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Bottlenose dolphin communities from the southern Brazilian coast: do they exchange genes or are they just neighbours?

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Abstract. The genetic structure of bottlenose dolphin communities found along the southern Brazilian coast is reported in this study. Genetic structure analysis using biopsy samples from free ranging dolphins and tissue samples from stranded dolphins revealed a fine-scale population structure among three distinct groups. The first genetically distinct group was composed of resident dolphins of Laguna with a high degree of site fidelity. The second group was composed of one photo-identified dolphin, previously recognised by its interaction with fishermen, and dolphins that stranded near the mouth of Tramandaí Lagoon. Moderate nuclear and low mitochondrial gene diversity was found in dolphins of those coastal communities, whereas most of the dolphins stranded along the coast showed markedly higher levels of gene diversity at both markers. These stranded dolphins of unknown origin formed the third distinct group, which may be part of a larger offshore community. These results demonstrate the presence of at least three bottlenose dolphin clusters along this portion of the Brazilian coast, with the coastal specimens appearing to be only neighbours of a larger offshore community that eventually strands along the coast, highlighting the importance of the establishment of management and conservation measures for the species at a local scale.

Additional keywords: gene flow, molecular markers, population structure, *Tursiops truncatus*.

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Introduction

The lack of visible geographic barriers in the marine environment and the wide distribution of some species over the oceans can often lead to different groups of the same cetacean species being considered as a single large population. However, in recent years, several studies based mainly on molecular and photo-identification data have demonstrated that communities of widely distributed cetacean species can be restricted to single areas or subdivided into multiple independent demographic units over small geographic scales (Rosel *et al.* 1994; Brown Gladden *et al.* 1997; Hoelzel *et al.* 1998; Parsons *et al.* 2002; Natoli *et al.* 2004; Martien *et al.* 2005; Natoli *et al.* 2005; Sanino *et al.* 2005; Waring *et al.* 2007; Baird *et al.* 2009). The genetic structure of common bottlenose dolphin (*Tursiops truncatus*)

communities appears to be highly dependent on the type of habitat occupied. Protected coastal habitats, such as embayment, lagoons and estuaries, are usually inhabited by genetically differentiated small groups with a high degree of site fidelity, local adaptation to different ecological conditions and differential resource use strategies. In contrast, open coastal waters are usually inhabited by larger communities (Wells *et al.* 1987; Hoelzel 1998; Defran and Weller 1999; Möller *et al.* 2007), presenting lower genetic differentiation and higher genetic diversity than those restricted in distribution.

The common bottlenose dolphin has a worldwide distribution, inhabiting a wide range of habitats. In Brazil, the species is distributed from the north-east to south-east coast living in lagoons, coastal bays or ocean waters (Pinedo *et al.* 1992; Ott *et al.* 2009; Gondim *et al.* 2013).

Specifically in southern Brazil, bottlenose dolphins have been commonly observed forming small associated communities within estuaries and river mouths in few areas (Simões-Lopes *et al.* 1998; Fruet *et al.* 2011; Daura-Jorge *et al.* 2013) such as the resident community of bottlenose dolphins of Santo Antônio dos Anjos Lagoon, in Laguna ($n = 54$; Simões-Lopes *et al.* 1998; Daura-Jorge *et al.* 2012). This dolphin community presents an apparent mutualistic interaction with artisanal fishermen: through synchronised behaviour, a subset (45%) of these dolphins drive mullet schools towards a shoreline of fishermen, and by ritualised signals, show when and where fishermen should throw the fishing nets (Simões-Lopes *et al.* 1998). A similar behavioural pattern was observed in resident dolphins around Tramandaí Lagoon (Tramandaí) and Mampituba River (Torres) (Simões-Lopes *et al.* 1998), which are the nearest neighbour estuarine communities located respectively 219 and 133 km south of Laguna, suggesting the complex behaviour is transmitted by matrilineal lines and social network (Simões-Lopes *et al.* 1998). Several long-term photo-identification studies explored population parameters and identified a considerable portion of individuals from these groups along the southern coast of Brazil. For the Laguna dolphins, the presence of high site fidelity of almost the entire community was verified with a low probability of dispersion of individuals to outside areas (Daura-Jorge *et al.* 2013). In contrast, dolphins from Tramandaí and Torres exhibited occasional movements between these areas, having been observed in coastal areas 219 km north and 314 km south of Tramandaí (e.g. Möller *et al.* 1994; Simões-Lopes and Fábian 1999; Hoffmann 2004). To date, the genetic relationships and the degrees of kinship of these resident coastal dolphins, and even among groups formed by transient individuals, are poorly understood.

With high level of site fidelity (Daura-Jorge *et al.* 2013), the small communities of Laguna ($n = 54$) (Simões-Lopes and Fábian 1999; Daura-Jorge *et al.* 2013), Tramandaí ($n = 9$) (Simões-Lopes and Fábian 1999; Giacomo 2010; Giacomo and Ott, in press) and Torres ($n = 7$) (Bernardi 2000; Hoffmann 2004) may be subjected to greater risks of extinction compared to populations with higher numbers of individuals and larger living areas (Thompson *et al.* 2000). Coastal dolphin populations are usually the most affected by anthropogenic actions. The increase of human activities can promote changes in habitat use, reduction in reproductive rates and higher mortality rates (Simões-Lopes and Daura-Jorge 2008; Viaud-Martinez *et al.* 2008).

Furthermore, genetic analysis have suggested that resident dolphins from Laguna have high maternal philopatry, restricted dispersal and low gene flow with coastal dolphin communities of southern Brazil (Fruet *et al.* 2014). According to those authors, such genetic differentiation is suggested to be due to the presence of a unique foraging technique observed only in Laguna. Fruet *et al.* (2014) analysed only biopsied samples of bottlenose dolphin communities where the mutualistic interaction with fishermen was not observed. In this sense, the present study aims to evaluate the genetic diversity and population structure among the specimens of *T. truncatus* inhabiting the estuary area of Laguna and the relationship of the resident bottlenose dolphins of Laguna with individuals that stranded along neighbouring areas in the southern Brazilian coast, where similar foraging technique is employed. This information is essential to support future viability analysis, from which conservation status can be assessed and may help to drive adequate conservation measures for each identified unit.

Methods

Sample collection and DNA extraction

A total of 41 specimens of *T. truncatus* were analysed in the present study. Skin tissues were obtained from photographically identified resident dolphins inhabiting the Santo Antônio dos Anjos Lagoon, Laguna, SC ($n = 10$), and the mouth of Mampituba River, Torres, RS ($n = 01$), using a biopsy dart system (Brown *et al.* 1991). Furthermore, we also used tissue samples of dolphins of unknown origin that were found stranded along the coasts of Santa Catarina (SC (28°29′S; 48°45′W)) and Rio Grande do Sul (RS (31°20′S; 51°00′W)), between 1993 and 2012 ($n = 30$). From these strandings, two samples were recognised as resident individuals of the Mampituba River (GEMARS 0333) and Tramandaí Lagoon (GEMARS 1259, a dolphin known by the local fishermen as ‘Lobisomen’).

The samples were stored in 70% ethanol or DMSO (Amos and Hoelzel 1991). Genomic DNA extractions were performed with standard phenol-chloroform (Sambrook *et al.* 1989) and NaCl protocols (Medrano and Aquilar-Cordova 1990) or using the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer’s instructions. The sex of stranded individuals was recorded, whenever possible, by inspection of the external genital slit, whereas the sex of free-ranging biopsied dolphins and stranded animals in advanced degrees of decomposition (whose sex determination was not possible by visual inspection) was identified by amplification of ZFX and ZFY introns (Palsbøll *et al.* 1992).

Microsatellite genotyping and analysis

A total of five nuclear microsatellite loci (EV37Mn (Valsecchi and Amos 1996); D08 (Shinohara *et al.* 1997); KWM9b; KWM12a (Hoelzel *et al.* 1998) and TexVet5 (Rooney *et al.* 1999)) were amplified following polymerase chain reaction (PCR) conditions in 10 μL reactions: 2 μL DNA (concentration ~20 ng μL^{-1}) was added to 0.13 μM of forward primer and 0.2 μM of reverse primer, 10 mM TRIS-HCl pH 8.3, 50 mM KCl, 0.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.02 U μL^{-1} *Taq* polymerase and 2 μM fluorescent marker (FAM). A M13-tail was added to the forward primer (5′-CACGACGTTGTAAAACGAC-3′),

which was combined with a fluorescent marker (FAM) (Boutin-Ganache *et al.* 2001). The PCR cycling profile was as follows: 5 min at 95°C, then 35 cycles of 40 s at 94°C, 1 min at the selected annealing temperatures (KWM12a: 46°C; KWM9b: 55°C; TexVet5: 54°C; EV37Mn: 57°C; D08: 57°C), 1 min at 72°C; then 10 min at 72°C. Approximately 2 μL of PCR product was diluted in ultrapure water and genotyped on an automated MegaBACE 1000 DNA sequencer (Amersham Biosciences, Uppsala, Sweden) at the Centro de Biologia Genômica e Molecular (Pontifícia Universidade Católica do Rio Grande do Sul). The allele sizes were estimated using Genetic Profiler 2.2 (Amersham Biosciences). Allele sizes were determined and genotyping errors checked using Allelogram (Manaster 2002).

The most probable number of populations (K) that best explains the pattern of genetic structure was estimated using the program STRUCTURE 2.0 (Pritchard *et al.* 2000). We assumed the admixture model and performed the analysis considering both independent and correlated allele frequency models with no prior information on sampling location letting K vary between one and four (according to the number of bottlenose dolphin communities with high levels of habitat residency in southern Brazil). Five independent runs were performed for each value of K, with a 1 000 000 burn-in period and 1 000 000 repetitions of the Markov Chain Monte Carlo (MCMC). The level of differentiation among populations was estimated as F_{st} (Wright 1978) using the program ARLEQUIN 3.1 (Excoffier *et al.* 2005).

Each microsatellite locus was checked for the presence of linkage disequilibrium and null alleles using GENEPOP 4.1.3 (Rousset 2008). Genetic diversity was estimated as the number of alleles per locus (A), number of private alleles (PA) and allele frequencies using GENALEX 6.41 (Peakall and Smouse 2006). Allelic richness (AR) was calculated using the program FSTAT 2.9.3 (Goudet 2001). Observed heterozygosity (Ho), expected heterozygosity (He) and the inbreeding coefficient F_{is} were calculated at each locus and population using ARLEQUIN 3.1. Deviations from the Hardy–Weinberg equilibrium (HWE) were tested using the Markov chain method (number of steps and dememorisation steps set at 10 000, Bonferroni correction applied) using ARLEQUIN 3.1. We calculated the relatedness between all individuals using RE-RAT online software (Schwacke *et al.* 2005). We performed 100 simulations using the Queller and Goodnight's (1989) pairwise index of relatedness. Pairs of individuals were considered closely related when relatedness values were higher than 0.45 ($r \geq 0.45$), following Rosel *et al.* (2009).

Mitochondrial DNA sequencing and analysis

A 316 bp fragment of the control region of the mitochondrial DNA (mtDNA) was amplified with universal primers Dlp-5 (5′-CCATCGWGATGTCTTATTTAAGRGGAA-3′) and Dlp-10 (5′-CCACAGTACTATGTCCGTATT-3′; Baker *et al.* 1993) following PCR conditions in 25-μL reactions: 1 μL of DNA (concentration ~100 ng μL^{-1}) was added to 1× PCR Master Mix DreamTaq (Fermentas, Lituania) and 1.6 pmol μL^{-1} of each primer. The PCR cycling profile was as follows: 1 min at 93°C, then 30 cycles of 30 s at 93°C, 30 s at 55°C, 45 s at 72°C; then 5 min at 72°C. PCR results were verified through electrophoresis of the amplicons on 1% agarose gels stained with ethidium bromide, visualised under UV transillumination, and purified by an ammonium acetate protocol. Amplicons were submitted to direct sequencing at Macrogen (Macrogen Inc., Seoul, Korea) and the Centro de Biologia Genômica e Molecular, with each sample sequenced in both directions. The quality of the sequencing was verified by CHROMASPRO (http://www.technelysium.com.au, accessed 21 October 2012), and its species identity was confirmed using BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed 6 June 2013). Sequence alignment was performed using CLUSTALX (Thompson *et al.* 1997), and manual edits were conducted using BIOEDIT 7.0.9 (Hall 1999).

For mtDNA sequence data, the number of haplotypes (h), nucleotides (π) and haplotype (Hd) diversities were estimated using DNASP 5.1 (Rozas *et al.* 2003). A median-joining network was generated to infer phylogenetic relationships among the mtDNA haplotypes, using the program NETWORK 4.6 (Bandelt *et al.* 1999). The program ARLEQUIN 3.1 was used to assess the degree of genetic differentiation among clusters identified by STRUCTURE using both F_{st} and φ_{st}. Significance was tested based on 10 000 permutations.

Results

Molecular sexing allowed the determination of the sex of all but seven individuals, with the incomplete sex determinations due to PCR amplification failure, caused by the degradation of the tissue sample. The results of the sexing revealed 26 males and 8 females. A total of 37 out of 41 specimens of *T. truncatus* were analysed using five microsatellite loci, and 40 out of 41 were analysed for the control region of the mtDNA.

Microsatellite analyses

The STRUCTURE analysis indicated that the observed genetic variability was best explained with a subdivision into three clusters (K = 3) (Fig. 1). Cluster 1 (light grey ($n = 11$)) comprised all dolphins biopsied inside the Santo Antônio dos Anjos Lagoon (Laguna) except for one (all dolphins of this area were determined to be residents according to photo-identification efforts), one dolphin biopsied in the mouth of Mampituba River and a dead dolphin found near the Tramandaí Lagoon. Cluster 2 (dark grey ($n = 8$)) comprised one dolphin biopsied inside the Santo Antônio dos Anjos Lagoon, one resident dolphin of the Tramandaí Lagoon (GEMARS 1259) found stranded dead (29°58′S; 50°07′W), and six dolphins that stranded near the mouth of the Tramandaí Lagoon (29°52′S; 50°04′W and 30°47′S; 50°32′W; within 21 km north and 91 km south of Tramandaí Lagoon). Cluster 3 (black ($n = 13$)) comprised half the dolphins (13 out of 26) that stranded along the coasts of Santa Catarina and Rio Grande do Sul (28°29′S; 48°45′W and 31°20′S; 51°00′W). The program also pointed out five individuals for which the group of origin was less clearly defined (i.e. mix of the three clusters and assignment probabilities less than 70%). Therefore, these individuals (referred to here as 'mixed clusters') were not assigned to any of the clusters cited above (Fig. 2). Pairwise F_{st} values between the three identified clusters showed significant genetic differentiation between all groups (Cluster 1 × Cluster 2: 0.27953; Cluster 1 × Cluster 3: 0.24498; Cluster 2 × Cluster 3: 0.23247; $P < 0.0001$ for all pairwise F-statistics). Pairwise F_{st} value was also performed considering

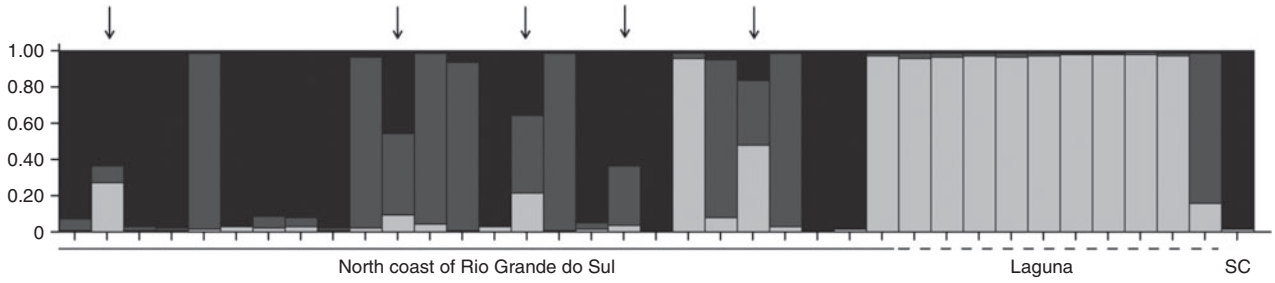

Fig. 1. Estimated proportions of each individual cluster (vertical bars) assigned to Cluster 1 (light grey), Cluster 2 (dark grey) and Cluster 3 (black). The arrows indicate the five individuals with genetic characteristics of the three clusters and assignment probabilities less than 70% (referred as 'mixed clusters'). The first 25 specimens stranded along the coast of Rio Grande do Sul and the 26th was biopsied in the mouth of the Mampituba River (north coast of Rio Grande do Sul; continued line), the next 10 individuals were biopsied inside the Santo Antônio dos Anjos Lagoon (Laguna; dashed line) and the last one stranded along the coast of Santa Catarina (SC).

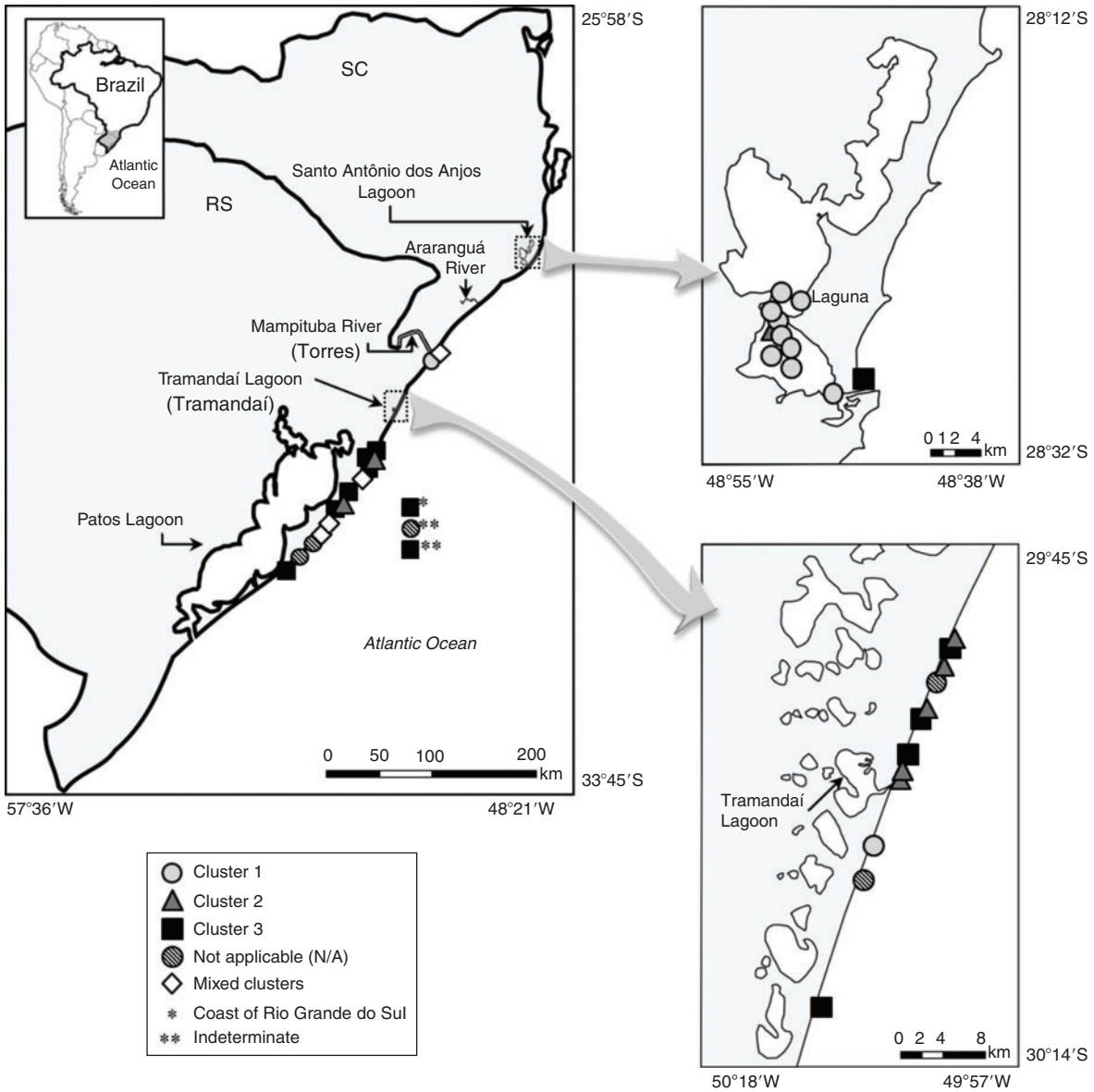

Fig. 2. Distribution of the specimens of three genetically distinct bottlenose dolphin clusters (Cluster 1, Cluster 2, and Cluster 3), the five individuals for whom the clusters of origin were less clearly defined ('mixed clusters'), and stranded samples with no genotype data (not applicable). Individuals without detailed information of the sampling location are denoted by asterisks: *, samples found on the coast of Rio Grande Sul; **, samples found in the study area.

the geographic location of the samples – photo-identified resident dolphins in Santo Antônio dos Anjos Lagoon ($n = 10$) *v.* stranded dolphins along the coast of Santa Catarina and Rio Grande do Sul ($n = 27$). The biopsied dolphin of Mampituba River and the resident dolphin of Tramandaí Lagoon were also included in the second group because they were not considered to belong to the former resident community. The five 'mixed clusters' individuals were also added in this second group: 0.12401; $P < 0.0001$.

No linkage between pairs of microsatellite loci and no evidence of null alleles were detected. Overall, all loci were polymorphic with 3–10 alleles per locus, and He and Ho ranging between 0.3679 and 0.8961 and 0.1000 and 0.7272 respectively. Levels of polymorphism varied among the three groups, with Cluster 3 showing the highest AR, He and number of PAs per locus. A significant positive value of F_{is} was observed only for Cluster 3 (F_{is}: 0.2437, $P = 0.000000$), suggesting a possible further subdivision within this population. He was greater than that observed for all loci of Cluster 3. Evidence of departure from expected Hardy–Weinberg proportions was detected in one locus of Cluster 3 and one of Cluster 2, even after the Bonferroni correction ($P < 0.01$) (Table 1).

Table 1. Genetic variability at five microsatellite loci in Cluster 1, Cluster 2, and Cluster 3

A, number of alleles; AR, allelic richness; He, expected heterozygosity; Ho, observed heterozygosity; *n*, number of individuals analysed; PA, private alleles; *, departure from Hardy–Weinberg equilibrium ($P < 0.01$); –, monomorphic locus

	D08	TexVet5	EV37Mn	KWM12a	KWM9b
Cluster 1 ($n = 11$)					
A	2	1	2	2	2
AR	2.000	1.000	2.000	1.996	1.990
PA	0	0	1	0	0
Ho	0.72727	–	0.10000	0.30000	0.45455
He	0.51948	–	0.47895	0.39474	0.36797
Cluster 2 ($n = 8$)					
A	3	3	3	4	1
AR	2.749	2.750	2.750	3.693	1.000
PA	1	2	2	0	0
Ho	0.62500	0.62600	0.12500*	0.50000	–
He	0.49167	0.54167	0.59167	0.59167	–
Cluster 3 ($n = 13$)					
A	7	7	8	7	9
AR	5.905	5.468	5.804	5.749	7.053
PA	5	6	7	3	6
Ho	0.70000	0.46154*	0.66667	0.53846	0.54545
He	0.83684	0.79692	0.77174	0.84615	0.89610

The relatedness analysis demonstrated that individuals of Clusters 1 and 2 were closely related between and among them. High relatedness values ($0.45 < r < 0.63$) were observed between dolphins from Cluster 1 ($n = 7$) and Cluster 2 ($n = 5$), or even between resident dolphins from Laguna ($n = 4$) and non-resident dolphins assigned to Clusters 1 and 2 ($n = 7$). Individuals of Cluster 3 were not closely related to any individual of other clusters (relatedness values ranging from 0 to 0.36).

Mitochondrial DNA analyses

A 316 bp fragment of the mtDNA control region was obtained from all but one individual (from Cluster 2). Comparison of aligned consensus sequences allowed the identification of 21 polymorphic sites and a total of eight different haplotypes. Moderate haplotype [Hd = 0.715 (±0.065)] and nucleotide [$\pi = 0.01688$ (±0.00159)] diversities were observed for the species. The lowest mtDNA diversity was found in Cluster 1 ($n = 11$), where only one haplotype (H3) was detected (Table 2). The most common haplotype in all clusters (H3) was found in 20 out of 40 individuals analysed. Cluster 2 ($n = 8$) presented the haplotypes H3 (50%), H7 (25%) and H8 (12.5%). Cluster 3 presented almost all of the haplotypes observed in this study (Table 2). The haplotypes H4 and H5 were found exclusively in Cluster 3 and in those specimens with 'mixed clusters' (i.e. no clear cluster defined). The H6 haplotype was observed in only one individual, with no cluster defined. In addition, GEMARS 1259 (known as a resident dolphin of Tramandaí) from Cluster 2 presented the H7 haplotype, whereas the specimen GEMARS 0333 (previously photo-identified in the mouth of Mampituba River) presented the H3 haplotype. However, it was not possible to assign the latter to any of the clusters defined by STRUCTURE (owing to PCR amplification failure), which also occurred for the stranded dolphins GEMARS 1337 (H4), GEMARS 0928 (H6) and GEMARS 1094 (H8), with no genotype data (Fig. 3, GenBank accession numbers: KP404604-KP404611). High levels of genetic structure between the three identified clusters were evident for both F_{st} and φ_{st} (Table 3). When comparing the resident bottlenose dolphins of Laguna with all the non-resident specimens analysed in this study, is also possible to observe high levels of genetic structure (F_{st}: 0.26549; φ_{st}: 0.32499; $P < 0.001$ for all pairwise *F*-statistics).

Discussion

Samples from cetacean carcasses do not always allow an inference of the origin of individuals with confidence because many cetaceans can strand far away from their habitats due to the action of ocean flows or winds (Peltier *et al.* 2012; Prado *et al.* 2013). However, genetic profiles can be a good proxy to

Table 2. Number of haplotypes (h), haplotype (Hd) and nucleotide (π) diversities, with their standard deviations (s.d.), for the three genetically distinct bottlenose dolphin clusters

n, number of individuals sequenced for a 316-bp mtDNA fragment. The haplotype codes are the same as those in Fig. 3

Population	*n*	h	Haplotype codes	Hd (s.d.)	π (s.d.)
Cluster 1	11	1	H3	0.000 (±0.000)	0.000 (±0.000)
Cluster 2	8	3	H3, H7, H8	0.667 (±0.160)	0.01356 (±0.00329)
Cluster 3	13	6	H1, H2, H3, H4, H5, H7	0.833 (±0.071)	0.01823 (±0.00310)

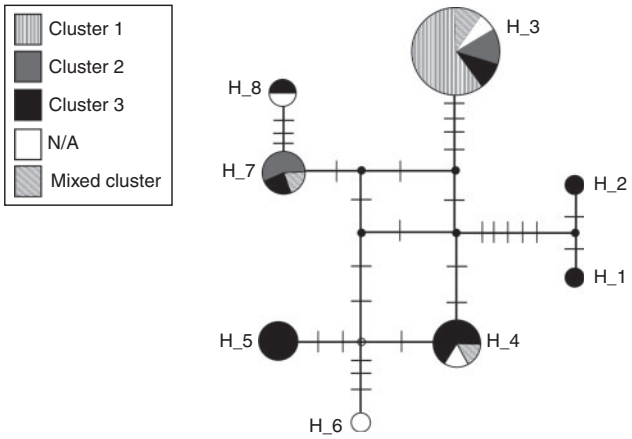

Fig. 3. Minimum spanning network among haplotypes of the bottlenose dolphin clusters. The size of the circles is proportional to the quantity of individuals sampled for each haplotype, and the sectors, defined by colours, are proportional to the haplotype frequency observed in each group. Mixed clusters: individuals for whom the clusters of origin were less clearly defined. N/A, not applicable, sample with no microsatellite data available due to PCR failure. Black little circles indicate either extinct or unsampled haplotypes.

Table 3. Estimation of pairwise F-statistics population differentiation for mtDNA databased on haplotype frequency

F_{st} (below diagonal) and nucleotide diversity – φ_{st} (above diagonal) for the three bottlenose dolphin clusters. *, $P < 0.05$

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	–	0.37769*	0.57061*
Cluster 2	0.31250*	–	0.24831*
Cluster 3	0.48226*	0.14726*	–

infer the source population when significant genetic differences are present. On a global scale, bottlenose dolphins of pelagic waters tend to be characterised by higher levels of genetic diversity than those inhabiting coastal waters (Parsons *et al.* 2002; Natoli *et al.* 2004). In the present study, the analysis revealed higher nuclear and mitochondrial gene diversity in dolphins of Cluster 3 ($n = 13$) than from individuals of Cluster 1 ($n = 11$) and Cluster 2 ($n = 8$), which had a moderate nuclear diversity and very low mitochondrial haplotype diversity. This suggests that dolphins from Clusters 1 and 2, which have individuals regularly sighted in coastal areas such as Santo Antônio dos Anjos Lagoon, the mouth of Mampituba River and Tramandaí Lagoon, belong to distinct local communities of the 'coastal type', whereas individuals comprising the Cluster 3 (composed only of stranded dolphins) could have originated from a larger offshore population.

The presence of distinct bottlenose dolphin ecotypes in the same geographic area is not unusual and has been recorded along several oceanic areas around the world (Duffield *et al.* 1983; Hersh and Duffield 1990; Van Waerebeek *et al.* 1990; Hoelzel *et al.* 1998; Torres *et al.* 2003; Kingston and Rosel 2004; Natoli *et al.* 2004; Sanino *et al.* 2005; Lowther 2006; Segura *et al.* 2006; Perrin *et al.* 2011), including the west coast of the South Atlantic Ocean (Simões-Lopes 1996; Barreto 2000; Oliveira *et al.* 2008).

The sharing of mtDNA haplotypes among the three clusters identified by STRUCTURE and the low genetic diversity of Clusters 1 and 2 is an indication that individuals from Cluster 3 possibly founded these local coastal communities. Offshore populations are probable founder sources, which have created independent discrete population segments in coastal areas as a possible result of philopatry or the emergence of some foraging technique specialisation (Hoelzel 1998; Natoli *et al.* 2004; Sellas *et al.* 2005; Tezanos-Pinto *et al.* 2009).

The high genetic structure implied by both molecular markers suggests reduced gene flow among the identified clusters despite the lack of visible geographic barriers, as well as between the resident bottlenose dolphins from Santo Antônio dos Anjos Lagoon (Laguna) and the non-resident dolphins. For cetaceans, patterns of genetic structure are not always related to merely geographic barriers. Frequently, complex behaviours, such as occupation of coastal areas, new foraging specialisation, philopatry to natal areas or social organisation, play a crucial role in shaping genetic structuring (Hoelzel 1998). Studies have demonstrated that bottlenose dolphins in southern Brazil exhibit high habitat philopatry and developed a unique foraging specialisation known as 'human–dolphin cooperative fishery' (Simões-Lopes *et al.* 1998). The occurrence of this specialised behaviour associated with high natal philopatry in areas like Santo Antônio dos Anjos Lagoon, Mampituba River and Tramandaí Lagoon (Simões-Lopes 1991; Simões-Lopes *et al.* 1998; Daura-Jorge *et al.* 2012), could potentially be an important component in promoting genetic structure in the study area.

Despite the high levels of dolphins' residency in the Santo Antônio dos Anjos and Tramandaí Lagoons (Simões-Lopes 1991; Simões-Lopes and Fábian 1999; Hoffmann 2004; Giacomo 2010; Daura-Jorge *et al.* 2013; Giacomo and Ott, in press) and the great genetic structure between resident dolphins from the former and dolphins from outside areas of this estuary verified in this study, the detection of migrants (i.e. female dolphin LG011 from Cluster 2 found in Laguna and male dolphin MP001 from Cluster 1 found in the mouth of Mampituba River) could be explained by the presence of occasional movements between these areas (Möller *et al.* 1994; Simões-Lopes and Fábian 1999; Hoffmann 2004) and lower gene flow between the coastal clusters. Specimens of Tramandaí ($n = 9$) and Torres ($n = 7$) do not remain permanently inside the estuaries, using the open coast waters more frequently than the mouth of the estuaries (Hoffmann 2004; Giacomo 2010; Giacomo and Ott, in press), with some of them (mostly males) having been already observed traveling along the coastal areas. There are records of the male dolphin GEMARS 1259 in both Santo Antônio dos Anjos and Tramandaí Lagoons, which are ~219 km apart (Möller *et al.* 1994; Simões-Lopes 1995; Simões-Lopes *et al.* 1998). This individual was recognised by its frequent interaction over the years (from 1992 until its death in 2005) with other dolphins, and also with the artisanal fishermen in Tramandaí Lagoon (Moreno *et al.* 2008).

Relatedness values demonstrated that the specimens of Cluster 3 are not closely related to other clusters ($r \leq 0.36$), whereas high relatedness values ($r \geq 0.45$) were observed between pairs of individuals from Cluster 1 and Cluster 2.

In this sense, is possible to suggest that both coastal bottlenose dolphin communities (Clusters 1 and 2) from southern Brazilian coast have low gene flow, as demonstrated by the *F*-statistic values. These coastal specimens appear to be only neighbours of a larger offshore population that eventually strands along the coast. However, the small number of loci used in our study may not provide accurate relatedness estimates. The results must be considered as preliminary, because an increase in number of loci may increase or decrease the apparent relatedness between individuals (Lewis *et al.* 2013). Furthermore, it is important to take in consideration the biology of dolphins when making assumptions about relatedness. Dolphins (i.e. bottlenose dolphins) give birth to only one calf per year, they usually do not reproduce every year, and female dolphins do not mate with the same males in every mating season.

Despite the small population sizes and lower number of loci, our results demonstrated remarkable genetic differentiation for bottlenose dolphins in southern Brazil at small spatial scale. This is in agreement with a recent work that have analysed the genetic structure of five bottlenose dolphins communities along the western South Atlantic Ocean, including dolphins of Laguna ($n = 11$) (Fruet *et al.* 2014). Fruet *et al.* (2014) found low genetic flow between Laguna and adjacent dolphin communities, suggesting that Laguna community may constitute a closed genetic unit. However, it is noteworthy to inform that Fruet *et al.* (2014) did not compare the community of Laguna with the stranded samples used in the present study.

The bottlenose dolphin community of Laguna can be divided into two groups according to the foraging technique employed (Daura-Jorge *et al.* 2012). A subset of 45% of these individuals cooperatively interacts with the artisanal fishermen (similar behaviour was observed in Torres and Tramandaí), also showing possible social and habitat use differentiation from the non-cooperative group. Unfortunately, due to the small number of samples to date (all the biopsied dolphins from Laguna seem to not cooperate with the fishermen) we could not infer if there is also a molecular distinction between these two groups. However, taking into consideration the relatedness between bottlenose dolphins from Laguna and individuals of Cluster 2 (found in areas neighbouring the Tramandaí Lagoon), which includes the cooperative dolphin GEMARS 1259, it is possible to suppose that this unique behaviour has a single origin and was passed from one individual to another by horizontal or vertical behaviour transmission. Therefore, further studies targeting both cooperative and non-cooperative dolphins of Laguna are needed to better understand the presence of individuals of Clusters 1 and 2 inside this estuary and the relatedness between both clusters.

On a small spatial scale, we demonstrated the presence of at least three bottlenose dolphin clusters along the southern coast of Brazil, with low gene flow between dolphins of Laguna and those from outside the estuary. Despite the movement of some individuals among the areas, significant genetic structure between dolphins was observed even among those from nearby estuaries (~219 km apart). Most of the stranded samples were revealed to be part of a possible offshore population (Cluster 3) with high levels of genetic diversity, whereas the other specimens were divided into two coastal groups (Cluster 1 and Cluster 2), which are clearly exposed to multiple human activities and surely facing threats. For example, several studies with both coastal communities indicated the existence of skin diseases such as lobomycosis (LLD), which may be derived from water contamination (e.g. Van Bressem *et al.* 2007; Reif *et al.* 2009). The first case of this disease in southern Brazil was recorded for a dolphin from the Santo Antônio dos Anjos Lagoon (Simões-Lopes *et al.* 1993). Currently, the LLD can be observed in 12% of individuals from this community (Daura-Jorge and Simões-Lopes 2011). LLD was also recorded for two specimens from Tramandaí Lagoon and one individual from the Mampituba River (Hoffmann 2004; Moreno *et al.* 2008). Additionally, the coastal bottlenose dolphins from southern Brazil are threatened by coastal gill-net fisheries, overfishing, habitat degradation, chemical and biological pollution, and boat traffic (Simões-Lopes and Daura-Jorge 2008; Zappes *et al.* 2011). In this sense, the coastal and estuarine communities of *Tursiops* from Rio Grande do Sul (RS) were recently considered as vulnerable in the regional red list (Decreto 51.797, 8 September 2014). The low demographic density of these coastal bottlenose dolphin communities, combined with their biological and ecological traits (e.g. high longevity, low reproductive rates, high degree of residency), as well as the genetic findings of this study (low genetic diversity and apparently moderate to high level of isolation) make them highly vulnerable to human impacts.

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