A note on divergent mtDNA lineages of bottlenose dolphins from coastal waters of southern Australia

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ABSTRACT

Bottlenose dolphins have a global distribution throughout tropical and temperate waters, both inshore and offshore. Many studies demonstrate the existence of at least two *Tursiops* species: *Tursiops truncatus*, consisting of inshore and offshore eco-types and *T. aduncus*, a coastal Indo-Pacific type known to extend south into temperate waters down the east coast of Australia. To clarify the taxonomic status of two populations (Port Phillip Bay and Gippsland Lakes) of coastal bottlenose dolphins along Australia's south coast (Victoria), a 346bp region of the mitochondrial-DNA (mtDNA) control region was sequenced from ten individuals and they were incorporated into phylogenetic analyses involving published sequences of other *Tursiops* spp., *Stenella* spp. and *Delphinus* spp., found worldwide. Both neighbour-joining and maximum parsimony trees place Victorian coastal haplotypes in a highly-supported group separate to those from the other dolphins, including those from the southern part of the Australian eastern coast. Victorian haplotypes are least divergent from *T. truncatus* (average 5.5%) and most divergent from *T. aduncus* (9.1%), with intermediate levels of divergence from *Stenella* and *Delphinus* spp. These data suggest that the Victorian coastal dolphins, similar to other world-wide coastal populations, are genetically unique, long isolated and therefore likely to be locally adapted. This has important implications for management and conservation.

KEYWORDS: GENETICS; TAXONOMY; CONSERVATION; BOTTLENOSE DOLPHIN; AUSTRALASIA

INTRODUCTION

Bottlenose dolphins (Family Delphinidae, Subfamily Delphininae, *Tursiops* genus) have a cosmopolitan distribution and show marked variation, despite being historically recognised as one species, the common bottlenose dolphin, *T. truncatus* (Montague 1821). Morphological and genetic studies have demonstrated the existence of several distinct *Tursiops* forms (inhabiting inshore and offshore regions) that differ in quantitative (and possible plastic) traits. Variable morphological traits include ventral spotting, beak length, body length (Ross and Cockcroft, 1990; Hale *et al.*, 2000; Wang *et al.*, 2000a), diet (Mead and Potter, 1995), haemoglobin type (Hersh and Duffield, 1990) and osteological characteristics (Wang *et al.*, 2000b). Genetic differentiation between 'types' has been observed using AFLP markers (Kingston and Rosel, 2004), cytochrome *b* sequences (LeDuc *et al.*, 1999) and mtDNA control region sequences (Möller and Beheregaray, 2001; Torres *et al.*, 2003). A smaller inshore form described as a separate species, *T. aduncus* (Ehrenberg 1932), occurs largely in warmer coastal waters of China and the Indo-Pacific region, but has recently been described (on the basis of mitochondrial haplotype) from the east coast of Australia (Möller and Beheregaray, 2001). Natoli *et al.* (2004) further suggest that an *aduncus*-type found in southern Africa may represent a third *Tursiops* species. While the coastal Indo-Pacific and distinct South African forms have both been described as species that are distinct from *T. truncatus*, the polytypic single-species perspective has been emphasised by others (e.g. Ross and Cockcroft, 1990). Based on several genetic markers, *T. aduncus* may be more closely related to *Stenella* and *Delphinus* species than to *T. truncatus* (LeDuc *et al.*, 1999; Natoli *et al.*, 2004). The often confusing taxonomic group has been named the '*Stenella-Tursiops-Delphinus-Lagenodelphis*' complex and the level of uncertainty regarding the taxonomy of the bottlenose dolphin worldwide has prompted its listing as a 'priority topic' for the International Whaling Commission (IWC) Scientific Committee's sub-committee on small cetaceans (Reeves *et al.*, 2004). What is clear however, is the emerging worldwide picture that coastal bottlenose dolphins often have local fine scale population structure with unique regional patterns of genetic differentiation and morphology. Historical founder events, long-term isolation and local and historical environmental effects, with reinforcement by philopatry, are the probable causal factors (Natoli *et al.*, 2004).

Australian bottlenose dolphins exhibit distinct regional morphological variation with respect to ventral spotting, body and beak length. One relevant factor may be that the resident populations assume an optimal body size for the local temperature regime, resulting in the formation of clines in body size. On this basis all Australian bottlenose dolphins were assigned to *T. truncatus* (Ross and Cockcroft, 1990). However, more recently *T. aduncus* mtDNA type has been reported from the bottlenose dolphins from coastal regions of eastern Australia (Möller and Beheregaray, 2001). To add to the complexity, Krützen *et al.* (2004) reported that the *Tursiops* population in Shark Bay on the northwest coast of Australia contains two distinct mtDNA lineages showing a level of sequence divergence similar to that seen between Chinese *T. truncatus* and *T. aduncus* (Wang *et al.*, 1999). Uncertainty remains about the taxonomy and population structure of bottlenose dolphins residing in coastal Victoria (southern Australia), in particular those in Port Phillip Bay (Hale, 2002; Scarpaci *et al.*, 2003). Their small physical size (average 2.5m) when compared to those found in Tasmania and further west along the south coast of Australia (3.05m and 2.83m respectively; Ross and Cockcroft, 1990), the absence of ventral spotting and reduced counter-shading, suggest that Port Phillip Bay dolphins may be *T. aduncus*,

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consistent with a recent prediction that *T. aduncus* may be continuously distributed around coastal waters of Australia (Möller and Beheregaray, 2001).

A resident Port Phillip Bay (PPB) population of 80-100 animals, at the southern end of the Bay, is considered to be vulnerable to extinction due to its small size, female natal philopatry, restricted home range and the large degree of associated human activity (Dunn *et al.*, 2001; Hale, 2002). In particular the population has shown high site fidelity to a region that has large amounts of boat traffic and a swim-with-dolphins tourism industry (Dunn *et al.*, 2001). Less direct human threats include urban development around this coastal region (pollution and vandalism), recreational and commercial fishing, channel dredging and heavy shipping traffic. While bottlenose dolphins are also known from one other Victorian coastal site, the Gippsland Lakes (Gips) around 320km east of PPB, little is documented about their population structure and biology.

To clarify their taxonomic status and population affinities, and thus contribute to improved population management, we report here the sequence a 346bp region of the mtDNA control region from ten dolphins from the PPB and Gips populations. These data are incorporated them into phylogenetic analyses involving published sequences of *T. aduncus, T. truncatus,* striped dolphin (*Stenella coeruleoalba*), long-beaked common dolphin (*Delphinus capensis*), and common dolphin (*D. delphis*) and the results discussed in the context of local and worldwide dolphin biology.

METHODS

Skin samples were collected via biopsy sampling (based on the system of Lambertson, 1987) from three individuals known[1] to be members of the local population in the southern end of PPB using a modified Junior Ranger Crossbow. Opportunistic sampling was also undertaken on dead dolphins washed ashore in either PPB (*n*=4) or Gips (*n*=3) as shown in Table 1 and Fig. 1 (additional data on all sampled animals is available from DRI).

Eight samples were preserved in a saline solution of 20% dimethyl-sulfoxide (DMSO), 0.25M EDTA, saturated with NaCl, pH7.5 (Suetin *et al.*, 1991) and two were stored in formaldehyde. Genomic DNA, from samples stored in the 20% DMSO solution, was extracted using a standard protocol (Sambrook *et al.*, 1989) following rinsing with RSB buffer (10mM Tris-Cl, 10mN NaCl, 25mM EDTA) (Davis *et al.*, 1986) to remove residual 20% DMSO solution. For the two samples stored in formaldehyde, DNA was extracted following the method of Rodriguez *et al.* (2002).

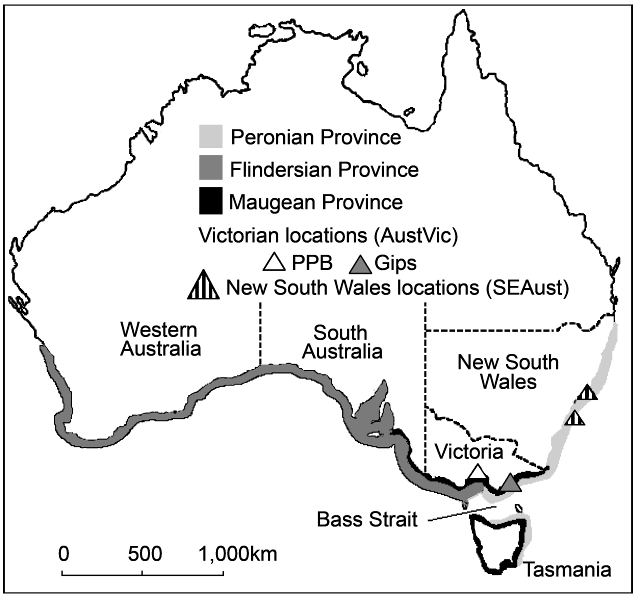

Fig. 1. Map of Australia, including marine bioregions for the south-east region and Australian sampled bottlenose dolphins.

A fragment of mtDNA control region was amplified by polymerase chain reaction (PCR) with primers Dlp 1.5 and Dlp 5 (Baker *et al.*, 1993). The PCR was carried out using *Expand High Fidelity PCR System* (Roche Molecular Biochemicals) to a final volume of 50μl. All PCRs were performed on an Applied Biosystems *GeneAmp PCR System 2700* using the regime reported by Möller and Beheregaray (2001). A *Wizard Purification System* (Promega) was used to purify the PCR product as per the manufacturer's instructions, which was used as template DNA in a cycle sequencing reaction. The thermal cycling conditions for the sequencing reaction consisted of a denaturing step for 30 seconds at 96°C, annealing step for 15 seconds at 50°C and an extension step for 4 minutes at 60°C. This cycle was repeated 25 times with a final hold at 4°C. Reagent concentrations and volumes used were; 6.0μl Terminator mix (Micromon), 100ng/μl template DNA, 5 μM Dlp 1.5 primer, and dH_2O to final volume of 20μl. Samples were analysed on an Applied Biosystems 3100 sequencer. Accuracy was confirmed by sequencing in both directions.

The 10 control region sequences from the Victorian dolphins were assigned to haplotypes (AustVic) (reduced to 346bp). They were aligned by eye with sequences of 4 *T. truncatus,* 4 *T. aduncus,* 2 *S. coeruleoalba,* 2 *D. capensis,* 2

Table 1

Skin samples collected.

Code	Haplolype	Location	Source of sample	Date of collection	Sex	Age
PPB1	AustVic1	PPB	Boat strike	29/11/2001	F	3yrs
PPB2	AustVic2	PPB	Mentone Beach	12/12/2001	M	Adult
PPB4	AustVic2	PPB	PPB biopsy	23/06/2003	-	-
PPB6	AustVic2	PPB	PPB biopsy	24/06/2003	-	-
PPB3	AustVic3	PPB	Geelong	25/03/2002	M	Adult
PPB5	AustVic4	PPB	PPB biopsy	24/06/2003	-	-
Gips2	AustVic5	Gips	Bairnsdale	16/04/2002	F	Calf
PPB7	AustVic5	PPB	Werribee South	26/02/2003	M	Adult
Gips1	AustVic6	Gips	Bairnsdale	07/08/2002	M	Adult
Gips3	AustVic7	Gips	Raymond Island	17/08/2003	M	Sub-adult

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[1] Identified by Dolphin Research Institute (Hastings, Victoria (DRI)) personnel from a photographic database of individuals collected over a ten year period.

Table 2

Control region sequences analysed.

Haplotype	Species	GenBank accession no.	Locality	Reference
Ttru6	*T. truncatus*	AF056224	Taiwan, Indo-Pacific	Wang *et al.* (1999)
Ttru10	*T. truncatus*	AF056228	Taiwan, Indo-Pacific	Wang *et al.* (1999)
Ttru13	*T. truncatus*	AF056231	Taiwan, Indo-Pacific	Wang *et al.* (1999)
Ttru22	*T. truncatus*	U20917	USA, NW Atlantic	Siemann (1994)
Tadu1	*T. aduncus*	AF056233	China and Taiwan, Indo-Pacific	Wang *et al.* (1999)
Tadu8	*T. aduncus*	AF056240	Taiwan, Indo-Pacific	Wang *et al.* (1999)
SEAust2	*T. aduncus*	AF287952	NSW, Australia, SW Pacific	Möller and Beheregaray (2001)
SEAust5	*T. aduncus*	AF287955	NSW, Australia, SW Pacific	Möller and Beheregaray (2001)
Lacu	*L. acutus*	AF113487	NW Atlantic	Cipriano (1997)
S.coer1	*S. coeruleoalba*	AY168600	-	Matzen *et al.*[1]
S.coer2	*S. coeruleoalba*	AY046549	Chinese waters	Yang *et al.*[2]
Dd10	*D. delphis*	AY168605	Azores Islands	Matzen *et al.*[3]
Z115	*D. delphis*	U02662	-	Rosel *et al.* (1994)
CDC2	*D. capensis*	AY185144	Chinese waters	Wang *et al.*[4]
CDC8	*D. capensis*	AY185142	Chinese waters	Wang *et al.*[4]
Oorca	*O. orca*	M60409	-	Hoelzel *et al.* (1991)
AustVic1		AY371171	Victoria, Australia, SW Pacific	This study
AustVic2		AY371172	Victoria, Australia, SW Pacific	This study
AustVic3		AY371173	Victoria, Australia, SW Pacific	This study
AustVic4		AY371174	Victoria, Australia, SW Pacific	This study
AustVic5		AY371175	Victoria, Australia, SW Pacific	This study
AustVic6		AY371176	Victoria, Australia, SW Pacific	This study
AustVic7		AY371177	Victoria, Australia, SW Pacific	This study

[1] Matzen Silva, J., Norberto, R., Matos, J., Mendonca, D., Simoes, F. and Azevedo, J. Direct sequence from GenBank accession number AY168600; [2] Yang, G., Ren, W.H., Niu, M.H. and Zhou, K. Sequence variability of the complete mitochondrial control region of striped dolphins (*Stenella coeruleoalba*). Direct sequence from GenBank accession number AY046549; [3] Matzen Silva, J., Norberto, R., Matos, J., Mendonca, D., Simoes, F. and Azevedo, J. Direct sequence from GenBank accession number AY168605; [4] Wang, J.Y., Yang, G., Liu, H., Zhou, K. and Wei, F.W. The preliminary application of mitochondrial DNA sequence variability in identification of common dolphins (genus Delphinus) in Chinese waters. Direct sequence from GenBank accession numbers AY185142 and AY185144.

D. delphis, 1 Atlantic white-sided dolphin (*Lagenorhynchus acutus)* and 1 killer whale (*Orcinus orca*) available on GenBank (Table 2).

Modeltest v3.5 (Posada and Crandall, 1998) was used to determine the most appropriate model and parameters for phylogenetic analysis of this data set. *PAUP* v4.0b10 (Swofford, 1998) was used to calculate sequence divergence values among haplotypes and to infer their phylogenetic relationships using both neighbour-joining (N-J) and maximum parsimony methods. All trees were generated using unweighted character analysis. A N-J tree was estimated using the HKY +G model (G=0.1156) (Hasegawa *et al.*, 1985) with gamma distribution (shape parameter = 0.2490) and observed ti/tv ratio (4.4082) as determined by *Modeltest* v3.5. All percentage differences cited are averages based on this model. Reliability of tree nodes for all trees was assessed using 1,000 bootstrap replicates. The *L. acutus* and *O. ocra* sequences were used as outgroups (Möller and Beheregaray, 2001; Pichler *et al.*, 2001).

RESULTS

Over the 346bp of the mtDNA control region, five polymorphic sites defined seven haplotypes among the 10 Victorian dolphin sequences. Four haplotypes (AustVic1-4) were only found in PPB, with AustVic2 having the highest frequency (three PPB individuals). AustVic 6 and 7 were each represented by a single Gips individual, while AustVic5 was recorded once in each location. When the AustVic sequences were aligned with the 15 from GenBank, there were a total of 52 variable sites and four fixed differences that characterise the Victorian coastal population (Table 3). All Victorian sequences diverged substantially from the *Tursiops* species sequences (Table 4), with the most similar being *T. truncatus*, from which they differed on average by 5.5% (Hasegawa *et al.*, 1985). Higher sequence divergence was observed between Victorian haplotypes and those of *T. aduncus* (9.1%). Regardless of the phylogenetic reconstruction method, the coastal Victorian sequences formed a strongly supported monophyletic grouping with respect to all other *Tursiops*, *Delphinus* and *Stenella* species (bootstrap values of 98% and 94% for the maximum parsimony and N-J trees, respectively; Fig. 2).

Table 4

Divergence from *Tursiops spp.* sequence (%).

	AustVic
AustVic	0.70%
SEAust (NSW)	9.70%
T. aduncus	9.10%
T. truncatus	5.50%
Stenella spp.	6.00%
Delphinus spp.	6.60%

DISCUSSION

The phylogenetic affinities of the resident PPB bottlenose dolphin population have been controversial, with authors variously describing them as, or predicting them to represent, *T. aduncus* and *T. truncatus* (Hale, 2002; Möller and Beheregaray, 2001; Scarpaci *et al.*, 2003). Our phylogenetic analyses suggest Victorian haplotypes do not cluster with those of other *Tursiops*, *Delphinus* or *Stenella* species. The average sequence divergence of these Victorian

Table 3

Relative position of variable nucleotide.

Haplotype	10	15	35	38	44	45	50	53	55	56	57	61	62	76	82	90	95	98	102	114	140	150	151	159	161	164	191	194	195	211	218	222	224	225	226	228	229	230	234	238	239	240	241	249	251	293	294	303	308	316	337	339	343
Ttru22	T	G	C	T	C	A	C	A	G	T	G	G	C	C	G	T	C	A	G	T	C	C	C	A	T	C	T	G	C	C	A	A	C	T	T	C	C	T	T	A	T	C	C	G	T	G	C	T	G	T	C	T	G
Ttru13	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.
Ttru10	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
Ttru6	.	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	T	.	T	.	.	.	C	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
AustVic1	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	C	T	.	.	.	T	.	.	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	T	A	.	.	.	C	.	.	T	.	.
AustVic2	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	C	T	.	.	.	T	.	T	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
AustVic3	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	C	T	.	.	.	T	.	.	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	.
AustVic4	G	.	.	.	.	.	.	G	A	.	.	.	.	.	.	C	T	.	.	.	T	.	T	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
AustVic5	.	.	.	.	.	.	.	G	A	.	.	.	G	.	.	C	T	.	.	.	T	.	.	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
AustVic6	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	C	T	.	.	.	T	.	.	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
AustVic7	.	.	.	.	.	.	.	G	A	.	.	.	.	T	.	C	T	.	.	.	T	.	.	.	C	.	C	.	A	A	.	.	T	.	.	T	A	.	.	.	.	.	.	A	.	.	.	C	.	.	T	.	.
Tadu1	.	.	.	C	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	T	T	.	T	.	T	C	.	.	G	T	.	T	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.
Tadu8	.	.	.	C	.	.	.	.	.	.	A	A	.	.	.	.	T	.	.	C	T	.	.	T	.	.	.	.	.	G	T	.	T	.	C	T	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
SEAust2	.	.	.	C	.	.	.	.	.	.	A	A	.	.	.	.	T	.	.	C	T	T	.	T	.	T	C	.	.	G	T	.	T	.	C	.	.	C	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.
SEAust5	.	A	.	C	.	.	.	.	.	.	A	A	.	.	.	.	T	.	.	C	T	.	.	T	.	T	C	.	.	G	T	.	T	.	C	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
S.atten	.	.	T	.	T	T	T	G	A	.	.	.	.	.	.	.	T	G	.	.	T	.	T	.	.	.	C	.	.	A	.	G	T	C	.	.	.	.	.	T	C	T	.	.	.	.	.	C	.	C	T	.	.
S.coer1	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	T	.	.	T	.	T	.	.	.	C	C	.	A	-	.	T	.	C	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
S.coer2	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	T	.	A	T	.	T	.	.	.	.	A	.	A	-	.	T	.	C	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
CDC2	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	T	T	.	.	T	.	T	.	.	.	C	A	.	A	-	.	T	.	C	T	.	.	.	.	C	.	.	A	.	.	.	.	.	.	T	.	.
CDC8	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	T	.	A	T	.	T	.	.	.	.	A	.	A	-	.	T	.	C	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
Dd10	.	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	T	T	.	.	T	.	T	.	.	T	C	C	.	A	-	.	T	.	C	T	T	C	.	.	C	.	.	.	.	A	G	.	C	.	T	.	A
Z115	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	A	.	T	.	T	.	.	.	C	.	.	A	-	G	T	.	C	T	.	C	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.

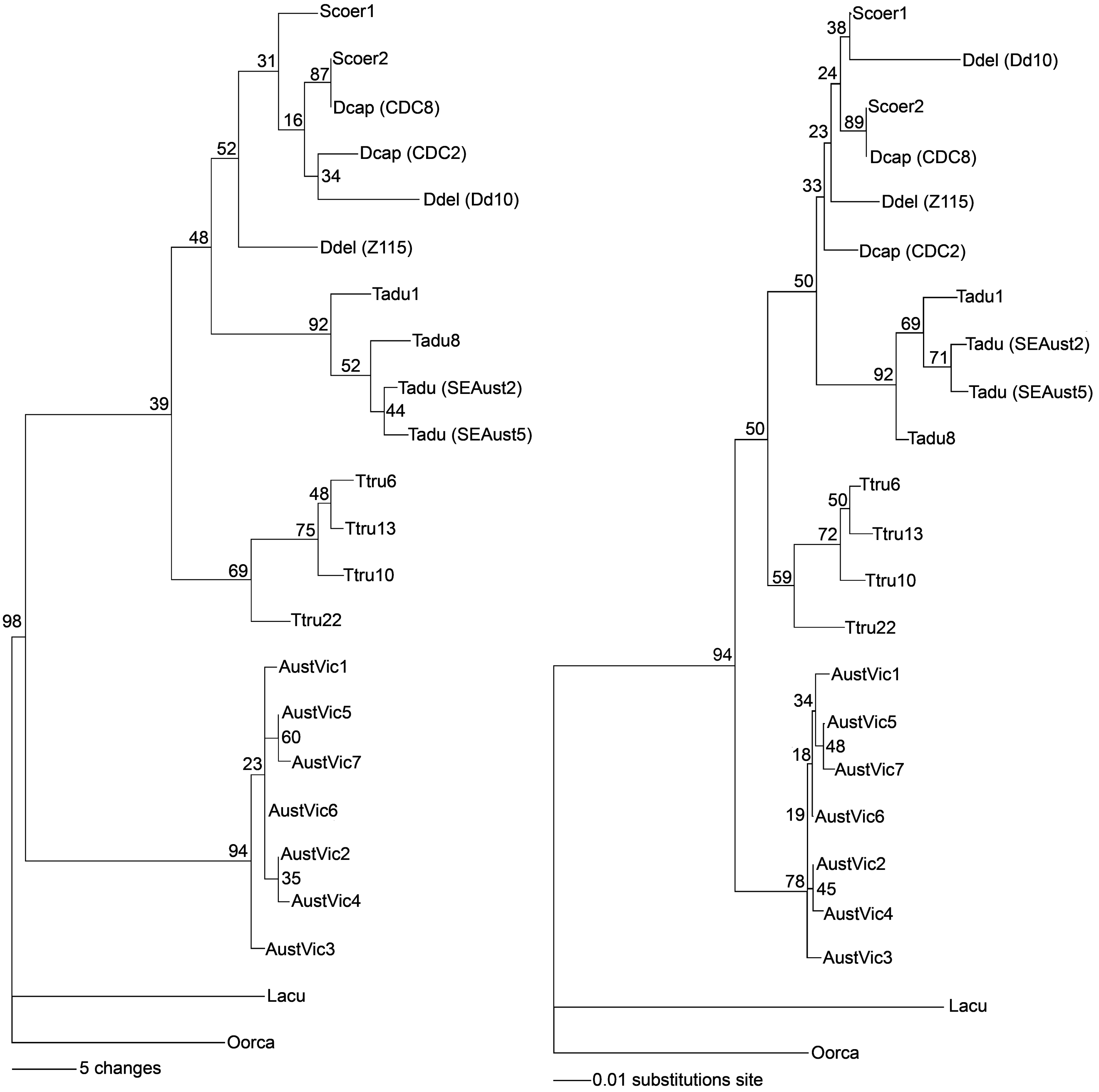

Fig. 2. Maximum parsimony (left) and N-J (right) bootstrap consensus trees based on mtDNA control region sequence of bottlenose dolphins from coastal Victoria (AustVic), and published *T. truncatus* (Ttru), *T. aduncus* (Tadu and SEAust), *S. coeruleoalba* (S.coer), *D. capensis* (CDC) and *D. delphis* (Z115 and Dd10) from different localities (50% majority-rule consensus). Outgroups *L. acutus* (Lacu) and *O. orca* (Oorca). Branch lengths are proportional to amount of genetic change and were calculated along strict consensus tree by PAUP (Swofford, 1998).

dolphins from the *T. truncatus* cluster is similar to that commonly observed between recognised species within each of the *Cephalorhynchus* (2.5-4%) and *Lagenorhynchus* (4.5-6.4%) genera (Pichler *et al.*, 2001), and higher than that between sympatric populations of short-beaked and long-beaked common dolphins, *Delphinus* sp. (1.09%, Rosel *et al.*, 1994). Our placement of taxa within the '*Stenella-Tursiops-Delphinus-Lagenodelphis*' complex agrees with that of LeDuc *et al.* (1999) using cytochrome b sequence, in that *T. aduncus* is more closely-related to *S. coeruleoalba* and *Delphinus* species than to *T. truncatus* (Fig 2). The overall level of mtDNA control region sequence divergence and presence of fixed polymorphisms in coastal Victorian dolphin haplotypes suggest that these populations may represent an undescribed taxon, requiring formal classification incorporating morphological and further genetic analysis.

How might such a divergent group have arisen? The establishment of coastal founder populations may be due to release of suitable habitat during inter-glacial periods (Natoli *et al.*, 2004). During glacial maxima, a Pleistocene landbridge connected Tasmania to mainland Australia, so PPB and Gips were formed only 18,000 years ago (CLIMAP, 1976; Waters and Roy, 2003). Resident dolphin population(s) therefore may have established relatively recently, during the postglacial period. The founders were unlikely to have been from recent ancestors of the eastern Australian coastal population, given the substantial contemporary haplotype divergence (9.7%). Comparable levels of sequence divergence observed between the genus *Lissodelphidae* and other members of its sub-family (7.7%-11.4%) lead Pichler *et al.* (2001) to suggest its early divergence in the history of the sub-family. In a similar way an early separation of Victorian coastal bottlenose dolphins may have occurred from the '*Stenella-Tursiops-Delphinus-Lagenodelphis*' complex. Our sampling has been neither widespread nor extensive and other dolphin groups with other affinity levels may occur in the region.

The distribution and divergence of the coastal Victorian population may be related to the occurrence of a number of marine bioregions that have been defined on the basis of physical and biotic parameters (Knox, 1963). The Maugean province (Fig. 1), which includes the area in which the study populations lie, is a cold-cool temperate region exhibiting a high level of diversity and endemism (Edyvane and Baker, 1995). Further sampling within and close-by on either side of this province will be important to see if and where dolphin phylogenetic barriers occur. The genetic uniqueness of coastal Victorian dolphins, and their possible origins from a cool-temperate bioregion, raises the question of if, and how well they are adapted to local environmental conditions. While the size of the coastal Victorian bottlenose dolphins may be a heritable trait related to its adaptation to water temperature (Ross and Cockcroft, 1990) it may also be a plastic developmental response, adaptive or otherwise, to the local environment. The possibility of local adaptation of cetaceans has been discussed in numerous reports where associations occur between population distributions or pod congregations and prey distributions, local marine habitat features (such as water depth and distance from shore), local currents, water temperatures, salinity changes and the presence of deep 'feeding' channels (Davis *et al.*, 2002; Selzer and Payne, 1988; Watts and Gaskin, 1985; Hastie *et al.*, 2004; Mead and Potter, 1995; Torres *et al.*, 2003; Wilson *et al.*, 1997). While many of these associations are likely to have an adaptive role, it is not known whether they are based on cultural (learned) behaviours or are long-term heritable adaptive characteristics of the populations. None-the-less, recent evidence of heritable and speedy adaptive divergence in many vertebrate species over latitudinal, altitudinal and environmental gradients (Stockwell *et al.*, 2003; Skelly, 2004), suggest that Victorian coastal bottlenose dolphins may be genetically well-adapted and hence an irreplaceable asset.

Given the extensive genetic divergence of the Victorian coastal bottlenose dolphins from other known *Tursiops* they arguably constitute a distinct entity worthy of separate management and conservation effort. The shared polymorphic sites and the existence of a shared haplotype among the PPB and Gips samples suggest close affinities between these locations, and relatively recent gene flow along this part of the coastline. However our sample size is insufficient to establish whether or not we are dealing with a large randomly mating group. The apparent small size of the Port Phillip Bay population, limited knowledge of the Gippsland Lakes population, and increasing anthropogenic threats make both populations vulnerable. Further sampling (including the southern Australian offshore dolphins, and more easterly and westerly populations), and analysis that incorporate morphology and nuclear genetic markers, is needed to elucidate local breeding structure and to determine the size and range of the population.

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