Syst. Biol. 0(0):1–13, 2013

DOI:10.1093/sysbio/syt051

Recent Diversification of a Marine Genus (*Tursiops* spp.) Tracks Habitat Preference and Environmental Change

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Received 3 May 2013; reviews returned 14 June 2013; accepted 2 August 2013
Associate Editor: Adrian Paterson

Abstract.—Understanding the evolution of diversity and the resulting systematics in marine systems is confounded by the lack of clear boundaries in oceanic habitats, especially for highly mobile species like marine mammals. Dolphin populations and sibling species often show differentiation between coastal and offshore habitats, similar to the pelagic/littoral or benthic differentiation seen for some species of fish. Here we test the hypothesis that lineages within the polytypic genus *Tursiops* track past changes in the environment reflecting ecological drivers of evolution facilitated by habitat release. We used a known recent time point for calibration (the opening of the Bosphorus) and whole mitochondrial genome (mitogenome) sequences for high phylogenetic resolution. The pattern of lineage formation suggested an origin in Australasia and several early divisions involving forms currently inhabiting coastal habitats. Radiation in pelagic environments was relatively recent, and was likely followed by a return to coastal habitat in some regions. The timing of some nodes defining different ecotypes within the genus clustered near the two most recent interglacial transitions. A signal for an increase in diversification was also seen for dates after the last glacial maximum. Together these data suggest the tracking of habitat preference during geographic expansions, followed by transition points reflecting habitat shifts, which were likely associated with periods of environmental change. [Climatic oscillations; marine mammal; pleistocene; radiation; speciation.]

In the marine environment, oscillations in global temperature during the Pleistocene likely caused dramatic changes in habitat availability due to fluctuations in sea levels and coastal topography. Together with changes in nutrient composition due to increased runoff from melting landlocked ice masses (Weaver et al. 2003) these factors provide potential mechanisms for ecological diversification, promoted by the occupation of newly formed habitats, or adaptation to changing environments. More broadly, patterns of diversification are thought to be determined by intrinsic biotic factors such as competition and adaptive potential (Van Valen 1973), or alternatively by extrinsic environmental changes (Barnosky 2001). However, these hypotheses are not mutually exclusive, and some have suggested that their relative influence is a matter of scale, with environmental factors best explaining patterns over a longer evolutionary time frame (Benton 2009). In the marine environment where boundaries that prevent movement (such as may be represented by rivers or mountain ranges in the terrestrial environment) are rare for species with strong dispersal potential, panmixia or isolation by distance is expected. Among the delphinid cetaceans, a group of species with a great capacity for dispersal, there is instead evidence for relatively fine-scale differentiation among populations within species (Hoelzel 2009), and the radiation of many apparently recent species (Steeman et al. 2009). This has in fact been observed for a diversity of marine taxa, and is referred to as the "marine speciation paradox" (Palumbi 1994; Bierne et al. 2003). Here we investigate the mechanisms that may be driving this pattern of diversification by focusing on the polytypic genus *Tursiops*.

Bottlenose dolphins (*Tursiops* spp.) are widespread and cosmopolitan, found in all major oceans except for polar oceanic regions (Folkens et al. 2002). Two species are commonly accepted within the genus: the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) distributed through coastal areas of east Africa, Asia, Australia, the Salomon Islands, and New Caledonia (Wang et al. 1999; Möller and Beheregaray 2001; Folkens et al. 2002; Kemper 2004; Perrin et al. 2007; Wang and Yang 2009); and the common bottlenose dolphin (*Tursiops truncatus*) widespread throughout all major oceans (Folkens et al. 2002), but the systematics of this group is in need of further clarification. There is also support for a third distinct species found in southern Australia based on consistent differences in both morphology and genetics (designated *Tursiops australis*; Charlton et al. 2006; Möller et al. 2008; Charlton-Robb et al. 2011). Among studies considering the phylogeny of the broader group Delphinidae, some support polyphyly for the genus *Tursiops* (LeDuc et al. 1999; Charlton et al. 2006; Nishida et al. 2007; Möller et al. 2008; Kingston et al. 2009; Xiong et al. 2009), while others support monophyly (McGowen et al. 2009; Steeman et al. 2009). Here we focus on differentiation within the genus as currently defined (see review in Charlton-Robb et al. 2011).

In Europe, the population found in the Black Sea is generally regarded as a valid subspecies (*Tursiops truncatus ponticus*) due to morphological and genetic differentiation (Viaud-Martinez et al. 2008). Within

T. aduncus, strong genetic differentiation between South African and Indo-Pacific populations led to the proposition that these might each represent valid species (Natoli et al. 2004). Both *T. truncatus* and *T. aduncus* can be divided into regional populations that sometimes show clear ecological differences. In the western North Atlantic, two populations of *T. truncatus* are known to have consistent differences in morphology, ecology (Mead and Potter 1995), genetics (Hoelzel et al. 1998), and habitat choice (Torres et al. 2003). In general, the distinction between populations occupying mainly offshore versus coastal habitats in this genus has been described in different regions of the world, in some cases supported by genetic differentiation at the population level (Hoelzel et al. 1998; Natoli et al. 2004; Segura et al. 2006; Tezanos-Pinto et al. 2008; Kingston et al. 2009). This observation led to the suggestion that pelagic populations may provide a source for the colonization of coastal habitats released during climatic oscillations (Hoelzel 1998a), similar to what has been proposed for species of both oceanic and lacustrine fish (Dynes et al. 1999; McKinnon and Rundle 2002).

In this study, we sequenced 75 whole mitochondrial genomes (mitogenomes) from samples representing several ecotypes and regional populations within the genus *Tursiops*, which we then integrated with other published cetacean mitogenomes to define deeper nodes within the phylogeny. The shared history within the mtDNA genome allows the use of a single mutation rate for the calculation of divergence times. Differentiation between the Black Sea and the Eastern Mediterranean populations was used to calibrate the mitogenomic substitution rate in *Tursiops*. The Black Sea is a semi-landlocked basin whose only connection to the adjacent Mediterranean Sea is achieved through the narrow Bosphorus Strait, which remained closed during the period between ∼10 Ma and ∼10 Ka (Gökaşan et al. 1997; Ryan et al. 1997; Hiscott et al. 2002; Nikishin et al. 2003; Kerey et al. 2004). Given that the earliest putative *Tursiops* fossils date to 5 Ma (when the Black Sea was still isolated; Fitzgerald 2005), this provides a biogeographical calibration point at ∼10 Ka to facilitate the estimation of the mtDNA substitution rate. Substitution rate estimates on a Holocene time frame (such as this) have been shown to be higher than those based on fossil calibration points (Ho et al. 2008 and further discussion below), so the assessment of dates based on both recent and fossil calibrations allows for a more accurate interpretation of the timing of especially the more recent events. Our data therefore provided the resolution and representation necessary to test hypotheses about the mechanisms that underlie the systematics of a polytypic marine genus with high dispersal potential.

In particular, we test the hypotheses that (i) high-resolution phylogenetic analysis will support multiple monophyletic lineages associated with either geographic distribution or habitat usage; (ii) inference from the phylogeny will indicate that there have been multiple transitions between pelagic and coastal forms; (iii) node dating will reveal an association between habitat release during interglacial periods and the founding of populations in coastal habitat; and (iv) an ancestral pelagic form founded the coastal populations, as suggested in earlier studies.

METHODS

Sample Collection and Laboratory Procedures

Samples were obtained from stranded and bycaught individuals as well as biopsies from free-ranging animals from worldwide locations (Fig. 1). These locations represent well-described regional populations, named species and/or ecotypes as detailed in Table 1. We chose to focus on the whole mtDNA genome for high resolution (many informative sites) and the ability to generate a mutation rate estimate without violating the assumption that all sites belong to the same gene region. The main limitation is that this then represents only one evolutionary history. For our objectives, the key limitation to the alternative strategy of including multiple nuclear genes is the difficulty with assessing an appropriate consensus mutation rate (to facilitate the accurate dating of recent nodes) among other complications associated with selection, congruence, and lineage sorting. The problems associated with the single-gene phylogeny from mtDNA can cause lineage topology to be disrupted relative to the real species phylogeny, however delphinid phylogenies including lineages with good data representation, often show consistent topologies between mtDNA and multiple nuclear loci (e.g., Steeman et al. 2009; Globicephalinae in Vilstrup et al. 2011). At the population level problems with lineage sorting and introgression can further affect resolution, but the lower effective population size of mtDNA relative to nuclear DNA means that population differentiation in haplotype frequencies is achieved at a faster rate. We thus consider that using mitogenomic sequences represents the best strategy for the accurate estimation of mutation rates and associated divergence times, but interpret the results in the context of the limitations of analysing single-gene histories.

Complete mitogenomes were sequenced for 75 samples (mean genome length 16,386 bp). DNA was extracted using a standard phenol-chloroform protocol (Hoelzel 1998b). Whole mitogenome sequences were generated using the Illumina sequencing platform, based upon two overlapping PCR amplicons that had been generated using LA-Takara long range PCR polymerase. Primers were designed using the PRIMER3 (Rozen and Skaletsky 2000) algorithm as implemented in GENEIOUS (Drummond et al. 2010). Details regarding the location and length of both fragments and the primers used are provided in Supplementary Table S1 (Supplementary Materials available at http://datadryad.org under doi: 10.5061/dryad.k501d), while standard PCR profiles are in Supplementary Tables S2 and S3. PCR products were purified using a PCR Purification Kit (Qiagen) and eluted in 1 X TE.

FIGURE 1. Geographic location of the *Tursiops* samples used in this study. Details on the species/ecotypes studied and the number of samples used are described in Table 1.

TABLE 1. Number of samples from each *Tursiops* species/ecotypes used in this study

Code	Location	Ecotype/Species	*N*	Reference
WNAP-Tt	Western North Atlantic	Pelagic *T. truncatus*	10	(Hoelzel et al. 1998)
WNAC-Tt	Western North Atlantic	Coastal *T. truncatus*	9	(Hoelzel et al. 1998)
SCO-Tt	Scotland	*T. truncatus* (coastal)	8	(Reid et al. 2003, Natoli et al. 2005)
EMED-Tt	Eastern Mediterranean	*T. truncatus* (coastal)	10	(Natoli et al. 2005)
BSEA-Ttp	Black Sea	*T. truncatus ponticus* (coastal)	10	(Natoli et al. 2005)
SA-Ta	South Africa	*T. aduncus* (coastal)	10	(Natoli et al. 2004)
IP-Ta	Eastern Australia	Indo-Pacific *T. aduncus* (coastal)	10	(Wang et al. 1999; Möller and Beheregaray 2001)
GC-Tt	Gulf of California	*T. truncatus* (coastal)	1	(Segura et al. 2006)
SABD	Southern Australia	Southern Australian bottlenose dolphin (coastal)	7	(Charlton et al. 2006; Möller et al. 2008)

Notes: The same code is used throughout this study, and reference corresponds to the publication where the species/ecotype was first described genetically and/or where habitat use has been determined. Designation in parenthesis indicates known habitat use rather than ecotype assignment based on genetic differentiation.

After purification, the concentration of each amplicon was quantified using a Nanodrop (Thermo Scientific), prior to pooling at equimolar ratio into a single pool per dolphin. Pooled DNA was fragmented using a Bioruptor (Diagenode), then converted into Illumina sequencing libraries following the manufacturer's protocol. Illumina libraries were PCR amplified using indexed primers, pooled, then sequenced using 100 bp SR chemistry over two lanes of the Illumina Hi-Seq2000 sequencing platform. Post sequencing, data were sorted by Illumina primer index into individual data files for each dolphin using a custom script, then trimmed for quality prior to mitogenome assembly.

Sequence Alignment and Phylogenetic Analysis

Sequencing reads were mapped to a *T. truncatus* mitogenomic reference sequence (GenBank accession number EU557093) or a *T. aduncus* mitogenomic sequence (GenBank accession number EU557092) as appropriate, using BWA version 0.5.8-r1536 (Li and Durbin 2009). The alignments were then sorted with ambiguous hits and PCR duplicates removed, all using SAMTOOLS version 0.1.8-r612 (Li et al. 2009). Consensus sequences were generated using the majority read base in each genomic position with a minimum depth of coverage threshold of 5, using a custom Perl script which parses the vcf file produced by the SAMTOOLS "mpileup" command. Discrepancies with the reference sequences were checked manually for accuracy. Sequences were then aligned with available mitogenomes representative of other delphinid species, using mitogenomes from the harbor porpoise (*Phocoena phocoena*), narwhal (*Monodon monoceros*) and four river dolphins (*Pontoporia blainvillei*, *Platanista minor*, *Inia geoffrensis* and *Lipotes vexilifer*) as outgroups (GenBank accession numbers in Supplementary Table S4). These alignments were done

using the MAUVE (Darling et al. 2010) algorithm as implemented in GENEIOUS (Drummond et al. 2010).

The best-fit model of sequence evolution was determined using TOPALI V2 (Milne et al. 2009), and a phylogenetic tree was estimated using MRBAYES (Huelsenbeck and Ronquist 2001). Four independent chains were run for 22,000,000 generations and a burn-in length of 2,200,000 generations, using a sampling frequency of 4000 generations. Three of the four chains were heated, and the analysis was run twice. A maximum-likelihood phylogeny was estimated using PHYML (Guindon and Gascuel 2003) as implemented in the software package GENEIOUS (Drummond et al. 2010), with branch support assessed through 10,000 bootstrap replicates.

The suitability of including full genome sequences for further analyses was assessed by comparing diversity among loci across the genome, and by assessing the level of saturation comparing the control region, all 13 protein-coding genes together, the third codon position only from all protein-coding genes, and all non-coding loci considered together (using the index described by Xia et al. 2003). This was further assessed by comparing the topology obtained by constructing Bayesian phylogenies as described above but using two different partitioning schemes: 1st, 2nd, and 3rd codon positions, with parameters for the substitution models estimated independently for each position; and the nine different protein-coding regions, with parameters for the substitution models estimated independently for each locus.

Calculation of Divergence Dates

Node age estimation was carried out using BEAST v1.6 (Drummond and Rambaut 2007). The initial phylogenetic tree was generated randomly, and the tree prior followed a Yule branching model. Given the intraspecific sampling in our dataset, we compared the Yule prior against coalescent priors using Bayes factors calculated with the TRACER package (Rambaut and Drummond 2003). The Yule prior received consistently better support, as was found for another delphinid study using comprehensive intraspecific sampling (Morin et al. 2010). The phylogenetic analyses showed that the separation between Eastern Mediterranean and Black Sea was visible in two independent lineages, suggesting that invasion of the Black Sea either happened in two colonization events, or that the individuals invading the Black Sea carried two different Mediterranean lineages by chance. The two terminal Eastern Mediterranean/Black Sea groups were thus constrained as being monophyletic independently in each lineage, and equal time to most recent common ancestor (TMRCA) priors within each lineage defined according to geological information regarding the opening of the Bosphorus Strait (Gökaşan et al. 1997; Ryan et al. 1997; Hiscott et al. 2002; Kerey et al. 2004), with a uniform distribution between 3 and 10 Ka. However, given that hard boundaries on priors can be unrealistic, we carried out trials imposing a lognormal distribution at this calibration point. This distribution places most of the probability between 0 and a mean of 10 Ka, but allows for a long tail of non-zero probability on older dates (see Supplementary Material).

A literature review on the diverse evidence for the recent origin of the connection between the Aegean and Black Seas indicates that although possibly not as recent as claimed by some (e.g., Ryan et al. 1997), the opening is unlikely to be older than ~10 Ka (Hiscott et al. 2002). Two fossil calibration points were also used. One represented the TMRCA for Delphinoidea (Delphinidae + Monodontidae), which was constrained as a defined lineage for the purpose of this analysis and the TMRCA prior set at 10 Ma based on fossil data (McGowen et al. 2009; Steeman et al. 2009; Xiong et al. 2009). The other represented the TMRCA for the clade including all named *Tursiops* species (together with the other dolphin genera that group in between different *Tursiops* lineages), set at 5 Ma based on the oldest known fossils that most closely resemble modern *Tursiops* (Barnes 1990; Fitzgerald 2005). Given the degree of uncertainty regarding the exact placement of these fossil calibration points (e.g., the oldest known fossil may not represent the earliest occurrence of that taxon), priors were set with a normal distribution with a standard deviation of 1.5 Ma (together with additional trials, see below).

MCMC analyses were run with 50,000,000 iterations, after a 5,000,000 burn-in, sampling every 5000 generations. The lognormal and exponential distribution of mutation models were compared for the uncorrelated relaxed clock model using Bayes factors, and by comparing ESS values. While the Bayes factor is not a test for statistical significance in itself, it can be used as an indication of relative model support following the criteria in Nylander et al. (2004). Additionally, three different models were run to test for the effects of using different calibration points and different priors, as well as a Bayesian random local clock model (BRLC; Drummond and Suchard 2010) using a hard minimum bound and soft maximum for the fossil calibration priors (details in Supplementary Table S5). Our objective was to both find the reconstruction with the best support, and to approximate the transition between effective mutation rates at different temporal scales.

The software IMa (Hey and Nielsen 2007) was run comparing all the Black Sea individuals against all the Eastern Mediterranean individuals. IMa is a two extant, one ancestor population model that derives inference from coalescent data using MCMC simulations. The coalescent model accounts for incomplete lineage sorting and can estimate both the population splitting time (division from ancestral population) and the TMRCA (oldest coalescent point for all included operational taxonomic units-OTU). Eighty different chains were run with a geometric heating scheme ($h1 = 0.99; \beta = 0.6$), until the maximum capacity of recorded trees was achieved after 1,000,000 iterations of burn-in. The generation time used was 21 years (Taylor et al. 2007). Preliminary runs

were carried out to fine tune the prior range on the test parameters effective population size, migration rate, and divergence time. In IMa, mutation rate is a free parameter used to scale the model parameters to real time units. Once the model parameter values were estimated using an arbitrary prior mutation rate, we altered the real time parameter values so that the splitting time matched the estimated date of the opening of the Bosphorus, thus providing an estimate of the mutation rate (Gökaşan et al. 1997; Ryan et al. 1997; Hiscott et al. 2002; Kerey et al. 2004). Because our phylogenetic data identified a deeper node separating two lineages, each lineage showing division between the Black Sea and the Eastern Mediterranean, IMa would be expected to identify both the deeper node (as the TMRCA), and the population division (as the splitting time). To help test and confirm this we repeated the analysis using samples from just one of the lineages showing division between the two populations (comprised of four samples from the Eastern Mediterranean and six from the Black Sea). This however was used only to confirm that in this case the TMRCA and splitting times would be essentially equivalent, with the full dataset (providing larger sample sizes) being used for further inference.

Analysis of Diversification Rates

The Likelihood Analysis of Speciation and Extinction Rates (LASER; Rabosky 2006) software package was used to describe diversification patterns and rates across the dated phylogeny from the BEAST analysis. The null hypothesis of a constant diversification rate was tested by calculating the difference between the Akaike Information Criteria (AIC) of different fixed-rate and variable-rate models (ΔAICrc). Specifically, we tested two fixed-rate models (pure-birth and birth–death), two density-dependent variable-rate models (DDL and DDX) and four Yule-n-rate variable-rate models (with number of rate classes from 2 to 5). Significance of the statistic was assessed by simulating 5000 trees under the better supported fixed-rate model to create a distribution of the ΔAICrc statistic under the null hypothesis of no rate variation, and fitting the observed ΔAICrc to this distribution. To help account for potential bias caused by the intraspecific sampling scheme artificially creating a pull-of-the-present effect, we repeated this analysis using a pruned dataset. Given that the exact taxonomy of the species is still uncertain, we achieved this by retaining a single randomly selected sample for each well-supported clade within *Tursiops*.

Ancestral Node Reconstruction

Statistical Dispersal–Vicariance Analysis was performed using S-DIVA (Yu et al. 2010) on a dataset containing only *Tursiops* mitogenomes and *Steno bredanensis* as an outgroup. The phylogenetic tree was built using MrBayes implemented in Geneious as described above. Distribution ranges were defined according to the sampling area of each population or ecotype, meaning all are designated unique present ranges, except Indo-Pacific *T. aduncus* (IP-Ta) and South Australian Bottlenose Dolphin (SABD) which were considered as occupying the same geographical region (Australasia). Although modern populations of IP-Ta are found along the eastern and western coasts of Australia, while SABD is found on the southern Australian coast, the broader IP-Ta lineage occupies the broader region of Australasia, and given that SABD has only been recently described, we cannot exclude its presence from this wider area as well. Within a broader geographic area we also distinguish between populations occupying coastal or offshore habitat. This assignment was done based on ecological and genetic studies when data were available (such as for the Western North Atlantic populations), or on observational data of habitat preferences alone as referenced in Table 3. For example, coastal designation for the Scottish population is based on extensive sighting surveys (Reid et al. 2003). Reconstruction of ancestral distribution ranges in the deeper nodes is expected to be less robust due to the higher number of ranges among daughter lineages. This effect can be minimized by limiting the maximum number of possible areas assigned to the ancestral nodes. After initial trials we set a maximum of four to allow resolution without greatly diminishing the level of support.

RESULTS

Phylogenetic Reconstruction

GenBank accession numbers for novel sequences are KF570315-KF570389. The model of evolution used in all phylogenetic analyses was the general time reversible with gamma distribution and a proportion of invariable sites (GTR+I+G) as determined in Topali. ESS values for the Bayesian analyses were all above 4000 and PSRF values were all equal to 1, and thus the number of generations used was considered appropriate. The Bayesian phylogenies produced by MrBayes and BEAST were strongly congruent, with minor differences found only in the placement of OTUs within species/ecotype lineages (Fig. 2; Supplementary Fig. S1). The same congruence in topology between species/ecotypes was confirmed with the maximum-likelihood phylogeny (support values shown in Supplementary Fig. S1; ML tree not shown), and with the partitioned Bayesian phylogenies, constructed as described in the methodology (see Supplementary Fig. S2 for phylogeny based on only the protein-coding genes).

The inclusion of all available mitogenome sequences shows *T. aduncus* in close affiliation with common (*Delphinus capensis*) and striped dolphins (*Stenella coeruleoalba*), as had been seen in earlier phylogenies (LeDuc et al. 1999; Charlton et al. 2006; Nishida et al. 2007; Möller et al. 2008; Kingston et al. 2009; Xiong et al. 2009; Vilstrup et al. 2011), however the objective of this study was to focus on taxa in the genus

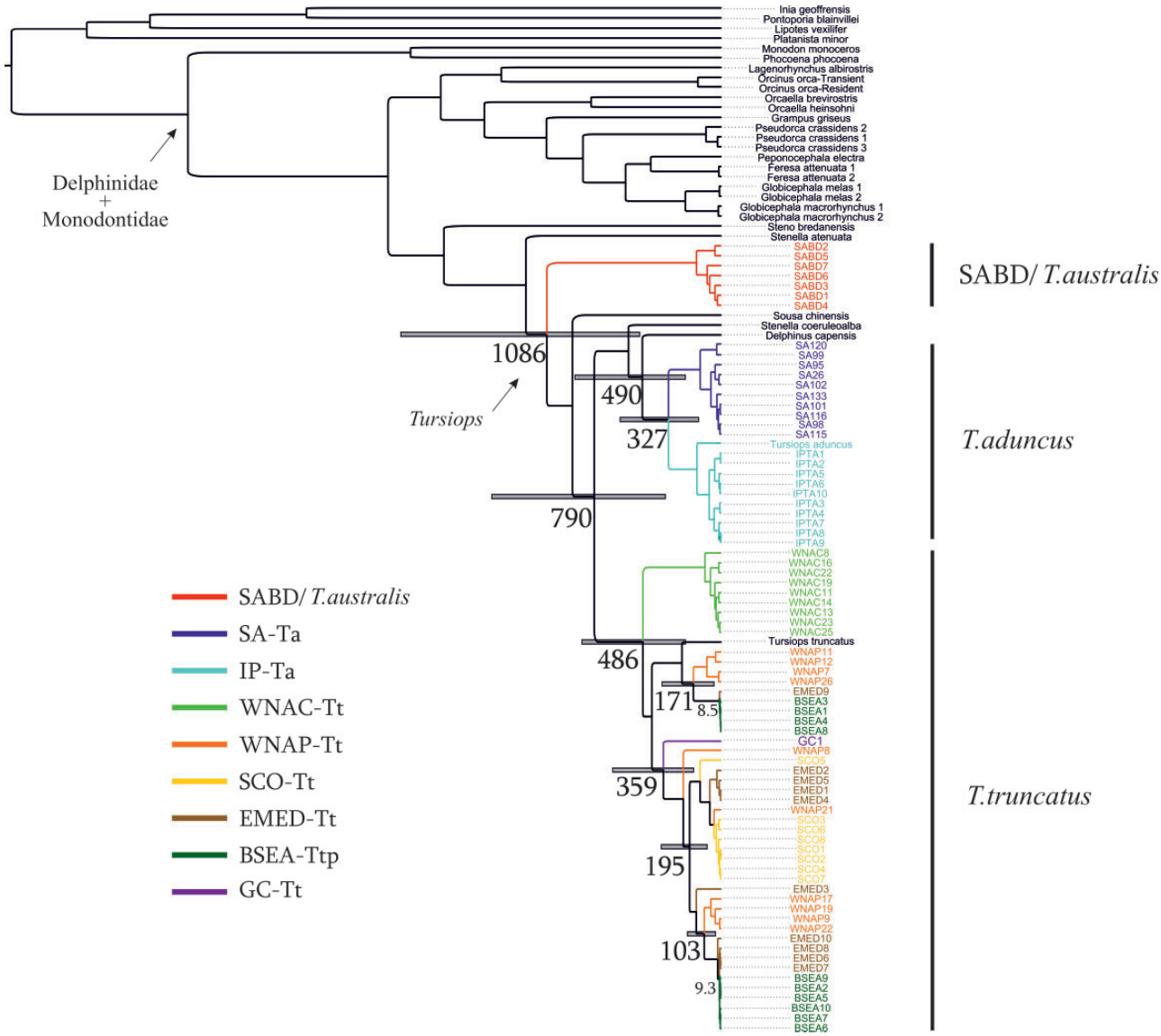

FIGURE 2. Phylogenetic tree estimated using the BEAST software. Divergence times for main species/ecotypes within *Tursiops* calculated using both fossil and biogeographical calibration points (check main text for details). Numbers indicate divergence times in one thousand years unit, while grey bars represent 95% HPD intervals. Details of sampling locations and accession numbers are given in Table 1 and Supplementary Table S4.

Tursiops, not the broader delphinid phylogeny. The morphological support for the inclusion and designation of species within the genus *Tursiops* is reviewed in Charlton-Robb et al. (2011). Topology of different *Tursiops* species/ecotypes/populations was also confirmed by the phylogenetic reconstruction using only *Tursiops* sequences and a single outgroup (*S. bredanensis*; Fig. 3). Some samples from defined ecotypes and regional populations within named *Tursiops* species grouped together in well differentiated lineages, however this was not the case for many of the sample sets representing *T. truncatus* (Fig. 2). Samples from the Mediterranean Sea, Black Sea, and western North Atlantic pelagic populations are all spread across multiple lineages, suggesting incomplete lineage sorting or dispersal, though data from Natoli et al. (2005) indicated low dispersal rates among European populations.

Comparison among loci across the mitochondrial genome showed that the most variable regions are not disproportionately more variable than the rest of the mitogenome. Pairwise percent similarity values within the genus *Tursiops* ranged from 97.1% to 99.3% (ND3-12S rRNA, respectively), with the control region value at 97.3%. When all taxa are included the range is from 89.1% to 97.7% (ND6-16S rRNA, respectively), with the control region at 93.1%. In each case the control region is within the range of diversity seen at other loci, as suggested earlier for cetacean mtDNA (Hoelzel et al. 1991). Saturation tests (Xia et al. 2003) including all taxa found no evidence for saturation

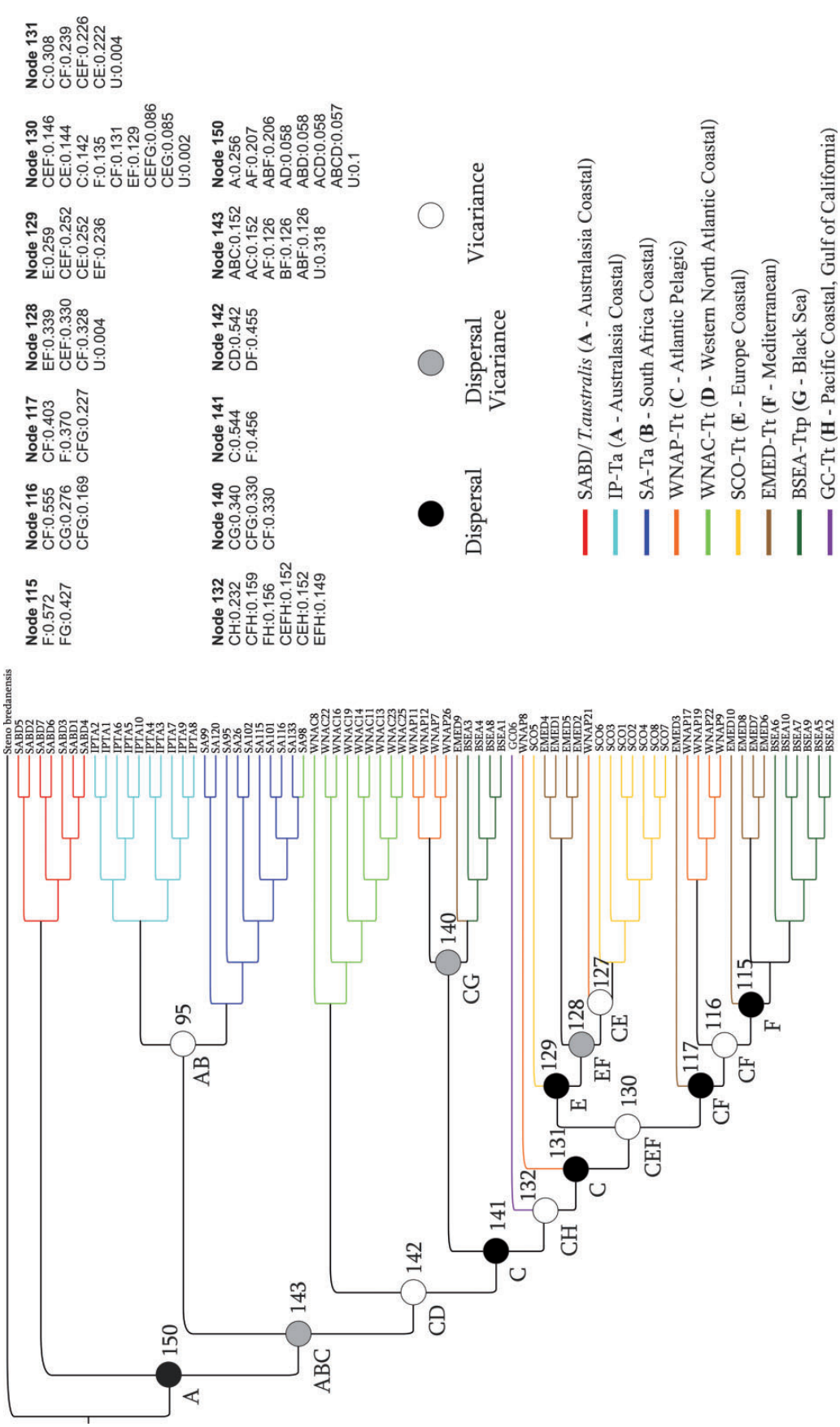

FIGURE 3. Results from the Statistical Vicariance–Dispersal analysis, carried out using the software S-Diva (Yu et al. 2010). Nodes of interest are marked by circles, assigned a unique number, and shaded according to the divergence process assigned. In this context, circles in gray represent nodes to which both vicariant and dispersal events were assigned. Note that incomplete lineage sorting and uncertainty regarding the ancestral node distribution will reduce the robustness of the assignment. Letters below circles represent the distribution range with the highest probability, but refer to text for discussion on the robustness of these inferences. Probabilities in nodes for which highest ancestral range probability were lower than 1 indicated in the top right.

in any of the four regions tested (control region, all protein genes, and the RNA genes), and no saturation was found in protein-coding 3rd codon positions. In each case the index of substitution saturation (I_{ss}) was significantly less than the critical value ($I_{ss.c}$; see Xia et al. 2003 for details of the methodology) and the significance level was $P < 0.0001$. Consistent topology was found for MrBayes phylogenies using only protein-coding genes (Supplementary Fig. S2), and for various partitioning trials (by codon positions and gene region). We therefore included the full mitogenome sequences in our phylogenetic reconstructions.

Patterns of Divergence

Runs carried out in IMa resulted in 6,093,088 iterations following burn-in, ESS values for both splitting time and TMRCA were all above 1,800,000 and no trends were visible in the likelihood plots, thus suggesting that convergence was achieved (posterior distributions in Supplementary Fig. S3). Using a credible maximum calibration date of 10 Ka defined by the collapse of the land bridge at the Bosphorus (Gökaşan et al. 1997; Ryan et al. 1997; Hiscott et al. 2002; Kerey et al. 2004) separating the eastern Mediterranean and Black Sea populations, we calculated the likely average substitution rate for the *Tursiops* mitochondrial genome using the program IMa (see Methods section). The best estimate was 0.0307 substitutions/site/Myr (95% HPD: 0.0161–0.0816 substitutions/site/Myr). Generating a linearized phylogeny and applying this rate provides dates for both nodes separating Eastern Mediterranean from Black Sea haplotypes (represented within two separate lineages in the phylogeny; see Supplementary Fig. S4) at ~10 Ka, consistent with the geological data. In IMa using this substitution rate, the splitting time (time at which the populations differentiated) between the Black Sea and Eastern Mediterranean populations calculated using the full dataset is 10.25 Ka (95% HPD: 5.27–26.6 Ka), but the TMRCA (deepest coalescent point of included OTUs) is considerably older, reflecting the node joining the two lineages as expected (estimated in IMa at 146 Ka ± s.d. 1.4 Ka; *c.f.* Fig. 2). When the same calculations are applied to the single-lineage dataset using the same rate, the splitting time is 4.55 Ka (95% HPD: 2.25–28.9 Ka) while the TMRCA is 9.19 Ka (± s.d. 0.21 Ka), well within the confidence limits for the splitting time. This confirms that the estimate of splitting time for the full dataset is still credible in spite of the much older TMRCA caused by the two independent lineages each incorporating differentiation between populations in the Eastern Mediterranean and Black Sea.

In order to estimate node dates within the phylogeny, we applied a Bayesian approach as implemented in the program BEAST (Fig. 2). Supplementary Tables S5, S6, and S7 summarize the various models and priors tested and their relative support. Model testing showed marginal support for the lognormal clock (Bayes Factor = 2.841), but ESS values for key statistics were all higher in the exponential clock (Supplementary Table S6), and the exponential clock was therefore applied in all subsequent analyses. The relaxed molecular clock was also much better supported than the BRLC model (Supplementary Table S7).

Comparison between models using different calibration points revealed that using only the fossil calibration points resulted in lower statistical support (Bayes Factor = –43.767) and dates for the divergence between Eastern Mediterranean and Black Sea *T. truncatus* clades that are incompatible with geological data (Supplementary Fig. S5). Using only the biogeographical calibration point leads to higher statistical support (Bayes Factor = 33.852) but dates of the older nodes are incompatible with previous studies and fossil data (Supplementary Fig. S6). Using uniform priors for both fossil and biogeographical calibration points results in divergence dates compatible with the literature for both recent and older nodes, but statistical support is lower (Bayes Factor = –26.295), and estimated substitution rates near the terminal nodes unrealistically slow. Using normal distributions at the fossil calibration points together with a lognormal distribution at the biogeographic calibration (lognormal due to the boundary at 0 years) gives support that is overall very low (Supplementary Tables S6 and S7), and again the dates for recent nodes become unrealistically old. Among all the different combinations tested, the best supported had a uniform prior at the biogeographic calibration, and normal distributions at both fossil calibrations (Supplementary Tables S5, S6, and S7), and the results from this analysis are shown in Figure 2. For that analysis, the mean clock rate was calculated as 0.021 substitutions/site/Myr (95% HPD: 0.011–0.034 substitutions/site/Myr) which fits within the range calculated in the IMa analysis, and is consistent with the rate calculated previously for cetacean protein-coding mtDNA (0.02 substitutions/site/Myr; Ho and Lanfear 2010).

Node dates suggest that divergence in the genus *Tursiops* occurred within the Pleistocene epoch (particularly since the lower Pleistocene). The earliest node defining the *Tursiops* lineage for the phylogeny shown in Figure 2 dated to ~1.086 Ma (95% HPD: 0.54–2.2 Ma), too recent based on the fossil date of ~5 Myr. When considered in the context of the Pleistocene climatic cycles, for terminations III–VI (243–533 Ka), eight nodes show periodicity broadly consistent with the 100 Kyr ± 20 Kyr Milankovitch climatic cycles, and are generally associated with these termination events, or the subsequent periods of fast global warming, however those dates may be underestimates (see below). Most recent nodes show no clear association with climate cycles apart from a subset associated with differentiation between coastal and offshore ecotypes. In particular, 3 of the 4 nodes clearly separating coastal from pelagic samples date closely to the Eemian interglacial period (171 Ka, 95% HPD 43–367; 153 Ka, 95% HPD 59–306; 103 Ka, 95% HPD 33–209). There is also an apparent increase in diversification at the start of

TABLE 2. Diversification rates and time periods to which they apply obtained using the best fit model as determined by LASER (Rabosky 2006)

Dataset/Model	Diversification rate	Time interval (Ka)
Full dataset/Yule-5-rate	0.711698	Root - 243
	3.022507	243 - 42
	11.90969	42 - 14
	31.25963	14 - 1.5
	6.034302	1.5 - Tips
Pruned dataset/ Yule-3-rate	0.2591858	Root - 523
	0.7596458	523 - 27
	5.186823	27 - Tips

the Holocene based on results from the LASER analysis (see below).

From the LASER analysis of the full dataset, the Yule-5-rate variable-rate model has the highest ΔAICrc (ΔAICrc = 21.778; $P < 0.0001$) when compared with the best-supported constant-rate model (Supplementary Table S8). This model indicates that the strongest rate increase occurred over a period starting at around 14 Ka, coinciding with termination I following the end of the last glacial period. This period was preceded by periods where the diversification rates were at least three times slower (Table 2). For the pruned dataset, the Yule-3-rate is the best-supported model (ΔAICrc = 7.049; $P = 0.013$; Table 2), and while the strongest rate increase is now dated to around 27 Ka (and lasts to present) it is still consistent with a recent increase incorporating the Holocene timeframe.

Biogeographic assessments using the program S-DIVA (Fig. 3) suggest an Australasian origin at the deepest *Tursiops* node. Inference in deeper nodes will necessarily be less robust with S-DIVA, and although for the deepest *Tursiops* node (Fig. 3, node 150) three different area combinations have similar probabilities, presence in coastal Australasia is common to all of them, with one comprising only coastal Australasia (with 25.6% support). All lineages until the start of the *T. truncatus* lineage are represented by the coastal ecotype. The node at the base of the *T. truncatus* lineage was undefined in S-DIVA, but offshore populations are represented across sub-lineages, suggesting an early transition from coastal ecotype to offshore, followed by later reversals back to the coastal type (e.g., in coastal habitat in Scotland and the Mediterranean Sea). These results suggest that the transition from a coastal to a pelagic ecotype occurred at the base of the *T. truncatus* lineage.

DISCUSSION

This study is based on whole mitogenome sequences (contributing 1.26 Mbp of novel data to compare with published mitogenomes), which provide sufficient informative sites to generate very high resolution phylogenetic reconstructions. Investigating a single non-recombining DNA molecule allows us to estimate an average mutation rate without violating the assumption of a single-gene region, however it can also lead to problems regarding the recovery of a single-gene phylogeny rather than the species phylogeny. While there are no earlier studies that include all of the same sample regions as we do, Amaral et al. (2012) constructed a phylogeny based on 13 nuclear loci and included two *T. truncatus* (from Portugal), two *T. aduncus* (from Australia), and two SABD (*T. australis*) samples (among 18 other samples from 12 other delphinid species). This phylogeny estimated a different overall topology, but the same relationship among the three named *Tursiops* species as we find from our mitogenome phylogeny, with the Australasian coastal samples splitting from more basal nodes relative to the *T. truncatus* samples. Our study suggests polyphyly consistent with previous studies based on mtDNA (LeDuc et al. 1999; Charlton et al. 2006; Möller et al. 2008; Xiong et al. 2009; Vilstrup et al. 2011) and suggested by some to be due to lineage sorting and introgression in mtDNA phylogenies (Amaral et al. 2012). However, some studies that include nuclear markers also show polyphyly (Nishida et al. 2007; Kingston et al. 2009; McGowen 2011). For our study, the key points of inference about the relative age of lineages within the group currently identified as *Tursiops* congeners appear well supported, and we suggest that the resolution of the polyphyly question in particular will require the inclusion of a balanced set of OTUs from the relevant taxa.

In our phylogenies, lineages representing coastal populations are generally well differentiated from pelagic populations, although between pelagic and European coastal lineages in *T. truncatus* incomplete lineage sorting is suggested. Deeper nodes correspond to coastal ecotypes (e.g., the population in the western North Atlantic), with the pelagic lineages exhibiting a more recent origin. Although not all details of phylogeography were well resolved from our assessment using the program S-DIVA, the deepest *Tursiops* node has strong support for an Australasian origin. Furthermore, an assessment of support for vicariance compared to dispersal at various nodes suggests dispersal as a dominant factor at the two deepest nodes, and a combination of dispersal and vicariance for later divisions, including dispersal for some divisions between nearshore and offshore populations (Fig. 3). In S-DIVA analysis, vicariance refers to a splitting where the distribution of the ancestral population represents the combined distributions of the derived lineages, while dispersal refers to a splitting where the distribution of at least one of the derived populations includes a region not occupied by the ancestral population (Ronquist 1997). In this case, putative dispersal may reflect the founding event of a new habitat or geographic region by a group of founders separating from the ancestral population.

Within the *T. truncatus* lineage the reconstruction of ancestral distributions suggests a pelagic ancestor, with occupation of coastal habitats occurring later in time, but relatively soon within the evolution of the group. If dispersal began in Australasia, following coastal

habitats around the Indian Ocean to Africa and then into the North Atlantic, a pelagic phase is then likely prior to the founding of the western North Atlantic coastal population, given the lack of contiguous coastal habitat between the North Atlantic coastal margins. In the eastern Pacific, although based on only one sample, separation of that haplotype occurs within the *T. truncatus* "pelagic" lineage (and so after the establishment of that lineage).

Overall, our data suggest that extant representatives of the genus *Tursiops* originated in Australasia (or more broadly within Oceania) from lineages occupying coastal habitats. Worldwide expansion of other *Tursiops* lineages would have occurred later, with expansion toward the Atlantic through Indo-Pacific coastal habitats first, and colonization of the pelagic environment later at the genesis of the *T. truncatus* lineage, followed by a regression to the ancestral coastal state accompanied by some of the corresponding morphological changes. Fossils representing putative ancestors to the *Tursiops* lineage have been found in various locations around the world, but include fossils from southern Australia (Fitzgerald 2005). Offshore *Tursiops* ecotypes found today in Australasia have been shown to group closely with WNAP *T. truncatus* lineages (e.g. Hoelzel et al. 1998; Natoli et al. 2004; Möller et al. 2008), and thus likely represent a later invasion of the Australasian region. Further investigation of samples from the eastern and central Pacific would be useful, but such samples are likely to fall within the *T. truncatus* lineage based on these earlier studies.

Our study has focused on recent systematic questions in order to better understand the mechanisms that underlie the diversification of the delphinids. Taxonomy within this genus is not well resolved, but we provide strong support for alpha taxonomic divisions between *T. truncatus* and *T. aduncus*, and biogeographic data suggesting that the ecological drivers are mainly ecotype division between coastal and offshore habitat. Alternatives associated with differentiation in allopatry or isolation by distance are less well supported by the data. We also strengthen the proposed classification of *T. australis* as a valid species (Charlton et al. 2006; Möller et al. 2008; Charlton-Robb et al. 2011), and show that it represents the sister group of all other *Tursiops* lineages. The South African *T. aduncus* (SA-Ta) and the Indo-Pacific *T. aduncus* (IP-Ta) remain reciprocally monophyletic as reported in an earlier study (Natoli et al. 2004), but our data suggest that the populations may have differentiated following the initial expansion of the IP-Ta population. Although these populations may represent incipient species, this should be further tested incorporating nuclear genes. With respect to the coastal ecotype in the western North Atlantic, this monophyletic lineage split from *T. truncatus* early on, and we suggest that it represents the first reversion from the pelagic type. Further transitions from the pelagic to the coastal ecotype likely occurred later (e.g., in the eastern North Atlantic). Among these transitions from pelagic to coastal ecotype, only the population in the western North Atlantic shows consistent mtDNA monophyly together with morphological and ecological differences from the pelagic ecotypes (Mead and Potter 1995; Torres et al. 2003) and support for monophyly from nuclear markers (Kingston and Rosel 2004) sufficient to suggest the possibility of incipient speciation.

Dating of Divergence Nodes

The results of our dating analysis suggest that divergence within *Tursiops* occurred during the last half of the Pleistocene, however our mean date estimates for the deeper nodes from the BEAST analysis were younger than the fossil estimates found in the literature (3.3 Ma compared to 10 Ma and 1.09 Ma compared to 5 Ma). This suggests that although the phylogeny presented was the best supported among the alternatives based on other models and priors, it did not successfully track the temporal change in substitution rate for the older nodes. DNA substitution rates and corresponding divergence times have been reported to change markedly depending on whether a fossil or a biogeographical calibration point is used, as first described in Ho et al. (2005) and subsequently supported (Genner et al. 2007; Waters et al. 2007; Burridge et al. 2008). The existence of this discrepancy has been controversial (together with the biological reasons behind it) and is the focus of debate (Woodhams 2006; Emerson 2007; Fagundes et al. 2008; Ho et al. 2008; Weir and Schluter 2008; Peterson and Masel 2009). It has been attributed to the segregation of slightly deleterious mutations (e.g., Ho et al. 2005), but also to inadequate sampling (Emerson 2007), uncertainty in the biogeographical calibration nodes used (Fagundes et al. 2008; Weir and Schluter 2008) and patterns of ancestral population structure and effective population size (Woodhams 2006; Peterson and Masel 2009).

In our study the sampling is robust and likely representative, and uncertainty in the biogeographical calibration point used is unlikely to bias the results obtained. As reviewed above, the geological data are now robust indicating a very recent opening between the Mediterranean and Black Sea (and an ancient earlier opening at no less than 5 Ma—Nikishin et al. 2003—too early to be a credible date of divergence between these lineages). Furthermore, the geologic date indicates a mutation rate that is consistent with independent estimates (Ho and Lanfear 2010). We cannot fully rule out the possibility that population differentiation already existed in the Eastern Mediterranean before the opening of the Bosphorus Strait. However, a previous study on *T. truncatus* population structure in coastal Europe showed clear differentiation between the Black Sea and Eastern Mediterranean for microsatellite loci, with no structuring found within either location. Even if there were such differentiation within the Eastern Mediterranean, it would be unlikely that one of these hypothetical populations would fully relocate to the Black Sea upon the opening of the Bosphorous strait, while the other did not enter it at all. At the same time,

mtDNA control region haplotypes were shared among all locations (Fig. 4 in Natoli et al. 2005), consistent with the incomplete lineage sorting indicated in the current study. Therefore the deeper lineage division reflected in the two lineages showing differentiation between the eastern Mediterranean and the Black Sea likely reflect the chance retention of those lineages in both locations.

From our data the most recent node dates become incompatible with the biogeographic date for the opening of the Bosphorus when we use only fossil calibration points (Supplementary Fig. S5), and the nodes representing the fossil dates become too recent when we use only the biogeographic calibration (Supplementary Fig. S6). This reflects the proposed transition between a faster recent and slower "phylogenetic" mutation rate, though we do not know when that transition occurs. The study that introduced the concept of time dependency for mutation rates suggested that the effect would decay over a period of 1–1.5 Myr in a pattern described as the "lazy-J" (Ho et al. 2005), however a later study reanalysing the same data suggested that there was no evidence for higher rates further back than 100 Kyr (Emerson 2007). Better resolution on the question was provided by studies that could compare multiple biogeographic calibration points over time, such as a study based on galaxiid fish species (Burridge et al. 2008) and another on birds (Ho et al. 2008), each of which suggest accelerated rates back to around 200 Ka. For this reason we focus on only the dates within the last 200 Kyr for our correlation against climate cycles, though this will only be an approximation, given that further biogeographic calibration is not possible for this lineage. Together with the results from LASER (indicating increased diversification after the last glacial maximum; LGM), these data suggest some association between diversification and at least the last two interglacial transitions. This was especially true for nodes within that time frame that defined a distinction between coastal and offshore ecotypes (though the confidence limits on these dates are broad). Such an association may be driven by habitat release during warmer interglacial periods when coastal habitat was made available by receding ice caps (as discussed previously; e.g. Hoelzel 1998a; Hoelzel et al. 1998, 2007). Possible alternatives would require some mechanism for the isolation of populations independent of habitat availability and the subsequent association of subdivided populations by habitat.

The latter part of the Pleistocene was characterized by strong climatic oscillations (Huybers 2011), the "Milankovitch cycles" with a periodicity of 100 Kyr $\pm$ 20 Kyr as determined from benthic $\delta^{18}O$ records (Lisiecki 2005). Diversification at timescales smaller than the Milankovitch cycles is thought to be best explained by intrinsic biotic factors (colloquially known as the Red Queen Hypothesis) rather than external environmental changes (Court Jester Hypothesis; Benton 2009). In this study both factors may play a role over this time scale, with biotic interactions being associated with divergent ecotypes dependent on differential habitat use or prey choice. However, the Red Queen hypothesis postulates that rates of speciation and extinction are constant through time, thus predicting that phylogenetic trees better fit a fixed-rate diversification model (Venditti et al. 2010), which is not supported in our data. This may suggest a greater role for environmental factors, at least over the longer timeframe within the Pleistocene. Earlier studies suggested that environmental changes associated with Pleistocene climate cycles may have led to a population bottleneck and subsequent population expansion in the killer whale (*Orcinus orca*; Hoelzel et al. 2002; Hoelzel et al. 2007). Although previous estimates on the most recent common ancestor of that group have varied according to the methodology used (Hoelzel et al. 2002; Morin et al. 2010), all reflect a recent radiation within the period of Pleistocene climate cycles (Supplementary Fig. S7).

Conclusions

Our data suggest an origin for the current *Tursiops* genus in coastal habitats in Australasia (or more broadly within Oceania), followed by expansion across the region and differentiation at the founding of the coastal population off Africa. Within the Atlantic, divergence of the coastal population in the Western North Atlantic occurred early in the *T. truncatus* lineage, but it appears to have been preceded by a pelagic state. The position of Atlantic pelagic and European coastal populations within the lineage suggests that more recent coastal populations originated from a pelagic stock, though our interpretation is likely affected by incomplete lineage sorting. Our data further suggests that much of the divergence within *Tursiops* occurred within the Pleistocene, indicating a rapid radiation within the species group. Differentiation of relatively recent ecotype divisions appears to correlate with periods of fast climatic change during the Eemian period and Holocene, suggesting a role for these climate oscillations in the diversification of the group. Changes in coastal topography promoted by temperature oscillations may provide empty niches and opportunity for divergence and speciation. This would have broader relevance for understanding the processes that generate diversity and lead to speciation in highly mobile marine taxa.

SUPPLEMENTARY MATERIAL

Data files and/or other supplementary information related to this article have been deposited at Dryad under doi: 10.5061/dryad.k501d.

FUNDING

This work was supported by the Portuguese Fundação para a Ciência e Tecnologia (PhD grant SFRH/BD/28012/2006) to A.E.M. and by the Danish

National Research Foundation "GeoGenetics" grant to M.T.P.G.

Acknowledgments

We thank everyone who provided samples for this study: Elena Politi, Giovanni Bearzi and Caterina Fortuna (Tethys Research Institute), Bob Reid (SAC Veterinary Science), Alexei Birkun (CIESM, ACCOBAMS Scientific Committee), Iris Segura (Durham University), and to Vic Peddemors and his staff at the National Shark Board for their support in the collection of South African samples. We also thank Andaine Seguine and the Danish National High-throughput DNA sequencing Centre for assistance in generating the mitogenomes.

References

Amaral A.R., Jackson J.A., Möller L.M., Beheregaray L.B., Manuela Coelho M. 2012. Species tree of a recent radiation: the subfamily Delphininae (Cetacea, Mammalia). Mol. Phylogenet. Evol. 64:243–253.

Barnes G.G. 1990. The fossil record and evolutionary relationships of the genus *Tursiops*. In: Leatherwood S., Reeves R.R., editors. The bottlenose dolphin. San Diego, CA: Academic Press Inc. p. 3–26.

Barnosky A. 2001. Distinguishing the effects of the Red Queen and Court Jester on miocene mammal evolution in the Northern Rocky Mountains. J. Vertebr. Paleontol. 21:172–185.

Benton M.J. 2009. The Red Queen and the Court Jester: species diversity and the role of biotic and abiotic factors through time. Science 323:728–732.

Bierne N., Bonhomme F., David P. 2003. Habitat preference and the marine-speciation paradox. P. Roy. Soc. Lond. B Bio. 270:1399–1406.

Burridge C.P., Craw D., Fletcher D., Waters J.M. 2008. Geological dates and molecular rates: fish DNA sheds light on time dependency. Mol. Biol. Evol. 25:624–633.

Charlton-Robb K., Gershwin L.A., Thompson R., Austin J., Owen K., McKechnie S. 2011. A new dolphin species, the burrunan dolphin *Tursiops australis* sp. nov., endemic to southern Australian coastal waters. PLoS ONE 6:e24047.

Charlton K., Taylor A.C., McKechnie S.W. 2006. A note on divergent mtDNA lineages of bottlenose dolphins from coastal waters of southern Australia. J. Cetacean Res. Manage. 8:173–179.

Darling A.E., Mau B., Perna N.T. 2010. ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS ONE 5:e11147.

Drummond A.J., Ashton B., Buxton S., Cheung M., Cooper A., Heled J., Kearse M., Moir R., Stones-Havas S., Sturrock S., Thierer T., Wilson A. 2010. Geneious v5.1. Available from: URL http://www.geneious.com.

Drummond A.J., Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Drummond A.J., Suchard M.A. 2010. Bayesian random local clocks, or one rate to rule them all. BMC Biol. 8:114.

Dynes J., Magnan P., Bernatchez L., Rodriguez M.A. 1999. Genetic and morphological variation between two forms of lacustrine brook charr. J. Fish Biol. 54:955–972.

Emerson B.C. 2007. Alarm bells for the molecular clock? No support for Ho et al.'s model of time-dependent molecular rate estimates. Syst. Biol. 56:337–345.

Fagundes N.J.R., Kanitz R., Bonatto Sandro L. 2008. Reply to Ho and Endicott. Am. J. Hum. Genet. 83:146–147.

Fitzgerald E.M.G. 2005. Pliocene marine mammals from the Whalers Bluff formation of Portland, Victoria, Australia. Memoirs of Museum Victoria 62:67–89.

Folkens P.A., Reeves R.R., Stewart B.S., Clapham P.J., Powell J.A. 2002. Guide to marine mammals of the world. New York: Alfred A. Knopf.

Genner M.J., Seehausen O., Lunt D.H., Joyce D.A., Shaw P.W., Carvalho G.R., Turner G.F. 2007. Age of cichlids: new dates for ancient lake fish radiations. Mol. Biol. Evol. 24:1269–1282.

Gökaşan E., Demirbag E., Oktay F.Y., Ecevitoglu B., Şimşek M., Yüce H. 1997. On the origin of the Bosphorus. Mar. Geol. 140:183–199.

Guindon S., Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52:696–704.

Hey J., Nielsen R. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. P. Natl Acad. Sci. USA 104:2785–2790.

Hiscott R.N., Aksu A.E., Yaşar D., Kaminski M.A., Mudie P.J., Kostylev V.E., MacDonald J.C., Işler F.I., Lord A.R. 2002. Deltas south of the Bosphorus Strait record persistent Black Sea outflow to the Marmara Sea since ~10 ka. Mar. Geol. 190:95–118.

Ho S.Y., Lanfear R. 2010. Improved characterisation of among-lineage rate variation in cetacean mitogenomes using codon-partitioned relaxed clocks. Mitochondr. DNA 21:138–146.

Ho S.Y., Phillips M.J., Cooper A., Drummond A.J. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol. Biol. Evol. 22:1561–1568.

Ho S.Y., Saarma U., Barnett R., Haile J., Shapiro B. 2008. The effect of inappropriate calibration: three case studies in molecular ecology. PLoS ONE 3:e1615.

Hoelzel A.R. 1998a. Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. J. Hered. 89:451–458.

Hoelzel A.R. 1998b. Molecular genetic analysis of populations: a practical approach. 2nd ed. Oxford: Oxford University Press

Hoelzel A.R. 2009. Evolution of population structure in marine mammals. In: Bertorelle G., Bruford M.W., Hauffe H.C., Rizzoli A., Vernesi C. editors. Population genetics for animal conservation. Cambridge: Cambridge University Press.

Hoelzel A.R., Hancock J.M., Dover G.A. 1991. Evolution of the cetacean mitochondrial D-Loop region. Mol. Biol. Evol. 8:475–493.

Hoelzel A.R., Hey J., Dahlheim M.E., Nicholson C., Burkanov V., Black N. 2007. Evolution of population structure in a highly social top predator, the killer whale. Mol. Biol. Evol. 24:1407–1415.

Hoelzel A.R., Natoli A., Dahlheim M.E., Olavarria C., Baird R.W., Black N.A. 2002. Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history. P. Roy. Soc. Lond. B Biol. 269:1467–1473.

Hoelzel A.R., Potter C.W., Best P.B. 1998. Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. P. Roy. Soc. Lond. B Biol. 265:1177–1183.

Huelsenbeck J.P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Huybers P. 2011. Combined obliquity and precession pacing of late Pleistocene deglaciations. Nature 480:229–232.

Kemper C.M. 2004. Osteological variation and taxonomic affinities of bottlenose dolphins, *Tursiops* spp., from South Australia. Aust. J. Zool. 52:29–48.

Kerey I.E., Meriç E., Tunoğlu C., Kelling G., Brenner R.L., Doğan A.U. 2004. Black Sea–Marmara Sea Quaternary connections: new data from the Bosphorus, İstanbul, Turkey. Palaeogeogr. Palaeocl. 204:277–295.

Kingston S.E., Rosel P.E. 2004. Genetic differentiation among recently diverged delphinid taxa determined using AFLP Markers. J. Hered. 95:1–10.

Kingston S.E., Adams L.D., Rosel P.E. 2009. Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). BMC Evol. Biol. 9:245.

LeDuc R.G., Perrin W.F., Dizon A.E. 1999. Phylogenetic relationships among the delphinid cetaceans based on full cytochrome b sequences. Mar. Mammal Sci. 15:619–648.

Li H., Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760.

Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079.

Lisiecki L.E. 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18O records. Paleoceanography 20:PA1003.
McGowen M.R. 2011. Toward the resolution of an explosive radiation—a multilocus phylogeny of oceanic dolphins (Delphinidae). Mol. Phylogenet. Evol. 60:345–357.
McGowen M.R., Spaulding M., Gatesy J. 2009. Divergence date estimation and a comprehensive molecular tree of extant cetaceans. Mol. Phylogenet. Evol. 53:891–906.
McKinnon J.S., Rundle H.D. 2002. Speciation in nature: the threespine stickleback model systems. Trends Ecol. Evol. 17:480–488.
Mead J.G., Potter C.W. 1995. Recognizing two populations of the bottlenose dolphin (*Tursiops truncatus*) on the Atlantic coast of North America: morphologic and ecologic considerations. IBI Reports 5:31–44.
Milne I., Lindner D., Bayer M., Husmeier D., McGuire G., Marshall D.F., Wright F. 2009. TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. Bioinformatics 25:126–127.
Möller L.M., Beheregaray L.B. 2001. Coastal bottlenose dolphins from Southeastern Australia are *Tursiops aduncus* according to sequences of the mitochondrial DNA control region. Mar. Mammal Sci. 17:249–263.
Möller L.M., Bilgmann K., Charlton-Robb K., Beheregaray L. 2008. Multi-gene evidence for a new bottlenose dolphin species in southern Australia. Mol. Phylogenet. Evol. 49:674–681.
Morin P.A., Archer F.I., Foote A.D., Vilstrup J., Allen E.E., Wade P., Durban J., Parsons K., Pitman R., Li L., Bouffard P., Abel Nielsen S.C., Rasmussen M., Willerslev E., Gilbert M.T., Harkins T. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Res. 20:908–916.
Natoli A., Birkun A., Aguilar A., Lopez A., Hoelzel A.R. 2005. Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). P. Roy. Soc. Lond. B Biol. 272:1217–1226.
Natoli A., Peddemors V.M., Hoelzel A.R. 2004. Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. J. Evol. Biol. 17:363–375.
Nikishin A.M., Korotaev M.V., Ershov A.V., Brunet M.-F. 2003. The Black Sea basin: tectonic history and Neogene–Quaternary rapid subsidence modelling. Sediment. Geol. 156:149–168.
Nishida S., Goto M., Pastene L.A., Kanda N., Koike H. 2007. Phylogenetic relationships among cetaceans revealed by Y-chromosome sequences. Zoolog. Sci. 24:723–732.
Nylander J.A., Ronquist F., Huelsenbeck J.P., Nieves-Aldrey J.L. 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53:47–67.
Palumbi S.R. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu. Rev. Ecol. Syst. 25: 547–572.
Perrin W.F., Robertson K.M., van Bree P.J.H., Mead J.G. 2007. Cranial description and genetic identity of the holotype specimen of *Tursiops aduncus* (Ehrenberg, 1832). Mar. Mammal Sci. 23:343–357.
Peterson G.I., Masel J. 2009. Quantitative prediction of molecular clock and ka/ks at short timescales. Mol. Biol. Evol. 26:2595–2603.
Rabosky D.L. 2006. LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. Evol. Bioinform. Online 2:273–276.
Rambaut A., Drummond A.J. 2003. Tracer [computer program]. Available from: URL http://tree.bio.ed.ac.uk/software/tracer/.
Reid J.B., Evans P.G.H., Northridge S.P. 2003. Atlas of Cetacean distribution in north-west European waters. Joint Nature Conservation Committee.
Ronquist F. 1997. Dispersal-Vicariance Analysis: a new approach to the quantification of historical biogeography. Sys. Biol. 46:195–203.
Rozen S., Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132:365–386.
Ryan W.B.F., Pitman W.C., Major C.O., Shimkus K., Moskalenko V., Jones G.A., Dimitrov P., Gorür N., Sakinç M., Yüce H. 1997. An abrupt drowning of the Black Sea shelf. Mar. Geol. 138:119–126.
Segura I., Rocha-Olivares A., Flores-Ramírez S., Rojas-Bracho L. 2006. Conservation implications of the genetic and ecological distinction of *Tursiops truncatus* ecotypes in the Gulf of California. Biol. Conserv. 133:336–346.
Steeman M.E., Hebsgaard M.B., Fordyce R.E., Ho S.Y., Rabosky D.L., Nielsen R., Rahbek C., Glenner H., Sorensen M.V., Willerslev E. 2009. Radiation of extant cetaceans driven by restructuring of the oceans. Syst. Biol. 58:573–585.
Taylor B.L., Chivers S.J., Larese J.P., Perrin W.F. 2007. Generation length and percent mature estimates for IUCN assessments of cetaceans. NOAA, NMFS, Southwest Fisheries Science Center Administrative Report LJ-07-01:21.
Tezanos-Pinto G., Baker C.S., Russell K., Martien K., Baird R.W., Hutt A., Stone G., Mignucci-Giannoni A.A., Caballero S., Endo T., Lavery S., Oremus M., Olavarria C., Garrigue C. 2008. A worldwide perspective on the population structure and genetic diversity of bottlenose dolphins (*Tursiops truncatus*) in New Zealand. J. Hered. 100:11–24.
Torres L.G., Rosel P.E., D'Agrosa C., Read A.J. 2003. Improving management of overlapping bottlenose dolphin ecotypes through spatial analysis and genetics. Mar. Mammal Sci. 19: 502–514.
Van Valen L. 1973. A new evolutionary law. Evol. Theor. 1:1–30.
Venditti C., Meade A., Pagel M. 2010. Phylogenies reveal new interpretation of speciation and the Red Queen. Nature 463:349–352.
Viaud-Martinez K., Brownell R. Jr., Komnenou A., Bohonak A. 2008. Genetic isolation and morphological divergence of Black Sea bottlenose dolphins. Biol. Conserv. 141:1600–1611.
Vilstrup J.T., Ho S.Y., Foote A.D., Morin P.A., Kreb D., Krutzen M., Parra G.J., Robertson K.M., de Stephanis R., Verborgh P., Willerslev E., Orlando L., Gilbert M.T. 2011. Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. BMC Evol. Biol. 11:65.
Wang J.Y., Chou L.S., White B.N. 1999. Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus:*Tursiops*) in Chinese waters. Mol. Ecol. 8:1603–1612.
Wang J.Y., Yang, S.C. 2009. Indo-Pacific bottlenose dolphin, *Tursiops aduncus*. In: Perrin W.F., Thewissen J.G.M., Würsig B., editors. Encyclopedia of marine mammals. 2nd ed. San Diego: Academic Press.
Waters J.M., Rowe D.L., Apte S., King T.M., Wallis G.P., Anderson L., Norris R.J., Craw D., Burridge C.P. 2007. Geological dates and molecular rates: rapid divergence of rivers and their biotas. Syst. Biol. 56:271–282.
Weaver A.J., Saenko O.A., Clark P.U., Mitrovica J.X. 2003. Meltwater pulse 1A from Antarctica as a trigger of the Bolling-Allerod warm interval. Science 299:1709–1713.
Weir J.T., Schluter D. 2008. Calibrating the avian molecular clock. Mol. Ecol. 17:2321–2328.
Woodhams M. 2006. Can deleterious mutations explain the time dependency of molecular rate estimates? Mol. Biol. Evol. 23:2271–2273.
Xia X., Xie Z., Salemi M., Chen L., Wang Y. 2003. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26:1–7.
Xiong Y., Brandley M.C., Xu S., Zhou K., Yang G. 2009. Seven new dolphin mitochondrial genomes and a time-calibrated phylogeny of whales. BMC Evol. Biol. 9:20.
Yu Y., Harris A.J., He X. 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. Mol. Phylogenet. Evol. 56:848–850.