing, and moving offshore in the afternoon, reaching the furthest offshore distance around midnight (Elwen *et al*. 2006). As these dolphins feed mainly on bottom-living prey such as juvenile hake that migrate towards the surface at night (Sekiguchi *et al.* 1992), it is believed that the offshore movement may be timed to coincide with this increase in availability of their primary prey.

The high degree admixture as indicated by our microsatellite analyses seems to challenge an earlier finding where 5 female Heaviside's dolphins tracked with satellite transmitters over 54 days showed a limited movement of up to 60 km along shore and 20 km offshore (Elwen *et al.* 2006). Whether this movement pattern indicates long-term site fidelity is not known, but similar spatial scales of movement have been observed for the Maui dolphin (Oremus *et al.* 2012), with occasional movements up to 80 km; and >400 km for Hector's dolphins (Hamner *et al.* 2014). The confounding factor may be the very different time scales of these 2 lines of evidence. Tagging studies operate in short time scales, showing the degree of movement of an individual(s) over the period of days, weeks or months. In contrast, genetic data are representative of long-term patterns of the entire population (or species) over evolutionary time scales. It may be possible that individuals are relatively philopatric in the short term, but over larger spatiotemporal scale the ranging pattern of a population may be far more dynamic. Resources are abundant along the southwest coast of Africa, with juvenile hake, the main prey of Heaviside's dolphins (Sekiguchi *et al.* 1992) are plentiful in relatively deep waters throughout the year (Prochaska *et al.* 2014). This may provide little need for individuals to engage in long-range movements along the coast or offshore, with the concomitant possibility for spatially restricted resource specialization (Hoelzel 1998) as a mechanism for genetic differentiation. Genetic differentiation reflecting resource specialization has been observed in a wide range of marine mammals, as a result of physical separation within a local environment (Hoelzel *et al.* 1991, 1993, 2007; Mead & Potter 1995; Natoli *et al.* 2008) and is considered a primary driver of population structure in marine environments.

When close inshore, Heaviside's dolphins predominantly socialize and rest, preferring sheltered coastline with abundant kelp beds (Behrmann 2011). However, as much of the southwestern African shore consists of high energy coastline, protected bays and sheltered stretches of coast may represent an important limiting environmental feature, as observed for other coastal dolphins off southeast Africa (e.g. Karczmarski 1999; Karczmarski *et al*. 2000). Given that TB and SHB are located on the southern edge of the species distribution range and are exposed to highly complex coastal dynamics (e.g. pattern of currents), the dolphins may exhibit relative-

ly stronger philopatry as a result of local conditions. Whether this leads to localized site fidelity and affects population structure, as has been suggested for other *Cephalorhynchus* species (Bejder & Dawson 2001; Parsons *et al.* 2006; Mirimin *et al.* 2011), remains to be tested.

A final consideration is the somewhat dissimilar patterns observed for the 2 molecular markers used in this study. One explanation is that F_{ST} estimators are subject to bias even though species with large population numbers (estimated at approximately 6345 individuals between TB and LB; Elwen *et al.* 2009) may not be in mutation–drift equilibrium. Following a population expansion event, as suggested for dolphins in Table Bay, mtDNA haplotype frequencies should return to equilibrium more quickly and show in general greater structure than microsatellite loci (Crow & Aoki 1984), potentially producing a disparity in the observed population structure. Furthermore, selective sweeps and genetic hitchhiking for mitochondrial DNA could cause differential patterns between markers (Meiklejohn *et al.* 2007; Galtier *et al.* 2009). Sample sizes can also be an issue, given that microsatellites require larger sample sizes to characterize allele frequencies (Kalinowski 2005), and homoplasy can reduce the signal of differentiation detected by the microsatellite markers (Balloux *et al.* 2000; Estoup *et al.* 2002).

In summary, the use of multiple genetic markers, wide geographic coverage and reasonably large sample sizes have unraveled the pattern of genetic diversity of this coastal delphinid. This study has for the first time covered a large geographic range of Heaviside's dolphins, and while a considerable assessment of the population genetic structure was achieved, further work is still needed to better understand the socio-demographic and historic processes that shape this population structure, and to establish proper management strategies and conservation measures. For that reason, utilizing population genetics in assessing the levels of risk faced by local populations is essential for monitoring the effectiveness of management and the long-term biological viability of this endemic species.

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Supplementary material

Table S1 Summary of genetic variation based on 16 microsatellite loci in the Heaviside's dolphin: *Ta* stands for annealing temperature, bp for allele sizes, and *Na* 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

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

Table S3 Summary of genetic variation based on 13 microsatellite loci in the Heaviside's dolphin: *Na* 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 (≤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}, H_O, H_S, mean assignment and variance assignment

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 S6 STRUCTURE clustering analysis: a. Proportion of Heaviside's dolphin individuals from each of the seven sampling locations assigned to each of the two clusters inferred from STRUCTURE 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* (*ΔK*) 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.

Appendix Ia Genetic differentiation between Heaviside's dolphins from 7 sampling sites off the Namibian and west South African coast, in terms of pairwise *F*-statistics for females and males separately

	N	Table Bay	St. Helena Bay	Lambert's Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
Females								
Table Bay	31	—	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.009 ± 0.009**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
St. Helena Bay	27	**0.12864**	—	**0.018 ± 0.033**	0.108 ± 0.033	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
Lambert's Bay	32	**0.21475**	**0.04457**	—	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
Hondeklipbaai	20	**0.10420**	0.03153	**0.14222**	—	0.054 ± 0.024	**0.000 ± 0.000**	**0.000 ± 0.000**
Port Nolloth	41	**0.10996**	**0.08200**	**0.13219**	0.03408	—	**0.000 ± 0.000**	**0.000 ± 0.000**
Luderitz	39	**0.22702**	**0.11773**	**0.21685**	**0.11438**	**0.10704**	—	**0.000 ± 0.000**
Walvis Bay	33	**0.17415**	**0.13407**	**0.17742**	**0.12345**	**0.08518**	**0.16335**	—
Males								
Table Bay	23	—	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
St. Helena Bay	28	**0.21135**	—	0.081 ± 0.034	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
Lambert's Bay	31	**0.30177**	0.02364	—	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
Hondeklipbaai	20	**0.24455**	**0.12045**	**0.15219**	—	0.063 ± 0.023	**0.000 ± 0.000**	**0.000 ± 0.000**
Port Nolloth	25	**0.19029**	**0.08065**	**0.12789**	0.03615	—	**0.000 ± 0.000**	0.072 ± 0.026
Luderitz	23	**0.28112**	**0.14831**	**0.21895**	**0.15520**	**0.08545**	—	**0.000 ± 0.000**
Walvis Bay	22	**0.20627**	**0.10958**	**0.15069**	**0.08020**	0.02449	**0.11301**	—

Below diagonal are the pairwise F_{ST} values and above the diagonal are the significant *P*-values estimated with AMOVA using mitochondrial DNA (mtDNA) frequency information obtained from the samples collected between 2009 and 2012. Values in bold font indicate significant estimates (≤ 0.05).

Appendix Ib Genetic differentiation between Heaviside's dolphins from 7 sampling sites off the Namibian and west South African coast, in terms of pairwise Φ_{ST} statistics for females and males separately

	Table Bay	St. Helena Bay	Lambert's Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
Females	31	27	32	20	41	39	32
Table Bay	—	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
St. Helena Bay	**0.25781**	—	0.189 ± 0.037	0.342 ± 0.040	0.162 ± 0.033	**0.000 ± 0.000**	**0.009 ± 0.009**
Lambert's Bay	**0.37095**	0.02035	—	**0.018 ± 0.012**	**0.009 ± 0.009**	**0.000 ± 0.000**	**0.009 ± 0.009**
Hondeklipbaai	**0.21293**	0.00093	**0.10291**	—	0.054 ± 0.015	0.108 ± 0.037	**0.000 ± 0.000**
Port Nolloth	**0.25171**	0.01853	**0.07175**	0.03518	—	**0.000 ± 0.000**	**0.027 ± 0.014**
Luderitz	**0.31627**	**0.10689**	**0.22909**	0.02395	**0.13008**	—	**0.000 ± 0.000**
Walvis Bay	**0.40208**	**0.07778**	**0.07409**	**0.14981**	**0.03504**	**0.26337**	—
Males	23	28	31	20	25	23	23
Table Bay	—	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
St. Helena Bay	**0.27591**	—	0.090 ± 0.023	0.054 ± 0.020	0.351 ± 0.053	**0.000 ± 0.000**	0.153 ± 0.030
Lambert's Bay	**0.44080**	0.03725	—	**0.009 ± 0.009**	**0.009 ± 0.009**	**0.000 ± 0.000**	**0.009 ± 0.009**
Hondeklipbaai	**0.42849**	0.05403	**0.14986**	—	**0.018 ± 0.012**	**0.000 ± 0.000**	**0.009 ± 0.009**
Port Nolloth	**0.24428**	-0.00073	**0.08949**	**0.07864**	—	**0.000 ± 0.000**	0.675 ± 0.031
Luderitz	**0.28010**	**0.11419**	**0.28581**	**0.23197**	**0.09771**	—	**0.000 ± 0.000**
Walvis Bay	**0.29664**	0.02276	**0.09096**	**0.09202**	–0.01429	**0.18708**	—

Below diagonal are the pairwise Φ_{ST} values while above the diagonal are the significant *P*-values estimated with AMOVA using mitochondrial DNA (mtDNA) haplotype frequency and genetic information obtained from samples collected between 2009 and 2012. Values in bold font indicate significant estimates (≤ 0.05).

Appendix II Pairwise comparison of Heaviside's dolphins sampled at 7 locations off the Namibian and west South African coast, based on 13 microsatellite loci. F_{ST} values are shown in the lower matrix and R_{ST} values in the upper matrix. Statistically significant results are shown in bold

	Table Bay	St. Helena Bay	Lambert's Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
Females							
Table Bay	—	0.00113	0.01132	**0.02947**	**0.04548**	**0.02667**	**0.01837**
St. Helena Bay	**0.01456**	—	0.00861	0.00595	0.01091	**0.02957**	0.00403
Lambert's Bay	**0.03070**	**0.02192**	—	0.00418	**0.02328**	0.01332	0.01487
Hondeklipbaai	**0.04808**	**0.03247**	**0.00460**	—	0.01018	0.01849	-0.01258
Port Nolloth	**0.03012**	**0.02081**	**0.00256**	**0.01557**	—	**0.05524**	**0.02242**
Luderitz	**0.02446**	**0.01716**	**0.00721**	**0.01019**	**0.01286**	—	**0.02546**
Walvis Bay	**0.03398**	**0.03146**	**0.01158**	**0.01557**	**0.01057**	**0.00878**	—
Males							
Table Bay	—	0.00692	**0.04948**	**0.03844**	**0.03497**	**0.03548**	0.00683
St. Helena Bay	**0.01411**	—	**0.02626**	**0.03166**	**0.03420**	0.00684	0.00950
Lambert's Bay	**0.03737**	**0.04354**	—	0.01371	**0.01972**	-0.00170	0.00463
Hondeklipbaai	**0.04371**	**0.04762**	**0.00415**	—	-0.00356	0.01120	-0.00454
Port Nolloth	**0.03157**	**0.02425**	**0.00868**	**0.00933**	—	**0.03596**	0.00819
Luderitz	**0.02665**	**0.04071**	**0.00440**	**0.00307**	**0.00717**	—	-0.00482
Walvis Bay	**0.03360**	**0.03793**	**0.01464**	**0.01271**	**0.01167**	**0.00729**	—

Appendix III Relationship between genetic distance ($F_{ST}/(1 - F_{ST})$ and log geographic distance in populations of Heaviside's dolphins off the southwest coast of southern Africa using Mantel Test: (a) mtDNA control region ($r = 0.34$; $P = 0.88$) and (b) microsatellite data ($r = -0.197$, $P = 0.23$).