
Oceanographic drivers of population differentiation in Indo-Pacific bottlenose (Tursiops aduncus) and humpback (Sousa spp.) dolphins of the northern Bay of Bengal

Ana R. Amaral1,2,*, Brian D. Smith3, Rubaiyat M. Mansur3, Robert L. Brownell, Jr4, Howard C. Rosenbaum3,1

1 Sackler Institute for Comparative Genomics, American Museum of Natural History, 79th Street and Central Park West, New York, NY 10024, United States of America.
3 Wildlife Conservation Society, Ocean Giants Program, 2300 Southern Boulevard, Bronx, New York 10460, United States of America
4 NOAA Fisheries, Southwest Fisheries Science Center, 34500 Highway 1, Monterey, California, 93940, USA

*Corresponding author

Address: Faculdade de Ciências da Universidade de Lisboa, Departamento de Biologia Animal, Edifício C2, Campo Grande, 1749-016, Lisbon, Portugal

Telephone number: +351217500000

E-mail: aramaral@fc.ul.pt
Abstract

The Bay of Bengal is one of the most productive ecosystems in the northern Indian Ocean and it harbours a rich community of cetaceans, including Indo-Pacific bottlenose (*Tursiops aduncus*) and humpback (*Sousa* spp.) dolphins. The taxonomy of these genera has been controversial, but within the Indian Ocean both seem to be divided into phylogenetically discrete units that range from the east to the west. Within the *Sousa* genus, *S. plumbea* is distributed in the western Indian Ocean while *S. chinensis* is distributed in the eastern Indian and western Pacific Ocean. *T. aduncus* has a discontinuous distribution throughout the Indo-Pacific Ocean and two different phylogenetic units are known to exist, one along the eastern African coast and another one in the eastern Indian and west Pacific Ocean. In this study we investigate the phylogeography of Indo-Pacific humpback and bottlenose dolphins in the northern Bay of Bengal. We sequenced the mitochondrial DNA control region for 17 bottlenose and 15 humpback dolphins and compared the results with previously published sequences within each genus. In both cases, we found that Bangladesh dolphins are genetically different from neighbouring populations. While the Bangladesh *T. aduncus* seem to be more closely related to the African *T. aduncus* form than the Pacific form, *Sousa* spp. seem to be more closely related to individuals from Australia. The genetic uniqueness of these populations has important evolutionary implications, due to their isolation, coastal distribution in a geographic cul-de-sac characterized by an extreme infusion, redistribution and recycling of biological productivity, and conservation implications since their survival is threatened in particular by fatal interactions with fisheries. We suggest that the
particular and extreme oceanographic conditions found in the Bay of Bengal may be driving speciation in these dolphins and other marine megafauna.

Keywords: Genetic structure; mtDNA; Indo-Pacific humpback dolphin; Indo-Pacific bottlenose dolphin; Bangladesh.

Introduction

Identifying environmental conditions driving population divergence and speciation is central for understanding ecological and evolutionary processes and for the establishment of effective conservation measures. In the marine environment, variables such as ocean currents, upwelling, bathymetry, sea surface temperature, primary productivity and salinity have been proposed as some of the factors that explain genetic diversity and structure in marine organisms, including small cetaceans (e.g. Selkoe et al. 2010; Mendez et al. 2011; Amaral et al. 2012).

Well described complex oceanographic systems are known to prevent gene flow between neighbouring populations of marine taxa, originating clear scenarios of vicariance and in some cases of speciation. Such examples include the Benguela and Agulhas currents and upwelling along the Southern African coast, the Humboldt Current marine ecosystem in the Southeast Pacific Ocean and the Northern Indian Ocean complex pattern of currents (e.g. Perrin 2007; Vogler et al. 2012; Henriques et al. 2014).

In the Northern Indian Ocean, the Bay of Bengal is a tropical ocean basin influenced by discharge from the third-largest river system in the world – the Ganges/
Brahmaputra/Meghna (GBM). This massive influx of freshwater and nutrient rich sediments sustains the world’s largest continuous mangrove forest (Hussain and Acharya 1994) and has eroded a submarine canyon leading to the world’s largest undersea sediment fan (Unger et al. 2003). Driven by regular flushing of rich silts and organic matter from mangrove litter falls (Islam 2003), the GBM supplies more than 1.5% of the total riverine input to the world’s oceans (Sarin et al. 1989). This enormous supply of freshwater, sediments and nutrients is circulated by a seasonally reversing, wind-driven, basin-scale gyre with adjacent meso-scale eddies (Somayajulu et al. 2003). These conditions combine to produce a highly stratified and productive sea-surface layer in shallow coastal waters that became available when the sea level rose 6,000 to 10,000 years ago. The minimum 50 m contour distance from shore is located in the far west where a 900+ metre-deep submarine canyon known as the Swatch-of-No-Ground (SoNG) incises to within about 35 km of edge of the Sundarbans mangrove forest. This canyon upwells nutrients supplied by the GBM system, which are concentrated at its head and then recycled back into the seasonally reversing current of euryhaline waters. The extreme infusion and redistributive dynamism of biological productivity in the northern Bay of Bengal is a rare ecological condition that supports cetaceans in numbers generally much larger than other populations in the region (Smith et al. 2008; Mansur et al. 2012).

Bottlenose dolphins

The waters at the head of the SoNG support one of the world’s largest populations of Indo-Pacific bottlenose dolphins, *Tursiops aduncus* (Mansur et al. 2012). However, these dolphins are potentially threatened as they are taken as bycatch in coastal fisheries. Their distribution strongly overlaps with gill net fisheries and a large portion
(28.2%) of individuals identified from dorsal fin photographs exhibits scars and mutilations clearly related to entanglements in fishing gear (Mansur et al. 2012).

*T. aduncus* is distributed in coastal waters of the Indian and western Pacific Oceans, although the continuity of its distribution is unknown (Wang 2009). Genetic studies have found strong differences between populations occurring along the coast of Africa and those occurring in the Indo-West Pacific (China, Japan, Korea, Melanesia, Australia). These may represent different taxonomic units at species or subspecies levels (Wang et al. 1999; Natoli et al. 2004; Sarnblad et al. 2011; Oremus et al. 2015).

Here we follow Oremus et al. 2015 by referring to these two forms as the “African” *T. aduncus* and the “Pacific” *T. aduncus*.

Little information is available on the population structure of Indo-Pacific bottlenose dolphins at smaller scales even though the species is believed to be composed of many small, fairly isolated populations due to their limited coastal distribution (but see Allen et al. 2006; Kopps et al. 2014). Sarnblad et al. 2011 found genetic differences between dolphins occurring in Northern and Southern Zanzibar, suggesting that differentiation may arise even across small geographic scales. Similar results were obtained with *T. aduncus* in Melanesia, where evidence of population structure was found between the Solomon Islands and New Caledonia (Oremus et al. 2015). Historical circumstances and local adaptation have been suggested as potential factors driving the divergence of *T. aduncus* populations (Natoli et al. 2004; Sarnblad et al. 2011).

**Humpback dolphins**

Humpback dolphins of the genus *Sousa* are distributed discontinuously in coastal waters of West Africa and in the Indian and Western Pacific Oceans (Parra and Ross...
A recent exhaustive review of multiple lines of evidence from skeletal morphology, external morphology, coloration, molecular genetics, and biogeography provided strong support for the recognition of four species (Mendez et al. 2013; Jefferson and Rosenbaum 2014). This scenario has been accepted by the Society of Marine Mammalogy Committee on Taxonomy (2014) with S. chinensis distributed in the Eastern Indian and Western Pacific Oceans, S. plumbea in the Western Indian Ocean, S. teuszii in the Eastern Atlantic Ocean along the west African coast and S. sahulensis in Northern Australia (Jefferson and Rosenbaum 2014). However, the exact ranges of S. chinensis and S. plumbea in the Bay of Bengal are poorly known (Jefferson and Smith 2016).

The most comprehensive genetic study conducted to date, which included samples from throughout the range of the genus, found high levels of divergence in both mitochondrial and nuclear DNA markers separating dolphins at the species level from West Africa (S. teuszii), Southeast Africa, Arabia-Oman and the Indian subcontinent (S. plumbea), Thailand and China (S. chinensis) and Australia (later described as S. sahulensis in Jefferson and Rosenbaum 2014) (Mendez et al. 2013). One dolphin from Bangladesh showed remarkable differences in the mitochondrial DNA, with its placement in phylogenetic trees as distant as those from Australia (Mendez et al. 2013).

Analyses conducted to date suggest strong genetic population structure within both S. plumbea and S. chinensis. Populations of S. plumbea in Oman and Tanzania show remarkable differentiation compared with populations in South Africa and Mozambique (Mendez et al. 2011; Mendez et al. 2013). Oceanographic features such as sea surface temperature and primary productivity were found to be among the drivers leading to differences between populations. S. chinensis populations in China
also show a high degree of divergence (Chen et al. 2008; Chen et al. 2010). Large portions of the range of both species have yet to be sampled. However, their distributional limit is believed to be the east coast of India.

Humpback dolphins observed along the west coast of India (Arabian Sea) have a large hump and appear dark grey, thus resembling *S. plumbea*, while those observed along the east coast of India in the Bay of Bengal do not have a distinct hump and are much lighter in color, thus resembling *S. chinensis* (Sutaria and Jefferson 2004). However, animals exhibiting the *plumbea*-type coloration, but without an obvious hump, have been observed as far east as the Mergui Archipelago, Myanmar (Smith and Tun 2008), suggesting that both forms may be sympatric in the Bay of Bengal. Given the genetic distinctiveness of the one animal sampled off Bangladesh (Mendez et al. 2013), it is particularly important to clarify the phylogenetic position of humpback dolphins in the northern Bay of Bengal.

Humpback dolphins are associated with shallow coastal waters generally near freshwater inputs. This makes them highly vulnerable to fatal entanglements in the densely distributed fisheries and to increasing degradation of their habitat. During a recent review both species *S. chinensis* and *S. plumbea* were proposed as Vulnerable according to IUCN Red List criteria (Jefferson and Smith 2016; Braulik et al. 2015).

Relating genetic differentiation and oceanography

A clear understanding of demographically isolated populations and the factors leading to their isolation is vitally important for determining biologically relevant conservation units and guiding efforts to protect them from extinction. This study aims to identify the phylogeographic affinity of Indo-Pacific bottlenose and humpback dolphins occurring in the waters of Bangladesh (northern Bay of Bengal),
and relate this to the extraordinary ecological and oceanographic conditions found in these waters. In particular we intend to understand if Indo-Pacific bottlenose and humpback dolphins from this region are genetically different from neighbouring populations. To accomplish this, we sequenced a fragment of the mitochondrial DNA control region and conducted relevant analyses with published sequences available in GenBank from other Indo-Pacific humpback and bottlenose dolphin populations. For humpback dolphins we included sequences from *S. teuszii* in the Atlantic Ocean. For *T. aduncus* we included sequences from South Africa (Natoli et al. 2004), Zanzibar (Sarnblad et al. 2011), India and Australia (Moller and Beheregaray 2001), Indonesia, and China (Wang et al. 1999), and Melanesia (Oremus et al. 2015).

**Material and Methods**

**Sampling**

A total of 17 Indo-Pacific bottlenose dolphin samples from Bangladesh were obtained through minimally invasive biopsy darting using a cross bow in coastal waters offshore the Sundarbans mangrove forest. A single tooth was also obtained from a mandible collected in the Andaman Islands (eastern part of the Bay of Bengal) in 1889. This mandible (3406) is in the collection of the Natural History Museum of the University of Florence, Italy (Museo di Storia Naturale dell’Università degli Studi di Firenze). To include these newly generated sequences in a broad phylogeographic analyses of *T. aduncus*, sequences encompassing different geographical regions corresponding to “African” *T. aduncus* and “Pacific” *T. aduncus* were retrieved from GenBank (Suppl. Table 1).

Similarly, a total of 15 humpback dolphin samples were obtained from the northern Bay of Bengal, Bangladesh, 14 from minimally invasive biopsy darting using a
crossbow in coastal waters offshore the Sundarbans mangrove forest and one from a stranding in Cox’s Bazaar in the far south of the country close to the border with Myanmar (Figure 1). These samples were included in a broader dataset comprising of 234 humpback dolphins that had already been analyzed by Mendez et al. (2013) and include the following regions: West Africa (WA, \(n=6\)), Southeast Africa (SEA, \(n=38\)), Oman (OM, \(n=58\)), Thailand (TH, \(n=8\)), India (\(n=3\)), China (CH, \(n=92\)), Australia (AUS, \(n=28\)) and Bangladesh (BAN, \(n=1\)).

Laboratory procedures

Genomic DNA was extracted from tissue samples using the QIAamp Tissue Kit (QIAGEN, Valencia, CA, USA), except for the single tooth from the humpback dolphin sampled in the Andaman Islands. For that we followed the protocol for DNA extraction as described in Morin et al. 2006. A fragment of the mitochondrial DNA control region was amplified and sequenced using primers Dlp-10 (5’-CCACAGTACTATGTCCGTATT-3’) and Dlp-5 (5’-CCATCGWGATGTCTTATTTAAGRGGAA-3’) (Baker et al. 1993). The PCR profile consisted of an initial denaturation for 3 min at 94ºC followed by 32 amplification cycles (30s at 94ºC, 30s at 52º, 1 min at 72ºC) and a final 5 min of extension at 72ºC. Both strands were directly sequenced (BigDye Terminator CycleSequencing; Applied Biosystems) on an ABI 3730 automated sequencer (Applied Biosystems).

Sequences were separated and subsequently analysed in two different sets, one corresponding to Indo-Pacific bottlenose dolphins and another one to humpback dolphins. Therefore, all the analyses mentioned below were applied to both datasets, with any exception detailed.
DNA sequences were inspected, edited and aligned by eye in Sequencher 5.0.1 (Gene Codes, Corp.). Sequences were collapsed into haplotypes using DNAsp v. 5.10 (Librado and Rozas 2009). Diversity measures (nucleotide and haplotype diversities and the average number of nucleotide differences) were estimated in DNAsp. Potential differences among populations in different geographical regions were tested by calculating pairwise $F_{ST}$ (using haplotype frequencies) and $\phi_{ST}$ (using genetic distance) in Arlequin v. 3.5. (Excoffier and Lischer 2010) for the humpback dolphin dataset. For the bottlenose dolphin dataset, since only haplotype sequences were available, we assessed genetic differences between the Bangladesh bottlenose dolphins and bottlenose dolphins from other regions, using the net average, $d_A$, and the mean gross, $d_{xy}$, distances as estimated by $d_A = d_{XY} - (d_X + d_Y)/2$, where $d_X$ and $d_Y$ are the mean within group distances, in the software MEGA v. 6.. with 5000 bootstrap replicates (Tamura et al. 2013). The best model of nucleotide substitution for the dataset was determined using the Akaike Information Criterion (AIC) as implemented in the program jModeltest v. 2.1.7 (Darriba et al. 2012; Guindon and Gascuel 2003). The model selected was HKY with a proportion of invariable sites and a gamma-shaped distribution of rates across sites ($G=0.429$). This model was therefore used to calculate $d_A$ and $d_{xy}$.

Phylogeographic analyses of both bottlenose and humpback dolphin datasets included a median-joining network of haplotypes constructed in NETWORK v. (Bandelt et al. 1999) and a Bayesian phylogenetic tree obtained in MrBayes v. 3.1.2. (Huelsenbeck and Ronquist 2001). For the latter analysis, four simultaneous MCMC chains were run for 2 million generations, with trees sampled at intervals of 100 generations. The first 3,000 trees were discarded as “burn-in”. A sequence of *Steno bredanensis* was used as outgroup (GenBank Accession Number KM260657). For the bottlenose
dolphin dataset a Maximum-likelihood method was also used to estimate a
phylogenetic tree using the program MEGA v. 6.0.6 (Tamura et al. 2013), with the
HKY model as the nucleotide substitution model and the Nearest-Neighbor-
Interchange Heuristic method with Branch Swap Filter. One thousand bootstrap
replicates were run to assess robustness of the phylogeny estimated.

Results

Genetic diversity

In total, 380 bp of the mitochondrial DNA control region were sequenced and
analysed for the 17 samples obtained for the Bangladesh bottlenose dolphins and for
the tooth sample from the Andaman Islands, which grouped into 7 haplotypes
(GenBank Accession Numbers KX364257-KX364263). The estimated haplotypic
diversity (0.699 ±0.117) was relatively low, but similar to values obtained for South
Africa, Zanzibar and Australia populations (Sarnblad et al. 2011). Conversely, the
estimated nucleotide diversity (0.009 ±0.005) was relatively high and similar to
values obtained for China/Taiwan and the Solomon Islands populations (Oremus et al.
2015).

A fragment of 456 bp of the mitochondrial DNA control region was sequenced for 15
humpback dolphins from Bangladesh. These sequences were aligned with an already
existent dataset comprising most of the genus distribution (Mendez et al. 2013).

Samples from Bangladesh grouped into 9 haplotypes (GenBank Accession Numbers
KX364242-KX364256) and showed the highest levels of genetic diversity when
compared to the other geographical regions analysed when haplotypic diversity and
the average number of nucleotide differences are considered (Table 1). There were no
shared haplotypes between samples from Bangladesh and the other regions.
The net average, $d_A$, and the mean gross, $d_{xy}$, genetic divergence estimates between the different bottlenose dolphin groups included in this study indicate differences between the Bangladesh population and all others (Table 2). Values for $d_A$ varied between 0.05 and 0.08 and $d_{xy}$ values varied between 0.06 and 0.09. Values of the same order of magnitude were also obtained in comparisons between “African” and “Pacific” T. aduncus, suggesting that the three bottlenose dolphins groups are genetically divergent. Comparisons among the different populations within the “Pacific” T. aduncus group showed much lower levels of divergence (Table 2).

Pairwise $F_{ST}$ and $\phi_{ST}$ values indicate significant differences among all geographical regions sampled for humpback dolphins. Samples from Bangladesh showed $F_{ST}$ values ranging from 0.195 to 0.531, with the lowest values observed in comparisons with regions from the Western Indian Ocean (SEA and OM) and the highest values observed in comparisons with the Eastern Atlantic and Indo-West Pacific (Table 3).

**Phylogeography**

The haplotype network and phylogenetic tree obtained for bottlenose dolphins showed the presence of three distinct clusters: one corresponding to the Bangladesh population (including the Andaman Islands specimen) and the other two to the “African” and “Pacific” T. aduncus populations that had already been reported in previous studies (e.g. Oremus et al. 2015). There were no shared haplotypes among these three groups (Figure 2 and Suppl. Fig.3). The only shared haplotypes were within the “Pacific” T. aduncus haplogroup. One haplotype was shared between China and Australia, one shared between Melanesia and Australia and the other one shared between Indonesia and China. There was one haplotype from Bangladesh that
grouped with the “Pacific” *T. aduncus*” group. In the phylogenetic trees obtained with Bayesian inference (not shown) and maximum-likelihood (Suppl. Fig. 3) methods, although there was no resolution of the sister taxa relationship among groups (i.e. it was not possible to infer which group originated first), a high posterior probability (>95%) and bootstrap value was found to support the distinction of the Bangladesh bottlenose dolphins.

The haplotype network obtained for humpback dolphins showed 6 distinct haplogroups, each corresponding to a different geographical region (Figure 3). The Bangladesh dolphins appear clearly distinct from all the other regions, with the exception of a single haplotype from the sample collected from a stranding in Cox’s Bazaar in the far south of Bangladesh close to the border with Myanmar that clustered with samples from Thailand. All other haplogroups had been reported before (Mendez et al. 2013). Of interest is also the distinct clustering of the assemblage from Southeast Africa and Oman. The relationships obtained in the Bayesian phylogenetic tree estimated with the mitochondrial control region sequences also showed distinct clusters that correspond to different geographical regions (Suppl. Fig. 4). Samples from Bangladesh appear as a distinct cluster, with a high posterior probability value (0.97) and as sister group to the Australian samples (*S. sahulensis*). The remaining geographical regions appear in separate distinct groups in a different clade, which is not well supported (posterior probability 0.53), suggesting that phylogenetic relationships among these assemblages are not fully resolved.

The alignment of the haplotype sequences displaying the variable sites clearly shows the differences in polymorphisms between the three groups mentioned above for *T. aduncus* (Suppl. Fig. 1). There are 5 fixed nucleotide differences that can diagnose all Bangladesh sequences (with the haplotype from the Andaman Islands included) from
“Pacific” and “African” *T. aduncus*, with the exception of one haplotype, which is the one that clustered with the “Pacific” *T. aduncus* samples in the haplotype network and phylogenetic tree. Conversely, only two fixed nucleotide differences can diagnose all “African” *T. aduncus* sequences.

For humpback dolphins, the character matrix of the fixed nucleotide positions that define the different haplotypes obtained for the mitochondrial DNA control region clearly shows marked differences among the different assemblages (Suppl. Fig. 2). When the sample from the stranding in Cox’s Bazaar is excluded, four diagnostic sites (positions 93G, 94A, 135T and 393G) allow the diagnosis of all the Bangladesh samples.

**Discussion**

In this study we analysed Indo-Pacific bottlenose and humpback dolphins from Bangladesh, a part of the range of these dolphins that has not been extensively included in earlier genetic studies even though these waters appear to be a transition point in the distribution of both the “African” and “Pacific” *T. aduncus*, and *S. plumbea* and *S. chinensis* populations. The results indicate that both bottlenose and humpback dolphins in Bangladesh are genetically distinct from neighbouring populations to east and the west. We suggest that the high biological productivity of this region may be driving or maintaining genetic differentiation in these species, even if historical events may have also played a role.

**Bottlenose dolphins**

Genetic differences between the Bangladesh, “African” and “Pacific” bottlenose dolphin populations, suggest little or no gene flow among all three populations and
that they constitute different phylogenetic units according to mtDNA, as has been previously suggested for the “African” and “Pacific” populations (Natoli et al. 2004; Sarnblad et al. 2011). The Bangladesh dolphins showed a relatively low level of haplotype diversity, but a high level of nucleotide diversity. This pattern is consistent with a scenario where a small population becomes isolated from a source population with high levels of genetic diversity and it has been previously described for Indo-Pacific bottlenose populations in other areas of their distribution (Natoli et al. 2004; Sarnblad et al. 2011; Oremus et al. 2015). These dolphins form small, isolated populations that can lead to lower levels of genetic diversity when compared to other more oceanic species like the common bottlenose dolphin, *T. truncatus* (Natoli et al. 2004). A lower haplotype diversity and higher nucleotide diversity can also indicate either a bottleneck or a founder event in the population when haplotypes were lost. The data analysed in this study do not allow for the distinction between these different scenarios. However, the localized occurrence of the bottlenose dolphins in Bangladesh straddles fairly shallow (19m) to deep-water (>200m) habitat about 30 km offshore at the head of the SoNG and they are absent in shallow water closer to shore despite extensive survey effort (Smith, unpublished sightings), the latter of which is more typical of habitat occupied by *T. aduncus* in other areas of their range (Wang 2009). This implies that the concentrated productivity created by upwelling currents found along the canyon edge may have promoted local adaptation and reproductive isolation.

**Humpback dolphins**

Levels of genetic divergence indicate that humpback dolphins from Bangladesh are as different from the other putative *Sousa* species as well as the recently described *S.*
The clearly distinct haplogroups seen in both the haplotype network and phylogenetic tree support this hypothesis. With the exception of the single sample from the far south outside of freshwater influence from the GBM River system (Cox’s Bazaar), four fixed sites in the mitochondrial control region diagnose all the Bangladesh samples from all the others groups that had been described in a previous publication (Mendez et al. 2013). Phylogenetically, the results from mitochondrial DNA analysis suggest that these dolphins are more closely related to *S. sahulensis* than to the other putative species, which group into a separate clade. While these mitochondrial DNA results are considerably well supported, pointing to species-level differentiation, additional information such as those from nuclear markers is required for a thorough evaluation of taxonomic status. Given the current findings, these animals are provisionally considered a highly differentiated group of animals within *S. chinensis*. Throughout their range, humpback dolphins are generally found in groups of less than 10 with a maximum group size of 30 individuals (Parra and Ross 2009). From dorsal fin photographs, 205 non-calf individuals were identified in a single group in Bangladesh. The actual group size was undoubtedly greater considering the estimated proportion of unmarked non-calf individuals (26%) plus the estimated proportion of calves (12%). This suggests that the actual group size was around 330. Other large groups were also occasionally observed in Bangladesh with 95 and 110 individuals estimated (Mansur et al., unpublished data.). The ecological and/or social reason(s) for these sporadic sightings of large groups are unknown but may be related to the clumped nature of estuarine prey driven by the complex dynamics of freshwater flow, and marine currents and tides.

Drivers of marine endemism
Extraordinary oceanographic, shallow water and ecological conditions in the geographic cul-de-sac of the northern Bay of Bengal include the intrusion of dynamic freshwater and sediment flow from among the world’s largest river systems, leaf litter and other bio-productivity from the world’s largest mangrove forest, a seasonally reversing current gyre with associated meso-eddies, and upwelling at the head of the SoNG submarine canyon. Together these relatively rare global conditions, both in terms of occurrence and size, almost certainly explain the genetic distinctiveness found in both bottlenose and humpback dolphin populations. Interestingly, a previously un-described likely new species of “river shark,” deep divergent from all other lineages in the Glyphis genus, was recently discovered (Li et al. 2015) occupying the same euryhaline waters as humpback dolphins in Bangladesh. This probably new species of “river shark” is located in the middle of the range of the Critically Endangered G. gangeticus (Compagno 2007), now known to extend both to the east and west in Myanmar and India, respectively, with another un-described species occurring in Sarawak, Malaysian Borneo as sister taxon to the “river sharks” in Bangladesh (Li et al. 2015). A maximum-likelihood tree, based on the protein-coding portion of the mitochondrial genome, suggests marine dispersal of these “river sharks” and that the northern Bay of Bengal was the original source of evolutionary radiation for the genus. Perhaps not surprisingly, mitochondrial control region sequences shown in the maximum likelihood tree for T. aduncus (see Suppl. Fig. 3) and the Bayesian phylogenetic tree for Sousa spp. (Suppl. Fig. 4) in Bangladesh indicate that both forms occupy a similarly basal, or near basal, position in their respective genera.

Several marine species that occur in the Indo-West Pacific Ocean have been found to have distinct genetic lineages in the east and the west, such as those seen in the
dolphin species studied here (e.g. Benzie et al. 2002, Keyse et al. 2014). This phylogeographic pattern may have resulted from restricted connectivity of populations across the Sunda shelf (southeast extension of the continental shelf of Southeast Asia comprising the Malay Peninsula, Sumatra, Borneo, Java and Bali) during periods of low sea level in the glacial periods of the Pleistocene (Voris 2000).

Oceanographic variables have been shown to drive population differentiation not only in humpback dolphins along the Western Indian Ocean (Mendez et al. 2011) but also in other cetacean species, such as bottlenose dolphins (Bilgmann et al. 2007), common dolphins (Amaral et al. 2012) and franciscana dolphins (Mendez et al. 2010).

The same situation has been observed in other high and low dispersal marine animals (e.g. Saha et al. 2015; Young et al. 2015; Liggins et al. 2016) suggesting that environmental factors lead to, and establish genetic structure in marine organisms leading to speciation and endemism in some cases.

Study limitations and potential biases

A limitation of our study was the relatively low number of tissue samples that were available to be analysed. This can be explained by the challenging logistics of conducting field surveys for cetaceans in the coastal waters of Bangladesh, an extremely short field season when surveys can be conducted in these waters (November to February) due to poor weather conditions and danger from cyclones, and funding constraints. For humpback dolphins, a particular challenge was finding animal groups (our mean encounter rate was only 0.16 groups/hour of survey effort) and then acquiring a sample from dolphin that exhibit erratic surfacing patterns, actively avoid boats and are only available on the surface for generally less than one second (as measured by camera motor drive photo sequences). Also, the turbid waters
where humpback dolphins are found in Bangladesh (12.8 nephelometric turbidity units measured during 56 sightings) make it impossible to anticipate their surfacing location before their body appears above the surface which is a major advantage when biopsy darting cetaceans. The success rate for obtaining a sample for humpback dolphins was 0.03/shot whereas for bottlenose dolphins, which occupy much clearer waters in the SoNG and often approach vessels to ride the bow, the success rate was 0.27/shot.

A potential bias of our study was the representativeness of the tissue samples especially if they were obtained from a single matrilineal group or if the same individual was sampled multiple times and therefore included in the genetic data set more than once. Although significant uncertainties exist about the social structure of humpback and bottlenose dolphins, to the best of the authors knowledge there is no evidence to suggest that any *Sousa* or *Tursiops* population that has been studied exhibits a strong matrilineal social structure. Also samples of bottlenose dolphins were obtained from eight groups with a mean group size of 73.5 individuals (SD=29.6, range=18-105) and of humpback dolphins from seven groups with a mean group size of 51.5 individuals (SD=59.9, range=2-160). This makes it highly unlikely that individuals were sampled more than once.

An additional limitation of this study is the sole use of mitochondrial DNA control region to infer phylogeographic patterns of the two studied species. This particular marker was chosen due to the availability of sequences from Indo-Pacific humpback and bottlenose dolphins from different geographic areas that could be used in a broader study. Given the strong and statistically highly significant patterns we obtained we consider that using additional markers would not have resulted in a different interpretation, although we acknowledge that the phylogeographic patterns
obtained represent those of the maternal lineages.

Conservation Implications

The discovery of the apparent new species of “river shark” together with the results of our genetic study on humpback and bottlenose dolphins imply that extraordinary oceanographic and habitat conditions in the northern Bay of Bengal have been driven megafauna endemism and they been a source of genetic radiation in the greater Indo-Pacific. These findings also imply that areas of the world exhibiting similar oceanographic features (large river mouths, offshore mangrove forests, embayment cul-de-sacs, reversing wind-driven currents, and river-eroded submarine canyons) should be prioritized for protecting phylogenetically distinct megafauna.

There is uncertainty about the range of these newly discovered phylogenetically unique dolphin populations in Bangladesh. However, robust mark-resight analyses of bottlenose (1,144 individuals) and humpback (468 individuals) dolphin photocatalogs from the head of the SoNG and estuarine waters offshore of the Sundarbans mangrove forest, respectively, indicate that both populations occupy a larger area than sampled during photoidentification (and biopsy collection) surveys in the northern Bay of Bengal, Bangladesh. This larger area almost certainly includes euryhaline waters to the east in Ganges-Brahmaputra-Meghna River mouth and to the west across the border with India also offshore of the Sundarbans mangrove forest and including the southwestern portion of the SoNG submarine canyon.

Declaration of Bangladesh’s first marine protected area in October 2014 to safeguard cetaceans and elasmobranches in 1,738 km² of coastal waters offshore the Sundarbans mangrove forest and at the head of the SoNG submarine canyon is a critical first step in protecting these threatened endemic, marine mammals occurring in a globally rare
oceanographic environment characterized by an extreme infusion, redistribution and recycling of biological productivity.

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References


Figure Captions

Fig. 1 Map showing the coastal waters of Bangladesh where the tissue samples of Indo-Pacific humpback and bottlenose dolphins were collected by biopsy darter offshore the Sundarbans mangrove forest and where one sample was collected from a stranding of a single humpback dolphin in Cox's Bazaar.

Fig. 2 Median-joining haplotype network of the mitochondrial control region sequences obtained for the Indo-Pacific bottlenose dolphin, T. aduncus. Circle size is proportional to the number of individuals exhibiting the corresponding haplotype and proportional of each population within each haplotype is coloured according to the legend. Length of lines is proportional to the number of mutational steps separating haplotypes. White circles indicate missing intermediate haplotypes. Insert map shows location of the different geographical region within the Indo-West Pacific Ocean analysed in this study.
**Fig. 3** Median-joining haplotype network of the mitochondrial control region sequences obtained for *Sousa* spp. Circle size is proportional to the number of individuals exhibiting the corresponding haplotype and proportional of each population within each haplotype is coloured according to the legend. Length of lines is proportional to the number of mutational steps separating haplotypes. White circles indicate missing intermediate haplotypes. Insert map shows location of the different geographical region within the Indo-West Pacific Ocean analysed in this study.
### Tables

**Table 1.** Genetic diversity measures for regional humpback dolphin samples (WA – West Africa; SEA – Southeast Africa; OM – Oman; BAN – Bangladesh; TH – Thailand; CH – China; AUS – Australia).  

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>h (SD)</th>
<th>Hd (SD)</th>
<th>π (SD)</th>
<th>k (SD)</th>
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<tbody>
<tr>
<td>WA</td>
<td>6</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>SEA</td>
<td>39</td>
<td>8</td>
<td>0.82 (0.03)</td>
<td>0.04 (0.02)</td>
<td>2.79 (1.51)</td>
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<tr>
<td>OM</td>
<td>58</td>
<td>10</td>
<td>0.79 (0.03)</td>
<td>0.11 (0.06)</td>
<td>7.29 (3.46)</td>
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<td><strong>BAN</strong></td>
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<td><strong>9</strong></td>
<td><strong>0.88 (0.06)</strong></td>
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<td>0.01 (0.01)</td>
<td>0.93 (0.71)</td>
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<td>8</td>
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<td>0.02 (0.01)</td>
<td>1.41 (0.87)</td>
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<tr>
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<td>4</td>
<td>0.55 (0.04)</td>
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<td>3.73 (1.94)</td>
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*N*, number of individuals; *h*, number of haplotypes; *Hd* – Haplotype diversity; *π*, nucleotide diversity; *k* – average number of nucleotide differences; SD – standard deviation.

**Table 2.** Net divergence (*dA*, below diagonal) and mean gross divergence (*dx,y*, above diagonal) estimated between the different *T. aduncus* geographical regions. All values were statistically significant (*P*<0.05). EAFR – East Africa; BAN – Bangladesh; IND – Indonesia; CH – China; MEL – Melanesia; and AUS – Australia. Grey area highlights comparisons within “Pacific” *T. aduncus* populations.

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Table 3. Pairwise $F_{ST}$ (below diagonal) and $\phi_{ST}$ (above diagonal) values for the different *Sousa* taxa from the geographical regions studied. (WA – West Africa; SEA – Southeast Africa; OM – Oman; BAN – Bangladesh; TH – Thailand; CH – China; AUS – Australia). All values were statistically significant ($P < 0.001$).

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<th></th>
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<th>OM</th>
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**Supplementary File Information:**

- **Suppl. Fig. 1.** Character matrix depicting the mitochondrial control region polymorphisms that define the different *T. aduncus* haplotypes.
- **Suppl. Fig. 2.** Character matrix depicting the mitochondrial control region polymorphisms that define the different *Sousa* spp. haplotypes.
- **Suppl. Fig. 3** Maximum-likelihood phylogenetic tree obtained for the mitochondrial DNA control regions sequences of *T. aduncus*. Values above branches correspond to bootstrap support values.
- **Suppl. Fig. 4** Bayesian phylogenetic tree obtained for the humpback dolphin mitochondrial control region sequences. Values above branches represent the posterior probability values. The different geographical assemblages are represented...
in different colours: purple – Bangladesh; Blue – Australia; Red – West Africa; light green – Southeast Africa; dark green – Oman; yellow – China; orange – Thailand.

Suppl. Table 1. Indo-Pacific bottlenose dolphin mitochondrial DNA control region sequences retrieved from GenBank.