# Genetic bottleneck and founder effect signatures in a captive population of common bottlenose dolphins *Tursiops truncatus* (Montagu 1821) in Mexico

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**Background.** The captive cetacean industry is very profitable and popular worldwide, focusing mainly on leisure activities such as "Swim-with-dolphins" (SWD) programs. However, there is a concern for how captivity could affect the bottlenose dolphin *Tursiops truncatus*, which in nature is a highly social and widespread species. To date, there is little information regarding to the impact of restricted population size on their genetic structure and variability.

**Methods.** The aim of this study was to estimate the genetic diversity of a confined population of *T. truncatus*, composed of wild-born (n=25) from Cuba, Quintana Roo and Tabasco, and captive-born (n=24) dolphins in southern Mexico, using the hypervariable portion of the mitochondrial DNA and ten nuclear microsatellite markers: TexVet3, TexVet5, TexVet7, D18, D22, Ttr19, Tur4_80, Tur4_105, Tur4_141 and GATA098.

**Results.** Exclusive mtDNA haplotypes were found in at least one individual from each wild-born origin populations and in one captive-born individual; total mean haplotype and nucleotide diversities were 0.912 (±0.016) and 0.025 (±0.013) respectively. At microsatellite loci, low levels of genetic diversity were found with a mean number of alleles per locus of 4 (±2.36), and an average expected heterozygosity over all loci of 0.544 (±0.163). Measures of allelic richness and effective number of alleles were similar between captive-born and wild-born dolphins. No significant genetic structure was found with microsatellite markers, whereas the mtDNA data revealed a significant differentiation between wild-born organisms from Cuba and Quintana ROO.

**Discussion.** Data analysis suggests the occurrence of a recent genetic bottleneck in the confined population probably because of a strong founder effect, given that only a small number of dolphins with a limited fraction of the total species genetic variation were selected at random to start this captive population. The results herein provide the first genetic baseline information on a captive bottlenose dolphin population in Mexico.

1    **Short title:** Améndola-Pimenta et al; Genetic drift in captive dolphins.

2

3    **Genetic bottleneck and founder effect signatures in a captive population of common**

4    **bottlenose dolphins *Tursiops truncatus* (Montagu 1821) in Mexico**

5

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21

22  **Abstract:**

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29  *T. truncatus*, composed of wild-born (n=25) from Cuba, Quintana Roo and Tabasco, and captive-

30  born (n=24) dolphins in southern Mexico, using the hypervariable portion of the mitochondrial

31  DNA and ten nuclear microsatellite markers: TexVet3, TexVet5, TexVet7, D18, D22, Ttr19,

32  Tur4_80, Tur4_105, Tur4_141 and GATA098.

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34  origin populations and in one captive-born individual; total mean haplotype and nucleotide

35  diversities were 0.912 ($\pm$0.016) and 0.025 ($\pm$0.013) respectively. At microsatellite loci, low

36  levels of genetic diversity were found with a mean number of alleles per locus of 4 ($\pm$2.36), and

37  an average expected heterozygosity over all loci of 0.544 ($\pm$0.163). Measures of allelic richness

38  and effective number of alleles were similar between captive-born and wild-born dolphins. No

39  significant genetic structure was found with microsatellite markers, whereas the mtDNA data

40  revealed a significant differentiation between wild-born organisms from Cuba and Quintana

41  ROO.

42  **Discussion.** Data analysis suggests the occurrence of a recent genetic bottleneck in the confined

43  population probably because of a strong founder effect, given that only a small number of

44  dolphins with a limited fraction of the total species genetic variation were selected at random to

45   start this captive population. The results herein provide the first genetic baseline information on a

46   captive bottlenose dolphin population in Mexico.

47

48   **Introduction**

49   According to the World Association of Zoos and Aquariums (WAZA), the most important

50   function of modern zoos and aquariums is to protect vulnerable and endangered species through

51   the promotion of activities in three broad fields: recreation, conservation and education/scientific

52   research (WAZA, 2006). Still, despite their potential to inspire positive emotions in visitors

53   (Bruni, Fraser & Schultz, 2008), and thereby to promote indirect actions to help the conservation

54   of endangered species, zoos and aquariums might have a negative effect on species at risk

55   because a limited group of organisms kept in captivity are often confined. The small effective

56   size of captive populations could contribute to a rapid loss of genetic variation within a few

57   generations, caused by founder effects and random genetic drift, associated with the increment of

58   inbreeding levels (Briscoe et al., 1992; Woodworth et al., 2002). As a result, captive populations

59   often have lower levels of genetic diversity than populations from natural habitats (Wisely,

60   McDonald & Buskirk, 2003). Their long-term sustainability depends on management measures

61   that could help to prevent the deterioration of genetic diversity (Frankel, 1983; Thévenon &

62   Couvet, 2002).

63

64   The common bottlenose dolphin *Tursiops truncatus* is a species worldwide distributed in tropical

65   and temperate waters (Leatherwood & Reeves, 1990; Wells & Scott, 1999; Reynolds, Wells &

66   Eide, 2000), with a complex and highly fluid population dynamics, exhibiting a fission-fusion

67   social structure (Tsai & Mann, 2013; Oudejans et al., 2015). The species is categorized as Least

68    Concern by the IUCN Red List (Hammond et al., 2012), but some local populations are

69    decreasing at a fast pace as a result of habitat disturbance, mostly induced by human activities

70    (Bearzi et al., 2008; Currey, Dawson & Slooten, 2009; Gaspari et al., 2015; Vermeulen &

71    Bräger, 2015). In this sense, the Mediterranean *T. truncatus* subpopulation has been classified as

72    Vulnerable by the IUCN due to habitat´s disturbance by human activities (Bearzi, Fortuna &

73    Reeves, 2012). Genetic variability and population structure of this species has been reported for

74    different regions along its geographic distribution, showing evidence of a strong genetic structure

75    even in small geographic distances, suggesting a limited gene flow among groups and philopatry

76    (Krützen et al., 2004; Natoli, Peddemors & Hoelzel, 2004; Parsons et al., 2006; Quérouil et al.,

77    2007; Caballero et al., 2011; Mirimin et al., 2011; Fruet et al., 2014; Gaspari et al., 2015;

78    Vermeulen & Bräger, 2015). Also, morphological, ecological and genetic studies in the North

79    Atlantic and the Gulf of Mexico, revelead two distinctive ecotypes: the inshore (coastal) and the

80    offshore (pelagic) forms (Hoelzel, Potter & Best, 1998; Sellas, Wells & Rosel, 2005; Caballero

81    et al., 2011; Oudejans et al., 2015). Inshore populations of bottlenose dolphins tend to show less

82    genetic variability than offshore populations (Hoelzel, Potter & Best, 1998; Natoli, Peddemors &

83    Hoelzel, 2004; Quérouil et al., 2007; Mirimin et al., 2011; Lowther-Thieleking et al., 2015).

84

85    In captivity, bottlenose dolphins are usually engaged in activities of "Swim-with-dolphins"

86    (SWD) programs, participating in a series of a interactions with visitors, such as hugs, kisses,

87    swirls, handshakes and footpushes (Curtin & Wilkes, 2007). The SWD programs represent a

88    very lucrative income derived from tourism. It has been estimated that one dolphin with special

89    training can produce almost US$1 million a year (Curtin & Wilkes, 2007). However, the

90    widespread implementation of these programs, there is raising concerns about the welfare of

91  these dolphins (Kyngdon, Minot & Stafford, 2003; Trone, Kuczaj & Solangi, 2005; Curtin &

92  Wilkes, 2007; Moorhouse et al., 2015). Captivity daily routines and the establishment of

93  artificial social groups produce high levels of social stress in dolphins that affect not only their

94  behavior but also their reproductive success and mates assessment (Gubbins et al., 1999; Waples

95  & Gales, 2002; Kyngdon, Minot & Stafford, 2003; Morgan & Tromborg, 2007; Marino &

96  Frohoff, 2011; Ugaz et al., 2013). Eventually, changes in the social structure of captive

97  populations could lead to an increment of non-random mating, producing an impact in the

98  distribution of genetic variability.

99

100  In Mexico, the dolphin captivity industry started in 1970 with exhibitions of few captive

101  dolphins in public shows held in Mexico City (Alaniz, 2010). This activity became very

102  profitable soon. By 2008, the last census performed during the national inspection of the

103  Mexican dolphinariums carried out by the Federal Attorney for Environmental Protection

104  (PROFEPA - Procuraduría Federal de Protección al Ambiente) recorded 270 captive bottlenose

105  dolphins (*Tursiops truncatus and T. aduncus*), 189 of them (70%) registred in the three leading

106  companies at the Yucatan Peninsula (southern Mexico) (PROFEPA, 2008; Alaniz, 2010). Apart

107  from the census of 2008, there is no new official information regarding the status of captive

108  dolphin populations in the country. All activities related to transport, exhibition and maintenance

109  of dolphins kept in captivity are regulated by a special Official Standard (NOM-135-

110  SEMARNAT-2004) (SEMARNAT, 2004), nevertheless this regulation does not include any

111  section on genetic management.

112

113  Genetic studies with wild Mexican dolphin populations are scarce (Segura et al., 2006; Caballero

114   et al., 2011), and there is no information about the genetic structure of confined dolphins used for

115   recreational purposes. The aim of our study was to evaluate the actual level of genetic diversity

116   in a captive population of common bottlenose dolphins conformed by founders (wild-born) and

117   captive-born individuals, to provide baseline information that can be used to monitor and prevent

118   eventual loss of gene diversity in future generations. For that, we estimated the genetic

119   variability and structure of *T. truncatus* kept in one of the largest dolphinariums in Mexican

120   territory (Delphinus – Via Delphi), using ten nuclear microsatellite loci and sequences of the

121   control region of mitochondrial DNA (mtDNA) as molecular markers.

122

123

124   **Material and methods**

125   All samples were collected by trained personnel of the dolphinarium, and all procedures were

126   done in compliance with the Mexican law, following the Ethical Guidelines for the performance

127   of Research on Animals by Zoo and Aquariums. No animals were directly targeted or killed for

128   this or any other associated study. Only trained vets (staff of the dolphinarium) collected a blood

129   sample from the tail of each dolphin. They followed the Mexican Official standard [NOM-059-

130   SEMARNAT-2010 enforced by the Secretariat of Environment and Natural Resources

131   (SEMARNAT)]. Experimental protocols were approved by the Institutional Animal Care and

132   Use Committee of the Center for Research and Advanced Studies (Centro de Investigacion y de

133   Estudios Avanzados del IPN) and comply with the applicable Mexican Official Norm (NOM-

134   062-ZOO-1999) "Technical Specifications for the Care and Use of Laboratory Animals".

135

136    "Comite Institucional para el Cuidado y uso de Animales" (CICUAL No. **0126-15**).

137

138

### *DNA extraction and amplification*

140 We analyzed samples from 49 common bottlenose dolphins (22 males and 27 females) of inshore

141 (coastal) form, confined in the main dolphinarium located in the Mayan Riviera along the

142 Caribbean Mexican Sea. A total of 25 individuals were wild-born and identified according to

143 their original site of capture: 7 from Tabasco, Mexico; 8 from Quintana Roo, Mexico and 10

144 from Cuba (Fig. 1), while the remaining 24 dolphins were first-generation captive-born

145 individuals. All captive-born dolphins analyzed were the offspring of wild-born parents.

146 **Figure 1**

147

148 Two ml of whole blood were collected from the caudal vein of each animal, using a BD

149 Vacutainer system® with EDTA as an anticoagulant, and stored at -20°C for subsequent DNA

150 extractions. Total gDNA was isolated from leukocytes using the Wizard® Genomic DNA

151 Purification kit (Promega, USA) and stored at -20°C.

152

153 A portion of the mtDNA including the 5' end of the control region was amplified using the pairs

154 of primers: L15824 (5'-CCT CAC TCC TCC CTA AGA CT-3') and H16265 (5'-GCC CGG

155 TGC GAG AAG AGG-3') (Sellas, Wells & Rosel, 2005). Polymerase chain reaction (PCR)

156 amplification was carried out in a volume of 20 µL containing: 1x PCR reaction buffer

157 (Promega), 150 µM of dNTPs, 0.3 µM of each primer, 1.5 mM $MgCl_2$, 0.5 U of Taq polymerase

158 (Promega) and approximately 100 ng of the DNA template. PCR amplification conditions

159 consisted of initial denaturation at 94°C for 30 seconds, followed by 30 cycles of denaturation

160  for 1 min at 94°C, annealing for 1 min at 52°C and extension for 1 min at 72°C, with a final

161  extension step at 72°C for 10 min, in a Gene Amp PCR System 2400 thermocycler (Perkin

162  Elmer). PCR products were purified and sequenced in both directions to ensure accuracy using

163  an ABI310 automatic sequencer (Applied Biosystem Inc., Foster City, CA) (Sanger, Nicklen &

164  Coulson, 1997).

165

166  Also, ten nuclear microsatellite loci were amplified, using primers designed for *T. truncatus*:

167  TexVet3, TexVet5, TexVet7 (Rooney, Merritt & Derr, 1999), D18, D22 (Shinohara, Domingo-

168  Roura & Takenaka, 1997), Ttr19 (Rosel, Forgetta & Dewar, 2005), AAT44 (Caldwell, Gaines &

169  Hughes, 2002); primers for *T. aduncus*: Tur4_80 and Tur4_105 (Nater, Kopps & Krützen, 2009);

170  and primers designed for baleen whales: GATA098 (Palsboll et al., 1997). Amplifications were

171  performed using a C-1000 Touch$^{TM}$ Thermal Cycler (BIO-RAD ®) under the following

172  conditions: initial denaturation at 94°C for 10 min, 35 amplification cycles (denaturing at 94°C

173  for 40s, annealing at specific temperature [TexVet5: 52°C; D22, GATA098: 54°C; TexVet3,

174  AAT44: 55°C; D18: 56°C; TexVet7: 57°C; Ttr19, Tur4_80, Tur4_105, Tur4_141: 60°C] for 40s,

175  and extension at 72°C for 1 min), with a final extension step at 72°C for 5 min. Each 15 µL

176  reaction volume contained 1X DreamTaq Green PCR Master Mix (Thermo Scientific®), 0.25

177  mM of each primer, and 3 µL of gDNA (50-100 ng). Negative controls were used in all

178  amplification series. PCR products were loaded for a capillary electrophoresis in the QIAxcel

179  Advanced System using a DNA High Resolution kit (QIAGEN Inc., Hilden, Germany). The

180  ScreenGel QIAxcel v1.4.0 program was used to determine the size (in bp) of amplified PCR

181  products.

182

183 ***Data analysis***

184 For statistical analyses, calculations were done for the whole captive population (including wild-

185 born and captive-born organisms) and separating sampled dolphins according to their place of

186 birth: wild-born group and captive-born group. For population structure analysis, wild-born

187 dolphins were further subdivided according to their original capture locations (Tabasco,

188 Quintana Roo, or Cuba).

189

190 The mtDNA sequences (forward and reverse) were aligned using ClustalW (Thompson, Higgins

191 & Gibson, 1994) implemented in MEGA version 6.0 (Tamura et al., 2013) and edited manually

192 to create a consensus sequence. The number of haplotypes, the haplotype diversity ($h$) and the

193 nucleotide diversity ($\pi$) were used to estimate genetic diversity and population structure and were

194 calculated using Arlequin v.3.5 (Excoffier, Laval & Schneider, 2005). A minimum spanning

195 network (MSN) was constructed applying Median-Joining (MJ) algorithm using NetWork v.

196 5.0.0.0 (Bandelt, Forster & Röhl, 1999) to visualize base pair changes and relationships between

197 the haplotypes.

198

199 For the nuclear microsatellite analyses, basic genetic analyses were performed using GENEPOP

200 v. 4.2 (Raymond & Rousset, 1995), FSTAT version 2.9.3.2 (Goudet, 1995) and POPGENE

201 v.1.32 (Yeh et al., 1997). Nuclear genetic diversity was measured as an average number of

202 alleles per locus ($Na$), the number of effective alleles ($Ne$), observed heterozygosity ($Ho$) and

203 Nei´s (1973) expected heterozygosity ($He$). As observed number of alleles is highly dependent

204 on some sampled dolphins at each group, we also calculated allelic richness ($A_R$), a measure of

205 allele diversity corrected for differences in sample sizes. We performed pairwise tests for linkage

206    disequilibrium (LD) for all pairs of loci. Deviations from Hardy–Weinberg equilibrium (HWE)

207    were assessed for each locus and over all loci, employing a Markov chain method for exact

208    probabilities tests, with 10,000 dememorizations, 1,000 batches and 10,000 interactions per batch

209    (Guo & Thompson, 1992). Inbreeding coefficient ($F_{IS}$) was calculated and their significance was

210    tested (Weir & Cockerham, 1984), and we adjusted *p*-values for multiple tests using Bonferroni

211    sequential correction (Rice, 1989). Since we found heterozygote deficiencies in the whole

212    captive population as well as in the groups formed by wild-born and captive-born individual (see

213    Results), we assessed possible scoring errors due to large allele dropout and to the presence of

214    null alleles using the software MICROCHECKER version 2.2.3(Van Oosterhout et al., 2004).

215    After that, we estimated null alleles frequencies for each locus using the Expectation

216    Maximization (EM) algorithm and generated a corrected dataset using FreeNA software

217    (Chapuis & Estoup, 2007).

218

219    The software package Arlequin v.3.5 (Excoffier, Laval & Schneider, 2005) was used to measure

220    population structure by pairwise $F_{ST}$ values for both microsatellite and mtDNA sequences data.

221    Significance tests were performed by 10,000 permutations and adjusted using Bonferroni

222    correction (Rice, 1989). As a complement, pairwise $F_{ST}$ values were calculated for microsatellite

223    data using the ENA (Excluding Null Allele) correction method with 1,000 bootstrap repetitions,

224    that corrects for positive bias on $F_{ST}$ estimation induced by the presence of null alleles (Chapuis

225    & Estoup, 2007).

226

227    The evidence for a possible cryptic population sub-structuring was tested with a Bayesian

228    approach using the software STRUCTURE v2.3.4 (Pritchard, Stephens & Donnelly, 2000). This

229    analysis applies a quantitative clustering method that allows the inference of true K (number of

230    populations) through the computation of the log likelihood for each K. Since the bias caused by

231    null alleles have a very low magnitude causing a slight reduction in the power to correctly

232    assigned individuals (0.2 to 1.0 percent units) suggesting that loci with null alleles can still be

233    used for assignment testing (Carlsson, 2008), we used all microsatellite markers for the analysis.

234    We ran 1,000,000 Markov chain Monte Carlo (MCMC) iterations with a burn-in period of

235    100,000 iterations, with 10 repetitions for each number of hypothetical genetic clusters

236    (K=number of populations), for a K ranging from 1 to 4 (considering the total number of origin

237    sites). Results were visualized using STRUCTURE HARVEST v0.6.94 (Earl & VonHoldt,

238    2012) and the most likely K was inferred using the Delta K method (Evanno, Regnaut & Goudet,

239    2005) analyzing the variance of Ln likelihood for each tested K.

240

241    To test for genetic evidence of a recent historical reduction in the effective population size, we

242    used the software BOTTLENECK 1.2.02 (Piry, Luikart & Cornuet, 1999). When a population

243    bottleneck occurs, there is a reduction in heterozygosity levels and the number of alleles at

244    polymorphic loci, but the allele loss is faster than the heterozygosity levels decrease. So, under

245    mutation-drift equilibrium, the expected heterozygosity (calculated based on the allele number)

246    become lower than the measured heterozygosity in the sense of Nei´s gene diversity (1987). We

247    performed a Wilcoxon sign-rank test to determine the significance of heterozygosity excess,

248    which is more appropriate than sign test to analyze a low number of individuals (Luikart &

249    Cornuet, 1998; Piry, Luikart & Cornuet, 1999). We estimated the gene diversity under three

250    models of molecular evolution: infinite allele model (IAM), stepwise mutation model (SMM),

251    and two-phase model (TPM), an intermediate between IAM and SMM which is adequate for

252    microsatellite data (Di Renzo et al., 1994). The proportion of alleles attributed to SMM under the

253    TPM tested was 70% with a variance of 12 and 10,000 iterations.

254

255    **Results**

256    The 373 bp fragment of the mtDNA control region amplified in 44 samples out of a total of 49

257    individuals. We found 35 polymorphic sites for a total of 13 unique haplotypes in the mtDNA

258    aligned sequences (Table S1). The sequences were deposited in the GenBank™ database with

259    the accession numbers KX151147 to KX151159. Exclusive haplotypes were found in Captive-

260    born (H3), and wild-born (H10: Cuba; H11 and H12: Quintana Roo; H13: Tabasco) dolphins and

261    the most common haplotype (H8) was shared among Tabasco, Cuba and Captive-born dolphins

262    (Table 1). Overall haplotype and nucleotide diversity were of 0.912 ($\pm$0.016) and 0.025 ($\pm$0.013),

263    respectively, with Captive-born dolphins presenting a slightly reduced haplotype diversity (0.900

264    $\pm$0.016).

265

266                                   **Table 1**

267

268    In the minimum spanning network (Fig. 2), three main haplogroups are observed: one with

269    haplotypes found in Cuba that are associated with haplotypes from Tabasco and captive-born

270    dolphins, another integrated mostly by dolphins from Quintana Roo and captive-born dolphins,

271    and one more formed by haplotypes from all locations. Haplotypes from captive-born dolphins

272    are distributed in the entire spanning network, while haplotype H13 from Tabasco is the most

273    differentiated one, separated by eight mutational steps from their closest related haplotype.

274

275                                    **Figure 2**

276

277    All microsatellite loci genotyped were polymorphic across the sampled populations. Exact tests

278    for linkage disequilibrium between pairs of loci were not significant, and all loci were

279    genetically independent. Overall low levels of genetic diversity were found; the total number of

280    alleles per locus ranged from 2 to 10, with a mean of 4 (±2.36), the mean effective number of

281    alleles was 2.57 (±1.29), the average expected heterozygosity over all loci was 0.544 (±0.163),

282    ranging from 0.300 to 0.829, and the average observed heterozygosity was 0.449 (±0.169),

283    ranging from 0.204 to 0.771 (Table 2). In general, genetic variability of wild-born and captive

284    born dolphins was highly similar, with no evidence of reduced diversity in the captive-born

285    group (Table 3).

286

287                                    **Table 2**

288                                    **Table 3**

289

290    Based on the original dataset including all individuals from captive population, a significant

291    departure from Hardy-Weinberg equilibrium (HWE) was observed over all loci after Bonferroni

292    adjustments (p<0.01,) with a heterozygote deficit at loci Ttr19 ($F_{IS}$=0.519, p<0.01), Tur4_80

293    ($F_{IS}$=0.545, p<0.01), Tur4_105 ($F_{IS}$=0.080, p<0.01) and GATA098 ($F_{IS}$=0.269, p<0.01). For

294    wild-born dolphins, significant departures from Hardy-Weinberg equilibrium were observed

295    after Bonferroni adjustments with heteorzygote deficit at loci Tur4_80 ($F_{IS}$=0.631, p>0.01), and

296    heterozygote excess at loci Tur4_105 ($F_{IS}$=-0.047, p<0.01), while for captive-born dolphins a

297    heterozygote deficit was observed at loci Ttr19 ($F_{IS}$=0.657, p<0.01), Tur4_80 ($F_{IS}$=0.464,

298     p<0.01), and Tur4_105 ($F_{IS}$=0.192, p<0.01) (Table 4). The MICROCHECKER analysis

299     suggested the presence of null alleles and their frequencies per locus were calculated by FreeNA,

300     ranging from 0 to 0.33 (Table S2).

301

302                                     **Table 4**

303

304     The analysis of population differentiation by pairwise $F_{ST}$ comparisons was done for both

305     microsatellite and mtDNA data. For mtDNA data, a significant differentiation ($F_{ST}$=0.302,

306     p<0.05) was found between individuals from Cuba and Quintana Roo (Table 5). For

307     microsatellites, results from pairwise $F_{ST}$ estimated from original dataset and from corrected

308     dataset for null alleles (ENA method correction, (Chapuis & Estoup, 2007), Table S3) were very

309     similar, suggesting that the presence of null alleles had very little influence on the estimates of

310     population differentiation, therefore all further tests were performed with uncorrected allele

311     frequencies. No significant genetic structure was detected, as pairwise $F_{ST}$ values were not

312     different from zero (Table 5), revealing an almost null genetic differentiation among common

313     bottlenose dolphins' origin sites.

314                                     **Table 5**

315

316     According to the pairwise $F_{ST}$ findings, the Bayesian analysis of population substructuring done

317     with STRUCTURE indicated that the most likely number of populations (K) was 1, since K=1

318     presented the lower variance of Ln likelihood (Table S4), pointing out that all common

319     bottlenose dolphins analyzed herein comprise a single population, regardless of their origin

320     location.

321

322

323    The one-tailed Wilcoxon test for evidence of population bottleneck was done excluding data

324    from microsatellite Tur4_80 which was the only microsatellite marker that presents an estimated

325    frequency of null alleles above 0.20 at global level (Table S2). When comparing measured Nei´s

326    heterozygosity (Nei, 1987) with heterozygosity under mutation-drift model, we detected a

327    significant heterozygosity excess ($p<0.05$) for both infinite allele model (IAM) and two-phased

328    model (TPM) for the global captive population (Table 6). When dolphins were separated

329    according to their site of birth, the signal for a population bottleneck was detected for IAM and

330    TPM in the group formed by captive-born individuals and for IAM in the group formed by wild-

331    born dolphins. Considering that TPM is the best model to fit empirical data regarding the

332    microsatellite mutation process (Di Renzo et al., 1994), our results suggest that the population of

333    captive bottlenose dolphins studied here presents a clear sign of a bottleneck event, and this

334    result could be a consequence of the already depleted genetic variability of the founder

335    organisms since wild-born dolphins also present a sign of a genetic bottleneck.

**Table 6**

337

338    **Discussion**

339    In thise study, overall low level of genetic diversity was found using nuclear microsatellite

340    markers, with a small number of alleles per locus and reduced heterozygosity, for both wild-born

341    and captive-born dolphins. Previous genetic studies with *T. truncatus* inhabitating open waters

342    from the Irish coasts, the Adriatic Sea and the Wider Caribbean region have reported higher

343    levels of allelic richness, observed heterozygosity and number of alleles than those found in our

344    captive population using the same nuclear microsatellites markers (Caballero *et al.*, 2011; Galov

345    *et al.*, 2011; Mirimin *et al.*, 2011). For example, data obtained using microsatellite D22 recorded

346    only 3 alleles with an allelic richness of 3.00 and observed heterozygosity of 0.449 for the

347    population at captivity, while for free ranging *T. truncatus* populations the number of alleles

348    ranged from 7 to 10, with an allelic richness from 6.14 to 7.90 and observed heterozygosity from

349    0.667 to 0.783. It has been previously established that coastal populations of *T. truncatus* present

350    less genetic variation compared to pelagic populations (Parsons et al., 2002; Natoli, Peddemors

351    & Hoelzel, 2004; Segura et al., 2006; Quérouil et al., 2007; Mirimin et al., 2011; Lowther-

352    Thieleking et al., 2015). The captive population evaluated here was all composed by individuals

353    captured in coastal waters and by individuals born in captivity from wild-born parents of coastal

354    form, so a low genetic diversity was expected. Whereas, at the mtDNA control region, the

355    genetic diversity of this confined population was similar to levels of nucleotide and haplotype

356    diversities from wild populations from the Gulf of Mexico and the Caribbean Sea using the same

357    molecular markers (Natoli, Peddemors & Hoelzel, 2004; Sellas, Wells & Rosel, 2005; Tezanos-

358    Pinto et al., 2009; Caballero et al., 2011).

359

360    For the dolphin captive population, we observed a heterozygote deficit for microsatellite loci

361    Ttr19, Tur4_80, Tur4_105 and GATA098. The heterozygote deficiency could be a result of

362    several factor such as inbreeding, the presence of null alleles or the Wahlund effect (sampling

363    more than one genetic population and treating it as one) (Thévenon & Couvet, 2002). In our

364    study, the Wahlund effect is not likely because the levels of population differentiation were

365    practically null, being the presence of null alleles the most probable explanation for the

366    deviations from Hardy-Weinberg expectations detected.  Null alleles are common in cross-

367    species PCR amplifications, such as the case of microsatellites Tur4_80, Tur4_105, developed

368    for *T. aduncus* (Nater, Kopps & Krützen, 2009)*,* and GATA098, developed for baleen whales

369    (Palsboll et al., 1997).

370

371    Previous genetic analyses using microsatellites and mtDNA data of wild bottlenose dolphin

372    populations across their distribution detected a strong spatial genetic structuring among

373    populations at local scale, suggested the existence of highly cohesive social groups at a fine

374    geographic scale (Natoli, Peddemors & Hoelzel, 2004; Sellas, Wells & Rosel, 2005; Viaud-

375    Martínez et al., 2008; Tezanos-Pinto et al., 2009; Mirimin et al., 2011). This finding is expected

376    in species with a stable social organization because of a differentiated use of habitat and

377    resources (Mirimin et al., 2011). However, in our study no genetic structure was observed

378    between origin populations of wild-born dolphins analyzed, even with correction of allele

379    frequencies for the presence of null alleles, suggesting that in this area, free-ranging dolphins are

380    part of one single population with constant gene flow among locations. In contrast, the analysis

381    of the maternally inherited mitochondrial markers reveals that wild-born *T. truncatus* from Cuba

382    and Quintana Roo had a signal of differentiation, although their haplotypes are distributed

383    through the entire haplotype network. One possible explanation for the pattern observed, with no

384    genetic structure at nuclear level but differentiation observed in matrilineal markers could be the

385    occurrence of female philopatry and male-biased dispersal, a tendency that has been already

386    observed in bottlenose dolphins (Krützen et al., 2004; Möller & Beheregaray, 2004).

387

388    Species kept in captivity suffer loss of genetic diversity, inbreeding depression, accumulation of

389    new mildly deleterious mutations, a genetic adaptation to captivity (Frankham, Ballou &

390    Briscoe, 2002; Armbruster & Reed, 2005; Frankham, 2008; Christie et al., 2012) and elevate

391    presence of null alleles that are scarce in wild populations (Leary, Allendorf & Knudsen, 1993).

392    Captive organisms show a reduced reproductive fitness when reintroduced to the wild, and this

393    reduction is proportional to the number of generations in captivity and sometimes just a single

394    generation is enough to reduce fitness (Frankham, 2008; Christie et al., 2012). (Natoli,

395    Peddemors & Hoelzel, 2004; Sellas, Wells & Rosel, 2005; Tezanos-Pinto et al., 2009; Caballero

396    et al., 2011). Also, captive populations have low number of individuals, and may suffer the Allee

397    effect, a declining in viability and reproductive success due to demographic reasons (negative

398    density-dependent processes) not related with genetic aspects (Lande, 1988).

399    Generally, the captivity establishment is made with a limited number of organisms, causing a

400    population bottleneck and a founder effect that reduce the genetic variability and increase the

401    differentiation among captive and wild populations (Launey et al., 2001; Muñoz-Fuentes, Green

402    & Sorenson, 2008; Biebach & Keller, 2009; Swatdipong, Primmer & Vasemägi, 2010; Yu et al.,

403    2011; Price & Hadfield, 2014). Even if the captive populations experience a recovery in the total

404    number of individuals, the genetic diversity is hardly restored to the original levels (Biebach &

405    Keller, 2009). In this study, we found evidence of a population bottleneck on a global scale  in a

406    captive population of bottlenose dolphins by analyzing nuclear microsatellite data; gene flow

407    could help to reduce the genetic impact of the genetic bottleneck and inbreeding (Biebach &

408    Keller, 2009; Swatdipong, Primmer & Vasemägi, 2010; Price & Hadfield, 2014), but there is no

409    documented evidence of implemented measures to increase the income of new genetic material

410    to this captive population.

411

412    The sporadic introduction of new specimens from the wild could help to minimize the impact of

413    genetic adaptation to captivity (Price & Hadfield, 2014), but in the particular case of captive

414    bottlenose dolphins in Mexico, this is not possible because Mexican legislation banned the

415    capture of wild individuals since 2002 and the importation and exportation of specimens of any

416    marine mammal species since 2006 (SEMARNAT, 2010). One alternative might be to establish

417    a program that enables regular translocations of captive dolphins among the dolphinariums for

418    breeding and allowing with this strategy, the introduction of new genetic material to small

419    captive populations to increase the gene flow. However, this strategy has serious limitations such

420    as the rivalry among different companies, the high cost of transportation, the elevated risk of

421    spreading infectious diseases and the exposure of translocated animals to accidents, injuries and

422    stress when they are being moved. Semen collection and artificial inseminations are regular

423    procedures at dolphinariums (Robeck & O'Brien, 2004) but they are usually done with the same

424    captive individuals and does not improve the captive population genetic pool.

425

426    In conclusion, this is the first study that evaluates the genetic diversity of a captive population of

427    *T. truncatus* in a major dolphinarium from southeast Mexico. It included wild-born dolphins

428    originally captured in the Gulf of Mexico and Cuba and captive-born dolphins. Considering the

429    reduced population size and because the Mexican law has banned the income of new individuals,

430    bottlenose dolphins at Mexican dolphinariums are kept in conditions of extremely restricted gene

431    flow where a strong founder effect is expected to occur because of the limited diversity of the

432    founders of this captive population and inbreeding in further generations might be very high.

433

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439

440    **References**

441    Alaniz Y. 2010. *Reporte sobre delfines cautivos en México y República Dominicana*. Heredia,

442         Costa Rica: WSPA - Sociedad Mundial para la Protección Animal.

443    Armbruster P., Reed DH. 2005. Inbreeding depression in benign and stressful environments.

444         *Heredity* 95:235–242. DOI: 10.1038/sj.hdy.6800721.

445    Bandelt HJ., Forster P., Röhl A. 1999. Median-joining networks for inferring intraspecific

446         phylogenies. *Molecular Biology and Evolution* 16:37–48. DOI:

447         10.1093/oxfordjournals.molbev.a026036.

448    Bearzi G., Agazzia S., Bonizzoni S., Costa M., Azzellino A. 2008. Dolphins in a bottle:

449         abundance, residency patterns and conservation of bottlenose dolphins *Tursiops truncatus*

450         in the semi-closed eutrophic Amvrakikos Gulf, Greece. *Aquatic Conservation: Marine and*

451         *Freshwater Ecosystems* 18:130–146. DOI: 10.1002/aqc.843.

452    Bearzi G., Fortuna C., Reeves R. 2012. *Tursiops truncatus* (Mediterranean subpopulation). *The*

453         *IUCN Red List of Threatened Species 2012*: e.T16369383A16369386. DOI:

454         10.2305/IUCN.UK.2012-1.RLTS.T16369383A16369386.en.

455    Biebach I., Keller LF. 2009. A strong genetic footprint of the re-introduction history of Alpine

456         ibex (*Capra ibex ibex*). *Molecular Ecology* 18:5046–5058. DOI: 10.1111/j.1365-

457         294X.2009.04420.x.

458    Bilgmann K., Möller LM., Harcourt RG., Gibbs SE., Beheregaray LB. 2007. Genetics

459         differentiation in bottlenose dolphins from South Australia: association with local

460         oceanography and coastal geography. *Marine Ecology Progress Series* 341:265–276. DOI:

461         10.3354/meps341265.

462    Briscoe DA., Malpica JM., Robertson A., Smith GJ., Frankham R., Banks RG., Barker JSF.

463      1992. Rapid loss of genetic variation in large captive populations of Drosophila flies -

464      Implications for the genetic management of captive populations. *Conservation Biology*

465      6:416–425. DOI: 10.1046/j.1523-1739.1992.06030416.x.

466 Bruni CM., Fraser J., Schultz PW. 2008. The value of zoo experiences for connecting people

467      with nature. *Visitor Studies* 11:139–150. DOI: 10.1080/10645570802355489.

468 Caballero S., Islas-Villanueva V., Tezanos-Pinto G., Duchene S., Delgado-Estrella A., Sanchez-

469      Okrucky R., Mignucci-Giannoni AA. 2011. Phylogeography, genetic diversity and

470      population structure of common bottlenose dolphins in the Wider Caribbean inferred from

471      analyses of mitochondrial DNA control region sequences and microsatellite loci:

472      conservation and management implications. *Animal Conservation* 15:95–112. DOI:

473      10.1111/j.1469-1795.2011.00493.x.

474 Caldwell M., Gaines MS., Hughes CR. 2002. Eight polymorphic microsatellite loci for

475      bottlenose dolphin and other cetacean species. *Molecular Ecology Notes* 2:393–395. DOI:

476      10.1046/j.1471-8286.2002.00270.x.

477 Carlsson J. 2008. Effects of microsatellite null alleles on assignment testing. *Journal of Heredity*

478      99:616–623. DOI: 10.1093/jhered/esn048.

479 Chapuis MP., Estoup A. 2007. Microsatellite null alleles and estimation of population

480      differentiation. *Molecular Biology and Evolution* 24:621–631. DOI:

481      10.1093/molbev/msl191.

482 Christie MR., Marine ML., French RA., Blouin MS. 2012. Genetic adaptation to captivity can

483      occur in a single generation. *Proceedings of the National Academy of Sciences of the United*

484      *States of America* 109:238–242. DOI: 10.1073/pnas.1111073109.

485 Currey RJC., Dawson SM., Slooten E. 2009. An approach for regional threat assessment under

486    IUCN Red List Criteria that is robust for uncertainty: The Fiordland bottlenose dolphins are

487    critically endangered. *Biological Conservation* 142:1570–1579. DOI:

488    10.1016/j.biocon.2009.02.036.

489 Curtin S., Wilkes K. 2007. Swimming with captive dolphins: current debates and post-

490    experience dissonance. *International Journal of Tourism Research* 9:131–146. DOI:

491    10.1002/jtr.599.

492 Earl DA., VonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for

493    visualizing STRUCTURE output and implementing the Evanno method. *Conservation*

494    *Genetics Resources* 4:359–361. DOI: 10.1007/s12686-011-9548-7.

495 Evanno G., Regnaut S., Goudet J. 2005. Detecting the number of clusters of individuals using the

496    software structure: a simulation study. *Molecular Ecology* 14:2611–2620. DOI:

497    10.1111/j.1365-294X.2005.02553.x.

498 Excoffier L., Laval G., Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for

499    population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47–50. DOI:

500    10.1177/117693430500100003.

501 Frankel OH. 1983. The place of management in conservation. In: Schoenwald-Cox CM,

502    Chambers SM, MacBryde B, Thomas L eds. *Genetics and conservation: A reference for*

503    *managing wild animal and plant populations*. Menlo Park, California:

504    Benjamin/Cummings, 1–14.

505 Frankham R. 2008. Genetic adaptation to captivity in species conservation programs. *Molecular*

506    *Ecology* 17:325–333. DOI: 10.1111/j.1365-294X.2007.03399.x.

507 Frankham R., Ballou JD., Briscoe DA. 2002. *Introduction to Conservation Genetics*. Cambridge,

508    UK.: Cambridge University Press.

509   Fruet PF., Secchi ER., Daura-Jorge FF., Vermeulen E., Flores PAC., Simoes-Lopes PC.,

510       Genoves RC., Laporta P., Di Tullio JC., Freitas TRO., Rosa LD., Valiati VH., Beheregaray

511       LB., Moller LM. 2014. Remarkably low genetic diversity and strong population structure in

512       common bottlenose dolphins (*Tursiops truncatus*) from coastal waters of the Southwestern

513       Atlantic Ocean. *Conservation Genetics* 15:879–895. DOI: 10.1007/s10592-014-0586-z.

514   Galov A., Kocijan I., Lauc G., Gomerčić MĐ., Gomerčić T., Arbanasić H., Šatović Z., Šeol B.,

515       Vuković S., Gomerčić H. 2011. High genetic diversity and possible evidence of a recent

516       bottleneck in Adriatic bottlenose dolphins (*Tursiops truncatus*). *Mammalian Biology -*

517       *Zeitschrift für Säugetierkunde* 76:339–344. DOI: 10.1016/j.mambio.2010.07.002.

518   Gaspari S., Holcer D., Mackelworth P., Fortuna C., Frantzis A., Genov T., Vighi M., Natali C.,

519       Rako N., Banchi E., Chelazzi G., Ciofi C. 2015. Population genetic structure of common

520       bottlenose dolphins (*Tursiops truncatus*) in the Adriatic Sea and contiguous regions:

521       Implications for international conservation. *Aquatic Conservation: Marine and Freshwater*

522       *Ecosystems* 25:212–222. DOI: 10.1002/aqc.2415.

523   Goudet J. 1995. FSTAT, version 1.2: a computer program to calculate Fstatistics. *Journal of*

524       *Heredity* 86:485–486. DOI: 0.1093/oxfordjournals.jhered.a111627.

525   Gubbins C., McCowan B., Lynn S. K., Hooper S., Reiss D. 1999. Mother-infant spatial relations

526       in captive bottlenose dolphins, *Tursiops truncatus*. *Marine Mammal Sscience* 15:751–765.

527       DOI: 10.1111/j.1748-7692.1999.tb00841.x.

528   Guo SW., Thompson EA. 1992. Performing the exact test of Hardy Weinberg proportion for

529       multiple alleles. *Biometrics* 48:361–372. DOI: 10.2307/2532296.

530   Hammond PS., Bearzi G., Bjørge A., Forney KA., Karkzmarski L., Kasuya T., Perrin WF., Scott

531       MD., Wang JY., Wells RS., Wilson B. 2012. *Tursiops truncatus*. *The IUCN Red List of*

532      *Threatened Species 2012*. e.T22563A17347397. DOI:

533      10.2305/IUCN.UK.2012.RLTS.T22563A17347397.en.

534 Hoelzel AR., Potter CW., Best PB. 1998. Genetic differentiation between parapatric "nearshore"

535      and "offshore" populations of the bottlenose dolphin. *Proceeding of the The Royal Society*

536      *B: Biological Sciences* 265:1177–1183. DOI: 10.1098/rspb.1998.0416.

537 Krützen M., Sherwin WB., Berggreb P., Gales N. 2004. Population structure in an inshore

538      cetacean revelead by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops* sp.)

539      in Shark Bay, Western Australia. *Marine Mammal Science* 20:28–47. DOI: 10.1111/j.1748-

540      7692.2004.tb01139.x.

541 Kyngdon DJ., Minot EO., Stafford KJ. 2003. Behavioural responses of captive common dolphins

542      *Delphinus delphis* to a "Swim-with-Dolphin" programme. *Applied Animal Behaviour*

543      Science 81:163–170. DOI: 10.1016/S0168-1591(02)00255-1.

544 Lande R. 1988. Genetics and demography in biological conservation. *Science* 241:1455–1460.

545      DOI: 10.1126/science.3420403.

546 Launey S., Barre M., Gerard A., Naciri-Graven Y. 2001. Population bottleneck and effective size

547      in *Bonamia ostreae*-resistant populations of *Ostrea edulis* as inferred by microsatellite

548      markers. *Genetical Research* 78:259–270. DOI: 10.1017/S0016672301005353.

549 Leary RF., Allendorf FW., Knudsen KL. 1993. Null allele heterozygosity at two lactate

550      dehydrogenase loci in rainbow trout are associated with decreased developmental stability.

551      *Genetica* 89:3–13. DOI: 10.1007/BF02424501.

552 Leatherwood S., Reeves RR. 1990. *The bottlenose dolphin*. San Diego, CA: Academic Press.

553 Lowther-Thieleking JL., Archer FI., Lang AR., Weller DW. 2015. Genetic differentiation among

554      coastal and offshore common bottlenose dolphins, *Tursiops truncatus*, in the eastern North

555     Pacific Ocean. *Marine Mammal Science* 31:1–20. DOI: 10.1111/mms.12135.

556     Luikart G., Cornuet JM. 1998. Empirical evaluation of a test for identifying recently

557     bottlenecked populations from allele frequency data. *Conservation Biology* 12:228–237.

558     DOI: 10.1111/j.1523-1739.1998.96388.x.

559     Marino L., Frohoff T. 2011. Towards a new paradigm of Non-Captive research on cetacean

560     cognition. *PLoS ONE* 6:e24121. DOI: 10.1371/journal.pone.0024121.

561     Mirimin L., Miller R., Dillane E., Berrow SD., Ingram S., Cross TF., Rogan E. 2011. Fine-scale

562     population genetic structuring of bottlenose dolphins in Irish coastal waters. *Animal*

563     *Conservation* 14:342–353. DOI: 10.1111/j.1469-1795.2010.00432.x.

564     Möller LM., Beheregaray LB. 2004. Genetic evidence for sex-biased dispersal in resident

565     bottlenose dolphins (*Tursiops aduncus*). *Molecular Ecology* 13:1607–12. DOI:

566     10.1111/j.1365-294X.2004.02137.x.

567     Moorhouse TP., Dahlsjö CAL., Baker SE., D'Cruze NC., Macdonald DW. 2015. The customer

568     isn't always right - Conservation and animal welfare implications of the increasing demand

569     for wildlife tourism. *PLoS ONE* 10:1–15. DOI: 10.1371/journal.pone.0138939.

570     Morgan KN., Tromborg CT. 2007. Sources of stress in captivity. *Applied Animal Behaviour*

571     *Science* 102:262–302. DOI: 10.1016/j.applanim.2006.05.032.

572     Muñoz-Fuentes V., Green AJ., Sorenson MD. 2008. Comparing the genetics of wild and captive

573     populations of white-headed ducks *Oxyura leucocephala*: consequences for recovery

574     programmes. *Ibis* 150:807–815. DOI: 10.1111/j.1474-919X.2008.00866.x.

575     Nater A., Kopps AM., Krützen M. 2009. New polymorphic tetranucleotide microsatellites

576     improve scoring accuracy in the bottlenose dolphin *Tursiops aduncus*. *Molecular Ecology*

577     *Resources* 9:531–534. DOI: 10.1111/j.1755-0998.2008.02246.x.

578    Natoli A., Peddemors VM., Hoelzel AR. 2004. Population structure and speciation in the genus

579        *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary*

580        *Biology* 17:363–375. DOI: 10.1046/j.1420-9101.2003.00672.x.

581    Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National*

582        *Academy of Sciences, USA*:3321–3323.

583    Nei M. 1987. *Molecular evolutionary genetics*. New York, USA: Columbia University Press.

584    Van Oosterhout C., Hutchinson WF., Wills DPM., Shipley P. 2004. Micro-checker: Software for

585        identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology*

586        *Notes* 4:535–538. DOI: 10.1111/j.1471-8286.2004.00684.x.

587    Oudejans MG., Visser F., Englund A., Rogan E., Ingram SN. 2015. Evidence for distinct coastal

588        and offshore communities of bottlenose dolphins in the north east Atlantic. *PLoS ONE*

589        10:1–15. DOI: 10.1371/journal.pone.0122668.

590    Palsboll PJ., Bérubé M., Larsen AH., Jorgensen H. 1997. Primers for the amplification of tri- and

591        tetramer microsatellite loci in baleen whales. *Molecular Ecology* 6:893–895. DOI:

592        10.1111/j.1365-294X.1997.tb00146.x.

593    Parsons KM., Durban JW., Claridge DE., Herzing DL., Balcomb KC., Noble LR. 2006.

594        Population genetics structure of coastal bottlenose dolphins (*Tursiops truncatus*) in the

595        northern Bahamas. *Marine Mammal Science* 22:276–298. DOI: 10.1111/j.1748-

596        7692.2006.00019.x.

597    Parsons KM., Noble LR., Reid RJ., Thompson PM. 2002. Mitochondrial genetic diversity and

598        population structuring of UK bottlenose dolphins (*Tursiops truncatus*): is the NE Scotland

599        population demographically and geographically isolated? *Biological Conservation*

600        108:175–182. DOI: 10.1016/S0006-3207(02)00103-9.

601    Piry S., Luikart G., Cornuet JM. 1999. BOTTLENECK: A computer program for detecting

602         recent reductions in the effective population size using allele frequency data. *Journal of*

603         *Heredity* 90:502–503. DOI: 10.1093/jhered/90.4.502.

604    Price MR., Hadfield MG. 2014. Population genetics and the effects of a severe bottleneck in an

605         ex situ population of critically endangered Hawaiian tree snails. *PloS One* 9:e114377. DOI:

606         10.1371/journal.pone.0114377.

607    Pritchard JK., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus

608         genotype data. *Genetics* 155:945–959. DOI: 10.1111/j.1471-8286.2007.01758.x.

609    PROFEPA. 2008. *Informe Anual PROFEPA 2008*. Procuraduría Federal de Protección al

610         Ambiente. DOI: 10.1017/CBO9781107415324.004.

611    Quérouil S., Silva MA., Freitas L., Prieto R., Magalhaes S., Dinis A., Alves F., Matos JA.,

612         Mendonca D., Hammond PS., Santos RS. 2007. High gene flow in oceanic bottlenose

613         dolphins (*Tursiops truncatus*) of the North Atlantic. *Conservation Genetics* 8:1405–1419.

614         DOI: 10.1007/s10592-007-9291-5.

615    Raymond M., Rousset F. 1995. Genepop (version 1.2): Population genetics software for exact

616         tests and ecumenicism. *Journal of Heredity* 86:248–249. DOI:

617         10.1093/oxfordjournals.jhered.a111573.

618    Di Renzo A., Peterson AC., Garza JC., Valdes AM., Slatkin M., Freimer NB. 1994. Mutation

619         processes of simple-sequence repeat loci in human populations. *Proceedings of the National*

620         *Academy of Sciences USA* 91:3166–3170. DOI: 10.1073/pnas.91.8.3166.

621    Reynolds JEI., Wells RS., Eide SD. 2000. *The bottlenose dolphin: Biology and conservation*.

622         University Press of Florida.

623    Rice WER. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225. DOI:

624     10.2307/2409177.

625  Robeck TR., O'Brien JK. 2004. Effect of cryopreservation methods and precryopreservation

626     storage on bottlenose dolphin (*Tursiops truncatus*) spermatozoa. *Biology of Reproduction*

627     70:1340–1348. DOI: 10.1095/biolreprod.103.025304.

628  Rooney AP., Merritt DB., Derr JN. 1999. Microsatellite diversity in captive bottlenose dolphins

629     (*Tursiops truncatus*). *Journal of Heredity* 90:228–231. DOI: 10.1093/jhered/90.1.228.

630  Rosel PE., Forgetta V., Dewar K. 2005. Isolation and characterization of twelve polymorphic

631     microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). *Molecular Ecology*

632     *Notes* 5:830–833. DOI: 10.1111/j.1471-8286.2005.01078.x.

633  Sanger F., Nicklen S., Coulson AR. 1997. DNA sequencing with chain terminating inhibitor.

634     *Proceedings of the National Academy of Sciences USA* 74:5463–5467. DOI:

635     10.1073/pnas.74.12.5463.

636  Segura I., Rocha-Olivares A., Flores-Ramírez S., Rojas-Bracho L. 2006. Conservation

637     implications of the genetic and ecological distinction of *Tursiops truncatus* ecotypes in the

638     Gulf of California. *Biological Conservation* 133:336–346. DOI:

639     10.1016/j.biocon.2006.06.017.

640  Sellas AB., Wells RS., Rosel PE. 2005. Mitochondrial and nuclear DNA analyses reveal fine

641     scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of

642     Mexico. *Conservation Genetics* 6:715–728. DOI: 10.1007/s10592-005-9031-7.

643  SEMARNAT. 2004. *Norma Oficial Mexicana NOM-135-SEMARNAT-2004*. Diario Oficial de la

644     Federación.

645  SEMARNAT. 2010. *Norma Oficial Mexicana NOM-059-ECOL-2010*. Diario Oficial de la

646     Federación.

647    Shinohara M., Domingo-Roura X., Takenaka O. 1997. Microsatellites in the bottlenose dolphin

648        *Tursiops truncatus. Molecular Ecology* 6:695–696. DOI: 10.1046/j.1365-

649        294X.1997.00231.x.

650    Swatdipong A., Primmer CR., Vasemägi A. 2010. Historical and recent genetic bottlenecks in

651        European grayling, *Thymallus thymallus. Conservation Genetics* 11:279–292. DOI:

652        10.1007/s10592-009-0031-x.

653    Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013. MEGA6: Molecular

654        evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729.

655        DOI: 10.1093/molbev/mst197.

656    Tezanos-Pinto G., Baker CS., Russell K., Martien K., Baird RW., Hutt A., Stone G., Mignucci-

657        Giannoni AA., Caballero S., Endo T., Lavery S., Oremus M., Olavarría C., Garrigue C.

658        2009. A worldwide perspective on the population structure and genetic diversity of

659        bottlenose dolphins (*Tursiops truncatus*) in New Zealand. *Journal of Heredity* 100:11–24.

660        DOI: 10.1093/jhered/esn039.

661    Thévenon S., Couvet D. 2002. The impact of inbreeding depression on population survival

662        depending on demographic parameters. *Animal Conservation* 5:53–60. DOI:

663        10.1017/S1367943002001075.

664    Thompson JD., Higgins DG., Gibson TJ. 1994. Clustal W: Improving the sensitivity of

665        progressive multiple sequence alignment through sequence weighting, position-specific gap

666        penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680. DOI:

667        10.1093/nar/22.22.4673.

668    Trone M., Kuczaj S., Solangi M. 2005. Does participation in Dolphin-Human Interaction

669        Programs affect bottlenose dolphin behaviour? *Applied Animal Behaviour Science* 93:363–

670    374. DOI: 10.1016/j.applanim.2005.01.003.

671    Tsai YJJ., Mann J. 2013. Dispersal, philopatry, and the role of fission-fusion dynamics in

672    bottlenose dolphins. *Marine Mammal Science* 29:261–279. DOI: 10.1111/j.1748-

673    7692.2011.00559.x.

674    Ugaz C., Valdez RA., Romano MC., Galindo F. 2013. Behavior and salivary cortisol of captive

675    dolphins (*Tursiops truncatus*) kept in open and closed facilities. *Journal of Veterinary*

676    *Behavior: Clinical Applications and Research* 8:285–290. DOI:

677    10.1016/j.jveb.2012.10.006.

678    Vermeulen E., Bräger S. 2015. Demographics of the disappearing bottlenose dolphin in

679    Argentina: a common species on its way out? *PLoS ONE* 10:1–19. DOI:

680    10.1371/journal.pone.0119182.

681    Viaud-Martínez KA., Brownell Jr. RL., Komnenou A., Bohonak AJ. 2008. Genetic isolation and

682    morphological divergence of Black Sea bottlenose dolphins. *Biological Conservation*

683    141:1600–1611. DOI: 10.1016/j.biocon.2008.04.004.

684    Waples KA., Gales NJ. 2002. Evaluating and minimising social stress in the care of captive

685    bottlenose dolphins (*Tursiops aduncus*). *Zoo Biology* 21:5–26. DOI: 10.1002/zoo.10004.

686    WAZA (World Association of Zoos and Aquariums). 2006. *Understanding Animals and*

687    *Protecting Them: About the World Zoo and Aquarium Strategy*. Liebefeld-Berne: WAZA.

688    Weir BS., Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure.

689    *Evolution* 38:1358–1370. DOI: 10.2307/2408641.

690    Wells RS., Scott MD. 1999. Bottlenose dolphin - *Tursiops truncatus* (Montagu, 1821). In:

691    Ridgway SH, Harrison SR eds. *Handbook of marine mammals: The second book of*

692    *dolphins and porpoises*. San Diego, CA: Academic Press, 137–182.

693    Wisely SM., McDonald DB., Buskirk SW. 2003. Evaluation of the genetic management of the

694        endangered black-footed ferret (*Mustela nigripes*). *Zoo Biology* 22:287–298. DOI:

695        10.1002/zoo.10089.

696    Woodworth LM., Montgomery ME., Briscoe DA., Frankham R. 2002. Rapid genetic

697        deterioration in captive populations: Causes and conservation implications. *Conservation*

698        *Genetics* 3:277–288. DOI: 10.1023/A:1019954801089.

699    Yeh FC., Yang RC., Boyle TBJ., Ye ZH., Mao JX. 1997. *Popgene, the user-friendly shareware*

700        *for population genetic analysis.* Molecular Biology and Biotechnology Center, University

701        of Alberta, Canada.

702    Yu D., Peng J., Hu S., Gao S., Fu M., Hu H., Zou J. 2011. Analysis of genetic variation and

703        bottleneck in a captive population of Siamese crocodile using novel microsatellite loci.

704        *Conservation Genetics Resources* 3:217–220. DOI: 10.1007/s12686-010-9326-y.

705

# Table 1(on next page)

Haplotype frequencies, ofindividuals (*n*), number of haplotypes (*k*), number or polymorphicsites (*PS*), and haplotype (*h*) and nucleotide (π) diversities forthe D-loop control region in *T. truncatus*.

1

| Haplotypes (Accession number) | Wild-born (origin site) | Captive-born | GLOBAL |
|---|---|---|---|
| H1 (KX151147) | 2 (1QR,1C) | 3 | 5 |
| H2 (KX151148) | 4 (2QR, 2C) | 1 | 5 |
| **H3 (KX151149)** | | **1** | **1** |
| H4 (KX151150) | 1 (QR) | 5 | 6 |
| H5 (KX151151) | 1 (QR) | 3 | 4 |
| H6 (KX151152) | 2 (1T, 1C) | 3 | 5 |
| H7 (KX151153) | 3 (2T, 1C) | 2 | 5 |
| H8 (KX151154) | 5 (1T, 4C) | 2 | 7 |
| H9 (KX151155) | 1 (1T) | 1 | 2 |
| **H10 (KX151156)** | **1 (1C)** | | **1** |
| **H11 (KX151157)** | **1 (1QR)** | | **1** |
| **H12 (KX151158)** | **1 (1QR)** | | **1** |
| **H13 (KX151159)** | **1 (1T)** | | **1** |
| $n$ | 23 | 21 | 44 |
| $k$ | 12 | 9 | 13 |
| $PS$ | 34 | 25 | 35 |
| $h \pm$ SD | $0.917 \pm 0.034$ | $0.900 \pm 0.036$ | $0.912 \pm 0.016$ |
| $\pi \pm$ SD | $0.025 \pm 0.0133$ | $0.025 \pm 0.013$ | $0.025 \pm 0.013$ |

2    QR= Quintana Roo, T=Tabasco, C=Cuba.

3

**Table 2**(on next page)

Overall genetic diversity of captive commonbottlenose dolphin population at nine nuclear microsatellite loci.

1

| Locus | Na | Ne | AR | Ho | $H_E$ |
|-------|-----|------|------|-------|-------|
| TexVet3 | 2 | 1.71 | 2.00 | 0.592 | 0.417 |
| TexVet5 | 3 | 1.63 | 2.93 | 0.449 | 0.387 |
| TexVet7 | 3 | 2.11 | 2.72 | 0.388 | 0.527 |
| D18 | 2 | 1.43 | 2.00 | 0.286 | 0.300 |
| D22 | 3 | 2.46 | 3.00 | 0.449 | 0.594 |
| Ttr19 | 3 | 1.72 | 2.93 | 0.204 | 0.418 |
| AAT44 | 5 | 3.07 | 5.00 | 0.564 | 0.674 |
| Tur4_80 | 4 | 2.61 | 4.00 | 0.286 | 0.618 |
| Tur4_105 | 10 | 5.83 | 9.81 | 0.771 | 0.829 |
| GATA098 | 5 | 3.07 | 4.98 | 0.500 | 0.675 |
| MEAN | 4 | 2.57 | 3.98 | 0.449 | 0.544 |
| Standard Dev. | 2.36 | 1.29 | 2.30 | 0.169 | 0.163 |

2    Na = number of alleles; Ne = effective number of alleles, AR = allelic richness; Ho = observed

3    heterozygosity, $H_E$ = Nei´s (1973) expected heterozygosity.

4

# Table 3(on next page)

Mean measures of microsatellite diversityof captive-born and wild-born bottlenose dolphins.

1

|  | Na (SD) | Ne (SD) | AR (SD) | Ho (SD) | $H_E$ (SD) |
|---|---|---|---|---|---|
| Captive-born | 3.9 (2.08) | 2.65 (1.41) | 3.84 (1.99) | 0.437 (0.171) | 0.549 (0.170) |
| Wild-born | 4.0 (2.36) | 2.37 (0.98) | 3.85 (2.14) | 0.459 (0.189) | 0.524 (0.154) |
| Global | 4.0 (2.36) | 2.57 (1.29) | 3.98 (2.30) | 0.449 (0.169) | 0.544 (0.163) |

2  Na = number of alleles; Ne = effective number of alleles, AR = allelic richness; Ho = observed

3  heterozygosity, $H_E$ = Nei´s (1973) expected heterozygosity.

4

# Table 4(on next page)

Wright´s fixation index ( F $_{IS}$ ) per locus ( Weir and Cockerham, 1984 ).

1

| Locus | Wild-born | Captive-born | Global |
|---|---|---|---|
| TexVet3 | -0.333 | -0.484 | -0.412 |
| TexVet5 | -0.211 | -0.086 | 0.152 |
| TexVet7 | 0.325 | 0.236 | 0.273 |
| D18 | 0.072 | 0.064 | 0.058 |
| D22 | 0.150 | 0.380 | 0.253 |
| Ttr19 | 0.405 | **0.657** | **0.519** |
| AAT44 | 0.178 | 0.165 | 0.176 |
| Tur4_80 | **0.631** | **0.464** | **0.545** |
| Tur4_105 | **-0.047** | **0.192** | **0.080** |
| GATA098 | 0.106 | 0.435 | **0.269** |

2    **\*\* p<0.01** after Bonferroni's correction

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# Table 5 (on next page)

Populationdifferentiation estimated by pairwise $F_{ST}$ values for microsatellite(below diagonal) and mtDNA (above diagonal) data

1

| | Cuba | Quintana Roo | Tabasco | Captive-born |
|---|---|---|---|---|
| **Cuba** | **** | **0.302*** | -0.031 | 0.031 |
| **Quintana Roo** | 0.009 | **** | 0.320 | 0.061 |
| **Tabasco** | 0.023 | -0.018 | **** | 0.07101 |
| **Captive-born** | 0.005 | -0.008 | -0.006 | **** |

2    **\* p<0.05** after Bonferroni's correction

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# Table 6(on next page)

Results of the one-tailed Wilcoxon test forheterozygosity excess for a population bottleneck under three models ofmolecular evolution.

SMM (stepwise mutation model), TPM (two-phased model) and IAM (infinite allele model).

1

| Mutation model | Proportion of SMM (%) | Wild-born | Captive-born | Global |
|---|---|---|---|---|
| SMM | | p = 0.590 | p = 0.326 | p = 0.097 |
| TPM | 70 (default) | p = 0.150 | **p = 0.014*** | **p = 0.001*** |
| IAM | | **p = 0.001*** | **p = 0.002*** | **p = 0.001*** |

2  **\* p < 0.05**

# Figure 1

**Original sampling sites of *Tursiopstruncatus* captive dolphins.**  Capture locationsof wild-born dolphins: Tabasco (T), Quintana Roo (QR) and Cuba (C). Thesample sizes are indicated in brackets

# Figure 2

**Minimum spanning network showing therelationship between haplotypes of captive *Tursiops truncatus*.** Circle sizes are proportional to haplotype frequency.

Haplotype names correspond to the same used in Table 1. Small black circles represent ancestral extinct or not sampled haplotypes, and each hash mark represents one mutational event.