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Delphinid systematics and biogeography with a focus on the current genus *Lagenorhynchus*: Multiple pathways for antitropical and trans-oceanic radiation

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ABSTRACT

The six species currently classified within the genus *Lagenorhynchus* exhibit a pattern of antitropical distribution common among marine taxa. In spite of their morphological similarities they are now considered an artificial grouping, and include both recent and the oldest representatives of the Delphinidae radiation. They are, therefore, a good model for studying questions about the evolutionary processes that have driven dolphin speciation, dispersion and distribution. Here we used two different approaches. First we constructed a multigenic phylogeny with a minimum amount of missing data (based on 9 genes, 11,030 bp, using the 6 species of the genus and their closest relatives) to infer their relationships. Second, we built a supermatrix phylogeny (based on 33 species and 27 genes) to test the effect of taxon sampling on the phylogeny of the genus, to provide inference on biogeographic history, and provide inference on the main events shaping the dispersion and radiation of delphinids. Our analyses suggested an early evolutionary history of marine dolphins in the North Atlantic Ocean and revealed multiple pathways of migration and radiation, probably guided by paleoceanographic changes during the Miocene and Pliocene. *L. acutus* and *L. albirostris* likely shared a common ancestor that arose in the North Atlantic around the Middle Miocene, predating the radiation of subfamilies Delphininae, Globicephalinae and Lissodelphininae.

1. Introduction

The processes that drive species radiations in the marine environment remain poorly understood, especially those involving species with high dispersal potential. The biogeography of these species can be difficult to interpret due to the frequent lack of obvious barriers to gene flow (e.g. Pastene et al., 2007). However, recent statistical approaches have improved inferences about biogeography from DNA sequences (Ronquist, 1997; Sanmartín et al., 2008; Nylander et al., 2008; Ree and Smith, 2008; Yu et al., 2010; Calvente et al., 2011; Ali et al., 2012). These methods provide inference about the ancestral distribution of species, and together with time-based phylogenies, on the impact of climatic and geological changes (e.g. Bocxlaer et al., 2006; Alexandre et al., 2009; Xie et al., 2009). Here we employ this methodology to consider the evolution of species within the delphinid radiation. We focus on the six species that had been classified in the genus *Lagenorhynchus*, because although the case for their classification based on morphology had been strong (e.g. Miyazaki and Shikano, 1997), genetic data suggested divergent origins (e.g. LeDuc et al., 1999). More data were needed to resolve these relationships, but beyond that, the radiation of these phenotypically similar species through the broader lineage is in itself informative.

Various studies have investigated the phylogenetic relationships among dolphin taxa using morphological characters (Messenger and McGuire, 1998; Geisler and Sanders, 2003; Kingston and Rosel, 2004; Price et al., 2005) and molecular data (LeDuc et al., 1999; May-Collado and Agnarsson, 2006; Harlin-Cognato and Honeycutt, 2006; McGowen et al., 2009; Xiong et al., 2009; Steeman et al., 2009; Vilstrup et al., 2011; McGowen, 2011). The fossil evidence suggests that the common ancestor of dolphins probably emerged around ~10–11 Ma in the mid-late Miocene (Fordyce, 2008). After this epoch, dolphins underwent a rapid radiation which gave rise to relatively few diagnostic characteristics among species. This led to difficulties in their taxonomic classification and controversy about the dating of divisions

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amongst subfamilies, genera and species (LeDuc et al., 1999; Pichler et al., 2001; Gygax, 2002).

The traditional classification of the family Delphinidae (Rice, 1998) includes 17 genera placed within six subfamilies: Lissodelphininae (genus *Lissodelphis*), Cephalorhynchinae, Orcaellinae, Stenoninae, Delphininae, and Globicephalinae (see LeDuc, 2008). Recent molecular studies have generated further discussion about this classification (LeDuc et al., 1999; Caballero et al., 2007; Vilstrup et al., 2011; McGowen, 2011) and identified several conflicting relationships, such as those amongst the genera *Delphinus*, *Tursiops* and *Stenella* (see Rosel et al., 1994; LeDuc et al., 1999; Kingston and Rosel, 2004; May-Collado and Agnarsson, 2006; Caballero et al., 2008), and those amongst species currently placed within the genus *Lagenorhynchus* (Cipriano, 1997; LeDuc et al., 1999; Harlin-Cognato and Honeycutt, 2006; McGowen, 2011) for which classification has been especially controversial.

Named *Lagenorhynchus* species (though see LeDuc et al., 1999; McGowen, 2011 for alternative classifications) have an antitropical distribution (see illustration in Fig. 1; Leatherwood et al., 1991; Gaskin, 1992). These species have been placed together because of similarities in coloration, skull and beak shape (Fraser, 1966; Mitchell, 1970), and because there are few craniometrical differences among them (Miyazaki and Shikano, 1997). The first molecular study to challenge this classification was presented by Cipriano (1997) using partial sequences of the mtDNA D-loop region and the Cytochrome b (*Cytb*) locus indicating that the two North Atlantic species (*L. acutus* and *L. albirostris*) are not closely related to other members of the genus.

LeDuc et al. (1999) using the entire *Cytb* sequence also suggested that the genus was polyphyletic. These authors recommend retaining the name *Lagenorhynchus* for *L. albirostris*, assigning *L. acutus* to a different genus, *Leucopleurus* (*Leucopleurus acutus*), and grouping *L. obliquidens*, *L. australis*, *L. obscurus* and *L. cruciger* into the genus *Sagmatias* (Cope 1866) within the subfamily Lissodelphininae. By contrast, Harlin-Cognato and Honeycutt (2006) using a multigenic phylogeny combining *Cytb*, the D-loop region, and two nuclear genes (actin and RAG2) suggested monophyly for *L. acutus* and *L. albirostris* and their placement as a sister group of the subfamily Delphininae. However, May-Collado and Agnarsson (2006) using Bayesian analysis of *Cytb* sequences and recent multilocus phylogenies (McGowen, 2011; Vilstrup et al., 2011) did not find a close relationship between *L. acutus* or *L. albirostris* and the subfamily Delphininae.

These initial studies provided new insight into phylogenetic relationships among dolphins, but could not fully resolve the relationships amongst *Lagenorhynchus* species in particular. Some recent multigenic studies (McGowen et al., 2009; Steeman et al., 2009; Xiong et al., 2009; McGowen, 2011; Vilstrup et al., 2011) agree with the placement of *L. acutus* and/or *L. albirostris* at the root of the Delphinidae phylogeny, although their position in the phylogenetic trees differ. Although Cetacean phylogeny has been extensively revised in recent years (e.g. Vilstrup et al., 2011; McGowen, 2011) and discussed elsewhere, '*Lagenorhynchus*' remains one of the most controversial classifications, and the fact that *L. acutus* and *L. albirostris* are basal in delphinid phylogenies, and have restricted ranges in the North Atlantic, poses interesting questions about the forces promoting the dispersal and speciation of ancestral delphinid populations.

Several hypotheses have been proposed regarding the causes of antitropical distribution in marine taxa. For example, Davies (1963) proposed that early cetaceans were mostly warm-water species, and that the first cold-water species evolved in the mid-Tertiary in response to the expansion of cold-water habitat. After this the tropical belt served as an "important but variable" barrier to dispersion between the poles. White (1986, 1989) considered antitropical distributions more generally and suggested that they were a consequence of global depression in temperatures, which allowed the spread of temperate-adapted organisms into low latitudes. However, Briggs (1987) disagreed and instead proposed the refugial hypothesis as an alternative. Other recent theories have

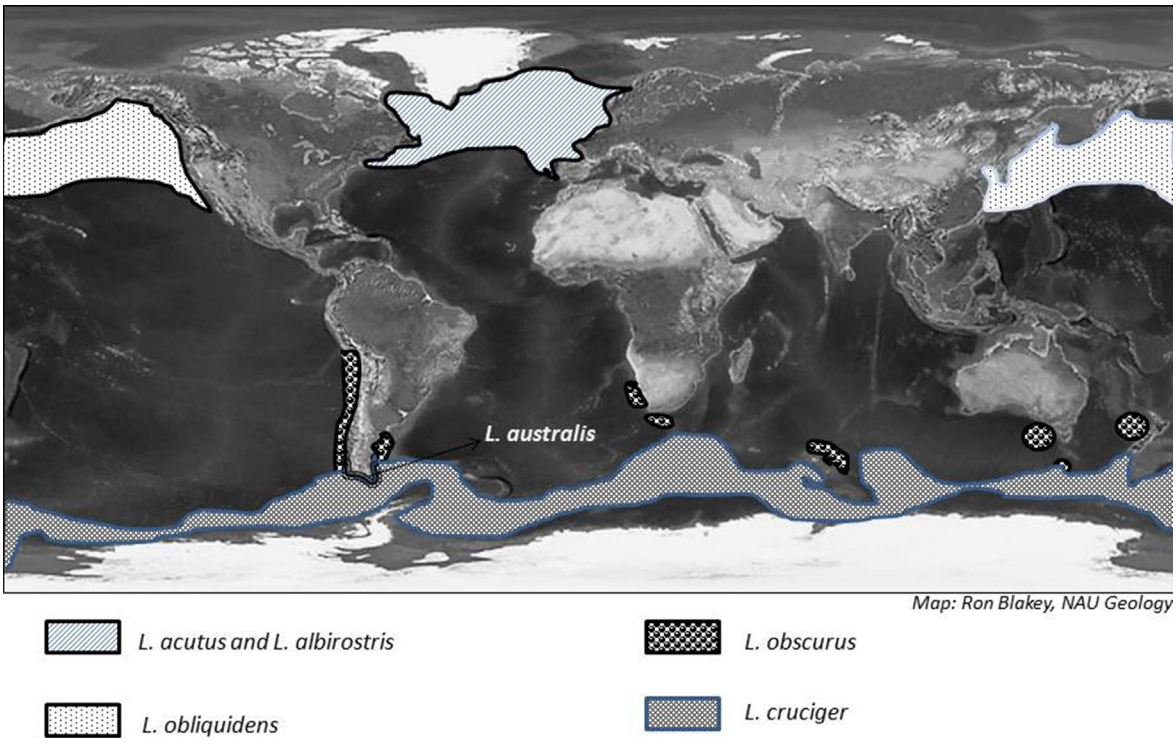

Fig. 1. World-wide distribution of study species. Distribution of *L. albirostris* is indicated as suggested by Dr. Peter Evans, personal communication. Note that the distribution of *L. cruciger* may extend into the southern Pacific Ocean.

proposed that the speciation, radiation and current distribution of cetaceans, including taxa distributed in different hemispheres, were a consequence of paleoceanographic changes, such as the establishment of new current systems and upwelling regions occurring during the Miocene, Pliocene and early Pleistocene epochs (Pichler et al., 2001; Berger, 2007; Pastene et al., 2007; Steeman et al., 2009; Marx and Uhen, 2010).

In this study we investigate the evolutionary history, phylogenetic relationships and biogeography both for the species historically classified within the genus *Lagenorhynchus*, and for the broader radiation. The broad geographic distribution of *Lagenorhynchus* spp. can provide inference about evolutionary process in multiple systems. We focus on the phylogenetic relationships among the six species of the genus using new sequence data, and further assess their phylogenetic and biogeography history using a time calibrated supermatrix phylogeny incorporating 25 delphinid species (representative of the lineages defined by the 32 species in the Family Delphinidae), and comparing five different methods for biogeographic inferences. We discuss how paleoceanographic and paleoclimatic changes may have influenced their dispersion, diversification and speciation and address the hypothesis that the processes that led to the evolution of *Lagenorhynchus* species reflect the processes leading to the broader radiation within the Delphinidae.

2. Methods

2.1. Samples

Six species of the genus *Lagenorhynchus*, one species of the genus *Cephalorhynchus* (*C. commersonii*), and three species of the subfamily Delphininae (*Delphinus delphis*, *Stenella coeruleoalba*, and *Tursiops truncatus*) were included for the initial analyses, together with *Phocoena phocoena* (Phocoenidae), *Delphinapterus leucas* (Monodontidae), *Hyperoodon ampullatus* (Ziphiidae) and *Physeter macrocephalus* (Physeteridae) as out-groups. *P. phocoena* and *D. leucas* were chosen as out-groups because they are sister taxa of Delphinidae (Fajardo-Mellor et al., 2006). *H. Ampullatus* and *P. Macrocephalus* were chosen so that we could estimate the age of the root node for Delphinoidea and compare it with the supermatrix analyses described below.

2.2. DNA extraction and gene amplification

Total genomic DNA was extracted following the procedure in Hoelzel and Green (1998). Published primers were then used to amplify five nuclear genes and one mitochondrial gene using Platinum®Taq Polymerase: (i) A section of Exon 1 of the inter-photoreceptor retinoid binding protein gene (*IRBP*; Stanhope et al., 1992; Springer et al., 1997); (ii) Exon 28 of the gene encoding the von Willebrand Factor (*vWF*; Porter et al., 1996); (iii) Two introns and two exons of the α lactalbumin gene (*LAC*; Waddell et al., 2000, see details in Milinkovitch et al., 1998); (iv) The Ca+ calmodulin-dependent kinase (*CAMK*) gene intron 6 (Bland et al., 1994; Lyons et al., 1997); (v) Intron 6 of the beta-hexosaminidase beta chain gene (*HEXB*; Lyons et al., 1997); and (vi) The mtDNA *16s rRNA* gene (Palumbi et al., 1997).

The genes *IRBP*, *16s rRNA*, *LAC*, *ACT* and *vWF* were amplified under the following conditions: 94 °C for 2 min followed by 32 cycles of 94 °C for 15 s, specific annealing temperature for 15 s, and an extension at 72 °C for 30 s. *CAMK* and *HEXB* were amplified using 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 30 s at specific annealing temperature, and 72 °C for 2 min. PCR products were sequenced in both directions on an ABI 377 automated sequencer. ACT1 and ACT1385H (5′-cttgtgaactgattacagtcc-3′) (Palumbi, unpublished, cited in Harlin-Cognato and Honeycutt, 2006) primers were used to amplify the *ACTIN* locus. *Cytb* and melanocortin-1 receptor (*MC1R*) sequences were obtained from the GenBank database (Tables 2 and S1).

2.3. Phylogenetic reconstruction

The dataset was aligned using the Clustal X programme v. 1.83 (Thompson et al., 1997), confirmed by eye, edited and compiled using the programme Chromas Pro (www.technelysium.co.au) resulting in 11,030 characters after alignment. All sequences were subjected to a Blast search in GenBank in order to verify sequence orthology. With the exception of two sequences from the *16s rRNA* gene in *L. obliquidens* from different geographic regions, all individuals from the same species had very similar or identical sequences (see Table S2). Multiple individuals, when different, were included in phylogenies, but the resulting trees did not differ from when one sample per species was included (data not shown). Therefore only one sequence per species was included in all subsequent analyses.

To allow the inclusion of different substitution models and test the effect of different datasets on the preliminary phylogenetic analyses, we used different partition schemes applying the evolutionary models suggested by Mr. Modeltest v2.2. (Nylander, 2004; see Table 1). Analyses were performed excluding and including gaps coded as a binary (0–1) state using the Fastgap v1.2 program (Borchsenius, 2009). We further performed Bayesian analyses for nuclear coding genes, non-coding genes and mitochondrial genes independently to determine whether or not different data sets could recover the same topology (see Fig. 2). The total evidence as well as the coding-gene phylogenies were also analyzed including and excluding the IRBP gene, which has been claimed to be under directional selection (Springer et al., 1997; Jansa et al., 2006). Models for each partition were selected and applied following the Akaike Information criterion (AIC) implemented in Mr. Model Test v. 2.2. (Nylander, 2004). The accuracy of combining different datasets was assessed using the partition homogeneity test (PHT/ILD test; Farris et al., 1994), in the programme PAUP* v. 4.0b10 (Swofford, 2002), using branch and bound searches with 1000 replicates. Analyses were performed excluding out-group taxa from the data.

Incongruence length difference (ILD) tests have been criticized, suggesting that they can falsely identify data partitions as incongruent (e.g. Yoder et al., 2001; Barker and Lutzoni, 2002), though others have questioned this interpretation and suggested that despite limitations it is the best understood alternative (Hipp et al., 2004; Planet, 2006). There is some consensus however that significant ILD test *p*-values should not be taken as a conclusive demonstration that combining the independent data partitions will produce misleading phylogenies. Therefore, for the preliminary dataset we also calculated Partitioned Bremer Support (PBS; Baker and DeSalle, 1997) to test inference from the ILD test using an independent method. PBS infers the relative contribution of each data partition for each node and detects conflict amongst data partitions. Positive values indicate support while negative values suggest conflict. PBS analyses were performed using 100 random addition replicates and the TBR branch swapping algorithm using the programs TreeRot v.3 (Sorenson and Franzosa, 2007) and PAUP* v. 4.0b10 (Swofford, 2002).

Bayesian analysis was implemented using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), using the above mentioned partitions and corresponding substitution models. For all schemes we used the following settings: nchains = 4, one cold and three heated chains. The number of steps was set between 1,000,000 and 10,000,000 depending on the complexity of the model, sampfreq = between 100 and 1000 and burnin = between 250 and 2500 steps. Convergence was assessed using the program Tracer v.1.5

Table 1
Partition schemes and evolutionary models.

	Partition	Description	Model	Gaps included/excluded	LnL
A	1 Partition	The whole sequence	GTR+I+G	y/y	−25967.957/−25471.693
B	3 Partition[a]	Nuclear-coding	HKY+I+G	y/y	−25104.768/24604.584
		Non-coding	GTR+G		
		Mitochondrial	GTR+I+G		
C	3 Partitions	Amino acids	Jones model	n/y	−/−19274.279
		Non-coding	GTR+G		
		16s	GTR+I+G		
D	8 Partition	Nuclear-coding and cytb	Partitioned (site specific) rate model	y/n	−25310.924/−
		Non-coding	GTR+G		
		16s	GTR+I+G		
E	9 Partition	Each gene under one model	See Table 2	y/y	−24971.106/24487.741

LAC was divided between exons and introns and a model was applied to each region. Coding genes includes vWF, IRBP, MC1-R and LAC exons and noncoding genes includes HEXB, CAMK, ACT and LAC introns. Gaps were analyzed as binary characters.
[a] These partitions were also evaluated independently to identify how each data type influence the phylogenetic hypothesis.

Table 2
Gene characteristics and genbank accession numbers.

Gene	HEXB	CAMK	ACT	IRBP	LAC	vWF	MCR-1	Cytb	16s
Type	Intron	Intron	Intron	Exon	Exon/Intron	Exon	Exon	mtDNA	mtDNA
Model of evolution (AIC criterion)	HKY+G	GTR+G	HKY+G	GTR+G	GTR+G	HKY+I	GTR	GTR+I+G	GTR+I+G
Length after alignment	2315[a]	2043	753	1080	1034	1218	936	1140	511
Species	Genbank accession numbers								
Lagenorhynchus acutus	KM101436	KM101448	*AF140825*	KM101407	KM101416	KM101425	*FJ773301*	*EF093022*	KM101399
Lagenorhynchus albirostris	KM101441	KM101449	*EF092978*	KM101408	KM101417	KM101426	*FJ773302*	*EF093018*	*AJ554061*
Lagenorhynchus australis	KM101440	KM101450	*EU121212*	KM101409	KM101418	KM101427	*NS*	*EF093035*	KM101400
Lagenorhynchus cruciger	KM101443	KM101451	*NS*	KM101410	KM101419	KM101428	*NS*	*AF084068*	KM101401
Lagenorhynchus obscurus	*AF140850*	*AF140819*	*AF140832*	*AF304078*	*AF228410*	KM101429	*FJ773299*	*EF093055*	*LOU13114*
Lagenorhynchus obliquidens	*AF140841*	KM101452	*AF140829*	KM101411	KM101420	KM101430	*FJ773300*	*EF093041*	KM101403-KM101404
Cephalorhynchus commersonii	KM101442	KM101453	*EU121213*	KM101412	KM101421	KM101431	*FJ773298*	*AF084073*	KM101402
Delphinus delphis	KM101447	KM101454	*EU121206*	*AF304077*	*AF304088*	KM101432	*FJ773288*	*EF093031*	*DDU13106*
Tursiops truncatus	KM101444	KM101455	*EF092989*	KM101413	KM101422	KM101433	*FJ773290*	*EF093029*	*AY770538*
Stenella coeruleoalba	KM101446	KM101456	KM101406	KM101414	KM101423	KM101434	*FJ773289*	*AF084081*	*AJ010816*
Phocoena phocoena	KM101439	KM101460	*EU121226*	*AF231340*	*AJ007811*	*AF061060*	*FJ773305*	*EF093010*	*PPU13121*
Delphinaterus leucas	KM101445	KM101457	*EU121227*	*AF231341*	*AF228409*	*AF231344*	*FJ773307*	*U72037*	*Z18639*
Hyperoodon ampullatus	KM101437	KM101458	*AY579499*	KM101415	KM101424	KM101435	*NS*	*X92539*	*AJ554056*
Physeter catodon	KM101438	KM101459	KM101405	*U50818*	*AF304098*	*AF108834*	*FJ773311*	*X75589*	*NC002503.2*

[a] Includes an insertion of 229 bp found only in *H. ampullatus*.

(Rambaut and Drummond, 2007), and also by examining the potential scale reduction factor (PSRF) values and standard deviation of split frequencies.

Maximum Likelihood (ML) analysis was performed using the PhyML v3.0 software (Guindon and Gascuel, 2003), excluding and including out-groups to avoid long branch attractions. The best substitution model was determined using MrModeltest (Nylander, 2004). Given that PhyML does not handle partitioned data, the 11,030 bp were analyzed as one single partition, and gaps were evaluated as missing data. Tree improvement was assessed using both Subtree Pruning and Regrafting topological moves (SPR), and simultaneous Nearest Neighbor Interchange (NNI) algorithms. Nonparametric bootstraps were assessed using 1000 replicates.

2.4. Supermatrix analyses

In order to compare the effect of taxon sampling in our phylogenetic analyses, and have a better representation of the distribution ranges of marine dolphins, we selected thirty-three species plus *Megaptera novaengliae* as an outgroup and built a supermatrix phylogeny using twenty-seven genes (including those amplified in this study). Data for these genes were downloaded from the Genbank database (mainly from McGowen, 2011).

A total of 16,815 characters were included in the analyses, and analyzed using different partition schemes. Most of them gave similar topologies, therefore we present the analysis obtained with data partitioned among protein genes, non-coding genes, and gaps, for which the best tree likelihood was obtained. Gaps were coded as a binary (0–1) state using the Fastgap Program v1.2 (Borchsenius, 2009), and the model for each partition was selected using Mr.Modeltest v2.2 (Nylander, 2004; see Table S1). The phylogenetic trees were constructed using the software MrBayes (Huelsenbeck and Ronquist, 2001) in the CIPRES Science gateways portal (http://www.phylo.org/portal2/) (Miller et al., 2010) and the Bayesian analyses were performed using four independent runs with 30 million generations, a burn-in of 25% using four chains (3 hot and one cold), and sampling every 1000 generations. Convergence for all parameters was tested as described for the preliminary phylogenetic analysis above.

Various studies have shown ambiguities for the placement of basal species within delphinid phylogenies when *O. orca* is included (e.g. among *L. acutis*, *L. albirostris* and *O. orca*; Steeman et al., 2009; McGowen et al., 2009; McGowen, 2011). We therefore repeat the above analyses including *O. orca* to test its influence our biogeographic interpretations. We expected little influence or resolution, given the world-wide distribution of this species.

2.5. Divergence time estimates

To calculate the divergence times in the preliminary phylogenetic tree we applied two different Bayesian approaches. First, the programs PAML/Multidivtime (Yang, 1997), which do not

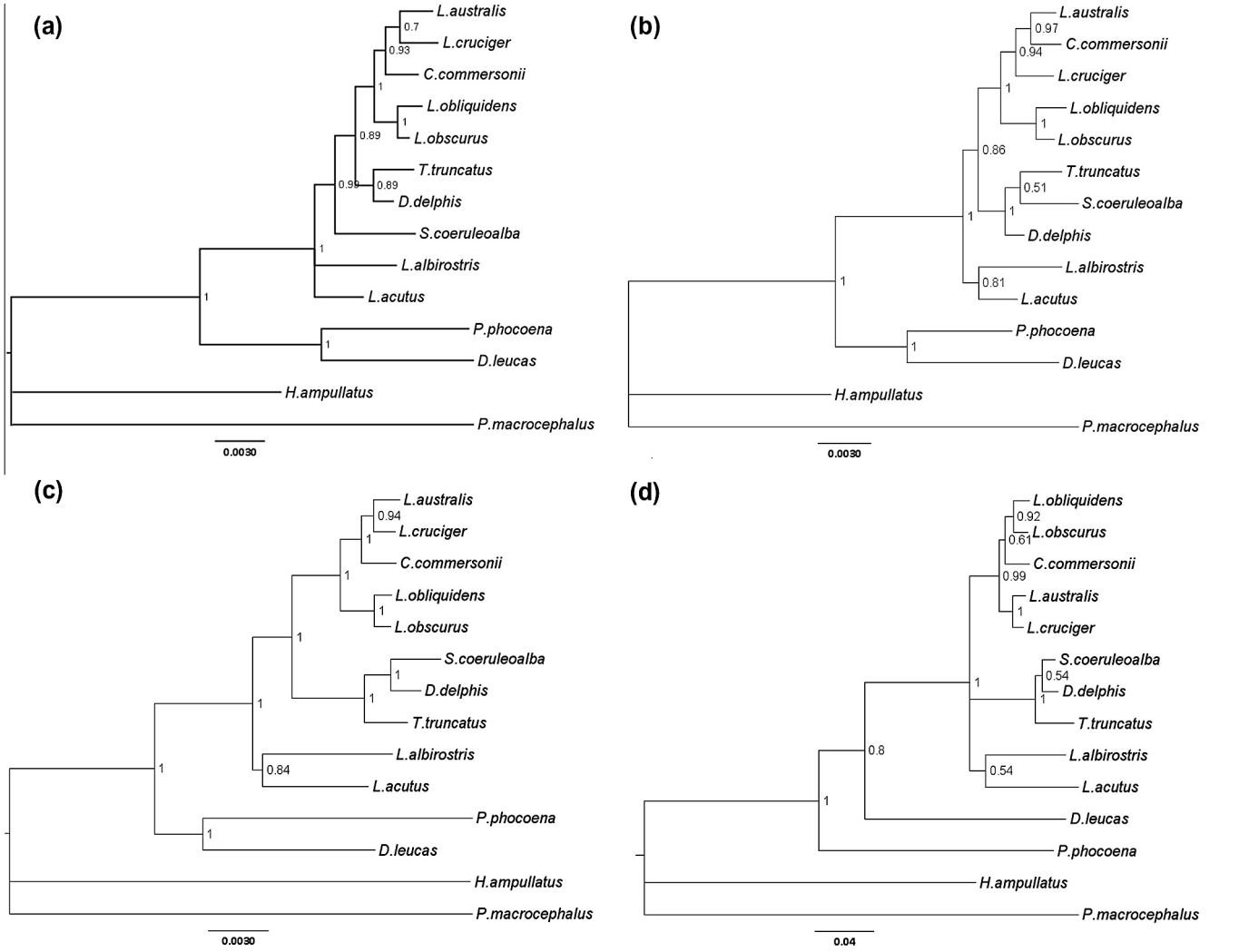

Fig. 2. (a) Bayesian tree topology using nuclear genes (IRBP, vWF, MRC1, LAC exons). (b) Bayesian tree topology using nuclear genes excluding IRBP. (c) Bayesian tree topology using non-coding genes (HEXB, CAMK, ACT, LAC introns). (d) Bayesian tree topology using mitochondrial genes (Cytb and 16s). Gaps were treated as missing data.

assume a molecular clock, were used for partitioned data, following the protocols described in Crawford (2008) and Rutschmann (2005). Secondly, we performed the analyses using BEAST v1.5.3 (Drummond and Rambaut, 2007) assuming a relaxed clock: uncorrelated Log-normal, which accounts for lineage-specific rate heterogeneity (Drummond et al., 2006). For the PAML/Multidivtime analyses we used a Bayesian consensus tree as the initial best topology. The data were partitioned by gene, and topologies for each gene were used for parameter estimation using the program baseml (which is part of the PAML package), with the F84+G substitution model (Felsenstein and Churchill, 1996). The program estbranches was then used to assess the ML estimates of the branch lengths and their variance–covariance matrix.

Posterior distributions of substitution rates and divergence times were calculated using the program Multidivtime, with the following settings: rttm = 2.35 rttmsd = 1.0, and rtrate = 0.0045. The MCMC analysis was run using 100,000 generations, retaining every 1000 samples and discarding the 10% burn-in. The analysis was repeated twice to ensure convergence. For calibration, the lower bound for the node formed by *P. phocoena–D. leucas* was set to 1.1 units, while the lower bound for the node formed by Delphinoidea was set to 2.35 units and the upper bound was set to 2.7 units (One unit is equivalent to 10 million years). The first calibration point (Phocoenidae–Monodontidae node) was based on the earliest fossil record of Phocoenidae (*Salumiphocaena stocktoni*) from the late Miocene (~11 Ma; Barnes, 1985) and the internal node for Delphinoidea was calibrated using the oldest fossil of the Odontoceti, Delphinidae (*Kentriodon* sp. of Ichishima et al., 1995), as recommended in Steeman et al. (2009).

For the Bayesian analysis using the program BEAST v1.5.3 (Drummond and Rambaut, 2007) data were analyzed as a single partition and also divided into three (see Table 1) and four partitions (nuclear-coding, non-coding, cytb and 16s) and a Yule model was used as the tree prior. We used the same two calibration points as above. For the first (Phocoenidae–Monodontidae node) we set a normal distribution centered at 11 Ma with a standard deviation of 1.0 (note that the choice of standard deviation is not critical since this is an unbounded prior). For the internal node (Delphinoidea) we used a normal distribution centered at 23.5 Ma (Steeman et al., 2009) with a standard deviation of 1.0. MCMC chain length was set to 10,000,000 and 50,000,000 with 10% burn-in. Four runs were performed for all analyses, and log files and tree files from the different runs were combined using LogCombiner v1.5.3 (Drummond and Rambaut, 2007). All parameters were analyzed for convergence using the program Tracer v1.5. The best tree was identified using TreeAnotator v. 1.5.3 (Drummond and Rambaut, 2007) and analyzed and edited using FigTree v. 1.3.1 (Rambaut, 2006).

Supermatrix phylogenies were calibrated using Bayesian analyses in the program BEAST (Drummond and Rambaut, 2007) with the same criteria as above, using 50 million generations and a non-partitioned analysis. Only BEAST was used because node dates were essentially the same for BEAST and PAML/Multidivtime in the previous tree (see results). An uncorrelated relaxed clock with a lognormal and an exponential prior distribution were tested, however data that are presented include only those from the lognormal distribution, given that after 50 million generations the exponential distribution did not reach convergence for most parameters. The program was run in the CIPRES Science gateways portal (http://www.phylo.org/portal2/).

2.6. Dispersal vicariance analyses and ancestral area reconstruction (using RASP)

Cetacean distributions are often difficult to establish (due to infrequent sightings, temporary range changes associated with climate, etc.; e.g., Ross and Leatherwood, 1994). Given that the distribution of most delphinids appears to be influenced by temperature (making southern and northern range boundaries difficult to establish), we used two main criteria to infer ancestral distribution areas. First, we set eight non-overlapping areas of distribution taking into account the geographical boundaries for tropical and temperate regions: A: northern North Atlantic/Northern Hemisphere above 50°N, B: central North Atlantic, C and D: tropical/temperate Atlantic, E: Southern Oceans, F: South Pacific, G: tropical/temperate Pacific, and H: North Pacific (see Fig. 1 for current species distributions). The North Pacific region and the tropical/temperate Pacific were initially divided into two areas, but since results were similar, we combined each of them into single regions.

Second we defined the distribution of each species in agreement with their main ranges of distribution and excluding extreme North and South ranges, since distribution outside the main ranges can be transient (e.g. Ross and Leatherwood, 1994). We performed different runs, first coding the species as present in an area when they occupied more than 50% of that area, and second coding the species as present even if they occupied only a small portion of that area. For some species such as *Phocoena phocoena*, *Delphinapterus leucas* and *Pseudorca crassidens* the extreme ranges in distribution were both included and then excluded in separate runs, considering that this expansion in range could be recent or transient. *Delphinus Delphis* distribution was coded as suggested by Jefferson et al. (2009). Results were similar in all runs, and therefore we present those obtained using the main distribution ranges for all species. Taxa were coded in agreement with their actual distribution following the distribution maps and distribution remarks produced by the IUCN Cetacean Specialist Group at http://www.cms.int/reports/.

To infer the main ancestral areas of distribution, we used the supermatrix tree and the program RASP (Reconstruct Ancestral State in Phylogenies) v. 2.1, which integrates five different approaches for reconstructing ancestral areas: S-DIVA analyses (Statistical Dispersal Vicariance; Yu et al., 2010), Bayesian Binary MCMC analyses (BBM; Yu et al., 2011), the Dispersal–Extinction–Cladogenesis model based upon a Maximum Likelihood approach (which allows inferences of the ancestral distribution of species taking into account dispersal), local extinction and cladogenesis (DEC model, Ree and Smith, 2008), the Maximum Parsimony Method (MP; Bremer, 1995; Hausdorf, 1998), and the Island Bayesian Analysis (IBA; which take into account complex dispersal models not assumed by the other approaches; Sanmartín et al., 2008). Discussion about the assumptions, advantages and disadvantages of these approaches can be found in Kodandaramaiah (2010) and Sanmartín (2007).

To avoid the well-recognized sensitivity of DIVA to the absence of sister taxa, which could cause the root node to exhibit a widespread distribution in several if not all ancestral areas (Ronquist, 1997), we included several outgroups in our supermatrix analyses to help infer the ancestral distribution areas of the ingroup. In S-DIVA optimization was performed using 50,000 trees generated by BEAST v.1.5 (Drummond and Rambaut, 2007) excluding the first 25,000 from the analyses. The consensus tree was calculated by the program and used as the tree for the BBM analyses. The BBM and the IBA analyses were performed using 10 chains, 5 million generations, sampling every 100 generations and a burn-in of 25%. The F81 model was chosen in order to allow different rate variation among ancestral areas, and distributions were set to null and outgroup and compared. The DEC Model was run using the consensus tree with branch lengths and divergence times generated by BEAST. For all approaches we constrained the analysis allowing the maximum areas occupied for the ancestor (Maxareas option) to be three or four.

3. Results

3.1. Lagenorhynchus phylogenies

3.1.1. Homogeneity test

The partition homogeneity test performed for both the preliminary analyses and the supermatrix showed that there was no conflict amongst the different data partitions. The null hypothesis of homogeneity between coding, non-coding and mitochondrial genes could not be rejected ($p = 0.442$). In the preliminary analyses a pair-wise comparison amongst genes showed that the IRBP gene conflicted with three other genes. Therefore Bayesian and ML analyses were performed both excluding and including the IRBP gene to evaluate the effect on topologies, and some differences were evident (Fig. 2). For the preliminary data we also applied the PBS test and found consistent results. The locus that caused the most conflict (negative PBS) with the other data for a given node was IRBP (5 out of 8 nodes). PBS also indicated that most of the support came from Cytb, while RAG and ACT neither supported nor rejected any of the nodes (PBS = 0). All Genbank accession numbers for sequences generated during this study are provided in Tables 2 and S1, and trees are available at TreeBase as submission 16101.

3.1.2. Bayesian and ML analysis

Our Bayesian and ML analyses generated different topologies for subsets of coding, non-coding nuclear regions and mitochondrial DNA genes. All three types of data supported a paraphyletic group formed by *L. obscurus*, *L. obliquidens*, *C. commersonii*, *L. australis* and *L. cruciger* differing only in the placement of *C. commersonii*. *L. acutus* and *L. albirostris*. These taxa were only placed outside the clade formed by the other delphinids in the nuclear coding and non-coding gene phylogenies. Differences among the three types of data were also found in the placement of *D. delphis*, *T. truncatus* and *S. coeruleoalba* (Fig. 2).

Nuclear-coding genes did not support the placement of *L. acutus* and *L. albirostris* as sister taxa when using a simple model of evolution (HKY+I+G). However, when these genes were analyzed by partitioning the data among first, second and third nucleotide positions, these two species formed a monophyletic clade, but with a low clade credibility support of 0.54. When the IRBP gene (putatively under directional selection) was excluded from the analysis, the topology, clade credibility support and bootstrap values were slightly different (Fig. 2b), providing increased support for the *L. acutus*, *L. albirostris* lineage. Our preliminary total-evidence tree using ML and Bayesian analyses resolved the phylogenetic relationships amongst all species in the '*Lagenorynchus*' phylogeny

and both partitioned and un-partitioned analyses yielded the same topologies, suggesting paraphyly of *Lagenorhynchus* species (i.e. *L. obscurus*, *L. obliquidens–L. australis* and *L. cruciger*) and monophyly of *L. acutus–L. albirostris*, similar to that suggested by the different partition schemes (see above). However, the support for *L. acutus–L. albirostris* monophyly was variable and dependent on gap exclusion/inclusion. For example, clade credibility support was between 0.95 and 1.0 in all analyses when gaps were included as binary characters (Table 3). These values decreased to between 0.56 and 0.88 in simple partitioned analyses and when gaps were treated as missing data. The ML analysis codifying gaps as missing data also showed variation in support values for this node, depending upon which test was used. The aLRT Sh-like branch support test gave values that were higher (0.84) than the aLRT Chi-square-based branch support test (0.76). The non-parametric bootstrap analysis for the same node was 59%, but when the IRBP gene was excluded, the value was higher. Fig. 3a shows the topology found using the Bayesian total evidence analysis together with the node dating inference from the program BEAST.

The supermatrix Bayesian analyses (Fig. 3b) recovered the same global relationships among members of the genus as obtained with the total evidence preliminary analyses, but with higher support for the monophyly of *L. acutus–L. albirostris* (1.0 posterior probabilities in the partitioned analyses). The inclusion of two species of *Lagenodelphis* and two species of *Cephalorynchus* in the analyses helped to corroborate the paraphyly of the genus as suggested by our initial analyses and also by McGowen (2011). The topologies obtained using BEAST with un-partitioned analyses (gaps treated as missing data) were similar to the MrBayes partitioned analyses, although posterior probabilities differed slightly between the two analyses for some nodes. Fig. 3b shows the phylogenetic tree constructed using the BEAST program with Posterior probability values for both analyses (partitioned and unpartitioned). The inclusion of *O. orca* (Fig. S1) disrupted the relationship between *L. acutus* and *L. albirostris* as seen earlier (Steeman et al., 2009; McGowen et al., 2009; McGowen, 2011), but did not alter the topology in other respects.

3.2. Divergence times and ancestral area reconstruction

Estimates of node ages obtained with PAML/Multidivtime (Yang, 1997; Thorne and Kishino, 2002) were similar to, and fell within the confidence intervals of those obtained using BEAST (Drummond and Rambaut, 2007). The standard deviation of the uncorrelated lognormal relaxed clock (ucld.stdev) calculated in BEAST was 0.4, indicating that our data are clock-like. These confidence intervals were also similar for the shared nodes between the preliminary tree and the supermatrix tree, and therefore discussion about divergence times in the context of biogeographic history will be based on the supermatrix analysis (Fig. 3 and Table 3).

Our BBM, IBA, and MP analyses gave similar results for most nodes, though results from S-DIVA and the DEC model differed in some respects (see Table 3). These analyses suggest that dispersal followed by a few vicariant events was the main force driving the speciation of marine dolphins. Extinction events are suggested to have little influence in the speciation of this group, with only one extinction event detected by the Island Bayesian analyses, and a different one in the DEC model (nodes 22 and 24; Table 3). Here

Table 3
Biogeographic patterns indicated by different analytical models. Node references are provided in Fig. 4a, bold letters refer to vicariance events and italics to extinctions. Locations with similar probabilities are separated by a forward slash. 'NR' indicates multiple areas with small probabilities (less than 10%), and so no resolution. The remaining entries suggest dispersal events. Model acronyms are defined in the text in the methods section.

	Nodes	Dates	95% HPD	Ancestral distribution				
				BBM	S-DIVA	MP	IBA	DEC
Delphininae	1	0.95	0.51–1.44	CDG	D	CDG	CDG	D
	2	1.79	1.17–2.43	CDG	D	CDG	CDG	D
	3	2.77	1.96–3.61	CDFG/BCDG	D	CDFG	CDFG	D
	4	2.96	2.1–3.84	CDFG	D	CDFG	CDFG	D
	5	3.73	2.7–4.76	CDFG	D	CDFG	CDFG	D
	6	4.52	3.32–5.74	CDFG	D	CDFG	CDFG	D
	7	5.24	3.93–6.68	CDFG	D	CDFG	CDFG	D
Globicephalinae	8	1.22	0.63–1.87	CDFG	**NR**	CDFG	CDFG	CDFG
	9	2.99	1.96–4.04	CDFG	D	CDFG	CDFG	C/D/G
	10	3.36	2.35–4.45	CDFG	D/DF	CDFG	CDFG	C/D/G
	11	4.33	3.12–5.6	CDFG	D	CDFG	CDFG	DC/G
	12	4.97	3.65–6.37	CDFG	D	CDFG	CDFG	NR
	13	7.3	5.54–9.14	CDFG	D	CDFG	CDFG	D/C
Delphininae + Globicephalinae	14	9.05	7.08–11.18	CDFG	D	CDFG	CDFG	C/D/G
Lissodelphininae (sensu LeDuc et al., 1999)	15	1.79	1.02–2.63	EF/E	E	EF	EF	E/EF
	16	3	1.95–4.15	EF	EF	EF	EF	**EF**
	17	3.5	2.43–4.68	EF/E	E	EF	EF	E/F
	18	2.56	1.43–3.73	**EF**	**EFH/EH**	**EF**	**EF**	**EFH**
	19	4.46	3.25–5.8	EF	E	EF	EF	E
	20	2.16	1.14–3.3	**EF**	**EFH/EH**	**EF**	**EF**	**EFH**
	21	5.31	3.91–6.84	EF	E	EF	EF	EFH
Delphininae + Globicephalinae + Lissodelphininae	22	10.29	8.06–12.59	NR	**DE**	F	***AB***	**NR**
L. acutus–L. albirostris	23	11.49	8.86–14.21	AB	A/B	AB	AB	A/AB
Delphinidae	24	12.46	9.83–15.38	**AB**	**AE/ADE/AD**	**ABF**	AB	***NR***
	25	4.38	2.8–6.12	AH	ABH/AH/H	ABH	ABH	ABH
	26	5.92	3.96–8.01	A	A	A	A	A
	27	12.95	11.13–14.82	AH/A	AH/A	ABH	AB	ABH
Delphinoidea	28	22.18	20.11–24.18	NR	NR	ABFH	AB	NR
	29	16.64	9.49–23.24	NR	NR	NR	AB	NR
	30	34.57	20.11–24.18	NR	**AB/B/A**	NR	AB	NR
	31	45.24		NR	B/A	NR	NR	ABCDFGH
	32	59.96		NR	NR	NR	NR	NR

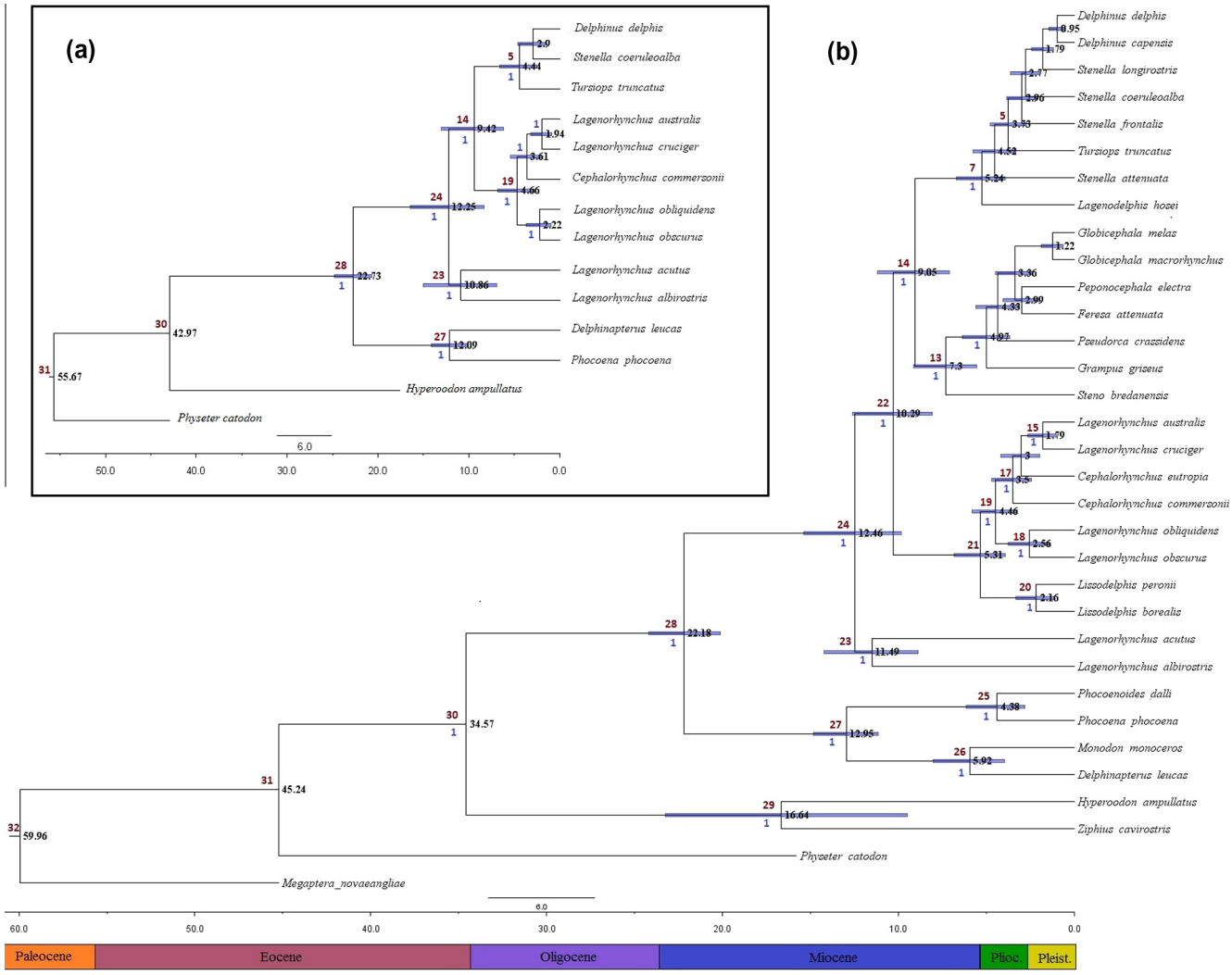

Fig. 3. (a) Pleliminary multigenic phylogeny and divergence times using Bayesian analysis with the program Beast. (b) Supermatix tree and divergence times based on analysis in BEAST. Node numbers are marked above the line (and match Table 3) and divergence times inside nodes. Date estimates are in Millions of years.

we focus on IBA, given that this approach incorporates dispersal as an important force in its calculations, unlike other methods which gave more weight to vicariance and extinction, and contrast these results with alternative possible scenarios (compared in Table 3). Fig. 4a, shows the area distribution obtained with IBA in a calibrated phylogeny.

According to our IBA and MP analyses, Delphinoidea ancestors were distributed mainly in the North Atlantic Ocean around 22.18 Ma (95% HPD 20.11–24.18 Ma; Fig. 4b, Table 3). Phocoenidae and Delphinidae probably evolved around 12.95 Ma (95% HPD 11.13–14.82 Ma) and 12.46 (95% HPD 9.83–15.38 Ma) respectively from ancestral populations inhabiting the North Atlantic during the early Miocene (Fig. 3b). *L. acutus* and *L. albirostris* ancestors inhabited this area around 11.49 Ma (95% HPD 8.86–14.21 Ma) and probably emerged from a different lineage than those giving rise to other delphinids. Dispersal from the North Atlantic toward the tropical and temperate Atlantic/Pacific and Southern hemisphere probably took place around 10.29 Ma (95% HPD 8.06–12.59 Ma), followed by the division of two dolphin lineages, one giving rise to the Delphininae and Globicephalinae ancestors in the tropical and temperate Atlantic/Pacific around 9.05 Ma (95% HPD 7.08–11.18 Ma), and the other to the Lissodelphinidae ancestors in the Southern hemisphere-South Atlantic around 5.31 Ma (95% HPD 3.91–6.84 Ma). Around the late Pliocene – early Pleistocene, vicariant events divided *L. obliquidens* from *L. obscurus* (2.56 Ma, 95% HPD 1.43–3.73 Ma) and *L. borealis* from *L. peronii* (2.16 Ma, 95% HPD 1.14–3.30 Ma) in southern and northern populations, respectively. The inclusion of *O. orca* did not change any of these interpretations (Fig. S1).

4. Discussion

4.1. Phylogenetic relationships

As has been discussed by various authors, a comparison of single gene phylogenies (from nuclear or mitochondrial genes) can reveal markedly different phylogenetic histories, mainly due to differences in evolutionary rates, inheritance pathways, selection pressures, responses to evolutionary processes, hybridization between lineages, homoplasy or lineage sorting (e.g. Palumbi and Baker, 1994; Moore, 1995; Shaw, 2002; Reyes et al., 2004; Ballard and Rand, 2005; Heath et al., 2008; Nabhan and Sarkar, 2011). While the use of longer sequences and an extensive set of characters can minimize the problem of taxon sampling and improve phylogenetic inference (Rosenberg and Kumar, 2001), care should be taken when inferring phylogenies with a considerable amount of missing data for some of the taxa of interest, which can decrease accuracy and recover uncertain relationships (e.g.,

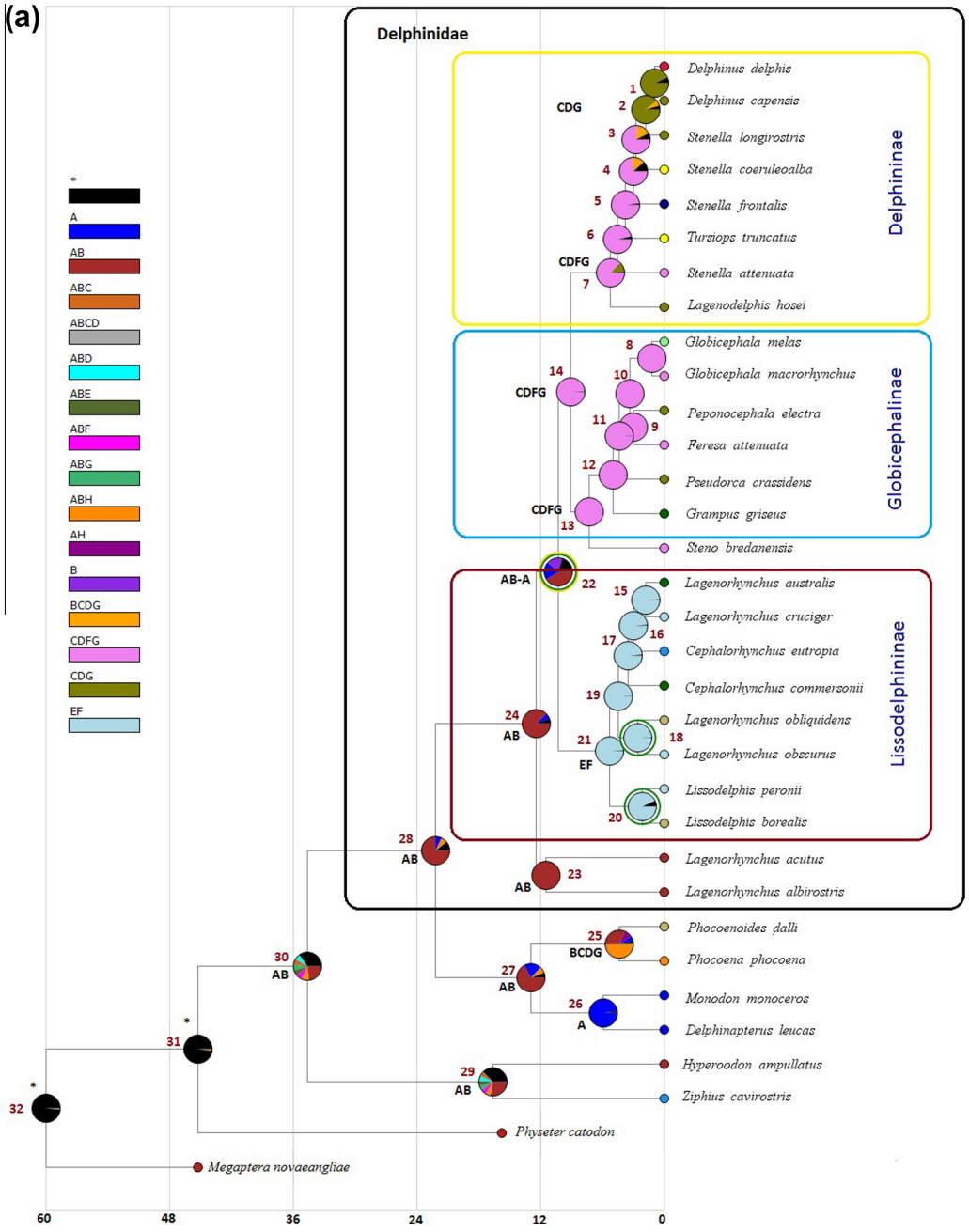

Fig. 4. (a) Linearized tree based in Bayesian trees from Beast (see Section 2) showing the estimated biogeography based on the Island Bayesian Analysis. The proportional support for different areas at a given node is represented by a pie chart (colour code provided for online version), and the corresponding area is indicated by the reference letters shown in black. Only the proportions with the highest probabilities are shown in association with an area letter (or letters). (b) Proposal for ancestral areas and migration routes for ancestral populations of dolphins included in this study. Area letters correspond with those given in Table 3 and Fig. 4a.

Huelsenbeck, 1991; Wilkinson, 1995; Kearney, 2002; Hartmann and Vision, 2008; Lemmon et al., 2009).

Consistent with this, our analyses showed that nuclear coding genes, mitochondrial genes and non-coding genes generated different topologies (Fig. 2). We also found an incidence of a gene (IRBP), possibly under directional selection (see Springer et al., 1997; Jansa et al., 2006), affecting the topology. Removing genes under selection from the analysis can increase the strength for some nodes (but see Jansa et al., 2006), as it did in this case (see *L. albirostris* and *L. acutus* discussion below). In addition, the inclusion of gaps in the phylogenetic analyses greatly improved the support for some difficult nodes in our phylogeny (i.e. *L. acutus* and *L. albirostris*), as has been reported for other groups (e.g., Graham et al., 2000; Bapteste and Philippe, 2002; Kawakita et al., 2003).

To help avoid uncertainty in our phylogenetic analyses of the genus *Lagenorhynchus* we initially used a multigene phylogeny of nine genes (11,030 characters) with few missing data. We then compared inference from that phylogeny with a supermatrix phylogeny (16,815 characters) using 33 odontocetes species plus one outgroup taxa. Our data for both phylogenies were concordant (Fig. 3) and suggested that taxon sampling did not affect the accuracy of the phylogenetic relationships recovered for this group. Our total evidence phylogenies provided sufficient congruence to increase the strength of inference about specific nodes, and to both support and refine earlier assessments (see below; c.f. Cipriano, 1997; LeDuc et al., 1999; Harlin-Cognato and Honeycutt, 2006; Steeman et al., 2009; McGowen et al., 2009; Xiong et al., 2009).

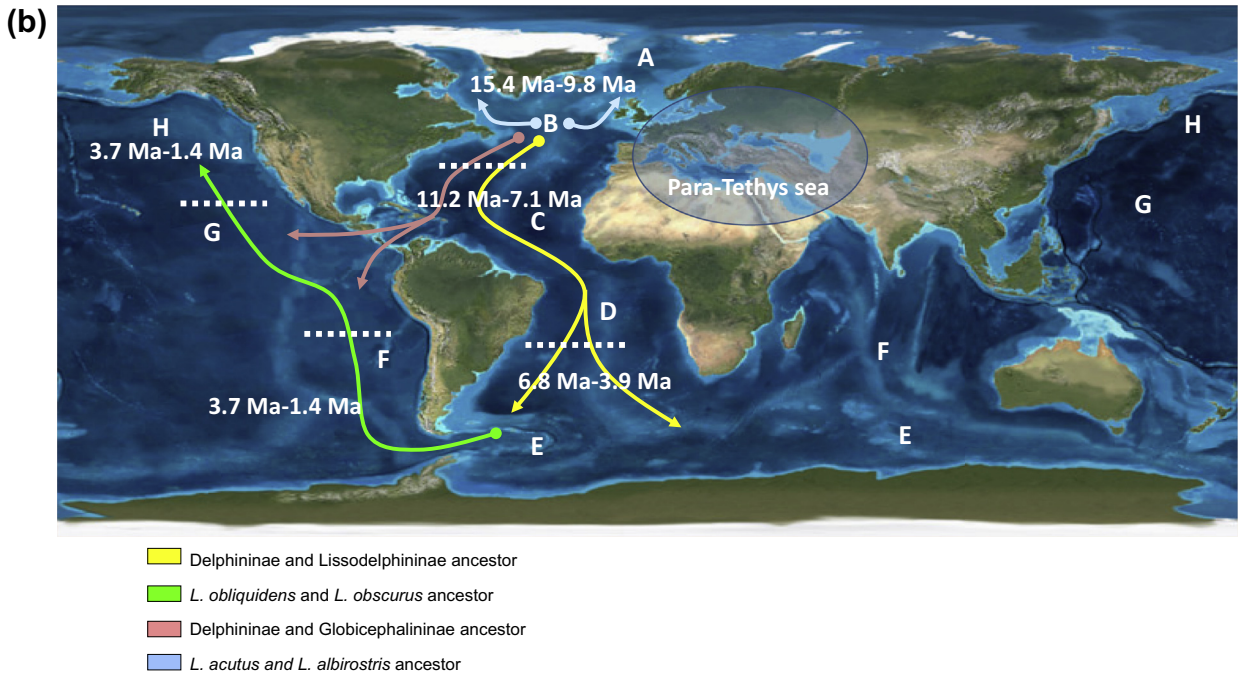

Fig. 4 (*continued*)

4.2. The current genus Lagenorhynchus

Our Bayesian analyses for both phylogenies and the ML analyses for the preliminary phylogeny, fully resolved the relationships among all species included in this study. Both the preliminary and the supermatrix phylogenies agreed with the placement of *L. australis*, *L. cruciger*, *L. obscurus* and *L. obliquidens* within the subfamily Lissodelphininae (sensu LeDuc et al., 1999), as suggested by earlier studies (e.g., Pichler et al., 2001; Harlin-Cognato and Honeycutt, 2006; May-Collado and Agnarsson, 2006; McGowen et al., 2009). However, unlike Harlin-Cognato and Honeycutt (2006), our analyses (Fig. 3a and b) support the placement of *Cephalorhynchus* in a monophyletic group with *L. cruciger* and *L. australis* with a high Bayesian posterior probability in both multigenic phylogenies.

Several studies including ours support the basal position of *L. acutus* and/or *L. albirostris* in the Delphinidae phylogeny (LeDuc et al., 1999; Price et al., 2005; May-Collado and Agnarsson, 2006; Xiong et al., 2009; Steeman et al., 2009; McGowen et al., 2009; Vilstrup et al., 2011) and we further propose the monophyletic origin of these two species. This node was well supported in most partitioned schemes (where gaps were included) with Bayesian posterior probabilities between 0.95 and 1.0. Support for this monophyletic lineage would merit their placement into a new subfamily, Lagenorhynchinae. Given the time of divergence between *L. acutus* and *L. albirostris*, they may further merit placing into two different genera, as suggested by LeDuc et al. (1999). The inclusion of *O. orca* disrupted the monophyly of these two species (Fig. S1), as seen previously, possibly due to missing taxa previously found to group with *O. orca*, such as *Orcaella* sp. (e.g. McGowen, 2011).

Our analyses also suggest a close relationship between *L. cruciger* and *L. australis* and a common ancestry between these two species and *C. commersonii* and *C. eutropia* (see biogeographic analysis below). May-Collado and Agnarsson (2006) suggested including *L. cruciger* and *L. australis* as members of the genus *Cephalorhynchus*, while LeDuc et al. (1999) proposed they be placed in the genus *Sagmatias*. McGowen (2011) included the four *Cephalorhynchus* species (*C. commersonii*, *C. heavisidii*, *C. eutropia* and *C. hectori*) and found support for this relationship. The final two species in the current genus *Lagenorhynchus*, *L. obliquidens* and *L. obscurus* group together and should remain congeneric, but in a separate genus from the other species currently classified in the genus *Lagenorhynchus*. These two species are included in the proposed genus *Sagmatias* by McGowen (2011) after LeDuc et al. (1999).

4.3. Evolutionary history of dolphins and biogeographic interpretation

We discuss biogeographic inference as supported concurrently by 5 different models (see Section 2), but note that stochastic events can be hard to capture in these analyses. Furthermore, all estimated dates are dependent on the accuracy of the calibration points, and accurate only within confidence limits and where different approaches agree. Our analyses suggest that the common ancestor of the family Delphinidae probably originated in the North Atlantic before or during the middle Miocene (Fig. 3). This origin is especially evident from the fact that two North Atlantic lineages split from the most basal node in the Delphinidae lineage. After a splitting event around 12.46 Ma (95% HPD 9.83–15.38 Ma), the common ancestor gave rise to two highly divergent lineages, one leading to the common ancestor of *L. acutus* and *L. albirostris* (11.49 Ma, 95% HPD 8.86–14.21 Ma), and the second to the common ancestor of the subfamilies Delphininae, Globicephalinae and Lissodelphininae (10.29 Ma, 95% HPD 8.06–12.59 Ma). The Lissodelphininae common ancestor may have evolved later in the Southern hemisphere during the late Miocene early Pliocene 5.31 Ma (95% HPD 3.91–6.84 Ma). This lineage probably originated from ancestral populations that migrated toward the southern hemisphere after the middle Miocene.

Dispersal events are the main force driving the evolution of delphinids according to these analyses (though there is some indication of vicariance and extinctions early on at nodes 22 and 24, and later during the radiation of the Lissodelphininae; see Table 3). The split of the ancestral lineages could be related to paleoclimatic and paleoceanographic changes in the Miocene seas, such as the abrupt cooling that occurred in middle and high latitudes after the Middle Miocene Climatic optimum – MMCO – 17–15 Ma (Zachos et al., 2001) and the "biogenic bloom" (Hermoyian and

Owen, 2001; Diester-Haass et al., 2005), which as suggested by other authors, could have influenced the radiation and speciation of cetaceans (Gingerich, 2005; Berger, 2007; Steeman et al., 2009; Marx and Uhen, 2010). However, our findings are in agreement with paleontological data showing that most delphinid fossils are of late Miocene origin or younger (Fordyce and Barnes, 1994).

4.4. L. acutus and L. albirostris

We propose that *L. acutus* and *L. albirostris* diverged early in the evolutionary history of marine dolphins (see above). The substantial differentiation between these two species is surprising, given their morphological similarities, but this is a consistent result of the molecular studies. Their persistence in sympatry in the North Atlantic, and apparent origin there suggests the possibility of an early divergence based on habitat specialization (*L. acutus* prefers offshore habitats, whilst *L. albirostris* is largely restricted to shelf areas; Evans and Smeenk, 2008a, 2008b), though we have no evidence that the specializations seen today for these species also existed ~10 Ma. More recent events suggest the possibility of this mechanism driving speciation or incipient speciation in other delphinid taxa (see Hoelzel et al., 1998; Natoli et al., 2005, 2006; Moura et al., 2013). Unlike other marine dolphin lineages, this lineage did not undergo further speciation after the Miocene. Therefore habitat restriction promoted by cooling events, likely a major driver for some other delphinid speciation events and for other species in the Northern Hemisphere (see Hewitt, 2004; Walteri et al., 2004; Carstens and Knowles, 2007), may not have been as important in this case.

4.5. Subfamily Lissodelphininae (sensu LeDuc et al., 1999)

Our data strongly suggest a South Atlantic/Southern Ocean origin for members of the subfamily *Lissodelphininae*. The subfamily probably evolved in this region in the early Pliocene (5.31 Ma, 95% HPD 3.91–6.84) after trans-equatorial dispersal of an ancestral population during the middle and/or late Miocene (10.29 Ma 95%HPD 8.06–12.59) from the North Atlantic into the Southern Hemisphere (Fig. 4b). The presence of members of this subfamily in Northern regions (i.e. *Lagenorhynchus obliquidens* and *Lissodelphis borealis*) could be explained by a later dispersion of ancestral populations toward the northern regions and subsequent break of genetic interchange due to vicariant events, as suggested below for *L. obliquidens*.

North–south faunal interchanges between marine biogeographic provinces during the Miocene and early Pliocene epochs have been suggested for several taxa (Vermeij, 2005). These dispersal events have been hypothetically correlated with the paleoceanographic changes in sea temperatures, current patterns and sea productivity (i.e. upwelling) during the Miocene-early Pliocene (e.g., Wares, 2002; Matul and Abelmann, 2005; Vermeij, 2005). Upwelling regions that arose as a consequence of the "biogenic bloom" and enhanced global marine productivity about 7.6–6.3 Ma (Zachos et al., 2001) have been recently proposed as a major factor promoting long-range dispersal in cetaceans and their subsequent speciation in allopatry (Berger, 2007; Steeman et al., 2009; Marx and Uhen, 2010).

Before the late Pliocene, subtropical upwelling regions are thought to have been the main source of abundant food for cetaceans, and the dispersion and distribution of many species in these regions probably were guided by the predictability of high productivity zones (Berger, 2007). Recently, Diester-Haass et al. (2005) reported high paleo-productivity values in the tropical Atlantic around 6.6–6.0 Ma and in the South Atlantic around 8.2 Ma and 6.2–5.4 Ma. Therefore, we suggest that the dispersal of ancestral populations from the North Atlantic towards the South Atlantic/Southern Oceans during the Middle Miocene-Late Miocene was guided by the availability of rich upwelling regions first in the tropical Atlantic and later in the Southern Ocean.

4.6. L. obliquidens and L. obscurus speciation and dispersal

The divergence between *L. obscurus* and *L. obliquidens* is placed at around 2.56 Ma (95% HPD 1.43–3.73 Ma) in the Late Pliocene. This is earlier than the divergence suggested by Hare et al. (2002) (0.74 Ma), while other divergence estimates (1.9–3.2, Cipriano, 1997; 1.9 Ma, 95% CI = 1.3–2.9; Harlin-Cognato et al., 2007) are consistent with our results. Our analyses all suggest that the most recent common ancestor of *L. obscurus* and *L. obliquidens* inhabited the Southern Ocean, South Pacific and North Pacific at the time of the splitting of these species (likely associated with a vicariance event). Given that our data suggest a southern origin for Lissodelphininae (Sensu LeDuc et al., 1999), this implies dispersal from the South Atlantic/Southern Ocean towards the North Pacific (Fig. 4b). These results are inconsistent with previous studies suggesting speciation following trans-equatorial dispersal from the North to the South Atlantic (Cipriano, 1997; Hare et al., 2002; Harlin-Cognato et al., 2007).

Several paleoceanographic and paleoclimatic changes could have promoted a broad distribution of the ancestor of *L. obliquidens* and *L. obscurus* in the Pacific Ocean, such as the presence of rich upwelling zones in this basin during the Pliocene, especially around 4.2 Ma (Kamikuri et al., 2009; Bolton et al., 2010). Other possibilities include the weak sea surface temperature gradient along the Equator, and the reduction of the meridional temperature gradient from the Equator to the mid latitudes (thought to have resulted in a uniform sea surface temperature between the Equator and the subtropics during the early Pliocene; Brierley et al., 2009; Fedorov et al., 2010). As suggested by Berger (2007), by the late Pliocene the availability of prey resources in subtropical upwelling zones probably had decreased, in contrast to the much greater predictability of resources in high-latitude feeding zones. This may have resulted in extensive high-latitude migrations during the late Pliocene so that populations that selected different migration routes may no longer have met, and consequently begun to diverge (Berger, 2007). This hypothesis, together with the cooling episodes between 2.9 and 2.4 Ma (Raymo, 1994; Raymo et al., 2006; Briggs, 2003), might explain the relatively recent split between *L. obscurus* and *L. obliquidens* around 2.56 Ma (95% HPD 1.43–3.73 Ma). Here we hypothesize that the ancestral population distributed across the Pacific began to diverge when individuals selected different migration routes toward the north (*L. obliquidens*) and south (*L. obscurus*). Ancestral populations could have been established in the extremes of their range, promoting divergence by peripatric speciation (see Mayr, 1982).

4.7. L. australis and L. cruciger

Our analysis suggests a common ancestor for *C. commersonii*, *C. eutropia*, *L. australis* and *L. cruciger* living in the Southern Hemisphere around 3.5 Ma (95%HPD 2.43–4.68 Ma). In contrast to the ancestor of *L. obliquidens* and *L. obscurus*, this ancestral population was probably restricted to the South Atlantic/Southern Ocean (Fig. 4b). *L. australis* is confined to the cold waters of southern South America (south of Chile and Argentina, around Tierra del Fuego, Beagle Channel and the Falkland Islands), while *L. cruciger* is a pelagic species distributed further south with a circumpolar distribution along the coasts of Antarctica and the Sub-Antarctic islands (Leatherwood et al., 1991; Goodall, 1997; Fig. 1). Speciation may have been related to adaptation to these different habitats; however our data provide no specific evidence in support of this hypothesis. The grouping of *L. cruciger* and *L. australis* into the genus *Cephalorhynchus* has recently been corroborated by multigenic

analyses including all four *Cephalorhynchus* species (McGowen, 2011).

4.8. Delphininae and Globicephalinae

The common origin of Delphininae and Globicephalinae has been discussed in several recent studies (e.g., McGowen et al., 2009; Vilstrup et al., 2011), and analysis on the origin of these two families is beyond the aims of this study. However, our biogeographic analyses suggest that ancestral populations of members of both families lived in sympatry in the Atlantic and Pacific Oceans during the late Miocene 9.05 Ma (95% HPD 7.08–11.18 Ma). Evolution in sympatry could have been promoted through habitat preferences, ecological interactions, and complex behavior as proposed for other marine taxa (see Palumbi, 1994; Runde and Nosil, 2005; Fontaine et al., 2007; Puebla, 2009; Norris and Hull, 2011).

5. Conclusions

Our data emphasize the importance of the North Atlantic during the early evolution of delphinid species, and probably of Odontocetes in general. Although there are few data from the relevant fossil record, what exists agrees well with this assessment (e.g. see Whitmore, 1994). One striking finding is the apparent lack of dispersal or further radiation for a lineage of two species that are currently found in the North Atlantic after originating there more than 10 Ma (*L. acutus* and *L. albirostris*). Data for specific speciation events among the other taxa included in the study are consistent with multiple routes of origin from the Atlantic to the Pacific, including dispersal first to equatorial waters, then into the South Atlantic and Southern Oceans, and from there into the Pacific, together with dispersal across the Isthmus of Panama (probably at a later date). These events can be correlated with major changes in the climate and ocean environment, and this provides new insight into the process of species radiation in this group.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.08.005.

References

Alexandre, A., Johan, A.A.N., Claes, P., Isabel, S., 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proc. Natl. Acad. Sci. USA 106, 9749–9754.
Ali, S., Yu, Y., Pfosser, M., Wetschnig, W., 2012. Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). Ann. Bot. 109, 95–107.
Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilipds. Syst. Biol. 46, 654–673.
Ballard, J.W.O., Rand, D.M., 2005. The population biology of mitochondrial DNA and its phylogenetic implications. Annu. Rev. Ecol. Syst. 36, 621–642.
Bapteste, E., Philippe, H., 2002. The potential value of indels as phylogenetic markers: position of trichomonads as a case study. Mol. Biol. Evol. 19, 972–977.
Barker, F.K., Lutzoni, F.M., 2002. The utility of incongruence length difference test. Syst. Biol. 51, 625–637.
Barnes, L.G., 1985. Evolution, taxonomy, and antitropical distributions of the porpoises (Phocoenidae, Mammalia). Mar. Mamm. Sci. 1, 149–165.
Berger, W.H., 2007. Cenozoic cooling, Antarctic nutrient pump, and the evolution of whales. Deep-Sea Res. (II Top. Stud. Oceanogr.) 54, 2399–2421.
Bland, M.M., Monroe, R.S., Ohmstede, C.A., 1994. The cDNA sequence and characterization of the Ca2+/calmodulin-dependent protein kinase-Gr from human brain and thymus. Gene 142, 191–197.
Bocxlaer, I.V., Roelants, K., Biju, S., Nagaraju, J., Bossuyt, F., 2006. Late Cretaceous vicariance in Gondwanan amphibians. PLoS ONE 1 (1), e74.
Bolton, C.T., Lawrence, K.T., Gibbs, S.J., Wilson, P.A., Cleaveland, L.C., Herbert, T.D., 2010. Glacial–interglacial productivity changes recorded by alkenones and microfossils in late Pliocene eastern equatorial Pacific and Atlantic upwelling zones. Earth Planet. Sci. Lett. 295, 401–411.
Borchsenius, F., 2009. FastGap 1.2. Department of Biological Sciences, University of Aarhus, Denmark. <http://192.38.46.42/aubot/fb/FastGap_home.htm>.
Bremer, K., 1995. Ancestral areas: optimization and probability. Syst. Biol. 44, 255–259.
Brierley, C.M., Fedorov, A.V., Liu, Z., Herbert, T.D., Lawrence, K.T., LaRiviere, J.P., 2009. Greatly expanded tropical warm pool and weakened Hadley circulation in the Early Pliocene. Science 323, 1714–1718.
Briggs, J.C., 1987. Antitropicality and vicariance. Syst. Zool. 36, 206–207.
Briggs, J.C., 2003. Marine centres of origin as evolutionary engines. J. Biogeogr. 30, 1–18.
Caballero, S., Trujillo, F., Vianna, J.A., Barrios-Garrido, H., Montiel, M.G., Beltran-Pedreros, S., Marmontel, M., Santos, M.C., Rossi-Santos, M., Santos, F.R., Baker, C.S., 2007. Taxonomic status of the genus Sotalia: species level ranking for 'tucuxi' (Sotalia fluviatilis) and 'costero' (*Sotalia guianensis*) dolphins. Mar. Mam. Sci. 23, 358–386.
Caballero, S., Jackson, J., Mignucci-Giannoni, A., Barrios-Garrido, H., Beltran-Pedreros, S., Montiel-Villalobos, M.G., Robertson, K., Baker, C.S., 2008. Molecular systematics of South American dolphins Sotalia: sister taxa determination and phylogenetic relationships, with insights into a multilocus phylogeny of the Delphinidae. Mol. Phylogenet. Evol. 46, 252–268.
Calvente, A., Zappi, D.C., Forest, F., Lohmann, L.G., 2011. Molecular phylogeny, evolution, and biogeography of South American epiphytic cacti. Int. J. Plant Sci. 172, 902–914.
Carstens, B.C., Knowles, L.L., 2007. Shifting distributions and speciation: species divergence during rapid climate change. Mol. Ecol. 16, 619–627.
Cipriano, F., 1997. Antitropical distribution and speciation in dolphins of the genus Lagenorhynchus: a preliminary analysis. In: Dizon, A.E., Chivers, S.J., Perrin, W.F. (Eds.), Molecular Genetics of Marine Mammals. Society for Marine Mammalogy, Lawrence, K.S, pp. 305–316.
Crawford. A.J., 2008. Pointers on running multidistribute (now with comments on "Thornian Time Traveler") for Bayesian MCMC approach to estimating divergence times from DNA data (AA option not covered here), (using Mac OSX/Darwin). <http://dna.ac/genetics.html>.
Davies, J.L., 1963. The antitropical factor in cetacean speciation. Evolution 17, 107–116.
Diester-Haass, L., Billups, K., Emeis, K.C., 2005. In search of the late Miocene-early Pliocene "biogenic bloom" in the Atlantic Ocean (Ocean Drilling Program Sites 982, 925, and 1088). Paleoceanography 20, 1–13.
Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.
Evans, P.G.H., Smeenk, C., 2008a. Atlantic white-sided dolphin *Leucopleurus acutus*. In: Harris, S., Yalden, D.W. (Eds.), Mammals of the British Isles. Handbook, fourth ed. The Mammal Society, Southampton, p. 800, pp. 727–731.
Evans, P.G.H., Smeenk, C., 2008b. Atlantic white-beaked dolphin *Lagenorhynchus albirostris*. In: Harris, S., Yalden, D.W. (Eds.), Mammals of the British Isles Handbook, fourth ed. The Mammal Society, Southampton, pp. 724–727.
Fajardo-Mellor, L., Berta, A., Brownell, R.L., Boy, C., Goodall, R.N., 2006. The phylogenetic relationships and biogeography of true porpoises (Mammalia:Phocoenidae) based on morphological data. Mar. Mam. Sci. 22, 910–932.
Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1994. Testing the significance of incongruence. Cladistics 10, 315–319.

Fedorov, A.V., Brierley, C.M., Kerry, E., 2010. Tropical cyclones and permanent El Niño in the early Pliocene epoch. Nature 463, 1066–1070.
Felsenstein, J., Churchill, G.A., 1996. A hidden Markov model approach to variation among sites in rate of evolution. Mol. Biol. Evol. 13, 93–104.
Fontaine, M.C., Baird, S.J., Piry, S., Ray, N., Tolley, K.A., Duke, S., Birkun, A., Ferreira, M., Jauniaux, T., Llavona, A., Öztürk, B., Öztürk, A., Ridoux, V., Rogan, E., Sequeira, M., Siebert, U., Vikingsson, G.A., Bouquegneau, J.M., Michaux, J.R., 2007. Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. BMC Biol. 5, 30.
Fordyce, R.E., 2008. Cetacean fossil record. In: Perrin, W.F., Würsig, B., Thewissen, H. (Eds.), Encyclopedia of Marine Mammals, second ed. Academic Press, San Diego, CA, pp. 207–214.
Fordyce, R.E., Barnes, G., 1994. The evolutionary history of whales and dolphins. Annu. Rev. Earth Plan. Sci. 22, 419–455.
Fraser, F.C., 1966. Comments on the Delphinoidea. In: Norris, K.S. (Ed.), Whales, Dolphins and Porpoises. University of California Press, Berkeley and Los Angeles, pp. 7–31.
Gaskin, D.E., 1992. Status of the Atlantic white-sided dolphin, *Lagenorhynchus acutus*, in Canada. Can. Field Nat. 106, 64–72.
Geisler, J.H., Sanders, A.E., 2003. Morphological evidence for the phylogeny of Cetacea. J. Mammal. Evol. 10, 23–129.
Gingerich, P.D., 2005. Cetacea. In: Rose, K.D., Archibald, J.D. (Eds.), Placental Mammals: Origin, Timing, and Relationships of the Major Extant Clades. Johns Hopkins University Press, Baltimore, pp. 234–252.
Goodall, R.N.P., 1997. Review of sightings of the hourglass dolphin, *Lagenorhynchus cruciger*, in the South American Sector of the Antarctic and sub-Antarctic. Rep. Int. Whal. Comm. 47, 1001–1014.
Graham, S.W., Reeves, P.A., Burns, A.C.E., Olmstead, R.G., 2000. Microstructural changes in noncoding chloroplast DNA: interpretation, evolution and utility of indels and inversions in basal angiosperm phylogenetic inference. Int. J. Plant Sci. 161, S83–S96.
Guindon, S., Gascuel, O., 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
Gygax, L., 2002. Evolution of group size in the superfamily Delphinoidea (Delphinidae, Phocoenidae and Monodontidae): a quantitative comparative analysis. Mammal. Rev. 32, 295–314.
Hare, M.P., Cipriano, F., Palumbi, S.R., 2002. Genetic evidence on the demography of speciation in allopatric dolphin species. Evolution 56, 804–816.
Harlin-Cognato, A.D., Honeycutt, R.L., 2006. Multi-locus phylogeny of dolphins in the subfamily Lissodelphininae: character synergy improves phylogenetic resolution. BMC Evol. Biol. 6 (87), 1–16.
Harlin-Cognato, A.D., Markowitz, T., Würsig, B., Honeycut, R.L., 2007. Multi-locus phylogeography of the dusky dolphin (Lagenorhynchus obscurus): passive dispersal via the west-wind drift or response to prey species and climate change? BMC Evol. Biol. 7, 1–17.
Hartmann, S., Vision, T., 2008. Can one accurately infer a phylogenetic tree from a gappy alignment? BMC Evol. Biol. 8, 95.
Hausdorf, B., 1998. Weighted ancestral area analysis and a solution of the redundant distribution problem. Syst. Biol. 47, 445–456.
Heath, T.A., Hedtke, S.M., Hillis, D.M., 2008. Taxon sampling and the accuracy of phylogenetic analyses. J. Syst. Evol. 46, 239–257.
Hermoyian, C.S., Owen, R.M., 2001. Late Miocene-early Pliocene biogenic bloom: evidence from low productivity regions of the Indian and Atlantic Oceans. Paleoceanography 16, 95–100.
Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. Roy. Soc. Lond. B. Biol. Sci. 359, 183–195.
Hipp, A.L., Hall, J.C., Sytsma, K.J., 2004. Congruence versus phylogenetic accuracy: revisiting the incongruence length difference test. Syst. Biol. 53, 81–89.
Hoelzel, A.R., Green, A., 1998. PCR protocols and population analysis by direct DNA sequencing and PCR-based fingerprinting. In: Hoelzel, A.R. (Ed.), Molecular Genetic Analysis of Population a Practical Approach, second ed. Oxford University Press.
Hoelzel, A.R., Potter, C.W., Best, P.B., 1998. Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. Proc. R. Soc. B 265, 1177–1183.
Huelsenbeck, J.P., 1991. When are fossils better than extant taxa in phylogenetic analysis? Syst. Zool. 40, 458–469.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Ichishima, H., Barnes, L.G., Fordyce, R.E., Kimura, M., Bohaska, D.J., 1995. A review of kentriodontine dolphins (Cetacea; Delphinoidea; Kentriodontidae): systematics and biogeography. The Island Arc 3, 486–492.
Jansa, S.A., Forsman, J.F., Voss, R.S., 2006. Different patterns of selection on the nuclear genes IRBP and DMP-1 affect the efficiency but not the outcome of phylogeny estimation for didelphid marsupials. Mol. Phylogenet. Evol. 38, 1055–7903.
Jefferson, T.A., Fertl, D., Bolanos-Jimenez, J., Zerbini, A.N., 2009. Distribution of common dolphins (*Delphinus* spp.) in the western Atlantic Ocean: a critical re-examination. Mar. Biol. 156, 109–1124.
Kamikuri, S.I., Motoyama, I., Nishi, H., Iwai, M., 2009. Neogene radiolarian biostratigraphy and faunal evolution rates in the eastern equatorial Pacific ODP sites 845 and 1241. Acta Palaeontol. Pol. 54, 713–742.
Kawakita, A., Sota, T., Ascher, J., Ito, M., Tanaka, H., Kato, M., 2003. Evolution and phylogenetic utility of alignment Gaps within intron sequences of three nuclear genes in bumble bees (*Bombus*). Mol. Biol. Evol. 20, 87–92.
Kearney, M., 2002. Fragmentary taxa, missing data, and ambiguity: mistaken assumptions and conclusions. Syst. Biol. 51, 369–381.
Kingston, S.E., Rosel, P.E., 2004. Genetic differentiation among recently diverged delphinid taxa determined using AFLP markers. J. Hered. 95, 1–10.
Kodandaramaiah, U., 2010. Use of dispersal–vicariance analysis in biogeography – a critique. J. Biogeogr. 37, 3–11.
Leatherwood, S., Grove, J.S., Zuckerman, A.E., 1991. Dolphins of the genus *Lagenorhynchus* in the tropical South Pacific. Mar. Mamm. Sci. 72, 194–197.
LeDuc, R.G., 2008. Delphinids overview. In: Perrin, W.F., Würsig, B., Thewissen, H. (Eds.), Encyclopedia of Marine Mammals, second ed. Academic Press, San Diego, CA, pp. 298–299.
LeDuc, R.G., Perrin, W.F., Dizon, A.E., 1999. Phylogenetic relationships among the delphinid cetaceans based on full cytochrome b sequences. Mar. Mamm. Sci. 15, 619–648.
Lemmon, A.R., Brown, J.M., Stanger-Hall, K., Lemmon, E.M., 2009. The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Syst. Biol. 58, 130–145.
Lyons, L.A., Laughlin, T.F., Copeland, N.G., Jenkins, N.A., Womack, J.E., O'Brien, S.J., 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nat. Genet. 15, 47–56.
Marx, F.G., Uhen, M.D., 2010. Climate, critters, and cetaceans: cenozoic drivers of the evolution of modern whales. Science 327, 993–996.
Matul, A., Abelmann, A., 2005. Pleistocene and Holocene distribution of the radiolarian Amphimelissa setosa Cleve in the North Pacific and North Atlantic: evidence for water mass movement. Deep-Sea Res. (II Top. Stud. Oceanogr.) 52, 2351–2364.
May-Collado, L., Agnarsson, I., 2006. Cytochrome b and Bayesian inference of whale phylogeny. Mol. Phylogenet. Evol. 38, 344–354.
Mayr, E., 1982. Processes of speciation in animals. In: Liss, A.R.I. (Ed.), Mechanisms of Speciation. Alan R. Liss Inc., New York, pp. 1–19.
McGowen, M.R., 2011. Toward the resolution of an explosive radiation—a multilocus phylogeny of oceanic dolphins (Delphinidae). Mol. Phylog. Evol. 60, 345–357.
McGowen, M.R., Spaulding, M., Gatesy, J., 2009. Divergence date estimation and a comprehensive molecular tree of extant cetaceans. Mol. Phylog. Evol. 53, 891–906.
Messenger, S.L., Mcguire, J.A., 1998. Morphology, molecules, and the phylogenetics of Cetaceans. Syst. Biol. 47, 90–121.
Milinkovitch, M.C., Bérubé, M., Palsbøll, P.J., 1998. Cetaceans are highly specialized artiodactyls. In: Thewissen (Ed.), The Emergence of whales: evolutionary patterns in the origin of Cetacea. Plenum, New York, pp. 113–131.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees". In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp. 1–8.
Mitchell, E., 1970. Pigmentation pattern evolution in delphinid cetaceans – an essay in adaptive coloration. Can. J. Zool. 48, 717.
Miyazaki, N., Shikano, C., 1997. Preliminary study on comparative skull morphology and vertebral formula among the six species of the genus *Lagenorhynchus* (Cetacea: Delphinidae). Mammalia 61, 573–587.
Moore, W.S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49, 718–726.
Moura, A.E., Nielsen, S.C.A., Vilstrup, J.T., Moreno-Mayar, J.V., Gilbert, M.T.P., Gray, H., Natoli, A., Moller, L., Hoelzel, A.R., 2013. Recent diversification of a marine genus (*Tursiops* spp.) tracks habitat preference and environmental change. Syst. Biol. 62, 865–877.
Nabhan, A.R., Sarkar, I.N., 2011. The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. Brief Bioinform. 13, 122–134.
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*). Proc. R. Soc. B 272, 1217–1226.
Natoli, A., Cañadas, A., Peddemors, V.M., Aguilar, C., Vaquero, C., Fernandez-Piqueras, P., Hoelzel, A.R., 2006. Phylogeography and alpha taxonomy of the common dolphin (*Delphinus* sp.). J. Evol. Biol. 19, 943–954.
Norris, R.D., Hull, P.M., 2011. The temporal dimension of marine speciation. Evol. Ecol. 26, 393–415.
Nylander, J.A., 2004. MrModeltest. version 2.2. Uppsala University, Uppsala.
Nylander, J.A.A., Olsson, U., Alstrom, P., Sanmartín, I., 2008. Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to dispersal–vicariance analysis of the thrushes (Aves: Turdus). Syst. Biol. 57, 257–268.
Palumbi, S.R., 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu. Rev. Ecol. Syst. 25, 547–572.
Palumbi, S.R., Baker, C.S., 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Mol. Biol. Evol. 7, 426–435.
Palumbi, S.R., Grabowsky, G.A., Duda, T., Geyer, L., Tachino, N., 1997. Speciation and population genetic structure in tropical pacific sea urchins. Evolution 51, 1506–1517.
Pastene, L.A., Goto, M., Kanda, N., Zerbini, D.K., Watanabe, K., Bessho, Y., Hasegawa, M., Nielsen, R., Larsen, F., Palsbøll, P.J., 2007. Radiation and speciation of pelagic organism during periods of global warming: the case of the common minke whale, Balaenoptera acutorostrata. Mol. Ecol. 16, 1481–1495.
Pichler, F.B., Robineau, D., Goodall, R.N., Meÿer, M.A., Olivarría, C., Baker, C.S., 2001. Origin and radiation of Southern Hemisphere coastal dolphins (genus *Cephalorhynchus*). Mol. Ecol. 10, 2215–2223.
Planet, P.J., 2006. Tree disagreement: measuring and testing incongruence in phylogenies. J. Biomed. Inform. 39, 86–102.

Porter, C.A., Goodman, M., Stanhope, M.J., 1996. Evidence on mammalian phylogeny from sequences of Exon 28 of the von Willebrand factor gene. Mol. Phylogenet. Evol. 5, 89–101.
Price, S.A., Bininda-Emonds, Olaf R.P., Gittleman, J.L., 2005. A complete phylogeny of the whales, dolphins and even-toed hoofed mammals (Cetartiodactyla). Biol. Rev. 80, 445–473.
Puebla, O., 2009. Ecological speciation in marine vs. freshwater fishes. J. Fish Biol. 75, 960–996.
Rambaut, A., 2006. Tree Figure Drawing Tool Version 1.3.1. Institute of Evolutionary Biology, University of Edinburgh. <http://tree.bio.ed.ac.uk/>.
Rambaut, A., Drummond, A.J., 2007. Tracer v1.5. <http://beast.bio.ed.ac.uk/Tracer>.
Raymo, M.E., 1994. The initiation of northern-hemisphere glaciation. Annu. Rev. Earth Planet. Sci. 22, 353–383.
Raymo, M.E., Lisiecki, E., Kerim, L.E., Nisancioglu, H., 2006. Plio-Pleistocene ice volume, Antarctic climate, and the global $\delta^{18}O$ record. Science 313, 492–495.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.
Reyes, A., Giss, C., Catzeflis, F., Nevo, E., Pesole, G., Saccone, C., 2004. Congruent mammalian trees from mitochondrial and nuclear genes using Bayesian methods. Mol. Biol. Evol. 21, 397–403.
Rice, D.W., 1998. Marine Mammals of the World: Systematics and Distribution. The Society for Marine Mammalogy. Special Publication 4, pp. 1–231.
Ronquist, F., 1997. Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rosel, P.E., Dizon, A.E., Heyning, J.E., 1994. Genetic-analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). Mar. Biol. 119, 159–167.
Rosenberg, M.S., Kumar, S., 2001. Incomplete taxon sampling is not a problem for phylogenetic inference. PNAS 98, 10751–10756.
Ross, G.J.B., Leatherwood, S., 1994. Pygmy killer whale *Feresa attenuata*. In: Ridgway, S.H., Harrison, S.R. (Eds.), Handbook of Marine Mammals (vol. 5: The first book of dolphins). Academic Press, London, pp. 387–404.
Runde, H.D., Nosil, P., 2005. Ecological speciation. Ecol. Let. 8, 336–352.
Rutschmann F., 2005. Bayesian molecular dating using PAML/multidivtime. A step-by-step manual. University of Zurich, Switzerland. <http://www.plant.ch>.
Sanmartín, I., 2007. Event-based biogeography: integrating patterns, processes, and time. In: Ebach, M.C., Tangey, R.S. (Eds.), Biogeography in a Changing World. Taylor & Francis, London, pp. 135–159.
Sanmartín, I., Van Der Mark, P., Ronquist, F., 2008. Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. J. Biogeogr. 35, 428–449.
Shaw, K.L., 2002. Conflict between mitochondrial and nuclear DNA phylogenies of a recent species radiation: what mitochondrial reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA 99, 16122–16127.
Sorenson, M.D., Franzosa, E.A. 2007. TreeRot, version 3. Boston University, Boston, MA (<http://people.bu.edu/msoren/TreeRot.html>).
Springer, M.S., Burk, A., Kavanagh, J.R., Waddell, V.G., Stanhope, M.J., 1997. The interphotoreceptor retinoid binding protein gene in therian mammals: Implications for higher level relationships and evidence for loss of function in the marsupial mole. Proc. Natl. Acad. Sci. USA 94, 13754–13759.
Stanhope, M.J., Czelusniak, J., Si, J.S., Nickerson, J., Goodman, M., 1992. A molecular perspective on Mammalian Evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for Bat monophyly. Mol. Phylogenet. Evol. 1, 148–160.
Steeman, M.E., Hebsgaard, M.B., Fordyce, E., Ho, S.Y., Rabosky, D.L., Nielsen, R., Rahbek, C., Glenner, H., Sørensen, Willerslev, E., 2009. Radiation of extant cetaceans driven by restructuring of the oceans. Syst. Biol. 58, 573–585.
Swofford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods), v. 4.0 β10. Sinauer Associates, Sunderland, MA.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689–702.
Vermeij, G.J., 2005. One way traffic in the western Atlantic: causes and consequences of Miocene to early Pleistocene molluscan invasions in Florida and the Caribbean. Paleobiology 31, 624–642.
Vilstrup, J.T., Ho, S.Y.W., Foote, A.D., Morin, P.A., Kreb, D., Krützen, M., Parra, G.J., Robertson, K.M., Stephanis, R., Verborgh, P., Willerslev, E., Orlando, L., Gilbert, T.M.P., 2011. Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. BMC Evol. Biol. 11, 65.
Waddell, V.G., Milinkovitch, M.C., Bérubé, M., Stanhope, M.J., 2000. Molecular phylogenetic examination of the Delphinoidea trichotomy: congruent evidence from three nuclear loci indicates that porpoises (Phocoenidae) share a more recent common ancestry with white whales (Monodontidae) than they do with true dolphins (Delphinidae). Mol. Phylogenet. Evol. 15, 314–318.
Walteri, E., Demboski, J.R., Klein, D.R., Cook, J.A., 2004. A molecular perspective on the historical biogeography of the northern high latitudes. J. Mammal. 85, 591–600.
Wares, J.P., 2002. Community genetics in the Northwestern Atlantic intertidal. Mol. Ecol. 11, 1131–1144.
White, B.N., 1986. The isthmian link, antitropicality and American biogeography: distributional history of the Atherinopsinae (Pisces: Atherinidae). Syst. Zool. 35, 176–194.
White, B.N., 1989. Antitropicality and vicariance: a reply to briggs. Syst. Zool. 38, 77–79.
Whitmore, F.C. Jr., 1994. Neogene Climatic Change and the Emergence of the Modern Whale Fauna of the North Atlantic Ocean. In: Berta, A., Demere, T.A. (Eds.), Contributions in Marine Mammal Paleontology Honoring Frank C. Whitmore, Jr. Proc. San Diego Soc. Nat. Hist. 29, pp. 223–228.
Wilkinson, M., 1995. Coping with abundant missing entries in phylogenetic inference using parsimony. Syst. Biol. 44, 501–514.
Xie, L., Wagner, W.L., Ree, R.H., Berry, P.E., Wen, J., 2009. Molecular phylogeny, divergence time estimates, and historical biogeography of Circaea (Onagraceae) in the Northern Hemisphere. Mol. Phylogenet. Evol. 53, 995–1009.
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.
Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. CABIOS 13, 555–556, <http://abacus.gene.ucl.ac.uk/software/paml.html>.
Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50, 408–424.
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.
Yu, Y., Downie, S.R., He, X.J., Deng, X.L., Yan, L., 2011. Phylogeny and biogeography of Chinese Heracleum (Apiaceae tribe Tordylieae) with comments on their fruit morphology. Plant Syst. Evol. 296, 179–203.
Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693.