Molecular Phylogenetics and Evolution 49 (2008) 674–681

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

Multi-gene evidence for a new bottlenose dolphin species in southern Australia

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ARTICLE INFO

Article history:
Received 18 April 2008
Accepted 20 August 2008
Available online 26 August 2008

1. Introduction

Molecular genetic studies can be very powerful for investigating species relationships and resolving taxonomic uncertainties. This is particularly true for cryptic or sibling species, which are discrete species that are difficult or sometimes impossible to distinguish morphologically (Knowlton, 1993). One group that has benefited from such studies is the Cetacea. For example, two new species have been recently proposed based on molecular data, the North Pacific right whale, *Eubalaena japonica* (Rosenbaum et al., 2000), and the Costero, *Sotalia guianensis* (Caballero et al., 2007).

The family Delphinidae is the largest family of cetaceans, comprising approximately 36 species of dolphins and small toothed whales (Rice, 1998), which appear to have diverged relatively recently (11 mya, Barnes et al., 1985). Due to its recent radiation there has been apparently little time for the development of diagnostic characters, making taxonomic classification within the family generally difficult (e.g. Pichler et al., 2001). Taxonomic affinities within one of its sub-families, the Delphininae, have been especially problematic to assess since there are no clear morphological characters that can be used to distinguish genera and a number of species in this sub-family (Perrin et al., 1981). In Delphininae, the taxonomy of bottlenose dolphins, genus *Tursiops*, has been among the most controversial (e.g. Ross and Cockcroft, 1990; Wang et al., 1999; Natoli et al., 2004). Several species were originally described in this genus but most were later considered as synonyms of the common bottlenose dolphin, *T. truncatus* (Rice, 1998). In addition to *T. truncatus*, the only other species that presently retains its specific status is the Indian Ocean or Indo-Pacific bottlenose dolphin, *T. aduncus* (Rice, 1998). In South Africa the two species were distinguished based on differences in snout length, ventral spotting and size, among other characters (Ross, 1977). However, the classification of *T. aduncus* as a separate species from *T. truncatus* was later retreated when latitudinal variation on size and morphological characters was found in Australian bottlenose dolphins (Ross and Cockcroft, 1990). It was not until a comprehensive cytochrome *b* phylogeny of the family Delphinidae was conducted that *T. aduncus* was re-considered as a valid species (LeDuc et al., 1999). This study showed that the genus *Tursiops* was polyphyletic, and that the Indo-Pacific bottlenose dolphin *T. aduncus* had closer affinities with some species of the genus *Stenella* (*S. frontalis*, *S. coeruleoalba* and *S. clymene*) and common dolphins *Delphinus* spp. than to the common bottlenose dolphin *T. truncatus*. The authors recommended that until a full taxonomy revision of the sub-family Delphininae is carried out, generic names should be maintained, while accepting *T. aduncus* as a valid species. Genetic evidence for taxonomic status of *T. aduncus* was then confirmed by a mtDNA control region phylogeny of sympatric *truncatus* and *aduncus*-type dolphins from Chinese waters (Wang et al., 1999), a finding corroborated by morphological and osteological studies (Wang et al., 2000a,b).

After *T. aduncus* was re-considered as a valid species, its presence in Australian waters was proposed based on both morphological (Hale et al., 2000; Kemper, 2004) and genetic studies (Möller and Beheregaray, 2001), although Hale et al. (2000) refers to *T. cf. aduncus*. *Tursiop cf. aduncus* was found in estuarine and near-coastal oceanic waters of eastern and northern Australia (Hale et al., 2000). *Tursiops aduncus* was proposed for coastal waters of New South Wales based on genetic data (Möller and Beheregaray, 2001) and for the state of South Australia based on osteological characters (Kemper, 2004).

Further advances and dispute revolving genetic affinities and the taxonomy of bottlenose dolphins nonetheless continued to emerge with additional phylogenetic studies of the mtDNA control region which included animals from other regions. Recently, results from a geographically broad phylogenetic study suggested that *aduncus*-type dolphins from South Africa may represent a

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1055-7903/$ - see front matter
doi:10.1016/j.ympev.2008.08.011

different species from *T. truncatus* and *aduncus*-type Chinese dolphins (Natoli et al., 2004). Similarly to the findings of Natoli et al. (2004), a mtDNA study of bottlenose dolphins from the state of Victoria, southern Australia, has suggested that animals in this area may represent an undescribed taxon, which appeared to be more related to *T. truncatus* than *T. aduncus* (Charlton et al., 2006). However, the mtDNA control region phylogeny in this study was reconstructed based solely on a few species of Delphininae, making uncertain the genetic affinities of this taxon to other members of this sub-family.

In this study we aim to further clarify the taxonomic status of southern Australian bottlenose dolphins based on a multi-gene approach and a much larger dataset in number of species, geographic range and population samples to investigate their phylogenetic relationships within the sub-family Delphininae. To achieve this aim we used mtDNA data to investigate phylogenetic relationships of these dolphins within the sub-family Delphininae and also used information from nuclear DNA to test for reproductive isolation between bottlenose dolphins found in southeastern and southern Australia.

2. Material and methods

2.1. Samples

A total of 182 samples of bottlenose dolphins were obtained in southeastern and southern Australia from the states of New South Wales (NSW), Tasmania (TAS), Victoria (VIC) and South Australia (SA) (Appendix Fig. S1). We used biopsy techniques (Krützen et al., 2002; Bilgmann et al., 2007) to sample coastal animals (defined as animals sampled up to 0.5 km from shore) in Jervis Bay and Twofold Bay/Merimbula, NSW ($n = 42$), Port Phillip Bay, VIC ($n = 3$) and in Southeast Spencer Gulf, North Spencer Gulf, Coffin Bay, Southwest Spencer Gulf and around St. Francis Island, SA ($n = 84$). We also biopsy sampled offshore animals (defined as animals biopsied >0.5 km from shore) off Byron Bay, Yamba, Forster and Sydney, NSW ($n = 22$) (Appendix Table S1). Coastal bottlenose dolphins from Jervis Bay were previously identified through a molecular phylogeny of the mtDNA control region to belong to the Indo-Pacific species, *T. aduncus* (Möller and Beheregaray, 2001). Samples of bottlenose dolphins were also collected from stranded individuals or animals entangled in nets in several locations in VIC ($n = 13$) and TAS ($n = 18$) (Appendix Table S1). In addition, samples of a closely related Delphininae species which occurs in the same geographic area—short-beaked common dolphins (*Delphinus delphis*) ($n = 52$) biopsied in the Great Australian Bight and Spencer Gulf Region in SA (Appendix Table S1)—were included for a comparison of levels of divergence and gene flow.

2.2. Genetic methods

DNA was extracted from samples using a salting-out protocol (Sunnucks and Hales, 1996). Samples from VIC and TAS were extracted using Gentra Systems, Puregene Tissue kit following manufacturer's instructions. Amplification of ca. 400-bp of the mtDNA control region was obtained following methods in Möller and Beheregaray (2001). Amplified fragments were then screened for sequence variation by the single-stranded conformation polymorphism (SSCP) analysis (Sunnucks et al., 2000), with the exception of samples from VIC and TAS, which were all sequenced. Representatives of all identified SSCP phenotypes were then sequenced in an ABI 377 DNA sequencing system according to manufacturer's instructions. All individuals with rare phenotypes were sequenced, whereas at least 15% of individuals with common phenotypes were sequenced. All different phenotypes confirmed as different sequences (i.e. haplotypes) and individuals with the same phenotype always had identical sequences.

In addition, sequence data of ca. 1000-bp of the mtDNA cytochrome *b* region of selected samples (see mtDNA data analysis below for explanation) was obtained through PCR using a primer located on the tRNA glutamine and a primer located on the tRNA threonine, as reported in LeDuc et al. (1999). PCR conditions consisted of an initial denaturation at 92°/10 min, followed by 40 cycles with 92 °C/1 min denaturation, 51 °C/1 min annealing and 72 °C/1 min extension, and a final extension at 72 °C/10 min. PCR products were separated by electrophoresis on 1% agarose gels, excised and purified with UltraClean 15 DNA purification kit (MO BIO, USA). PCR conditions for the VIC and TAS samples consisted of an initial denaturation at 94°/4 min, followed by 3 cycles with 40 cycles with 94 °C/45 s denaturation, 50 °C/1 min annealing and 72 °C/1 min extension, followed by 32 cycles of 94 °C/20 s denaturation, 50 °C/1 min annealing and 72 °C/1 min extension, and a final extension at 72 °C/3 min. Sequencing was done directly from the purified PCR. Both strands of the cytochrome *b* were sequenced in an ABI 377 DNA sequencing system according to the manufacturer's instructions, using the primers above.

Six nuclear markers (microsatellite loci EV1, EV37, Valsecchi and Amos, 1996; KW12, Hoelzel et al., 1998; MK5, MK6, MK8, Krützen et al., 2001) were also PCR-amplified for all samples as described in Möller and Beheregaray (2004). VIC and TAS samples amplified with an initial denaturation at 95°/2 min, followed by a 15-cycle 'touchdown' (94 °C for 30 s, 58–50 °C for 30 s and 72 °C for 30 s) and 22 cycles of 94 °C/30 s, 50 °C/30 s and 72 °C/30 s, and a final extension at 72 °C/2 min; except EV37 and KW12 consisting of an initial denaturation at 94°/2 min, followed by 35 cycles of 94 °C/30 s, 55 °C/30 s, 72 °C/30 s and a final extension at 72 °C/2 min.

2.3. Molecular phylogenetics analysis based on mitochondrial DNA genes

Mitochondrial DNA control region and cytochrome *b* sequences were edited and aligned using Sequencher 4.1.2 (Gene Codes Corporation, MI). Mitochondrial DNA control region data were used to estimate gene genealogies based on the statistical parsimony method (Templeton et al., 1992) implemented in TCS 1.06 (Clement et al., 2000). Gaps were used as a fifth character state for this analysis.

From the mtDNA control region network we selected samples representing abundant and/or divergent control region haplotypes for the cytochrome *b* sequence analysis ($n = 20$, see Appendix Table S1). Sequence divergence of the cytochrome *b* haplotypes was calculated and their phylogenetic relationships within the sub-family Delphininae were inferred by Neighbour-Joining (NJ) and Maximum Parsimony (MP) methods implemented in PAUP* 4.0b10 (Swofford, 2003). For these analyses, in addition to our samples, we used between one to four representative sequences of each of the species of the sub-family Delphininae deposited in GenBank by LeDuc et al. (1999) ($n = 25$, Appendix Table S2). We used the Akaike information criterion (AIC) implemented in ModelTest 3.04 (Posada and Crandall, 1998) to determine the model of nucleotide substitution that best fitted the data. The NJ tree was then estimated with the selected Tamura-Nei model (Tamura and Nei, 1993) taking into account the proportion of invariable sites ($P_{inv} = 0.3876$) and gamma correction (shape = 0.5732). Unweighted MP trees were obtained with the heuristic search with 100 random sequence-addition replicates and tree-bisection-reconnection branch swapping. Statistical support of tree nodes for NJ and MP phylogenies was assessed by bootstrap analysis (Felsenstein, 1985) with 1000 replicates. Values equal or higher than 70% were assumed to indicate strong support for a clade (e.g. Hillis

and Bull, 1993). Two delphinid species (*Orcinus orca*, GenBank #AF084061; *Lagenorhynchus acutus*, GenBank #AF084061; LeDuc et al., 1999) were used as outgroups for the analyses.

2.4. Species assignment and gene flow analysis based on microsatellites

Genetic variation was estimated by calculating number of alleles, and expected (He) and observed (Ho) heterozygosities in Genepop 3.4 (Raymond and Rousset, 1995). Tests for Hardy-Weinberg equilibrium (HWE), using an exact test and based on 1000 iterations, and tests for linkage disequilibrium were also conducted in Genepop, with the significance level Bonferroni-corrected (Rice, 1989).

We used a Bayesian clustering method implemented in Structure 2.1 (Pritchard et al., 2000) to test the assignment of individual samples to genetic clusters. The number of clusters (K) was inferred from the posterior probability distribution Pr (K/X) calculated from the posterior probability of the data Log Pr (X/K). For this analysis we used a burn in period of 100,000 iterations, runs of 10^6, values of K between 1 and 4, series of 5 independent runs for each value of K, with the admixture and correlated frequency models.

In addition, a factorial correspondence analysis implemented in Genetix 4.0, which graphically projects individuals on the factor space defined by the similarity of their allelic states, was used to detect the degree of similarity between bottlenose and common dolphin samples.

Rates of recent migration were estimated using a Bayesian approach implemented in BayesAss 1.3 (Wilson and Rannala, 2003), which accounts for unequal migration rates and do not require populations to be in migration-drift or Hardy-Weinberg equilibrium. To infer posterior probability distributions a burn in period of 100,000 iterations and runs of 3×10^6 were carried out, with a thinning interval of 2000 iterations.

3. Results

3.1. Cytochrome b phylogeny and sequence divergence

We unambiguously obtained cytochrome *b* sequences of 971 bp for 18 bottlenose dolphin samples, including coastal and offshore individuals from NSW, coastal and stranded animals from VIC, stranded dolphins from TAS and coastal individuals from SA (Appendix Table S1). Among these samples a total of 17 haplotypes were identified (Appendix Table S3). In addition, we also obtained equivalent sequence data for two common dolphins, from which two haplotypes were identified (Appendix Table S3). There were 81 polymorphic sites in our Australian bottlenose dolphin cytochrome *b* sequences, 95 sites including the two common dolphin sequences, and 233 variable sites when all sequences used to reconstruct the phylogeny were considered (Appendix Table S3). From these 152 characters were parsimony informative. The Delphininae phylogenetic relationships reconstructed in this study (Fig. 1) were concordant with those presented by LeDuc et al. (1999). However, three instead of two monophyletic clades of bottlenose dolphins were identified in this phylogeny (Fig. 1). The first one is represented by LeDuc et al.'s reference sequences of common bottlenose dolphins, *T. truncatus*, and some sequences of Australian bottlenose dolphins stranded in TAS and VIC, and biopsied offshore in NSW, and is hereafter considered the *T. truncatus*-cyt*b* clade. The second clade is represented by reference sequences of Indo-Pacific bottlenose dolphins, *T. aduncus*, and the two sequences of coastal bottlenose dolphin from NSW, and is hereafter considered the *T. aduncus*-cyt*b* clade. The third clade, not present in LeDuc et al.'s (1999) phylogeny, comprised of sequences of coastal bottlenose dolphins biopsy sampled in SA and VIC, and some individuals stranded in VIC and TAS. All three bottlenose dolphin clades were supported by very high bootstrap values (NJ and MP, 100%; Fig. 1). The novel bottlenose dolphin clade showed a sister relationship to reference sequences of Fraser's dolphins, *Lagenodelphis hosei*. This relationship also showed high bootstrap support in the tree (NJ, 80%; MP, 80%; Fig. 1). In addition, sequences of the two common dolphins from SA clustered with reference sequences of short-beaked common dolphins *D. delphis* (Fig. 2).

Corrected sequence divergence between *T. truncatus* and *T. aduncus* haplotypes ranged between 3.2% and 5.8%. Sequence divergence between these two recognized *Tursiops* species and the novel bottlenose dolphin clade was higher, ranging from 5.5% to 7.7% between the novel clade and *T. truncatus*, and 4.8% to 6.4% between the new clade and *T. aduncus*. By comparison, the novel clade showed sequence divergence between 3.8% and 4.7% to its putative sister taxon, *L. hosei*.

The novel bottlenose dolphin clade also showed three diagnostic nucleotide substitutions at positions #589, #661 and #910 relative to all other members of the sub-family Delphininae and the two outgroup species (Appendix Table S3). This novel clade is considered hereafter as the Southern Australian bottlenose dolphin (SABD) cyt*b* clade.

3.2. Control region gene genealogies

We resolved the control region haplotypes for all bottlenose dolphin samples (Appendix Table 1S). Among these sequences 45 unique haplotypes were identified. In addition, we also resolved the haplotypes for all common dolphin samples, with 22 haplotypes recognized. Four haplogroups not linked to each other were reconstructed in the haplotype network (Fig. 2). These haplogroups were not linked because their probability of linkage was rejected at the 5% level (Templeton et al., 1992). The first haplogroup is represented by *T. truncatus*-cyt*b* clade samples and all sequences of animals biopsied offshore in NSW, most individuals stranded in TAS (13 of 18), and two stranded in VIC. This haplogroup is considered the *T. truncatus* control region (cr) haplogroup. The second haplogroup is represented by *T. aduncus*-cyt*b* clade samples and includes all coastal dolphins biopsied in NSW, and is referred to as the *T. aduncus* cr haplogroup. The third haplogroup is represented by the SABD-cyt*b* clade samples and includes all coastal dolphins biopsied in SA and VIC, most individuals stranded in VIC (11 of 13) and five of the animals stranded in TAS, and it is considered the SABD cr haplogroup. The fourth haplogroup is represented by the *D. delphis*-cyt*b* clade samples and all other common dolphins sampled in SA, and it is referred to as the *D. delphis* cr haplogroup.

3.3. Microsatellite genetic structure and migration rates

Number of alleles, expected and observed heterozygosities as well as results of tests for Hardy-Weinberg equilibrium is presented in Table 1. The Bayesian clustering method implemented in Structure showed that all individuals belonging to the four mtDNA cr haplogroups had very high probability of membership to its own group based on nuclear DNA data (Fig. 3a). When determining the most likely number of distinct groups in the data set, the highest probability was obtained when $K = 4$ groups [P(K/X) = 1 for $K = 4$ groups and P(K/X) = ~0 for $K = 1$, 2 and 3 groups]. When we tested for the existence of additional groups in the dataset ($K > 4$) further genetic subdivision was suggested within the four groups (data not shown, but see Bilgmann et al., 2007 for an example of population-level structure in Southern Australian bottlenose dolphins).

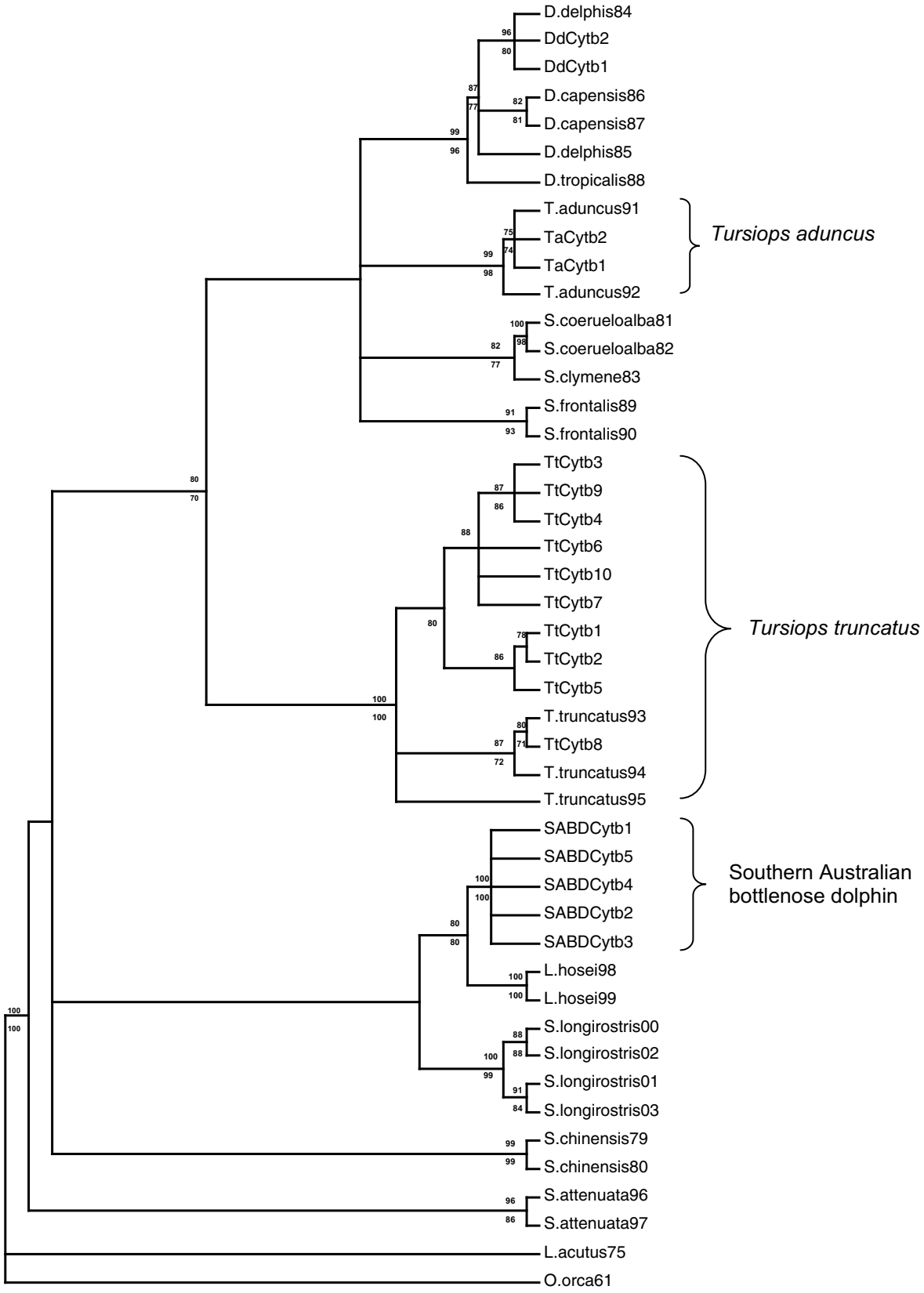

Fig. 1. Maximum parsimony tree (50% majority-rule consensus) based on sequences of 971 bp of the mtDNA cytochrome *b* of bottlenose dolphins and common dolphins from southern and southeastern Australia and representatives of the sub-family Delphininae from LeDuc et al. (1999) (See Appendix Table S1–S3 for abbreviations). Maximum parsimony and neighbor-joining bootstrap support are shown below and above branches, respectively (bootstraps higher than 70% are reported). Clades representing common bottlenose dolphins, *Tursiops truncatus*, Indo-Pacific bottlenose dolphins, *T. aduncus*, and the novel bottlenose dolphin clade (named Southern Australian bottlenose dolphin) are shown in parentheses.

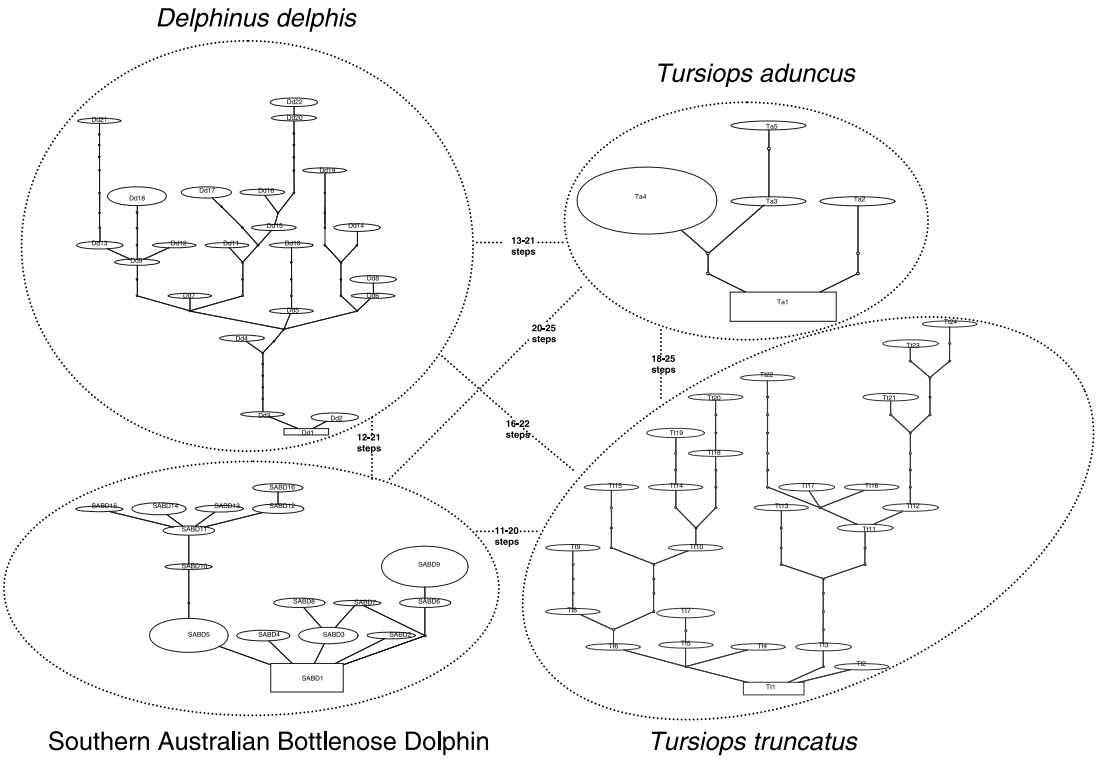

Fig. 2. Haplotype parsimony network depicting genealogical relationships among mtDNA control region lineages of bottlenose dolphins and common dolphins from southern and southeastern Australia. The size of ovals is proportional to the haplotype frequency. Single lines indicate one mutation between haplotypes and small circles represent missing haplotypes. Four haplogroups not linked to each other were obtained. These were identified as representing common bottlenose dolphins, *Tursiops truncatus*, Indo-Pacific bottlenose dolphins, *T. aduncus*, Southern Australian bottlenose dolphins and short-beaked common dolphins, *Delphinus delphis*, based on the mtDNA cytochrome *b* phylogeny.

In addition, a factorial correspondence analysis based on the degree of similarity of individuals' allelic states supports the distinctive nuclear genetic composition of the four mtDNA cr haplogroups (Fig. 3b).

Bayesian analysis of recent migration rates implemented in BayesAss indicated extremely low migration rates between the four groups. The estimated proportion of migrants between groups ranged from 0.001 to 0.003 (Table 2).

Table 1
Number of alleles, observed (Ho) and expected (He) heterozygosities at microsatellite loci

Loci	SABD (n = 102)	*Tursiops aduncus* (n = 42)	*Tursiops truncatus* (n = 36)	*Delphinus delphis* (n = 52)	Total
EV1					
No. Alleles	17	7	8	11	22
Ho	0.626*	0.675	0.528*	0.755	
He	0.802	0.705	0.831	0.770	
EV37					
No. Alleles	17	7	21	8	26
Ho	0.608	0.561	0.842*	0.346	
He	0.623	0.650	0.935	0.329	
KW12					
No. Alleles	6	6	8	9	13
Ho	0.495	0.810	0.556*	0.558	
He	0.557	0.711	0.790	0.630	
MK5					
No. Alleles	6	3	12	10	14
Ho	0.465*	0.488	0.714*	0.588	
He	0.692	0.512	0.893	0.620	
MK6					
No. Alleles	15	5	14	12	22
Ho	0.706	0.463	0.730	0.808	
He	0.849	0.559	0.881	0.829	
MK8					
No. Alleles	7	7	10	7	12
Ho	0.816	0.732	0.694	0.680	
He	0.800	0.718	0.825	0.653	

SABD, Southern Australian Bottlenose Dolphin. Asterisks denote loci with significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction.

4. Discussion

This study provides evidence for three genealogically distinct, reciprocally monophyletic, cytochrome *b* and control region mtDNA lineages of bottlenose dolphins in southeastern and southern Australia. It also provides evidence for reproductive isolation among these lineages, which is evident by the apparent absence of gene flow at nuclear DNA between them. Individuals from one of these lineages clustered with reference samples of common bottlenose dolphins *Tursiops truncatus* used in a comprehensive mtDNA cytochrome *b* phylogeny of the family Delphinidae by LeDuc et al. (1999). Dolphins from the second lineage clustered to reference samples of Indo-Pacific bottlenose dolphins *T. aduncus*. The third lineage, represented by Southern Australian bottlenose dolphins, was not identified in LeDuc et al.'s (1999) study. In a recent mtDNA study of bottlenose dolphins from waters of the state of Victoria, southern Australia, Charlton et al. (2006) alerted to the potential presence of an undescribed taxon of bottlenose dolphin in this region, which was more related to the common bottlenose dolphin than to the Indo-Pacific species. That study, however, used only a few representative species of the sub-family Delphininae. Here we included all representative species of the sub-family Delphininae in a mtDNA cytochrome *b* phylogeny and provide evidence for a sister relationship of this undescribed taxon (referred to as the Southern Australian bottlenose dolphin) to the Fraser's dolphin *L. hosei*. Our result reinforces the idea proposed by LeDuc

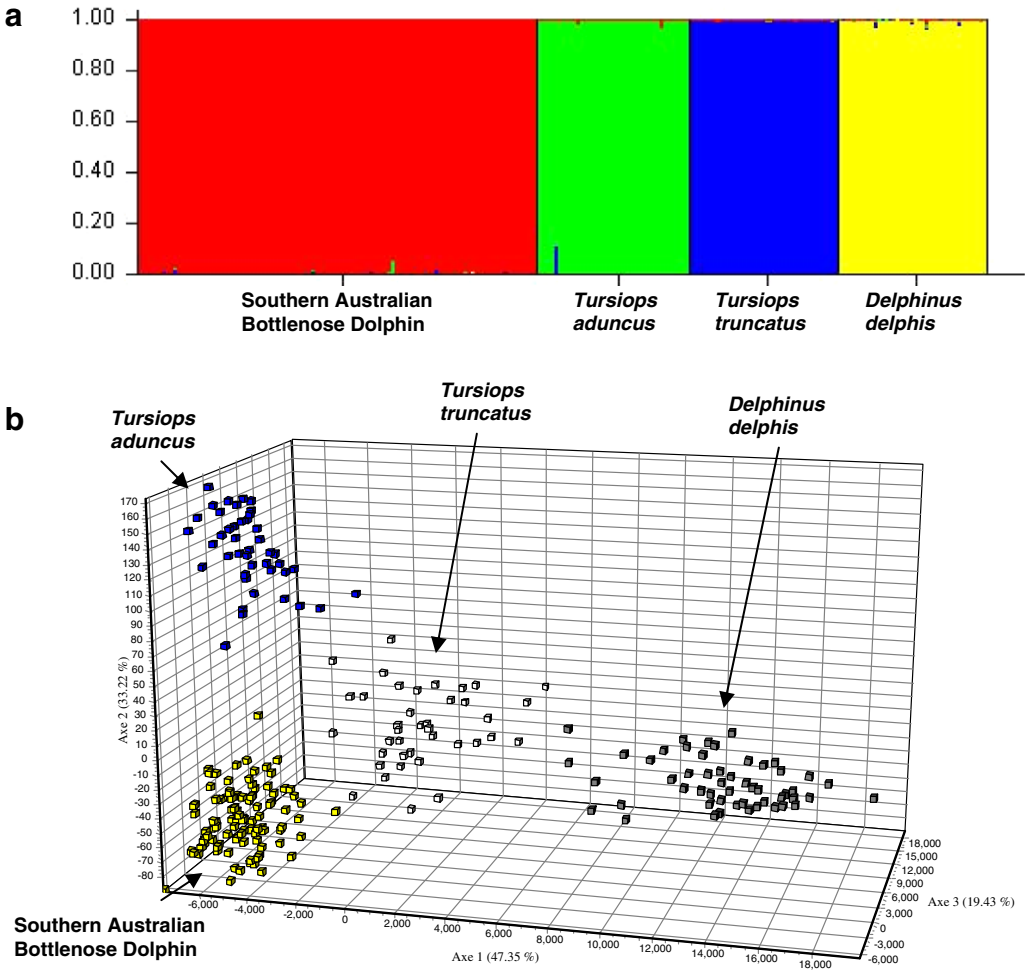

Fig. 3. (a) Bayesian clustering of the four mtDNA control region haplogroups of bottlenose and common dolphins from southern and southeastern Australia based on six microsatellite loci. Each individual is represented by a vertical column partitioned into four colored segments, with the length proportional to the individual's estimated membership coefficient into the four groups. (b) Three-dimensional representation of a factorial correspondence analysis of the four mtDNA control region haplogroups of bottlenose and common dolphins from southern and southeastern Australia based on six microsatellite loci. The four haplogroups were identified as representing common bottlenose dolphins, *Tursiops truncatus*, Indo-Pacific bottlenose dolphins, *T. aduncus*, Southern Australian bottlenose dolphins and short-beaked common dolphins, *Delphinus delphis*, based on the mtDNA cytochrome *b* phylogeny.

Table 2
Recent migration rates among the four dolphin mitochondrial control region haplogroups based on six microsatellite loci

	SABD	*Tursiops aduncus*	*Tursiops truncatus*	*Delphinus delphis*
SABD	0.996	0.001	0.001	0.001
Tursiops aduncus	0.003	0.992	0.003	0.003
Tursiops truncatus	0.003	0.003	0.991	0.003
Delphinus delphis	0.002	0.002	0.002	0.994

Means of the posterior distribution of the migration rate into each dolphin haplogroup (m) are shown. The haplogroups from which each dolphin belong are listed in the rows, while the haplogroups from which they migrated are listed in the columns. Values along the diagonal are the proportions of dolphins derived from their own haplogroups each generation. Standard deviations for all distributions were <0.01. SABD, Southern Australian bottlenose dolphin.

et al. (1999) that bottlenose dolphins are part of a polyphyletic group. More importantly, based on the phylogenetic species concept, this study provides evidence for fixed and diagnosable genetic differences between these lineages, suggesting the presence of a new species of bottlenose dolphin in Southern Australia. This is different from the *aduncus*-type dolphins from South Africa as shown by a phylogenetic analysis of the mtDNA control region (Appendix Fig. S2). The *aduncus*-type dolphins from South Africa were suggested to represent a different species from the Chinese *aduncus*-type by Natoli et al. (2004).

In our study southern Australian bottlenose dolphins were sampled in coastal waters of South Australia, Victoria and Tasmania, but not in New South Wales. By contrast, Indo-Pacific bottlenose dolphins (*T. aduncus*) were found in coastal waters of New South Wales but not detected in Victoria, Tasmania or South Australia. Common bottlenose dolphins (*T. truncatus*), on the other hand, were sampled in offshore waters of New South Wales, and stranded in Victoria and Tasmania. Based on our sampling effort we tentatively propose that Southern Australian bottlenose dolphins are distributed in coastal waters of South Australia, Victoria, Tasmania and potentially south and south-western Western Australia (see below). The presence of both *T. aduncus* and *T. truncatus*, was previously proposed to South Australia based on a morphological study of stranded animals (Kemper, 2004). This study, however, distinguished two morphological groups of South Australian bottlenose dolphins which were subsequently aligned with *T. truncatus* and *T aduncus* (Kemper, 2004). We believe that one of these groups may have been incorrectly identified and in fact represent the Southern Australian bottlenose dolphin. Additional morphological studies including samples from Southern Australian bottlenose dolphins, Fraser's dolphins, common bottlenose dol-

phins and Indo-Pacific bottlenose dolphins are needed for clarifying morphological affinities of South Australian bottlenose dolphins.

It has been suggested that historical glacial cycles (Dartnall, 1974; Burridge, 2000; Waters et al., 2004), contemporary latitudinal temperature gradients, and/or oceanographic patterns (Bennet and Pope, 1953, 1960; O'Hara and Poore, 2000) are associated with the presence of different marine biogeographic provinces in Australia's temperate region. For example, the land bridge connecting Tasmania and the mainland during the Plio-Pleistocence glaciations was proposed to have promoted separation and divergence of coastal taxa, such as of species of the six-rayed sea-stars of the genus *Patiriella* (Waters et al., 2004) and gastropods of the genus *Nerita* (Waters et al., 2005). The disappearance of the land bridge has been a relatively recent event and therefore the resident populations along the Victorian coastline are likely to be a result of niche filling with the release of suitable habitat. The potential ancestor is unlikely to have been from eastern Australian *T. aduncus* populations, given the substantial genetic divergence of this group, as previously discussed by Charlton et al. (2006). In the case of the wider southern Australian bottlenose dolphin, it seems unlikely that the Tasmanian land bridge was responsible for allopatric speciation of coastal animals, as neither the Indo-Pacific bottlenose dolphin or the common bottlenose dolphin were identified as a sister taxon in the cytochrome *b* phylogeny. Rather, the Plio-Pleistocene glaciations may have given opportunity for colonization of coastal waters west and east of the barrier by two different dolphin taxa. In addition, contemporary ocean currents may be responsible for maintaining the apparent allopatric distribution displayed by these taxa. The distribution of Indo-Pacific bottlenose dolphins in our study area coincides with the influence of the East Australian Current, which flows southward along eastern Australia and deflects away from the Australian continent as it reaches southern New South Wales (Godfrey et al., 1980). By contrast, two current systems dominate in Southern Australia, the Coastal Current which flows east along the coast in South Australia and Victoria, and the Zeehan Current which flows southeast around Southern Tasmania (Cirano and Middleton, 2004). Interestingly, the distribution of the Southern Australian bottlenose dolphins in South Australia, Victoria and Tasmanian coastal waters is in agreement with the distribution of these currents. On the other hand, in Western Australia, where the major oceanographic system is the Leeuwin Current (Waite et al., 2007), a bottlenose dolphin population genetics study in Shark Bay identified only control region haplotypes characteristic of *T. aduncus* and *T. truncatus* (Krützen et al., 2004). This current flows southward along Western Australia and then eastward into south-western Australia in winter, with the system partially reverted in summer (Cirano and Middleton, 2004; Waite et al., 2007). The south-western region of Australia would be an interesting area for further study as it may represent the western boundary for the distribution of southern Australian bottlenose dolphins.

Our study adds to the escalating number of DNA-based reports of cryptic species in charismatic megafauna (e.g. Beheregaray and Caccone 2007) and highlights the importance of an adequate sampling of geographic populations, taxa, and gene markers for taxonomic research.

Acknowledgments

Funding and logistic support was provided by Graduate School of the Environment, Macquarie University, Linnean Society of New South Wales, South Australian Research and Development Institute, Dolphin Research Institute, Tasmanian Department of Primary Industries, Water and Environment. Samples, fieldwork or technical support was provided by Rob Harcourt, Steve McKechnie, Andrea Taylor, Cath Kemper, Sue Gibbs, Simon Allen, Anika Warren-Smith, Joanna Wiszniewski, Rosemary Gales, Shannon Corrigan. This research was carried out under permits by the Department of the Environment and Conservation (NSW), the Department of Environment and Heritage (SA), the Victorian Department of Sustainability and Environment (VIC) and animal ethics approvals by DEH, Macquarie University and the Dolphin Research Institute.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.08.011.

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