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RESEARCH ARTICLE

Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (*Tursiops truncatus*) from coastal waters of the Southwestern Atlantic Ocean

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Abstract Knowledge about the ecology of bottlenose dolphins in the Southwestern Atlantic Ocean is scarce. Increased by-catch rates over the last decade in coastal waters of southern Brazil have raised concerns about the decline in abundance of local dolphin communities. Lack of relevant data, including information on population structure and connectivity, have hampered an assessment of the conservation status of bottlenose dolphin communities in this region. Here we combined analyses of 16 microsatellite loci and mitochondrial DNA (mtDNA) control region sequences to investigate genetic diversity, structure and connectivity in 124 biopsy samples collected over six communities of photographically identified coastal bottlenose dolphins in southern Brazil, Uruguay and central Argentina. Levels of nuclear genetic diversity were remarkably low (mean values of allelic diversity and heterozygosity across all loci were 3.6 and 0.21, respectively), a result that possibly reflects the small size of local dolphin communities. On a broad geographical scale, strong and significant genetic differentiation was found between bottlenose dolphins from southern Brazil–Uruguay (SB–U) and Bahía San Antonio (BSA), Argentina (AMOVA mtDNA $\Phi_{ST} = 0.43$; nuclear $F_{ST} = 0.46$), with negligible contemporary gene flow detected based on Bayesian estimates. On a finer scale, moderate but significant differentiation (AMOVA mtDNA $\Phi_{ST} = 0.29$; nuclear $F_{ST} = 0.13$) and

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asymmetric gene flow was detected between five neighbouring communities in SB–U. Based on the results we propose that BSA and SB–U represent two distinct evolutionarily significant units, and that communities from SB–U comprise five distinct Management Units (MUs). Under this scenario, conservation efforts should prioritize the areas in southern Brazil where dolphins from three MUs overlap in their home ranges and where by-catch rates are reportedly higher.

Keywords Cetacean · Conservation · Connectivity · Population genetics · Microsatellite · Mitochondrial DNA

Introduction

Bottlenose dolphins (*Tursiops* spp.) are cetaceans able to explore, occupy and adapt to different marine environments, with the exception of polar regions. Many genetic studies of bottlenose dolphins around the globe have reported moderate genetic differentiation among regional populations, despite some reproductive exchange (Sellas et al. 2005; Rosel et al. 2009; Tezanos-Pinto et al. 2009; Urian et al. 2009; Mirimin et al. 2011). Over large spatial scales, genetic discontinuities appear to coincide with ecological and topographic breaks, such as distinct water masses, currents and depth contours (Hoelzel et al. 1998a; Natoli et al. 2004; Bilgmann et al. 2007). On the other hand, habitat selection (e.g. open coast vs. estuarine ecosystems) and local adaptation to prey resources are believed to shape population structure over small spatial scales (Möller et al. 2007; Wiszniewski et al. 2010). Therefore, a combination of environmental, geomorphological and evolutionary factors appears to influence the genetic structure of bottlenose dolphin populations, although some may represent cryptic species-level differences (e.g. Natoli et al. 2004; Rosel et al. 2009).

Despite being extensively studied in many regions of the world, limited information is available for bottlenose dolphins of the Southwestern Atlantic Ocean (SWA); particularly scarce are details of their genetic diversity and population structure. Understanding population sub-divisions and connectivity provides information critical to the identification of relevant biological units to be conserved. These include evolutionary significant units (ESUs)—a group of historically isolated populations with unique genealogical and adaptive legacy—and Management Units (MUs)—demographically distinct populations that should be managed separately to ensure the viability of the larger metapopulation (see Funk et al. 2012 for definitions and a recent perspective on ESUs and MUs). This is especially important in cases where populations are restricted in distribution, have small population sizes and are subject to human induced mortality, which is the case for bottlenose dolphins of the SWA. It has been reported that in the SWA coastal bottlenose dolphins are mainly found between Santa Catarina State, in southern Brazil, and Central Argentina—and particularly along a narrow coastal corridor between southern Brazil and Uruguay (SB–U) (Laporta et al. in press). In this region, bottlenose dolphins occur in bays and estuaries, and between the surf zone and 2 km from the coastline when in the open-coast, with occasional records between 2 and 4 km (Laporta 2009; Di Tullio 2009). The distribution of coastal and offshore bottlenose dolphins apparently does not overlap and their feeding ecology is distinct, at least in part of the SWA (e.g. Botta et al. 2012). Concerns about the conservation of coastal bottlenose dolphins in SWA has recently emerged due to their relatively small population sizes (Laporta 2009; Fruet et al. 2011; Daura-Jorge et al. 2013), vulnerability to by-catch (Fruet et al. 2012) and substantial coastal development, particularly in southern Brazil (Tagliani et al. 2007). A long-term study of dolphin strandings has revealed high levels of mortality along Brazil's southernmost coastline, mainly in areas adjacent to the Patos Lagoon estuary where by-catch seems to be the main cause of death (Fruet et al. 2012).

Systematic photo-identification studies have shown that coastal bottlenose dolphins of the SWA consist of small communities with high site fidelity to estuaries and river mouths (and each community not exceeding 90 individuals, Fruet et al. in press a). These are often bordered by other small bottlenose dolphin communities that show more extensive movements along the coast, in contrast to estuarine communities (Laporta et al. in press). Photo-identification efforts in the two main estuaries of southern Brazil suggest that bottlenose dolphins exhibit long-term residency in these areas (Fruet et al. 2011; Daura-Jorge et al. 2013). Although there is distribution overlap of dolphins from these estuarine-associated and the adjacent coastal communities, no information is available on the levels of genetic connectivity among them. For example, social network analyses has revealed the existence of at least three distinct communities, which partially overlap in range near the Patos Lagoon estuary, in southern Brazil (Genoves 2013). This includes the year-round resident community of the Patos Lagoon estuary and two coastal communities: one that regularly moves from Uruguay to southern Brazil during winter and spring (Laporta 2009) and another which appears to inhabit the adjacent coastal waters of the Patos Lagoon estuary year-round. Such range overlap suggests potential for interbreeding among individuals of these communities, which would have implications for MUs classification and conservation management efforts. Given the assumption of demographic independence between different MUs, their delineation requires a

direct or indirect estimate of current dispersal rates (Palsbøll et al. 2007). However, dispersal rates can be difficult to estimate, particularly in the marine environment, which lacks marked physical barriers and where many organisms are not easily accessible for long-term field studies of identifiable or tagged individuals. In these cases, genetic methods generally offer a suitable alternative to assess dispersal rates and other indicators of demographic independence, as well as for estimating genetic diversity.

In this study we investigate the genetic diversity and population structure of bottlenose dolphins along the SWA coast using data from nuclear microsatellite markers and mtDNA control region sequences. We use this information to assess the strength and directionality of genetic connectivity over a range of spatial scales. Our sampling design allows comparisons among neighbouring coastal communities in southern Brazil-Uruguay (SB–U), and between these and a community inhabiting Bahía San Antonio (BSA) in the Patagonian coast—the most southern resident bottlenose dolphin community known for the SWA and located in a different marine biogeographical region to southern Brazil-Uruguay. We hypothesize that specialization for, or association with particular habitat types such as estuaries and open coasts may promote genetic differentiation on small spatial scales, while the biogeographical disjunction may influence differentiation at broad scale. The adjacent dolphin communities sampled in SB–U include two estuarine and three open coast communities. If habitat type specialization or, association with, drives genetic structure, we might expect to find lower genetic differentiation between communities inhabiting the contiguous open coast habitat than those living in sheltered estuarine environments, irrespective of geographical distances. We also expect that greater differentiation would characterize communities from different biogeographical regions. By delineating conservation units for coastal bottlenose dolphins in the SWA we expect to provide scientific support to guide strategies for population monitoring efforts, conservation status assessment and short-term management goals.

Methods

Sampling scheme

The study area covers approximately 2,112 km of linear distance along the coast. It extends from Florianópolis, in southern Brazil, to Bahía San Antonio, in the Patagonian Argentina. Along this region we surveyed six locations between 2004 and 2012 and collected 135 samples (Fig. 1). Samples consisted primarily of skin tissue obtained from free-ranging coastal bottlenose dolphins (common bottlenose dolphins, *Tursiops truncatus*—see Wang et al. (1999) for southern Brazil bottlenose dolphins molecular taxonomic identification) belonging to communities inhabiting a variety of habitat types: Florianópolis (FLN, coastal, $n = 9$), Laguna (LGN, estuarine, $n = 11$), north of Patos Lagoon (NPL, coastal, $n = 21$), Patos Lagoon estuary (PLE, estuarine, $n = 71$), south of Patos Lagoon/Uruguay (SPL/URU, coastal, $n = 14$) and Bahía San Antonio, Argentina (BSA, coastal bay, $n = 12$) (Table 1). Samples were collected using a crossbow with 150 lb (68 kg) draw weight and darts and tips especially designed for sampling small cetaceans (Ceta-Dart, Copenhagen, Denmark). We attempted to individually identify sampled dolphins through simultaneous photo-identification (see Fruet et al. in press b for details). Samples were grouped according to the sampled location. For those collected in the adjacent coastal areas of Patos Lagoon estuary, where three distinct communities live in close proximity and overlap in their range, identified individuals were grouped according to the social unit to which they were previously assigned based on social network analysis (Genoves 2013). Our dataset also included four samples from freshly stranded carcasses, two collected in La Coronilla, Uruguay, and two in southern Brazil from animals known to belong to the NPL community as photo-identified based on their natural marks prior to their death. Samples were preserved in 20 % dimethyl sulphoxide (DMSO) saturated with sodium chloride (Amos and Hoelzel 1991) or 98 % ethanol.

Genetic methods

Genomic DNA was extracted from all samples following a salting-out protocol (Sunnucks and Hales 1996). Sex of each biopsy sample was determined by the amplification of fragments of the *SRY* and *ZFX* genes through the polymerase chain reaction (PCR) (Gilson et al. 1998), with PCR conditions described in Möller et al. (2001). Samples were genotyped at 16 microsatellite loci (Online Resource 1) and a fragment of approximately 550 bp of the control region was sequenced using primers Dlp-1.5 and Dlp-5 (Baker et al. 1993) on an ABI 3730 (Applied Biosystems) with GenScan 500 LIZ 3130 internal size standard. Procedures for microsatellite PCR and genotyping are found in Möller and Beheregaray (2004), and for mtDNA PCR and sequencing in Möller and Beheregaray (2001). For microsatellites, bins for each locus were determined and genotypes scored in GeneMapper 4.0 (Applied Biosystems). Rare alleles (i.e. frequency < 0.05) or alleles that fell in between two bins were re-genotyped. Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) was used to check for potential scoring errors, the presence of null alleles, stuttering and large allelic drop out. Genotyping error rates were estimated by re-genotyping 30 randomly selected samples, representing 22 % of the total sample size used in this study. We used GenALEx 6.5

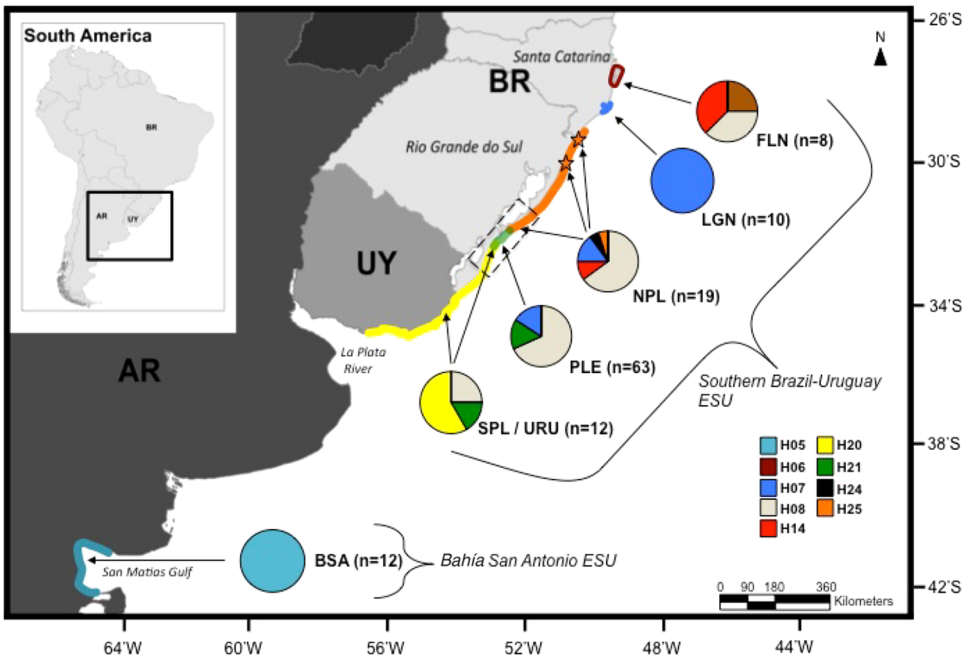

Fig. 1 Study area in the Southwestern Atlantic Ocean showing the proposed evolutionary significant units (ESUs) and management units (MUs) (*color counter lines*) for coastal common bottlenose dolphins (*Tursiops truncatus*), and the respective frequencies of mitochondrial control region haplotypes (*pie charts*). *Arrows* indicate the main sampling locations for each dolphin community. Approximate geographic boundaries of management units were built combining the results of this study with current knowledge on residency, social structure and movement patterns of bottlenose dolphins along this region. Specifically for NPL, the genetic assignment of some individuals regularly sighted approximately 400 km north of Patos Lagoon estuary (represented by *stars*) to NPL community were used as a proxy to define the northern limit of the community range. The *dashed rectangle* highlights the area of heightened conservation concern proposed by this study (see "Conservation implications" section for details). *FLN* Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio. (Color figure online)

(Peakall and Smouse 2012) to find potential matches between genotypes and to estimate the probability of identity as an indicator of the power of the 16 markers to distinguish between two sampled individuals. Samples matching at all genotypes or those mismatching at only a few alleles (1–2) were double-checked for potential scoring errors. Sequences of the mtDNA were edited using SEQUENCHER 3.0 (Gene Codes Corporation, Ann Arbor, MI) and aligned using the ClustalW algorithm in MEGA 5.05 (Tamura et al. 2011). Haplotypes were defined using DNASP 5.0 (Librado and Rozas 2009). After careful examination, samples sharing identical genotypes at all loci, same mtDNA haplotype and sex were considered as re-sampled individuals and one of each pair was removed. Re-sampled individuals identified by photo-identification ($n = 7$) were also confirmed through genetic methods.

Data analysis

Population structure

We used 10,000 permutations in SPAGEDI to test for the relative importance of a stepwise mutation model as a contributor to genetic diversity and structure (Hardy and Vekemans 2002). This provides a way to assess whether F_{ST} or R_{ST} potentially provides a more appropriate statistic to estimate genetic structure since R_{ST} accounts for divergence times between microsatellite alleles and is thus expected to better reflect older divergences (Hardy et al. 2003). Allele size permutation test in SPAGEDI were non significant for all loci. This suggests that F_{ST} is likely the most appropriate estimator, and only F_{ST} values are therefore reported hereafter. ARLEQUIN 3.5.1.2 was used for an analysis of molecular variance (AMOVA) to evaluate differentiation between SB–U and BSA dolphins, and among SB–U communities, for both nuclear and mtDNA datasets. Degree of genetic differentiation among locations was also assessed using ARLEQUIN to calculate F_{ST} (Weir and Cockerham 1984) for microsatellites, and both F_{ST} and Φ_{ST} measures for mtDNA. For each of these measures we used the Tamura and Nei (1993) model with a gamma correction of 0.5. Significance was tested based on 10,000 permutations. We also estimated the statistical power to detect nuclear differentiation using POWSIM (Ryman and Palm 2006) by simulating six populations with samples sizes of each sampled community (8, 10, 19, 63, 12, 12) with F_{ST} of 0.05 (combining generation, time

Table 1 Ecological information and summary of genetic diversity for the six communities and the two proposed evolutionary significant units (ESUs) of coastal common bottlenose dolphins (*Tursiops truncatus*) based on mtDNA control region sequences and 15 microsatellite loci

		N (f:m)	Pop. size (95 % CI)	Habitat type	mtDNA		Microsatellites							
					h	π	PA	NA	AR	H_E	H_O	F_{IS}	PI_U	PI_{SIBS}
Southern Brazil–Uruguay ESU	FLN	8 (6:2)	Unknown	Coastal	0.7500 (0.0965)	0.0045 (0.0032)	0	1.6	1.6	0.19	0.23	−0.22	1.5×10^{-3}	4.3×10^{-2}
	LGN	10 (2:8)	59 (49–72)[a]	Estuarine	0.0000 (0.0000)	0.0000 (0.0000)	0	1.6	1.5	0.21	0.15	0.28*	1.3×10^{-3}	3.6×10^{-2}
	NPL	19 (8:11)	Unknown	Coastal	0.5425 (0.1231)	0.0067 (0.0041)	2	2.3	1.9	0.20	0.19	0.06	7.5×10^{-4}	3.5×10^{-2}
	PLE	63 (38:25)	86 (78–95)[b]	Estuarine	0.4808 (0.0621)	0.0072 (0.0042)	9	3.0	2.0	0.26	0.26	−0.01	4.6×10^{-5}	9.7×10^{-3}
	SPL/URU	12 (5:7)	Unknown	Coastal	0.6484 (0.1163)	0.0067 (0.0041)	5	2.1	1.9	0.20	0.23	−0.02	3.5×10^{-4}	2.4×10^{-2}
	Total	112 (59:53)	–	–	0.6457 (0.0404)	0.0096 (0.0053)	16	3.7	2.2	0.22	0.22	0.02	–	–
Bahía San Antonio ESU	BSA	12 (2:10)	76 (70–97)[c]	Coastal Bays	0.0000 (0.0000)	0.0000 (0.0000)	1	1.76	1.76	0.19	0.18	0.08	2.6×10^{-3}	5.4×10^{-2}
	Total	124 (61:63)	–	–	0.7022 (0.0352)	0.0195 (0.0100)	–	3.6	–	0.28	0.23	0.194*	–	–

N total number of individuals (separated by sex); *PA* number of private alleles; *NA* mean number of alleles per locus; *AR* mean allelic richness; H_E mean expected heterozygosity; H_O mean observed heterozygosity; F_{IS} inbreeding coefficient; PI_U, PI_{SIBS} probabilities of identity for unbiased samples and samples of full-sibs, respectively

* Significant multi-locus *P* value ($P < 0.001$)

[a] Daura-Jorge et al. (2013), [b] Fruet et al. (2011), [c] Vermeulen and Cammareri (2009)

$t = 25$ with effective population size, $N_e = 500$), which approximates the lowest empirical fixation index found based on 15 loci (see "Results" section). The α (Type I) error was assessed running the same simulated scenario, but sampling directly from the base population (i.e. setting drift time $t = 0$). A thousand replicates were run and the significance of the tests was assessed with Fisher's exact tests and Chi square tests.

The Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard et al. 2000) was also used for inferring population structure based on the microsatellite data. We assumed correlated allele frequencies and an admixture model using sampling location as prior information (LOCPRIOR function) (Hubisz et al. 2009). Simulations were performed using a 200,000 step burn-in period and 10^6 repetitions of the Markov Chain Monte Carlo (MCMC) search, assuming number of clusters (K) varying between 1 and 6. We performed 20 independent runs to limit the influence of stochasticity, to increase the precision of the parameter estimates, and to provide an estimate of experimental reproducibility (Gilbert et al. 2012). The most likely K was explicitly determined by examining ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and vonHoldt 2012). Following the recommendations of Evanno et al. (2005), we ran an iterative process where, for each most likely K detected by STRUCTURE, we independently re-analyzed the data to test for further sub-division. This process was repeated until the most likely K was 1.

Isolation by distance (IBD) was assessed by conducting Mantel tests (Mantel 1967) between matrices of F_{ST} genetic distances and geographical distances measured as the shortest marine coastal distance between two locations. Given the large geographical distance between the southernmost sampling site (BSA) and others, we excluded BSA from the IBD analysis. We also used partial Mantel tests to test for an association between habitat type (estuarine versus coastal) and genetic distance, while controlling for the effect of geographical distance. Both tests were run with 1,000 random permutations in GENODIVE 2.0.

Gene flow

Magnitude and direction of contemporary gene flow among the six sampled communities was estimated using BAYESASS 3.0 (Wilson and Rannala 2003). The software uses a MCMC algorithm to estimate the posterior probability distribution of the proportion of migrants from one population to another. This was conducted with ten independent MCMC runs of 10^7 steps, with the first 10^6 repetitions discarded as burn-in. To reach the recommended acceptance rates of total iterations between 20 and 40 % we adjusted the values of continuous parameters such as

migration rates (Δ_M), allele frequencies (Δ_A) and inbreeding coefficient (Δ_F) to 0.9, 0.6 and 0.8, respectively. Samples were collected every 200 iterations to infer the posterior probability distributions of parameters. Trace files were monitored for convergence and runs with potential problems were discarded. Additionally, convergence was checked by comparing the migration rate profile between the runs according to their average total likelihood and associated credible confidence interval (CI).

Genetic diversity

For microsatellites, genetic diversity, expressed as number of alleles (NA), expected (H_E) and observed (H_O) heterozygosity, as well as the inbreeding coefficient (F_{IS}) were estimated for each community in GENODIVE 2.0 (Meirmans and Van Tienderen 2004). Departures from Hardy–Weinberg equilibrium and linkage disequilibrium were tested using the Fisher's exact test and a Markov chain method with 1,000 iterations in GENEPOP 4.2 (Rousset 2008). Allelic richness (AR) was estimated in FSTAT 2.9.3.2 (Goudet 1995). All statistical tests followed sequential Bonferroni correction to address type I errors associated with multiple comparisons (Rice 1989). For the mtDNA sequences, we used ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) to estimate haplotypic and nucleotide diversities. A median-joining network from the mtDNA haplotypes was constructed using NETWORK 4.6.1.1 (Bandelt et al. 1999).

Results

Summary statistics

A total of 134 biopsy samples and four samples from stranded carcasses were used. All samples were successfully amplified at 16 microsatellite loci and sequenced for approximately 550 bp of the mtDNA control region. Only eight out of 450 repeated genotypes (1.7 %) did not match but were resolved by re-genotyping. The probability of two unrelated individuals or siblings sharing the same genotypes was very low for all communities (Table 1). Multiple lines of evidence (identical genotype, same mtDNA sequence and sex) suggested that 14 biopsied individuals were sampled twice, including seven individuals that were suspected re-samples based on photo-identification. All re-sampled animals were biopsied in the same location: eight in PLE, two in SPL/URU, two in NPL, one in LGN, and one in FLN. After removal of duplicates, 124 samples were included in the final dataset analyzed. From these, 61 samples were males and 63 were females (Table 1).

The microsatellite locus Tur91 was monomorphic and therefore excluded from further analysis. We found no evidence for effects of large allelic dropout in any locus. Null alleles were detected for two loci but these were not consistent among sampled locations (locus TUR80 in PLE and Ttr04 in BSA), and therefore the loci were kept for all analyses. One locus pair (TUR105 and EV37) showed evidence of linkage disequilibrium. However, because similar results were obtained when analyses were run both with and without TUR105 this locus was kept in the dataset. Laguna was the only sample location that showed significant deviation from Hardy–Weinberg equilibrium when averaged across all loci, likely due to inbreeding ($F_{IS} = 0.28$) in this small community. Inbreeding coefficient was low and non-significant for all other communities (Table 1).

Genetic structure

The AMOVA results showed strong differentiation between SB–U and BSA for both microsatellites ($F_{ST} = 0.46$, $P < 0.001$) and mtDNA ($\Phi_{ST} = 0.43$, $P < 0.0001$). On a smaller spatial scale, the AMOVA indicated moderate differentiation among SB–U communities, for both microsatellites ($F_{ST} = 0.13$, $P < 0.0001$) and mtDNA ($\Phi_{ST} = 0.29$, $P < 0.0001$). Accordingly, significant differentiation was observed for all pairwise comparisons using microsatellites (Table 2), but over a wide range of F_{ST} values (0.066–0.617). Excluding BSA, which was by far the most differentiated (average F_{ST} of 0.51 for all comparisons with other communities), moderate but significant differentiation was found between all other pairwise comparisons, with the two geographically closest communities (PLE and NPL) having the lowest value of F_{ST} ($F_{ST} = 0.06$; $P < 0.001$). POWSIM simulations for 15 microsatellite loci and the sample sizes used in this study suggested a 100 % probability of detecting differentiation above the lowest empirical F_{ST} level of differentiation, indicating satisfactory statistical power for our analyses. The estimated type I error varied from 0.041 with Fisher's exact tests to 0.083 with χ^2 tests, which approximates the conventional 5 % limit for significance testing.

Results of pairwise comparisons using mtDNA were generally congruent with results from the microsatellite analyses, albeit with higher levels of differentiation between communities. The exceptions were NPL and PLE (for both F_{ST} and Φ_{ST}), and NPL and FLN (for Φ_{ST} only), which showed no significant differentiation (Table 3). All three of these communities are dominated by the most common mtDNA haplotype (H08). Pairwise significant F_{ST} values ranged between 0.097 (NPL–FLN) to 1 (LGN–BSA), with BSA the most differentiated community across all comparisons.

Mantel tests revealed a positive and significant correlation between microsatellites and mtDNA fixation indices

Table 2 Estimates of microsatellite differentiation among six coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along the Southwestern Atlantic Ocean

	FLN	LGN	NPL	PLE	SPL/URU	BSA
FLN	–					
LGN	0.131**	–				
NPL	0.147**	0.169**	–			
PLE	0.144**	0.101**	0.066**	–		
SPL/URU	0.289**	0.250**	0.156**	0.101**	–	
BSA	0.617**	0.502**	0.538**	0.423**	0.477**	–

Differentiation is expressed as F_{ST} based on 15 microsatellites loci

FLN Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio

* $P < 0.05$; ** $P < 0.01$

and geographical distances, suggesting a pattern of IBD (Fig. 2). For the mtDNA data, the correlation was not as strong ($r^2 = 0.428$) as for the microsatellites ($r^2 = 0.934$), but still significant. Results of partial Mantel tests (details not shown) suggested that differentiation was more likely influenced by distance than by habitat type (estuarine versus coastal). When controlling for geographical distances, non-significant relationships between locations and clusters (cluster 1 and 2: estuarine and coastal communities, respectively) were found for both microsatellites ($r^2 = -0.437$; $P = 0.51$) and mtDNA ($r^2 = -0.525$; $P = 0.52$).

Bayesian posterior probabilities indicated that the dataset is best explained by the clustering of samples into two genetic populations ($K = 2$), with all individuals from BSA placed in one cluster and remaining individuals sampled in SB–U placed in a second cluster (Fig. 3a). Negligible admixture appears to exist between these two clusters, with assignment estimates of all individuals to their respective clusters above 0.99 and 0.98, respectively. Testing for further sub-division by running STRUCTURE for the set of northern communities led to the identification of additional partitioning within SB–U most consistent with five populations (Fig. 3b–d). No sub-division was detected within BSA (data not shown).

Gene flow

Estimates of contemporary gene flow inferred in BayesAss suggested very low gene flow from BSA to SB–U communities (2.2 %) and negligible gene flow in the opposite direction (0.3 %). Within the SB–U region, BayesAss revealed moderate and complex asymmetrical migration rates (Table 4; Fig. 4) consistent with the inferred pattern of IBD. Generally, higher migration occurred between neighbouring communities than between those separated by greater geographic distances, with the exception of LGN, which seems to exchange more migrants with more distant communities than with its closest neighbouring community (FLN). Migration estimates between sampling locations at the extremities of the sampling distribution was low. Estimated migration rates from FLN to NPL and from SPL/URU to PLE were at least twice the rates between all other community pairs (Fig. 4). For the estuarine communities, PLE seems to act as a sink with a considerable rate of migrants coming from LGN, NPL and SPL/URU, and negligible migration in the opposite direction. In contrast, LGN seems to be more closed to immigration while contributing genetic migrants to PLE and NPL.

Genetic diversity

Levels of genetic variation were remarkably low for all samples as measured by both allelic richness (AR) and expected heterozygosity (H_E) (Table 1; Appendix). Observed heterozygosity (H_O) ranged from 0.15 to 0.26, with a mean across all loci of 0.21. AR ranged from 1.5 to

Table 3 Estimates of mitochondrial differentiation among six coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along the Southwestern Atlantic Ocean

	FLN	LGN	NPL	PLE	SPL/URU	BSA
FLN	–	0.659**	0.100*	0.209**	0.249**	0.687**
LGN	0.893**	–	0.622**	0.572**	0.666**	1.000**
NPL	0.040	0.744**	–	0.009	0.297**	0.679**
PLE	0.198*	0.489**	0.06	–	0.329**	0.638**
SPL/URU	0.531**	0.466**	0.392**	0.230**	–	0.689**
BSA	0.639**	1.000**	0.399**	0.340**	0.609**	–

Differentiation is expressed as Φ_{ST} (above diagonal) and F_{ST} (below diagonal) based on 457-bp of the mtDNA control region

FLN Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio

* $P < 0.05$; ** $P < 0.01$

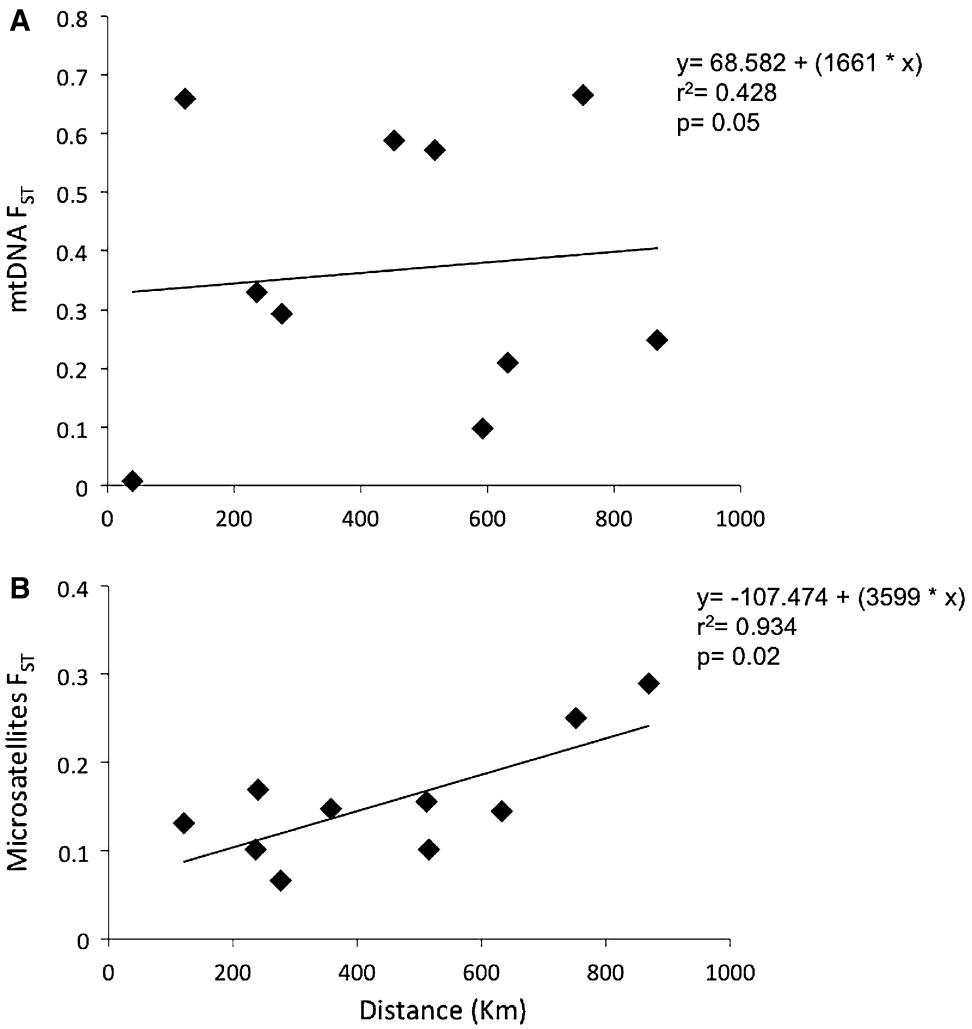

Fig. 2 Isolation by distance plots using Euclidean distance (km) and genetic distance (F_{ST}) among five coastal communities of common bottlenose dolphins (*Tursiops truncatus*) inhabiting southern Brazil–Uruguay based on **a** mtDNA control region and **b** 15 microsatellite loci (*lower box*)

2.0, being higher in PLE, NPL and SPL/URU, and lower in LGN and BSA. Number of alleles per locus ranged from two to seven (Appendix) with a mean across all loci of 3.6, while the mean number of alleles per community was two. Out of 17 "private" (unique) alleles identified, nine were found in PLE, five in SPL/URU, two in NPL and one in BSA (Table 1). The only private allele in BSA was found in high frequency in that community, while in all other communities unique alleles had low frequencies.

After sequence alignment and editing, 457 bp of the mtDNA control region could be analyzed for the same 124 individuals used for the microsatellite analysis. Thirteen polymorphic sites (all transitional mutations) revealed nine distinct haplotypes. The number of haplotypes detected in each sampled location varied from one to five, and haplotype diversity ranged from 0 to 0.75. Overall, nucleotide diversity among all individuals was low ($\pi = 0.009$), and haplotype diversity moderate ($h = 0.712$), although values varied among communities. FLN community displayed the highest level of haplotype diversity, while PLE had the highest nucleotide diversity (Table 1). The most common and widely dispersed haplotype (H8) was found in 49.6 % of the individuals and across all locations, except in LGN and BSA where all dolphins shared the same haplotypes (H7 for LGN and H4 for BSA). Private haplotypes were found in four of the six communities (FLN, $n = 1$; NPL, $n = 1$; SPL/URU, $n = 2$; BSA, $n = 1$) (Fig. 1).

The median-joining network showed two main groups of haplotypes separated by a minimum of five mutational steps (Fig. 5). Individuals from PLE, NPL and SPL/URU communities were present in both groups while individuals from LGN, BSA and FLN were represented in only one of the groups. Bahía San Antonio retains a unique haplotype (H05), which is fixed for this location and differs from the most common haplotype (H08) by one mutational step.

Discussion

This study comprises the first comprehensive assessment of population structure and genetic diversity of coastal

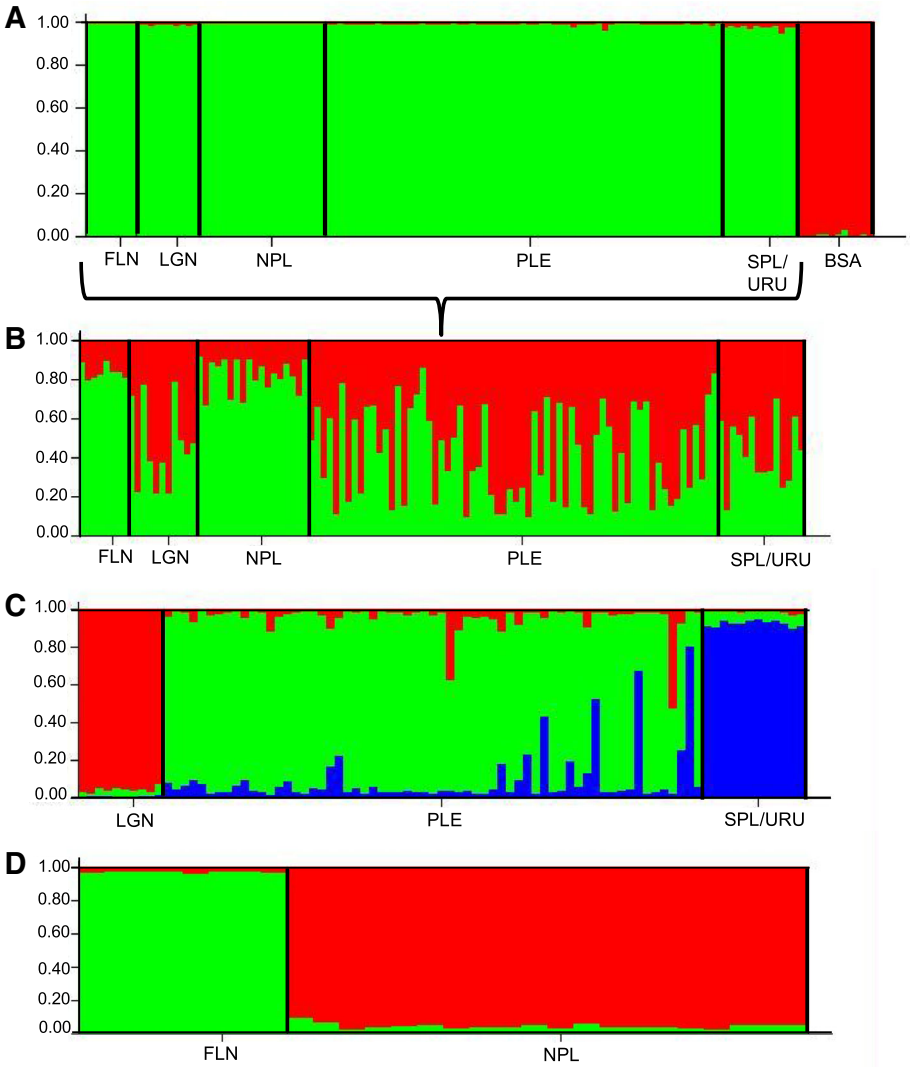

Fig. 3 STRUCTURE Bayesian assignment probabilities for common bottlenose dolphins (*Tursiops truncatus*) based on 15 microsatellite loci. *Each vertical line* represents one individual dolphin and *vertical black lines* separate the sampled communities. We run an iterative process where for each most likely K detected by STRUCTURE we independently re-analyzed the data to test for further sub-division (Evanno et al. 2005; Pritchard et al. 2007). This process was repeated iteratively until the highest likelihood values resulted in K = 1. When all samples were analyzed together, STRUCTURE clearly separated individuals sampled in BSA from all those sampled in southern Brazil/Uruguay, resulting in K = 2 (**a**). The highest ΔK for the next run within southern Brazil/Uruguay communities was for K = 2, clustering LGN, PLE and SPL/URU, and FLN and NPL (**b**). When we run STRUCTURE independently for the above-mentioned clusters, the highest ΔK resulted for K = 3 (**c**) and K = 2 (**d**), respectively. *FLN* Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio. (Color figure online)

bottlenose dolphins (*Tursiops truncatus*) along the SWA. On a large spatial scale, we report on two genetic populations (SB–U and BSA) that are highly differentiated and show very low level of gene flow. On a smaller spatial scale, we detected low to moderate levels of asymmetric gene flow between communities within the SB–U population and an influence of geographic distance in shaping patterns of connectivity, perhaps with the exception of Laguna. Here we also show that coastal bottlenose dolphins in the SWA have very low levels of genetic diversity. This reduced gene flow and genetic diversity, combined with the small size and probable demographic independence of communities, limit the likelihood of replenishment if they undergo a genetic or demographic decline, highlighting the need to implement local-based monitoring and conservation plans.

Large-scale population structure in SWA bottlenose dolphins

On a broad geographical scale, our results indicate that bottlenose dolphins in coastal Argentinean Patagonia (BSA

Table 4 Estimates of recent migration rates among six coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along the Southwestern Atlantic Ocean

From	To					
	FLN	LGN	NPL	PLE	SPL/URU	BSA
FLN	**0.6915 (0.646–0.736)**	0.0232 (0.019–0.066)	0.2152 (0.133–0.296)	0.0237 (0.019–0.067)	0.0232 (0.019–0.065)	0.0232 (0.019–0.063)
LGN	0.0209 (0.017–0.058)	**0.6887 (0.648–0.728)**	0.1289 (0.016–0.241)	0.1197 (0.007–0.232)	0.0209 (0.017–0.058)	0.0210 (0.017–0.059)
NPL	0.0126 (0.011–0.036)	0.0127 (0.011–0.036)	**0.8454 (0.738–0.952)**	0.1036 (0.001–0.208)	0.0127 (0.012–0.037)	0.0129 (0.010–0.036)
PLE	0.0050 (0.004–0.015)	0.0054 (0.004–0.015)	0.0455 (0.003–0.094)	**0.9343 (0.883–0.985)**	0.0049 (0.010–0.019)	0.0049 (0.004–0.014)
SPL/URU	0.0181 (0.015–0.051)	0.0179 (0.016–0.052)	0.0237 (0.029–0.076)	0.2367 (0.141–0.331)	**0.6855 (0.621–0.749)**	0.0180 (0.015–0.051)
BSA	0.0182 (0.015–0.051)	0.0183 (0.015–0.051)	0.0182 (0.015–0.052)	0.0185 (0.015–0.052)	0.0183 (0.015–0.052)	**0.9084 (0.841–0.975)**

Bold denotes the proportion of non-migrants in each dolphin community. 95 % CI values are given in brackets

FLN Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPL/URU* south of Patos Lagoon/Uruguay; *BSA* Bahía San Antonio

community) are highly differentiated from those sampled along the southern Brazil–Uruguay (SB–U) coast, likely reflecting a combination of IBD and environmental differentiation. Several studies have argued that bottlenose dolphins are capable of specialization for a variety of habitats and prey types, and that such specialization could promote genetic divergence (Hoelzel et al. 1998a; Natoli et al. 2004; Möller et al. 2007; Tezanos-Pinto et al. 2009; Wiszniewski et al. 2010; Möller 2012). Bahía San Antonio is located in the San Matías Gulf (Fig. 1), which is part of the Northern Patagonian gulfs of Argentina. Geomorphological characteristics (bathymetry and coastal complexity), oceanographic processes (upwelling, nutrient input, sea surface temperature regimes and currents), and biological community structure biogeographically distinguishes the Patagonian region from the rest of the Atlantic coast (Balech and Ehrlich 2008; Tonini 2010). For example, archaeozoological evidence suggests that one of the main prey species of bottlenose dolphins in SB–U, the white croaker (*Micropogonias furnieri*) (Pinedo, 1982; Mehsen et al. 2005), is currently absent from BSA (Scartascini and Volpedo 2013), which is the northernmost limit for many prey species confirmed to be part of the diet of bottlenose dolphins in Patagonia (e.g. pouched lamprey (*Geotria australis*), Patagonian octopus (*Octopus tehuelchus*), Argentine Hake (*Mercluccius hubbsi*) (Crespo et al. 2008), as it is located at the boundary between two biogeographic regions (Galván et al. 2009). Regional differences in prey distribution and abundance are thought to play a role on the genetic structuring of bottlenose dolphins elsewhere (e.g. Bilgmann et al. 2007). Therefore, BSA bottlenose dolphins may have different foraging adaptations compared to SB–U bottlenose dolphins. The high degree of differentiation at neutral markers and the results from the Bayesian analysis of migration rates imply negligible gene flow between bottlenose dolphin communities of these two regions. Future studies combining morphological, genetic, environmental, and ecological data are needed to better clarify the taxonomic status between BSA and SB–U coastal bottlenose dolphins.

Fine-scale population structure in SWA bottlenose dolphins

In spite of their high dispersal potential, several empirical studies have shown that coastal bottlenose dolphins often form discrete population units, even at very small geographical scales (e.g. Sellas et al. 2005; Möller et al. 2007; Rosel et al. 2009; Ansmann et al. 2012). Our results from both fixation indices and the Bayesian clustering analysis confirmed that the five studied communities within the SB–U population are genetically distinct, indicating higher genetic differentiation than expected over small

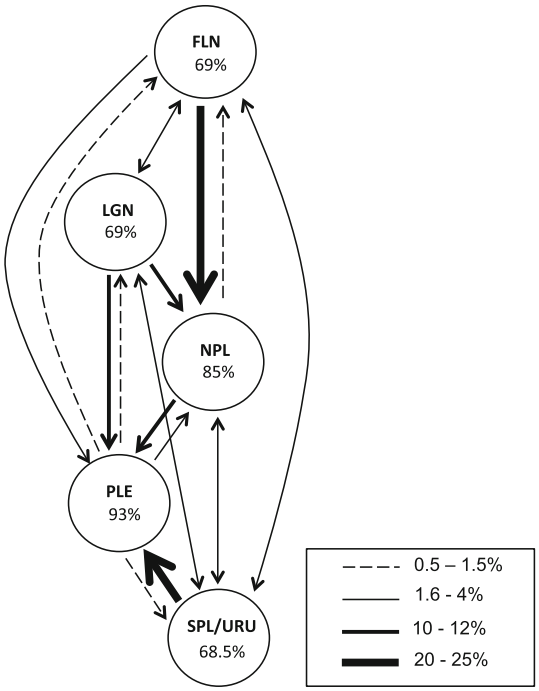

Fig. 4 Schematic diagram showing the recent asymmetric migration rates estimated between five coastal communities of common bottlenose dolphins (*Tursiops truncatus*) sampled along southern Brazil and Uruguay. The width of the *arrows* corresponds to the rates of gene flow between putative populations

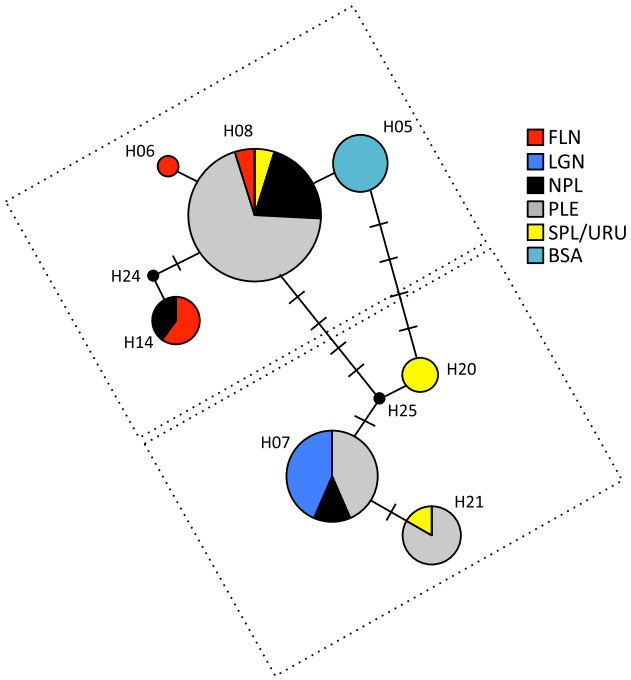

Fig. 5 Median-joining network of mtDNA control region haplotypes in coastal common bottlenose dolphins (*Tursiops truncatus*). The size of the *circles* is proportional to the total number of individuals bearing that haplotype. *Dashed lines* separate the two main groups of haplotypes. *Different colors* denote the different sampled communities: *FLN* Florianópolis, *LGN* Laguna, *NPL* north of Patos Lagoon, *PLE* Patos Lagoon estuary, *SPE/URU* south of Patos Lagoon/Uruguay, *BSA* Bahía San Antonio. *Dashes* represent extinct or unsampled haplotypes. (Color figure online)

geographical scales. Relatively lower degrees of nuclear genetic differentiation are commonly reported for bottlenose dolphins over comparable spatial scales with the exception of the high differentiation found among the neighbouring communities of *T. truncatus* in Irish coastal waters (Shannon estuary and Connemara–Mayo communities $F_{ST} = 0.179$; Mirimin et al. 2011). For instance, lower differentiation was found between neighbouring communities of *T. truncatus* along the coast of the western North Atlantic (minimum and maximum reported F_{ST} values of 0.002 and 0.015, respectively; Rosel et al. 2009) and Bahamas ($F_{ST} = 0.048$; total distance between two sampling sites was 116 km; Parsons et al. 2006).

For highly mobile, long-lived animals with low reproductive rates such as cetaceans, it is well accepted that a combination of mechanisms including habitat selection, specialized foraging behaviours, social structure and natal philopatry can drive population differentiation across small spatial scales (Hoelzel 2009; Möller 2012). For a closely related species, the Indo-Pacific bottlenose dolphins, restricted gene flow between some coastal and estuarine communities appears to have occurred after coastal dolphins colonized the embayment, as a consequence of high site fidelity and resource and behavioural specializations (Möller et al. 2007). In our study, however, we actually found similar levels of genetic differentiation when comparing coastal and estuarine communities or among coastal communities of the common bottlenose dolphin in SWA. This pattern is contrary to what would be expected if habitat type was a main driver of bottlenose dolphin population structure in the region. Instead, for most communities, structure appeared to follow an isolation-by-distance model, where exchange of individuals seems to more likely occur between adjacent communities, irrespective of habitat type. The only exception was Laguna, which appeared as an outlier to the IBD model. In Laguna, a unique foraging tactic involving cooperative interactions between dolphins and beach-casting fishermen has evolved. It has been suggested that the propagation of such behaviour through social learning has a matrilineal origin, where the mother–calf relationship might create conditions suitable for behavioural information exchange (Daura-Jorge et al. 2012). In such special conditions, the costs to individuals of leaving a suitable habitat is likely greater than the risk of searching for more profitable locations. In contrast, some

PLE dolphins frequently interact with animals from other communities in the coastal zone, and there is no evidence of particular feeding specializations compared to LGN. Thus, it appears that feeding specializations (LGN) and sociality (PLE), instead of habitat type *per se*, may play a role in shaping genetic structure of bottlenose dolphins in these regions.

The contemporary asymmetric gene flow found in our study system suggests moderate levels of connectivity among communities in SB–U ESU, which are consistent with a metapopulation. Gene flow is particularly mediated by coastal communities, especially FLN and SPL/URU, although estuarine communities exchange genes as well. It seems that PLE potentially acts as a sink, receiving low to moderate number of migrants while not contributing substantially to other communities. In contrast, LGN showed much lower gene flow with adjacent communities, apparently constituting a more closed genetic unit. This pattern is also supported by mitochondrial data, which suggested high connectivity between PLE and the adjacent coastal community (NPL), but high maternal philopatry and restricted dispersal of LGN dolphins.

Remarkably low levels of genetic diversity in SWA bottlenose dolphins

Low genetic variation was detected with both mitochondrial and nuclear DNA markers across all communities. Levels of variation at the mtDNA control region were similar to those reported for *T. truncatus* in other parts of the world. In contrast, nuclear DNA variation for all communities was much lower than that reported for other local coastal communities elsewhere (see Online Resource 2 for comparisons with studies of Parsons et al. 2006; Rosel et al. 2009; Tezanos-Pinto et al. 2009; Mirimin et al. 2011; Caballero et al. 2012). This is supported by the low numbers of alleles, reduced allelic richness and reduced heterozygosity. For LGN and BSA communities in particular, the remarkably low variation at both marker types fall within the range observed for cetaceans with extremely small populations sizes (i.e. <100 individuals), such as the subspecies of Hector's dolphins, *Cephalorhyncus hectori mauii* (Hamner et al. 2012), and the Black Sea subspecies of the harbour porpoise, *Phocoena phocoena relicta* (Rosel et al. 1995). These findings are consistent with the current abundance estimates of less than 90 individuals for the BSA, PLE, and LGN communities (Vermeulen and Cammareri 2009; Fruet et al. 2011; Daura-Jorge et al. 2013) and may also reflect the potential small size of the other communities (such as FLN, NPL and SPL/URU) for which estimates of abundance are not currently available. Several authors have suggested that coastal populations of bottlenose dolphin elsewhere might have originated via independent founder events from offshore populations, followed by local adaptation and natal philopatry (Hoelzel et al. 1998a; Natoli et al. 2004; Sellas et al. 2005; Möller et al. 2007; Tezanos-Pinto et al. 2009), leading to a reduction in genetic diversity.

Conservation implications

On a large geographical scale our results strongly support that SB–U and BSA dolphins constitute at least two distinct ESUs, and these warrant separate conservation and management strategies. The SB–U ESU comprises a set of communities (or sub-populations) distributed along a narrow strip of the coast between Florianopolis (27°21′S) in southern Brazil, and the southern limit of the Uruguayan coast (34°55′S). The BSA ESU geographical range goes possibly from the northern border of Rio Negro Province, at the Rio Negro estuary (41°01′S), to southern Golfo Nuevo (43°05′S), as suggested by sightings of bottlenose dolphins in northern Patagonia (Vermeulen and Cammareri 2009; Coscarella et al. 2012). Our results indicate that these two ESUs are genetically isolated which has important implications for future conservation plans. It is fundamental that managers design appropriate conservation strategies for each ESU, taking into account their respective threats, genetic and ecological processes shaping structure, and geographical distribution in space and time, as their responses to future environmental changes may possibly differ. This is of particular relevance for BSA dolphins since they apparently constitute the only population within that ESU with reduced abundance and signs of historical decline (Bastida and Rodríguez 2003; Coscarella et al. 2012).

The most serious and continuous threats for bottlenose dolphins along the SWA coast are found within the SB–U ESU, where they have experienced increased rates of human-related mortalities during the past decade (Fruet et al. 2012). These animals also face considerable coastal habitat degradation as a consequence of ongoing industrial and port development activities (Tagliani et al. 2007). Based on this study we suggest that these dolphin communities within SB–U are functionally independent, and therefore should be treated as separate MUs for conservation purposes. We advocate for managers to adopt the proposed MUs reported here (see Fig. 1), while

recognizing that their boundaries may change as more information on dolphin home ranges and population genetic structure becomes available. Under this proposed management scenario, conservation programs should be directed towards the Patos Lagoon estuary and adjacent coastal waters where dolphins from distinct communities (PLE, NPL and SPL/URU) show overlapping home ranges, and where by-catch rates are reportedly higher (Fig. 1). Protecting dolphins in this region would reduce the risk of disrupting connectivity between MUs and increase the chances of long-term viability. Strategies should reduce the impact of by-catch and maximize the protection of "corridors" in coastal areas for maintaining connectivity between adjacent dolphin communities.

The very low levels of genetic diversity in coastal bottlenose dolphins from SWA could be a source for concern. The importance of genetic variation relates to multiple aspects of population resilience and persistence, and is usually assumed to be critical for long-term fitness and adaptation (Franklin 1980; Charlesworth and Willis 2009), although some studies have shown that minimal genetic variation is not always a reliable predictor of extinction risk in wild populations (e.g. Schultz et al. 2009). We propose, however, the adoption of a precautionary approach for coastal bottlenose dolphins in SWA. Although there is no evidence of inbreeding depression for bottlenose dolphins in this region, the possibility of inbreeding in the small LGN community (Table 1) may, in the long-term, be detrimental to its viability since inbreeding can increase vulnerability to environmental stressors (O'Brien et al. 1985; Frankham 1995; Spielman et al. 2004; Hale and Briskie 2007). Bottlenose dolphins from Laguna and their neighbouring community (FLN) are being affected by a chronic dermal infection, the fungal Lobomycosis, and Lobomycosis-like disease (LLD) (Van Bressen et al. 2007, Daura-Jorge and Simões-Lopes 2011), with evidence of an increase in the number of affected animals in recent years (Daura-Jorge and Simões-Lopes 2011). While our results suggest restricted dispersal of LGN dolphins, which may limit the spread of the disease, the isolated nature of this community can potentially accelerate fungal transmission among resident dolphins.

Conclusions

Common bottlenose dolphins from coastal waters of the SWA are characterized by unprecedentedly low mitochondrial and nuclear DNA diversity. Moderate to strong levels of population differentiation at both marker types were also disclosed and are likely associated with a combination of geographical, environmental and social factors. The pattern of genetic differentiation and the negligible migration rates detected suggest two distinct lineages, or evolutionarily significant units, one in Argentina and the other in southern Brazil-Uruguay. In addition, five distinct communities, or Management Units, characterized by low to moderate asymmetrical gene flow were identified in southern Brazil–Uruguay—a region where human activities negatively impact upon common bottlenose dolphins. We propose that policies and practices relevant to conservation management of common bottlenose dolphins in coastal waters of the SWA should recognize the existence of two lineages, as well as promote connectivity between the estuarine and open-coast populations in southern Brazil and Uruguay to ensure their long-term persistence.

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Appendix

See Table 5.

Table 5 Genetic diversity screened at 16 microsatellite loci in six coastal communities of common bottlenose dolphin sampled along the Southwestern Atlantic

	FLN ($n = 8$)				LGN ($n = 10$)				NPL ($n = 19$)				PLE ($n = 63$)				SPL/URU ($n = 12$)				BSA ($n = 12$)			
	NA	H_O	H_E	P	NA	H_O	H_E	P	NA	H_O	H_E	P	NA	H_O	H_E	P	NA	H_O	H_E	P	NA	H_O	H_E	*P*
Tur4_142[a]	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	2	0.01	0.01	1.00	1	0.00	0.00	NA	1	0.00	0.00	NA
Tur4_91[a]	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA
Tur4_141[a]	2	0.25	0.23	1.00	1	0.00	0.00	NA	1	0.00	0.00	NA	2	0.06	0.06	1.00	2	0.08	0.08	1.00	2	0.08	0.08	1.00
Tur4_F10[a]	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	3	0.06	0.09	0.05	2	0.08	0.08	1.00	2	0.25	0.23	1.00
Tur4_E12[a]	3	0.75	0.66	0.77	3	0.30	0.59	0.02*	3	0.45	0.53	0.15	4	0.68	0.65	0.85	3	0.67	0.68	0.21	2	0.33	0.39	1.00
Tur4_105[a]	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	4	0.04	0.04	1.00	1	0.00	0.00	NA	2	0.25	0.23	1.00
Tur4_80[a]	1	0.00	0.00	NA	2	0.10	0.10	1.00	2	0.05	0.05	1.00	5	0.03	0.08	0*	2	0.08	0.23	0.13	1	0.00	0.00	NA
Tur4_87[a]	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	3	0.03	0.03	1.00	1	0.00	0.00	NA	1	0.00	0.00	NA
Mk6[b]	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	1	0.00	0.00	NA	2	0.58	0.52	1.00
Mk8[b]	3	0.62	0.62	0.73	2	0.60	0.53	1.00	5	0.50	0.45	0.13	4	0.43	0.46	0.03*	4	0.75	0.69	0.45	2	0.42	0.43	1.00
Kw2[c]	2	0.75	0.50	0.43	2	0.20	0.50	0.08	5	0.60	0.62	0.92	5	0.55	0.67	0.15	3	0.08	0.70	0.55	1	0.00	0.00	NA
Kw12a[c]	1	0.00	0.00	NA	2	0.30	0.39	0.48	2	0.15	0.14	1.00	2	0.46	0.39	0.20	1	0.00	0.00	NA	2	0.08	0.08	1.00
Ev37mn[d]	2	0.62	0.46	0.48	2	0.20	0.50	0.08	3	0.25	0.23	1.00	3	0.44	0.43	1.00	4	0.17	0.30	0.09	1	0.00	0.00	NA
TexVet5[e]	2	0.12	0.12	1.00	1	0.00	0.00	NA	2	0.05	0.05	1.00	1	0.00	0.00	NA	2	0.08	0.08	1.00	2	0.25	0.23	1.00
Ttr63[f]	2	0.12	0.12	1.00	1	0.00	0.00	NA	3	0.35	0.50	0.23	3	0.63	0.51	0.06	2	0.33	0.29	1.00	1	0.00	0.00	NA
Ttr04[f]	2	0.50	0.40	1.00	3	0.70	0.65	0.37	4	0.65	0.66	0.37	5	0.78	0.75	0.69	4	0.58	0.47	1.00	3	0.42	0.68	0.28

NA number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, *P* *P*-value of exact test using Markov chain, *NA* not available

* Significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$)

[a] Nater et al. (2009), [b] Krützen et al. (2001), [c] Hoelzel et al. (1998b), [d] Valsecchi and Amos (1996), [e] Rooney et al. (1999), [f] Rosel et al. (2005)

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