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

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37 **Abstract**

38

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

61 particular and extreme oceanographic conditions found in the Bay of Bengal may be  
62 driving speciation in these dolphins and other marine megafauna.

63

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

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## 68 **Introduction**

69

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

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

84 In the Northern Indian Ocean, the Bay of Bengal is a tropical ocean basin influenced  
85 by discharge from the third-largest river system in the world – the Ganges/

86 Brahmaputra/Meghna (GBM). This massive influx of freshwater and nutrient rich  
87 sediments sustains the world's largest continuous mangrove forest (Hussain and  
88 Acharya 1994) and has eroded a submarine canyon leading to the world's largest  
89 undersea sediment fan (Unger et al. 2003). Driven by regular flushing of rich silts and  
90 organic matter from mangrove litter falls (Islam 2003), the GBM supplies more than  
91 1.5% of the total riverine input to the world's oceans (Sarin et al. 1989). This  
92 enormous supply of freshwater, sediments and nutrients is circulated by a seasonally  
93 reversing, wind-driven, basin-scale gyre with adjacent meso-scale eddies (Somayajulu  
94 et al. 2003). These conditions combine to produce a highly stratified and productive  
95 sea-surface layer in shallow coastal waters that became available when the sea level  
96 rose 6,000 to 10,000 years ago. The minimum 50 m contour distance from shore is  
97 located in the far west where a 900+ metre-deep submarine canyon known as the  
98 Swatch-of-No- Ground (SoNG) incises to within about 35 km of edge of the  
99 Sundarbans mangrove forest. This canyon upwells nutrients supplied by the GBM  
100 system, which are concentrated at its head and then recycled back into the seasonally  
101 reversing current of euryhaline waters. The extreme infusion and redistributive  
102 dynamism of biological productivity in the northern Bay of Bengal is a rare ecological  
103 condition that supports cetaceans in numbers generally much larger than other  
104 populations in the region (Smith et al. 2008; Mansur et al. 2012).

105

#### 106 Bottlenose dolphins

107 The waters at the head of the SoNG support one of the world's largest populations of  
108 Indo-Pacific bottlenose dolphins, *Tursiops aduncus* (Mansur et al. 2012). However,  
109 these dolphins are potentially threatened as they are taken as bycatch in coastal  
110 fisheries. Their distribution strongly overlaps with gill net fisheries and a large portion

111 (28.2%) of individuals identified from dorsal fin photographs exhibits scars and  
112 mutilations clearly related to entanglements in fishing gear (Mansur et al. 2012).

113 *T. aduncus* is distributed in coastal waters of the Indian and western Pacific Oceans,  
114 although the continuity of its distribution is unknown (Wang 2009). Genetic studies  
115 have found strong differences between populations occurring along the coast of  
116 Africa and those occurring in the Indo-West Pacific (China, Japan, Korea, Melanesia,  
117 Australia). These may represent different taxonomic units at species or subspecies  
118 levels (Wang et al. 1999; Natoli et al. 2004; Sarnblad et al. 2011; Oremus et al. 2015).  
119 Here we follow Oremus et al. 2015 by referring to these two forms as the “African” *T.*  
120 *aduncus* and the “Pacific” *T. aduncus*.

121 Little information is available on the population structure of Indo-Pacific bottlenose  
122 dolphins at smaller scales even though the species is believed to be composed of  
123 many small, fairly isolated populations due to their limited coastal distribution (but  
124 see Allen et al. 2006; Kopps et al. 2014). Sarnblad et al. 2011 found genetic  
125 differences between dolphins occurring in Northern and Southern Zanzibar,  
126 suggesting that differentiation may arise even across small geographic scales. Similar  
127 results were obtained with *T. aduncus* in Melanesia, where evidence of population  
128 structure was found between the Solomon Islands and New Caledonia (Oremus et al.  
129 2015). Historical circumstances and local adaptation have been suggested as potential  
130 factors driving the divergence of *T. aduncus* populations (Natoli et al. 2004; Sarnblad  
131 et al. 2011).

132

### 133 Humpback dolphins

134 Humpback dolphins of the genus *Sousa* are distributed discontinuously in coastal  
135 waters of West Africa and in the Indian and Western Pacific Oceans (Parra and Ross

136 2009). A recent exhaustive review of multiple lines of evidence from skeletal  
137 morphology, external morphology, coloration, molecular genetics, and biogeography  
138 provided strong support for the recognition of four species (Mendez et al. 2013;  
139 Jefferson and Rosenbaum 2014). This scenario has been accepted by the Society of  
140 Marine Mammology Committee on Taxonomy (2014) with *S. chinensis* distributed in  
141 the Eastern Indian and Western Pacific Oceans, *S. plumbea* in the Western Indian  
142 Ocean, *S. teuszii* in the Eastern Atlantic Ocean along the west African coast and *S.*  
143 *sahulensis* in Northern Australia (Jefferson and Rosenbaum 2014). However, the  
144 exact ranges of *S. chinensis* and *S. plumbea* in the Bay of Bengal are poorly known  
145 (Jefferson and Smith 2016).

146 The most comprehensive genetic study conducted to date, which included samples  
147 from throughout the range of the genus, found high levels of divergence in both  
148 mitochondrial and nuclear DNA markers separating dolphins at the species level from  
149 West Africa (*S. teuszii*), Southeast Africa, Arabia-Oman and the Indian subcontinent  
150 (*S. plumbea*), Thailand and China (*S. chinensis*) and Australia (later described as *S.*  
151 *sahulensis* in Jefferson and Rosenbaum 2014) (Mendez et al. 2013). One dolphin from  
152 Bangladesh showed remarkable differences in the mitochondrial DNA, with its  
153 placement in phylogenetic trees as distant as those from Australia (Mendez et al.  
154 2013).

155 Analyses conducted to date suggest strong genetic population structure within both *S.*  
156 *plumbea* and *S. chinensis*. Populations of *S. plumbea* in Oman and Tanzania show  
157 remarkable differentiation compared with populations in South Africa and  
158 Mozambique (Mendez et al. 2011; Mendez et al. 2013). Oceanographic features such  
159 as sea surface temperature and primary productivity were found to be among the  
160 drivers leading to differences between populations. *S. chinensis* populations in China

161 also show a high degree of divergence (Chen et al. 2008; Chen et al. 2010). Large  
162 portions of the range of both species have yet to be sampled. However, their  
163 distributional limit is believed to be the east coast of India.

164 Humpback dolphins observed along the west coast of India (Arabian Sea) have a large  
165 hump and appear dark grey, thus resembling *S. plumbea*, while those observed along  
166 the east coast of India in the Bay of Bengal do not have a distinct hump and are much  
167 lighter in color, thus resembling *S. chinensis* (Sutaria and Jefferson 2004). However,  
168 animals exhibiting the *plumbea*-type coloration, but without an obvious hump, have  
169 been observed as far east as the Mergui Archipelago, Myanmar (Smith and Tun  
170 2008), suggesting that both forms may be sympatric in the Bay of Bengal. Given the  
171 genetic distinctiveness of the one animal sampled off Bangladesh (Mendez et al.  
172 2013), it is particularly important to clarify the phylogenetic position of humpback  
173 dolphins in the northern Bay of Bengal.

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

179

#### 180 Relating genetic differentiation and oceanography

181 A clear understanding of demographically isolated populations and the factors leading  
182 to their isolation is vitally important for determining biologically relevant  
183 conservation units and guiding efforts to protect them from extinction. This study  
184 aims to identify the phylogeographic affinity of Indo-Pacific bottlenose and  
185 humpback dolphins occurring in the waters of Bangladesh (northern Bay of Bengal),

186 and relate this to the extraordinary ecological and oceanographic conditions found in  
187 these waters. In particular we intend to understand if Indo-Pacific bottlenose and  
188 humpback dolphins from this region are genetically different from neighbouring  
189 populations. To accomplish this, we sequenced a fragment of the mitochondrial DNA  
190 control region and conducted relevant analyses with published sequences available in  
191 GenBank from other Indo-Pacific humpback and bottlenose dolphin populations. For  
192 humpback dolphins we included sequences from *S. teuszii* in the Atlantic Ocean. For  
193 *T. aduncus* we included sequences from South Africa (Natoli et al. 2004), Zanzibar  
194 (Sarnblad et al. 2011), India and Australia (Moller and Beheregaray 2001), Indonesia,  
195 and China (Wang et al. 1999), and Melanesia (Oremus et al. 2015).

196

## 197 **Material and Methods**

### 198 Sampling

199 A total of 17 Indo-Pacific bottlenose dolphin samples from Bangladesh were obtained  
200 through minimally invasive biopsy darting using a cross bow in coastal waters  
201 offshore the Sundarbans mangrove forest. A single tooth was also obtained from a  
202 mandible collected in the Andaman Islands (eastern part of the Bay of Bengal) in  
203 1889. This mandible (3406) is in the collection of the Natural History Museum of the  
204 University of Florence, Italy (Museo di Storia Naturale dell'Università degli Studi di  
205 Firenze). To include these newly generated sequences in a broad phylogeographic  
206 analyses of *T. aduncus*, sequences encompassing different geographical regions  
207 corresponding to “African” *T. aduncus* and “Pacific” *T. aduncus* were retrieved from  
208 GenBank (Suppl. Table 1).

209 Similarly, a total of 15 humpback dolphin samples were obtained from the northern  
210 Bay of Bengal, Bangladesh, 14 from minimally invasive biopsy darting using a

211 crossbow in coastal waters offshore the Sundarbans mangrove forest and one from a  
212 stranding in Cox's Bazaar in the far south of the country close to the border with  
213 Myanmar (Figure 1). These samples were included in a broader dataset comprising of  
214 234 humpback dolphins that had already been analyzed by Mendez *et al.* (2013) and  
215 include the following regions: West Africa (WA,  $n=6$ ), Southeast Africa (SEA,  $n=38$ ),  
216 Oman (OM,  $n=58$ ), Thailand (TH,  $n=8$ ), India ( $n=3$ ), China (CH,  $n=92$ ), Australia  
217 (AUS,  $n=28$ ) and Bangladesh (BAN,  $n=1$ ).

218

#### 219 Laboratory procedures

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

232 Sequences were separated and subsequently analysed in two different sets, one  
233 corresponding to Indo-Pacific bottlenose dolphins and another one to humpback  
234 dolphins. Therefore, all the analyses mentioned below were applied to both datasets,  
235 with any exception detailed.

236 DNA sequences were inspected, edited and aligned by eye in Sequencher 5.0.1 (Gene  
237 Codes, Corp.). Sequences were collapsed into haplotypes using DNAsp v. 5.10  
238 (Librado and Rozas 2009). Diversity measures (nucleotide and haplotype diversities  
239 and the average number of nucleotide differences) were estimated in DNAsp.  
240 Potential differences among populations in different geographical regions were tested  
241 by calculating pairwise  $F_{ST}$  (using haplotype frequencies) and  $\phi_{ST}$  (using genetic  
242 distance) in Arlequin v. 3.5. (Excoffier and Lischer 2010) for the humpback dolphin  
243 dataset. For the bottlenose dolphin dataset, since only haplotype sequences were  
244 available, we assessed genetic differences between the Bangladesh bottlenose  
245 dolphins and bottlenose dolphins from other regions, using the net average,  $d_A$ , and  
246 the mean gross,  $d_{xy}$ , distances as estimated by  $d_A = d_{XY} - (d_X + d_Y)/2$ , where  $d_X$  and  $d_Y$   
247 are the mean within group distances, in the software MEGA v. 6.. with 5000 bootstrap  
248 replicates (Tamura et al. 2013). The best model of nucleotide substitution for the  
249 dataset was determined using the Akaike Information Criterion (AIC) as implemented  
250 in the program jModeltest v. 2.1.7 (Darriba et al. 2012; Guindon and Gascuel 2003).  
251 The model selected was HKY with a proportion of invariable sites and a gamma-  
252 shaped distribution of rates across sites ( $G=0.429$ ). This model was therefore used to  
253 calculate  $d_A$  and  $d_{xy}$ .

254 Phylogeographic analyses of both bottlenose and humpback dolphin datasets included  
255 a median-joining network of haplotypes constructed in NETWORK v. (Bandelt et al.  
256 1999) and a Bayesian phylogenetic tree obtained in MrBayes v. 3.1.2. (Huelsenbeck  
257 and Ronquist 2001). For the latter analysis, four simultaneous MCMC chains were  
258 run for 2 million generations, with trees sampled at intervals of 100 generations. The  
259 first 3,000 trees were discarded as “burn-in”. A sequence of *Steno bredanensis* was  
260 used as outgroup (GenBank Accession Number KM260657). For the bottlenose

261 dolphin dataset a Maximum-likelihood method was also used to estimate a  
262 phylogenetic tree using the program MEGA v. 6.0.6 (Tamura et al. 2013), with the  
263 HKY model as the nucleotide substitution model and the Nearest-Neighbor-  
264 Interchange Heuristic method with Branch Swap Filter. One thousand bootstrap  
265 replicates were run to assess robustness of the phylogeny estimated.

266

## 267 **Results**

### 268 Genetic diversity

269 In total, 380 bp of the mitochondrial DNA control region were sequenced and  
270 analysed for the 17 samples obtained for the Bangladesh bottlenose dolphins and for  
271 the tooth sample from the Andaman Islands, which grouped into 7 haplotypes  
272 (GenBank Accession Numbers KX364257-KX364263). The estimated haplotypic  
273 diversity ( $0.699 \pm 0.117$ ) was relatively low, but similar to values obtained for South  
274 Africa, Zanzibar and Australia populations (Sarnblad et al. 2011). Conversely, the  
275 estimated nucleotide diversity ( $0.009 \pm 0.005$ ) was relatively high and similar to  
276 values obtained for China/Taiwan and the Solomon Islands populations (Oremus et al.  
277 2015).

278 A fragment of 456 bp of the mitochondrial DNA control region was sequenced for 15  
279 humpback dolphins from Bangladesh. These sequences were aligned with an already  
280 existent dataset comprising most of the genus distribution (Mendez et al. 2013).  
281 Samples from Bangladesh grouped into 9 haplotypes (GenBank Accession Numbers  
282 KX364242-KX364256) and showed the highest levels of genetic diversity when  
283 compared to the other geographical regions analysed when haplotypic diversity and  
284 the average number of nucleotide differences are considered (Table 1). There were no  
285 shared haplotypes between samples from Bangladesh and the other regions.

286 Genetic Differentiation

287 The net average,  $d_A$ , and the mean gross,  $d_{xy}$ , genetic divergence estimates between  
288 the different bottlenose dolphin groups included in this study indicate differences  
289 between the Bangladesh population and all others (Table 2). Values for  $d_A$  varied  
290 between 0.05 and 0.08 and  $d_{xy}$  values varied between 0.06 and 0.09. Values of the  
291 same order of magnitude were also obtained in comparisons between “African” and  
292 “Pacific” *T. aduncus*, suggesting that the three bottlenose dolphins groups are  
293 genetically divergent. Comparisons among the different populations within the  
294 “Pacific” *T. aduncus* group showed much lower levels of divergence (Table 2).

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

300

301 Phylogeography

302 The haplotype network and phylogenetic tree obtained for bottlenose dolphins showed  
303 the presence of three distinct clusters: one corresponding to the Bangladesh  
304 population (including the Andaman Islands specimen) and the other two to the  
305 “African” and “Pacific” *T. aduncus* populations that had already been reported in  
306 previous studies (e.g. Oremus et al. 2015). There were no shared haplotypes among  
307 these three groups (Figure 2 and Suppl. Fig.3). The only shared haplotypes were  
308 within the “Pacific” *T. aduncus* haplogroup. One haplotype was shared between  
309 China and Australia, one shared between Melanesia and Australia and the other one  
310 shared between Indonesia and China. There was one haplotype from Bangladesh that

311 grouped with the “Pacific” *T. aduncus*” group. In the phylogenetic trees obtained with  
312 Bayesian inference (not shown) and maximum-likelihood (Suppl. Fig. 3) methods,  
313 although there was no resolution of the sister taxa relationship among groups (i.e. it  
314 was not possible to infer which group originated first), a high posterior probability  
315 (>95%) and bootstrap value was found to support the distinction of the Bangladesh  
316 bottlenose dolphins.

317 The haplotype network obtained for humpback dolphins showed 6 distinct  
318 haplogroups, each corresponding to a different geographical region (Figure 3). The  
319 Bangladesh dolphins appear clearly distinct from all the other regions, with the  
320 exception of a single haplotype from the sample collected from a stranding in Cox’s  
321 Bazaar in the far south of Bangladesh close to the border with Myanmar that clustered  
322 with samples from Thailand. All other haplogroups had been reported before (Mendez  
323 et al. 2013). Of interest is also the distinct clustering of the assemblage from  
324 Southeast Africa and Oman. The relationships obtained in the Bayesian phylogenetic  
325 tree estimated with the mitochondrial control region sequences also showed distinct  
326 clusters that correspond to different geographical regions (Suppl. Fig. 4). Samples  
327 from Bangladesh appear as a distinct cluster, with a high posterior probability value  
328 (0.97) and as sister group to the Australian samples (*S. sahulensis*). The remaining  
329 geographical regions appear in separate distinct groups in a different clade, which is  
330 not well supported (posterior probability 0.53), suggesting that phylogenetic  
331 relationships among these assemblages are not fully resolved.

332 The alignment of the haplotype sequences displaying the variable sites clearly shows  
333 the differences in polymorphisms between the three groups mentioned above for *T.*  
334 *aduncus* (Suppl. Fig. 1). There are 5 fixed nucleotide differences that can diagnose all  
335 Bangladesh sequences (with the haplotype from the Andaman Islands included) from

336 “Pacific” and “African” *T. aduncus*, with the exception of one haplotype, which is the  
337 one that clustered with the “Pacific” *T. aduncus* samples in the haplotype network and  
338 phylogenetic tree. Conversely, only two fixed nucleotide differences can diagnose all  
339 “African” *T. aduncus* sequences.

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

346

#### 347 **Discussion**

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

357

#### 358 Bottlenose dolphins

359 Genetic differences between the Bangladesh, “African” and “Pacific” bottlenose  
360 dolphin populations, suggest little or no gene flow among all three populations and

361 that they constitute different phylogenetic units according to mtDNA, as has been  
362 previously suggested for the “African” and “Pacific” populations (Natoli et al. 2004;  
363 Sarnblad et al. 2011). The Bangladesh dolphins showed a relatively low level of  
364 haplotype diversity, but a high level of nucleotide diversity. This pattern is consistent  
365 with a scenario where a small population becomes isolated from a source population  
366 with high levels of genetic diversity and it has been previously described for Indo-  
367 Pacific bottlenose populations in other areas of their distribution (Natoli et al. 2004;  
368 Sarnblad et al. 2011; Oremus et al. 2015). These dolphins form small, isolated  
369 populations that can lead to lower levels of genetic diversity when compared to other  
370 more oceanic species like the common bottlenose dolphin, *T. truncatus* (Natoli et al.  
371 2004). A lower haplotype diversity and higher nucleotide diversity can also indicate  
372 either a bottleneck or a founder event in the population when haplotypes were lost.  
373 The data analysed in this study do not allow for the distinction between these different  
374 scenarios. However, the localized occurrence of the bottlenose dolphins in  
375 Bangladesh straddles fairly shallow (19m) to deep-water (>200m) habitat about 30  
376 km offshore at the head of the SoNG and they are absent in shallow water closer to  
377 shore despite extensive survey effort (Smith, unpublished sightings), the latter of  
378 which is more typical of habitat occupied by *T. aduncus* in other areas of their range  
379 (Wang 2009). This implies that the concentrated productivity created by upwelling  
380 currents found along the canyon edge may have promoted local adaptation and  
381 reproductive isolation.

382

### 383 Humpback dolphins

384 Levels of genetic divergence indicate that humpback dolphins from Bangladesh are as  
385 different from the other putative *Sousa* species as well as the recently described *S.*

386 *sahulensis*. The clearly distinct haplogroups seen in both the haplotype network and  
387 phylogenetic tree support this hypothesis. With the exception of the single sample  
388 from the far south outside of freshwater influence from the GBM River system (Cox's  
389 Bazaar), four fixed sites in the mitochondrial control region diagnose all the  
390 Bangladesh samples from all the others groups that had been described in a previous  
391 publication (Mendez et al. 2013). Phylogenetically, the results from mitochondrial  
392 DNA analysis suggest that these dolphins are more closely related to *S. sahuensis*  
393 than to the other putative species, which group into a separate clade. While these  
394 mitochondrial DNA results are considerably well supported, pointing to species-level  
395 differentiation, additional information such as those from nuclear markers is required  
396 for a thorough evaluation of taxonomic status. Given the current findings, these  
397 animals are provisionally considered a highly differentiated group of animals within  
398 *S. chinensis*. Throughout their range, humpback dolphins are generally found in  
399 groups of less than 10 with a maximum group size of 30 individuals (Parra and Ross  
400 2009). From dorsal fin photographs, 205 non-calf individuals were identified in a  
401 single group in Bangladesh. The actual group size was undoubtedly greater  
402 considering the estimated proportion of unmarked non-calf individuals (26%) plus the  
403 estimated proportion of calves (12%). This suggests that the actual group size was  
404 around 330. Other large groups were also occasionally observed in Bangladesh with  
405 95 and 110 individuals estimated (Mansur et al., unpublished data.). The ecological  
406 and/or social reason(s) for these sporadic sightings of large groups are unknown but  
407 may be related to the clumped nature of estuarine prey driven by the complex  
408 dynamics of freshwater flow, and marine currents and tides.

409

410 Drivers of marine endemism

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

434 Several marine species that occur in the Indo-West Pacific Ocean have been found to  
435 have distinct genetic lineages in the east and the west, such as those seen in the

436 dolphin species studied here (e.g. Benzie et al. 2002, Keyse et al. 2014). This  
437 phylogeographic pattern may have resulted from restricted connectivity of  
438 populations across the Sunda shelf (southeast extension of the continental shelf of  
439 Southeast Asia comprising the Malay Peninsula, Sumatra, Borneo, Java and Bali)  
440 during periods of low sea level in the glacial periods of the Pleistocene (Voris 2000).  
441 Oceanographic variables have been shown to drive population differentiation not only  
442 in humpback dolphins along the Western Indian Ocean (Mendez et al. 2011) but also  
443 in other cetacean species, such as bottlenose dolphins (Bilgmann et al. 2007),  
444 common dolphins (Amaral et al. 2012) and franciscana dolphins (Mendez et al. 2010).  
445 The same situation has been observed in other high and low dispersal marine animals  
446 (e.g. Saha et al. 2015; Young et al. 2015; Liggins et al. 2016) suggesting that  
447 environmental factors lead to, and establish genetic structure in marine organisms  
448 leading to speciation and endemism in some cases.

449

#### 450 Study limitations and potential biases

451 A limitation of our study was the relatively low number of tissue samples that were  
452 available to be analysed. This can be explained by the challenging logistics of  
453 conducting field surveys for cetaceans in the coastal waters of Bangladesh, an  
454 extremely short field season when surveys can be conducted in these waters  
455 (November to February) due to poor weather conditions and danger from cyclones,  
456 and funding constraints. For humpback dolphins, a particular challenge was finding  
457 animal groups (our mean encounter rate was only 0.16 groups/hour of survey effort)  
458 and then acquiring a sample from dolphin that exhibit erratic surfacing patterns,  
459 actively avoid boats and are only available on the surface for generally less than one  
460 second (as measured by camera motor drive photo sequences). Also, the turbid waters

461 where humpback dolphins are found in Bangladesh (12.8 nephelometric turbidity  
462 units measured during 56 sightings) make it impossible to anticipate their surfacing  
463 location before their body appears above the surface which is a major advantage when  
464 biopsy darting cetaceans. The success rate for obtaining a sample for humpback  
465 dolphins was 0.03/shot whereas for bottlenose dolphins, which occupy much clearer  
466 waters in the SoNG and often approach vessels to ride the bow, the success rate was  
467 0.27/shot.

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

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

486 obtained represent those of the maternal lineages.

487

#### 488 Conservation Implications

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

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

507 Declaration of Bangladesh’s first marine protected area in October 2014 to safeguard  
508 cetaceans and elasmobranchs in 1,738 km<sup>2</sup> of coastal waters offshore the Sundarbans  
509 mangrove forest and at the head of the SoNG submarine canyon is a critical first step  
510 in protecting these threatened endemic, marine mammals occurring in a globally rare

511 oceanographic environment characterized by an extreme infusion, redistribution and  
512 recycling of biological productivity.

513

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527

528

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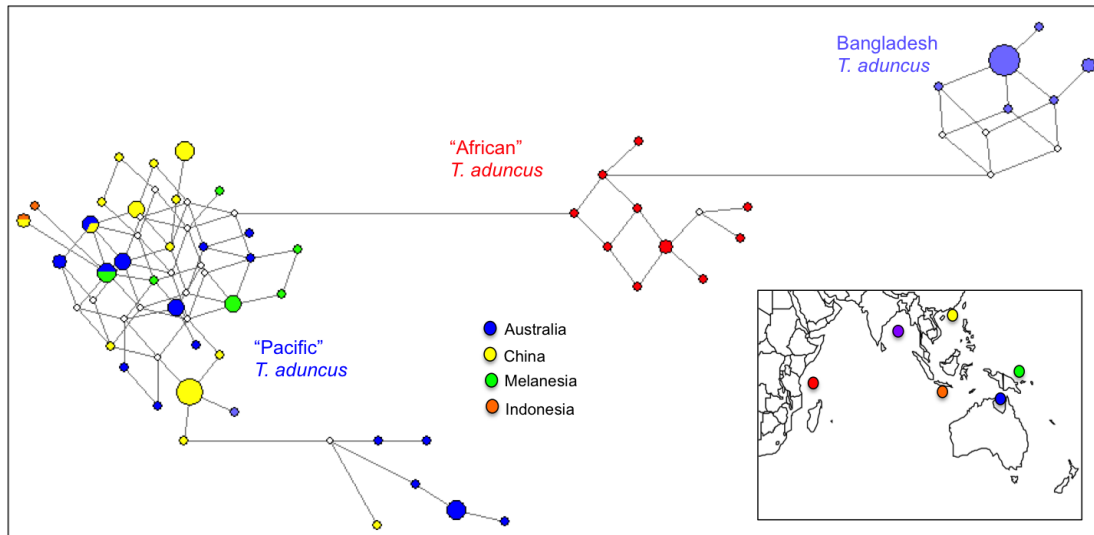
705 **Figure Captions**

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



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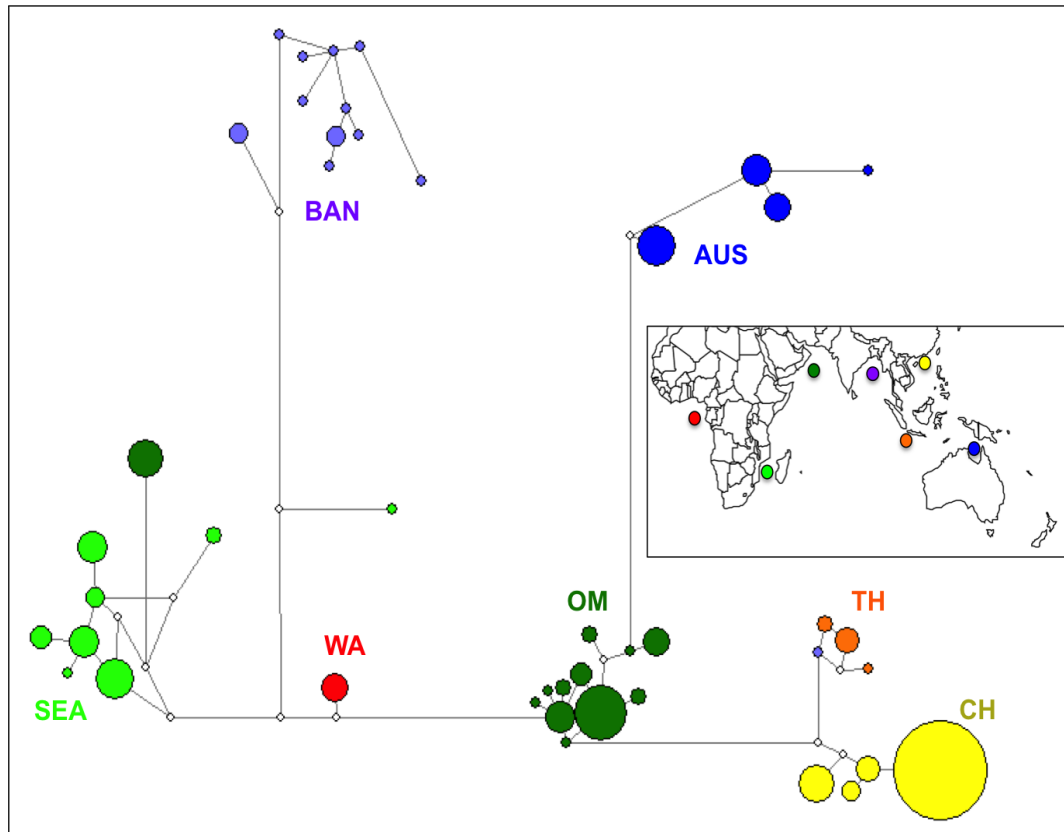
711 **Fig.2** Median-joining haplotype network of the mitochondrial control region  
712 sequences obtained for the Indo-Pacific bottlenose dolphin, *T. aduncus*. Circle size is  
713 proportional to the number of individuals exhibiting the corresponding haplotype and  
714 proportional of each population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



719

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

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729

730 **Tables**

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

Region	<i>N</i>	<i>h</i> (SD)	<i>Hd</i> (SD)	$\pi$ (SD)	<i>k</i> (SD)
WA	6	1	0 (0)	0 (0)	0 (0)
SEA	39	8	0.82 (0.03)	0.04 (0.02)	2.79 (1.51)
OM	58	10	0.79 (0.03)	0.11 (0.06)	7.29 (3.46)
<b>BAN</b>	<b>16</b>	<b>9</b>	<b>0.88 (0.06)</b>	<b>0.02 (0.00)</b>	<b>7.71 (1.80)</b>
TH	8	3	0.61 (0.16)	0.01 (0.01)	0.93 (0.71)
CH	91	8	0.34 (0.06)	0.02 (0.01)	1.41 (0.87)
AUS	23	4	0.55 (0.04)	0.05 (0.03)	3.73 (1.94)

734 *N*, number of individuals; *h*, number of haplotypes; *Hd* – Haplotype diversity;  $\pi$ , nucleotide  
 735 diversity; *k* – average number of nucleotide differences; SD – standard deviation.

736

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

742

African *T.*  
*aduncus*

Pacific *T. aduncus*

		EAFR	BAN	IND	CH	MEL	AUS
African <i>T. aduncus</i>	EAFR		0.064	0.079	0.058	0.062	0.062
	BAN	0.052		0.095	0.079	0.080	0.083
	IND	0.065	0.080		0.026	0.020	0.028
Pacific <i>T. aduncus</i>	CH	0.042	0.063	0.007		0.018	0.025
	MEL	0.050	0.068	0.005	0.002		0.023
	AUST	0.043	0.063	0.005	0.001	0.003	

743

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

	WA	SEA	OM	BAN	TH	CH	AUS
WA		0.793	0.483	<b>0.470</b>	0.700	0.744	0.835
SEA	0.445		0.496	<b>0.744</b>	0.859	0.861	0.803
OM	0.479	0.255		<b>0.544</b>	0.623	0.689	0.660
BAN	<b>0.470</b>	<b>0.195</b>	<b>0.242</b>		<b>0.263</b>	<b>0.531</b>	<b>0.326</b>
TH	0.700	0.278	0.326	<b>0.263</b>		0.632	0.432
CH	0.744	0.494	0.508	<b>0.531</b>	0.632		0.604
AUS	0.595	0.325	0.360	<b>0.326</b>	0.432	0.604	

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752 **Supplementary File Information:**

753 **Suppl. Fig. 1.** Character matrix depicting the mitochondrial control region

754 polymorphisms that define the different *T. aduncus* haplotypes.

755 **Suppl. Fig. 2.** Character matrix depicting the mitochondrial control region

756 polymorphisms that define the different *Sousa* spp. haplotypes.

757 **Suppl. Fig.3** Maximum-likelihood phylogenetic tree obtained for the mitochondrial

758 DNA control regions sequences of *T. aduncus*. Values above branches correspond to

759 bootstrap support values.

760 **Suppl. Fig.4** Bayesian phylogenetic tree obtained for the humpback dolphin

761 mitochondrial control region sequences. Values above branches represent the

762 posterior probability values. The different geographical assemblages are represented

763 in different colours: purple – Bangladesh; Blue – Australia; Red – West Africa; light  
764 green – Southeast Africa; dark green – Oman; yellow – China; orange – Thailand.

765

766 **Suppl. Table 1.** Indo-Pacific bottlenose dolphin mitochondrial DNA control region  
767 sequences retrieved from GenBank.  
768