



HAL
open science

Harbor porpoise losing its edges: genetic time series suggests a rapid population decline in Iberian waters over the last 30 years

Yacine Ben Chehida, Tjibbe Stelwagen, Jeroen P A Hoekendijk, Marisa Ferreira, Catarina Eira, Andreia T Pereira, Lidia Nicolau, Julie Thumloup, Michael C. Fontaine

► To cite this version:

Yacine Ben Chehida, Tjibbe Stelwagen, Jeroen P A Hoekendijk, Marisa Ferreira, Catarina Eira, et al.. Harbor porpoise losing its edges: genetic time series suggests a rapid population decline in Iberian waters over the last 30 years. *Ecology and Evolution*, In press, 10.1101/2021.08.19.456945 . hal-03415287v2

HAL Id: hal-03415287

<https://hal.science/hal-03415287v2>

Submitted on 23 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 Harbor porpoise losing its edge: genetic time series suggests a rapid 2 population decline in Iberian waters over the last 30 years

3
4 Yacine Ben Chehida^{1,2,3}, Tjibbe Stelwagen^{1,4}, Jeroen P. A. Hoekendijk^{1,5,6}, Marisa Ferreira⁷,
5 Catarina Eira^{7,8,9}, Andreia Torres-Pereira^{7,8,9}, Lidia Nicolau⁷, Julie Thumloup¹, Michael C.
6 Fontaine^{1,10*}

7 Affiliations

8 ¹ Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The
9 Netherlands

10 ² Department of Biology, University of York, Heslington, United Kingdom

11 ³ Ecology and Evolutionary Biology, School of Biosciences, University of Sheffield, Western Bank,
12 Sheffield, S10 2TN, United Kingdom

13 ⁴ BirdEyes, Centre for Global Ecological Change at the Faculties of Science & Engineering and Campus
14 Fryslân, University of Groningen, Zaailand 110, 8911 BN Leeuwarden, The Netherlands

15 ⁵ NIOZ Royal Netherlands Institute for Sea Research, Department of Coastal Systems, Utrecht University,
16 Texel, The Netherlands

17 ⁶ Wageningen University & Research Centre, Wageningen, The Netherlands

18 ⁷ MATB-Portuguese Wildlife Society (SPVS), Figueira da Foz, Portugal

19 ⁸ ECOMARE, Universidade de Aveiro, 3810-193 Aveiro, Portugal

20 ⁹ Centre for Environmental and Marine Studies CESAM, University of Aveiro, Portugal

21 ¹⁰ MIVEGEC, Univ. Montpellier, CNRS, IRD, Montpellier, France

22
23 * **Correspondence to** Michael C. Fontaine (michael.fontaine@ird.fr)

24 MIVEGEC (U. Montpellier, CNRS, IRD)

25 Institut de Recherche pour le Développement (IRD),

26 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5,

27 France.

28 29 ORCID ID

30 Yacine Ben Chehida: <https://orcid.org/0000-0001-7269-9082>

31 Tjibbe Stelwagen: <https://orcid.org/0000-0003-0296-1270>

32 Jeroen P. A. Hoekendijk: <https://orcid.org/0000-0002-9308-7429>

33 Marisa Ferreira: <https://orcid.org/0000-0002-0733-3452>

34 Andreia Torres-Pereira: <https://orcid.org/0000-0002-7189-0771>

35 Catarina Eira: <https://orcid.org/0000-0003-2735-6034>

36 Lidia Nicolau: <https://orcid.org/0000-0001-5714-162X>

37 Michael C. Fontaine: <https://orcid.org/0000-0003-1156-4154>

38

Abstract

39 Impact of climate change is expected to be especially noticeable at the edges of a species'
40 distribution, where they meet sub-optimal habitat conditions. In Mauritania and Iberia, two
41 genetically differentiated populations of harbor porpoises (*Phocoena phocoena*) form an
42 ecotype adapted to local upwelling conditions and distinct from other ecotypes further north
43 on the NE Atlantic continental shelf and in the Black Sea. By analyzing the evolution of
44 mitochondrial genetic variation in the Iberian population between two temporal cohorts (1990-
45 2002 vs. 2012-2015), we report a substantial decrease in genetic diversity. Phylogenetic
46 analyses including neighboring populations identified two porpoises in southern Iberia
47 carrying a divergent haplotype closely related to those from the Mauritanian population, yet
48 forming a distinct lineage. This suggests that Iberian porpoises may not be as isolated as
49 previously thought, indicating possible dispersion from Mauritania or an unknown population
50 in between, but none from the northern ecotype. Demo-genetic scenario testing by approximate
51 Bayesian computation showed that the rapid decline in the Iberian mitochondrial diversity was
52 not simply due to the genetic drift of a small population, but models support instead a
53 substantial decline in effective population size, possibly resulting from environmental
54 stochasticity, prey depletion, or acute fishery bycatches. These results illustrate the value of
55 genetics time series to inform demographic trends and emphasize the urgent need for
56 conservation measures to ensure the viability of this small harbor porpoise population in
57 Iberian waters.

58

59 **KEYWORDS:** cetacean conservation genetics, genetic time series, genetic diversity,
60 bycatch, approximate Bayesian computation, population genetic modelling

61 **1. Introduction**

62 The impact of climate change on species is expected to be especially noticeable at their
63 distribution edges, where species meet the limits of their ecological tolerance. Marginal
64 populations of temperate species at the rear (warm) distribution edge are often considered more
65 susceptible to warming than central populations because of the warmer ambient temperatures
66 they experience, but this overlooks the potential for local adaptation (Bennett et al., 2015; Vilà-
67 Cabrera et al., 2019).

68 Cetaceans are particularly vulnerable to climate change (MacLeod, 2009) due to their
69 crucial function as apex predators in marine ecosystems (Bowen, 1997). Climate change
70 influences the availability of their prey and modifies the habitat, thereby impacting several (if
71 not all) cetacean species and may even put some of them at a high risk of extinction (Learmonth
72 et al., 2006). As a response, several cetacean species are shifting their distribution ranges to
73 track suitable habitats or adapt locally by switching to different food resources (Lambert et al.,
74 2011; Learmonth et al., 2006; MacLeod, 2009; Williamson et al., 2021). For example,
75 temperate and subpolar small cetaceans like the harbor porpoises (*Phocoena phocoena*) are
76 likely to show a poleward shift (Heide-Jørgensen et al., 2011; MacLeod, 2009). Due to an
77 elevated metabolic rate and relatively short generation time, this small cetacean is heavily
78 dependent on a continuous food supply (Hoekendijk et al., 2018; Wisniewska et al., 2016).
79 This species is thus expected to be particularly sensitive to climate changes (Heide-Jørgensen
80 et al., 2011). In addition, due to their coastal distribution, thousands of harbor porpoises are
81 also killed accidentally each year by commercial fisheries. This by-catch mortality is
82 threatening local populations to a level that is still hard to quantify, but it is likely unsustainable,
83 especially with a highly fluctuating environment (Carlén et al., 2021; IMR-NAMMCO, 2019;
84 Vingada & Eira, 2018).

85 Harbor porpoises are officially subdivided into three distinct subspecies: *P. p. vomerina*
86 in the Pacific, *P. p. phocoena* in the North Atlantic, and *P. p. relicta* in the Black Sea (Fontaine,
87 2016; Read, 1999; Rosel et al., 1995). A fourth lineage or Evolutionary Significant Unit (ESU)
88 has been suggested for the Afro-Iberian harbor porpoises (IBMA; currently unnamed sub-
89 species, but possibly *P. p. meridionalis* following Fontaine et al. 2014) owing to their
90 distinctive ecology, morphology (Donovan & Bjørge, 1995; Smeenk et al., 1992), and genetic
91 divergence (Fontaine et al., 2014) (Figure 1). However, no formal description or subspecies
92 taxonomic assignment has been made for this distinct lineage so far (Pierce et al., 2020). IBMA
93 porpoises are found around the relatively cold and productive upwelling waters off the coasts
94 of Iberia and on the NW Atlantic coasts of Africa from southern Morocco, to Mauritania, and
95 Senegal (Fontaine, 2016; Gaskin, 1984; IMR-NAMMCO, 2019; Read, 1999). They are
96 significantly larger in term of skull morphology, growth curve, and adult body size (typically
97 ≥ 2 m long) compared to the two other ecotypes (or subspecies) (~ 1.5 m long) (Galatius &
98 Gol'din, 2011; Read, 1999; Sequeira, 1996; Smeenk et al., 1992). Beside their distinctive
99 morphological features, the IBMA porpoises belong to the Eastern Canary Coastal province
100 with equatorward surface circulation in the form of the Canary current, rich in eddies and large
101 upwellings that are permanent along the NW African coast and seasonal along the Iberian coast
102 (Álvarez-Salgado et al., 2003; Fraga, 1981; Moustahfid et al., 2021; Pierce et al., in revision).
103 While *P. p. phocoena* in the northern Bay of Biscay and further north in the North Atlantic are
104 generally considered opportunistic feeder relying primarily on bottom dwelling on the shallow
105 continental shelf (i.e. in waters of < 200 m depth), the IBMA porpoise feeding ecology is still
106 poorly known. It is hypothesized that they exhibit similar foraging behaviors as their
107 counterparts on the continental shelf to the north, but with a greater emphasis on pelagic fish
108 (Pierce et al., in revision; Pinela et al., 2010; Santos & Pierce, 2003). From a genetic
109 perspective, the Iberian and Mauritanian porpoises are closely related but they were found

110 genetically differentiated from each other and form two distinct populations separated by at
111 least 2500 km (Ben Chehida et al., 2021; Fontaine, 2016; Fontaine et al., 2014).

112 The upwelling habitat of the Iberian porpoise (IB) population is at the border of a
113 biogeographical transition zone between temperate and subtropical waters where species with
114 different affinities co-occur and reach their respective ecological limits (Bowen, 1997;
115 MacLeod, 2009). Because species' habitat suitability at the margins between biogeographic
116 zones are often suboptimal, populations like the Iberian harbor porpoises are expected to be
117 among the first to show the impacts of environmental changes. This is particularly true with
118 the small-scale Iberian upwelling system, which is known to fluctuate in response to ongoing
119 climate change (Casabella et al., 2014; Pires et al., 2013). Such environmental fluctuation may
120 impact the Iberian harbor porpoises in various ways, possibly leading to dramatic impacts on
121 the population demography and genetic diversity. Porpoises may adapt locally, for example,
122 by adapting to various prey species (Heide-Jørgensen et al., 2011), by migrating to more
123 suitable environments, or the population may also become extirpated if environmental changes
124 outpace their ability to adapt. Previous population genetic studies (Ben Chehida et al., 2021;
125 Fontaine et al., 2014; Fontaine et al., 2017; Fontaine et al., 2010), based on samples collected
126 between 1990 and 2002, showed that the Iberian population was a source population with
127 highly asymmetric gene flow, sending more migrants to the North into the *P. p. phocoena*
128 populations on the northern side of the Bay of Biscay and to the South into the Mauritanian
129 population than in the other direction (Figure 1). In fact, a tension zone was detected in the
130 northern side of the Bay of Biscay with Iberian genetic ancestry decreasing sharply with the
131 increasing latitude (Fontaine et al., 2007; Fontaine et al., 2014; Fontaine et al., 2017). If
132 recruitment does not keep up with the mortality and migration rate, the census size of the
133 Iberian population may decrease, leading to a gradual reduction in number of reproducing
134 individuals, in genetic diversity, and thus in effective population size over time.

135 Unlike previous studies, which inferred population structure between the Iberian
136 population and other populations (Ben Chehida et al., 2021; Fontaine et al., 2014; Fontaine et
137 al., 2017; Fontaine et al., 2010), the goal of the present study is to assess how mtDNA variation
138 evolved over time in the Iberian population, comparing sampling cohorts spanning about 3
139 decades and how this information can guide conservation of the Iberian population. More
140 precisely, using genetic diversity as a proxy for effective population size variation, we tested
141 if mitochondrial genetic diversity decreased through time by comparing the genetic
142 composition of two cohorts of porpoises, one sampled in the 1990's (1990-2002), and the other
143 one from the 2010's (2012-2015).

144

145 **2. Material and methods**

146 *Sampling and molecular analyses.* Tissue samples from 52 by-caught or stranded
147 individuals collected along the Portuguese coast between 2012 and 2015 were obtained from
148 the Portuguese Marine Animal Tissue Bank (MATB). These samples form the 2010's cohort
149 of the Iberian population (also called the 'new' cohort or IB_N in this study) (**Figure 1, Table**
150 **1 and S1**). Tissues samples were stored in 70% ethanol and kept at -20°C until molecular
151 analyses. Genomic DNA was extracted with the DNeasy Blood and Tissue kit (QIAGEN Inc.)
152 following the manufacturer's protocol. Following Fontaine et al. (2014), we screened five
153 mitochondrial coding regions (*ATP-6*, *ATP-8*, *Cyt-b*, *ND5*, and *COI*) for genetic
154 polymorphisms using Sanger sequencing. Target regions were PCR amplified, quality
155 controlled, and purified following the protocol described in Fontaine et al. (2014). Sanger
156 sequencing was outsourced to GATC Biotech Ltd. The resulting forward and reverse sequences
157 were visually inspected, cleaned, and assembled with *Geneious* 10.0.9 (Kearse et al., 2012).

158 We augmented the dataset with the 82 previously published sequences from Fontaine et al.
159 (2014) encompassing the same five mitochondrial genes. These sequences were obtained for

160 82 harbor porpoises collected between 1990 and 2002 (Figure 1, Table 1 and S1). These
161 included: 14 individuals collected along the French coast of the Bay of Biscay (BB), 23 further
162 north in the North Atlantic (NAT) from the Channel to the Nordic waters of Norway and
163 Iceland; 19 from the Iberian coast (*a.k.a.* the 'old' Iberian cohort or IB_O), 14 from Mauritania
164 (MA); and 12 from the Black Sea (BS). The 'old' Iberian cohort from Fontaine et al. (2014)
165 obtained between 1990 and 2002 included 19 stranded and bycaught porpoises from the
166 Galician coasts of Spain and Portugal (Figure 1 and Table S1). Previous studies based on
167 mtDNA data and nuclear microsatellite data demonstrated that the Spanish and Portuguese
168 samples were part of a same panmictic genetic group (Ben Chehida et al., 2021; Fontaine et
169 al., 2007; Fontaine et al., 2014; Fontaine et al., 2017). The main focus of the present study thus
170 consists in comparing the temporal evolution of the mtDNA variation between the two Iberian
171 cohorts of porpoises: *i.e.* the 'new' [2010's: 2012-2015, n=52] *versus* the 'old' [1990's:1990-
172 2002, n=19]). The temporal coverage of this serial sampling of the Iberian population is thus
173 at most 25 years, which corresponds to 2.5 generations, assuming a generation time of 10 years
174 (Read, 1999). The mtDNA sequences from the other populations of Fontaine et al. (2014)
175 neighboring the Iberian population were included here to provide a broader perspective to
176 interpret the phylogenetic analyses.

177 One additional sequence from the closest outgroup species, the Dall's porpoise
178 (*Phocoenoides dalli*) also generated in Fontaine et al. (2014), was included in the present
179 dataset to root the mitochondrial phylogeny. All mtDNA sequences (n=135) were aligned with
180 *MUSCLE* (Edgar, 2004).

181

182 *Phylogenetic and population genetic analyses.* In order to place the Iberian porpoises into
183 a global phylogeographic context, we first reconstructed a maximum-likelihood (ML)
184 phylogeny with all the 135 mtDNA sequences (including the outgroup). Unique mtDNA

185 haplotypes were identified using *DnaSP* v.5.10.1 (Librado & Rozas, 2009). Then, we used
186 *PhyML* (Guindon et al., 2010) implemented in *Geneious* to estimate the ML tree based on all
187 available unique haplotypes identified in the dataset using a nearest neighbor interchange
188 topology search. We used the Tamura and Nei (1993)'s (or TN93) model of nucleotide
189 evolution as identified as the best-fitting model for our sequence alignment using the Akaike
190 Information Criterion (AIC) in jModelTest 2.1.10 (Darriba et al., 2012). Each node's support
191 was evaluated by conducting 5,000 iterations of bootstrap resampling. The phylogenetic tree
192 was rooted with the sequence from a Dall's porpoise. In addition to the phylogenetic trees, we
193 also reconstructed a median-joining haplotype network (Bandelt et al. 1999) using *PopART*
194 (<http://popart.otago.ac.nz>).

195 Various statistics informative on the mtDNA genetic variation of the two cohorts of
196 Iberian porpoises ("old" vs. "new") were estimated using the C library *libdiversity*
197 (<https://bioinfo.mnhn.fr/abi/people/achaz/cgi-bin/neutraltytst.c>) and the R package *pegas*
198 v.1.2 (Paradis, 2010) (**Table 1**). Genetic diversity was estimated using the number of
199 haplotypes (H), haplotype diversity (H_d), nucleotide diversity (π), and Watterson's theta (θ_W).
200 Differences in sample size can significantly impact the values of some genetic diversity
201 estimators (Goodall-Copestake et al., 2012). Therefore, a rarefaction approach was applied to
202 estimate measures of genetic diversity while correcting for difference in sample size (Sanders,
203 1968) using a custom python script. More precisely, for the two cohorts of Iberian porpoises,
204 for each statistic and each sample size increment k , we randomly subsampled 5,000 times with
205 replacement a number of k sequences available in each group, with k ranging between 2 and
206 the k_{max} available sequences (up to a maximum of 50). At each sample size increment k , we
207 estimated the mean and standard error values per statistic and per group. Subsequently, genetic
208 differentiation between the two cohorts was assessed using the Hudson's estimator of F_{ST}
209 (Hudson et al., 1992) and ϕ_{ST} (Excoffier et al., 1992) using respectively *the libdiversity C-*

210 *library* and the R-package *haplotypes v.1.1.3.1*. The significance levels of F_{ST} and ϕ_{ST} were
211 assessed by randomization tests (1,000 permutations). Finally, we investigated evidence of
212 changes in mtDNA effective population size (N_e) between the two cohorts (old vs. new) by
213 calculating values of Tajima's D (Tajima, 1989) and Achaz's Y (Achaz, 2008) accounting for
214 differences in sample sizes by implementing the same rarefaction approach as described for
215 the diversity statistics. The Achaz's Y statistic is analogous to the *Tajima's D*, but accounting
216 for potential sequencing errors (see details in Achaz, 2008).

217

218 *Approximate Bayesian Computation (ABC)*. In order to evaluate the support for different
219 demographic scenarios and assess whether there is a signal of a recent population decline in
220 the Iberian population based on the mtDNA data, we applied an ABC approach relying on
221 coalescent simulations (Lopes & Beaumont, 2010). More precisely, we compared six
222 demographic scenarios: (1) a population with a large constant effective population size, (2) a
223 small constant effective population size, (3) an old expansion (≥ 150 generations, gen.), (4) an
224 old decline (≥ 150 gen.), (5) a recent decline (≤ 3 gen.), and (6) an old expansion (> 150 gen.)
225 followed by a recent decline scenario (≤ 3 gen.) (see **Figure 2 and Table S2** for details). In the
226 coalescent simulations, we leverage the time series data information available and specified
227 explicitly at which time the samplings of the two cohorts were performed.

228 For each of the six scenarios, we ran 10,000 coalescent simulations using *fastsimcoal2*
229 *v2.6* (Excoffier & Foll, 2011). Details on the coalescent simulations and the mutation model
230 used are provided in the supplementary **Table S2**. We used the program *Arlsumstat v3.5.2*
231 (Excoffier & Lischer, 2010) to compute a set of 55 summary statistics listed in **Table S3**,
232 augmented with the principal axes obtained from a linear discriminant analysis (LDA) on the
233 summary statistics (Pudlo et al., 2016), to describe and summarize the genetic variation of each
234 simulation.

235 We employed a random forest classification (RF) procedure for the model selection (Pudlo
236 et al., 2016). RF is a machine-learning algorithm that uses bootstrapped decision trees to
237 classify and select the best scenario using the summary statistics as a set of predictor variables
238 (Breiman, 2001). RF was therefore applied to compare the likelihood of the six competing
239 scenarios (Figure 2). This procedure provides classification votes that accounts for the number
240 of times a scenario is selected as the best one among the trees used to constructed the random
241 forest. Based on a forest of a 1,000 decision trees, the scenario that gathered the highest number
242 of votes was selected as the most likely.

243 The *prior* error rate and the type-II error rate were estimated using the out-of-bag
244 simulations (i.e. simulations not used to build the decision trees). In addition, we computed the
245 confusion matrix, which provides a more global assessment of the performance of our RF
246 procedure (Schridder & Kern, 2018). The convergence of the results was evaluated over 10
247 independent RF runs, as recommended by Fraimout et al. (2017).

248 Finally, to check whether the formulated scenarios and associated priors were compatible
249 with the observed dataset, we plotted both the simulated datasets from the training set and the
250 observed dataset on the two first principal axes of a Linear Discriminant Analysis (LDA) and
251 of a Principal Component Analysis (PCA), following the recommendation of Pudlo et al.
252 (2016). We visually checked whether the summary statistics from the observed data was
253 located within the clouds formed by those from the simulated data for at least one demographic
254 scenario. In addition, we assessed the goodness-of-fit between the simulated and the observed
255 data following Gelman et al. (2004): the summary statistics for which the cumulative simulated
256 distribution was larger or smaller than 95% CI of the distribution compared to the observed
257 value were considered as significantly different from the observed data. All ABC-RF analyses
258 were performed using the R package *abcrf* v.1.8.1 (Pudlo et al., 2016) in R 4.1.2 (R Core Team,
259 2016).

260

261 **3. Results and Discussion**

262 In total, we successfully sequenced 52 Iberian harbor porpoises from the new cohort
263 (2012-2015) for fragments of five mitochondrial genes including Cytochrome b (Cyt-b: 1,111
264 base-pairs, bp), and ATP-6 and -8 (842bp), NADH dehydrogenase 5 (ND5: 707bp), and the
265 Cytochrome c oxidase subunit I (COI: 1,515 bp), When aligned with the 82 previous sequences
266 of harbor porpoises from Fontaine et al. (2014) and an additional one of the Dall's porpoise
267 used here as outgroup, the final alignment of 4,175 base-pairs (pb) and included 135 sequences
268 and contained 384 segregating sites with 267 singletons and 117 parsimony informative sites
269 defining 61 distinct haplotypes. Without the outgroup, the 134 mtDNA sequences of harbor
270 porpoise contained 170 segregating sites with 65 singletons and 105 parsimony informative
271 sites defining 60 distinct haplotypes ([Table 1](#)).

272 Consistent with previous studies, the phylogenetic relationships among mtDNA
273 haplotypes depicted by the ML tree ([Figure 3 and S1](#)) and by the haplotype network ([Figure](#)
274 [S2](#)) revealed the three main monophyletic lineages corresponding to the three previously
275 identified subspecies or ESU's (Fontaine et al., 2014): (i) *P. p. relicta* in the Black Sea; (ii) *P.*
276 *p. phocoena* composed of the porpoises from the North Sea to the waters of Norway; and (iii)
277 the Afro-Iberian (IBMA) porpoises (unnamed *ssp.* possibly *P. p. meridionalis*), which
278 comprises two distinct monophyletic sub-lineages in the upwelling waters of Iberia and
279 Mauritania, respectively.

280 Evidence of migration from the Iberian to the Mauritanian populations has been previously
281 documented (Ben Chehida et al., 2021; Fontaine et al., 2014) and is also observed here with
282 two haplotypes (Hap_55 and Hap_59) found in the Mauritanian population, but clustering with
283 the Iberian lineage ([Figure 3, S1 and S2](#)). However, we also identified one haplotype (Hap_10
284 or Haplotype 10, see [Figure 3, S1 and S2](#)) present in two porpoises from the new Iberian

285 cohort, but clustering with the Mauritanian lineage. This haplotype was carried by two
286 individuals in the southernmost part of the Iberian sampling (red arrow in **Figure 1**). This result
287 provides the first evidence of possible dispersal (or migration) from the Mauritanian population
288 into Iberian waters, suggesting that these two populations may not be as isolated as previously
289 thought. Haplotype 10 could also belong to a distinct unknown population as it is relatively
290 divergent from the other Mauritanian haplotypes (**Figure 3, S1 and S2**). Indeed, the ML tree
291 (**Figure 3 and S1**) shows that this haplotype forms a strongly supported (bootstrap support
292 >70%) distinct lineage from the other Mauritanian porpoises. The haplotype network also
293 illustrates this divergence (**Figure S2**): while the Mauritanian haplotypes are separated from
294 each other with at most two mutational steps, at least ten mutational steps separate this
295 haplotype 10 from the other Mauritanian haplotypes (**Figure S2**). Notably, both porpoises
296 carrying this haplotype 10 were found on the southern extremity of the Portuguese coasts
297 (8.78W-37.07N; Praia do Burgau, Portugal), at least 400km south from all other Iberian
298 sampling locations (**Figure 1**). Stranding records of harbor porpoises were previously reported
299 in Morocco, on the east coasts of Gibraltar, and in Cadiz (Spain; Rojo–Nieto et al., 2011). If
300 unsampled populations occur between the Iberian Peninsula and the Mauritanian waters, they
301 are most likely confined to a narrow and seasonal upwelling area. This relatively divergent
302 haplotype 10 closely related to the Mauritanian rather than the Iberian porpoises could be
303 evidence of such unsampled populations. This hypothesis of such unsampled population could
304 be plausible since harbor porpoise sightings, stranding, and bycatches have been reported from
305 the North of Morocco to the Gambia (Boisseau et al., 2010; IMR-NAMMCO, 2019).
306 Haplotype 10 was excluded from subsequent analyses of genetic diversity and coalescent
307 simulations because it could be part of a distinct population and therefore would artificially
308 inflate measures of genetic diversity (**Table 1 and S1**). Future studies with additional samples
309 from these relatively unsampled regions (southern Iberian coast and Morocco) and data from

310 the nuclear genome are needed to confirm whether these two porpoises are genetically distinct
311 from the other Iberian porpoises, possibly forming a distinct population, and whether or not
312 they are admixing with the other Iberian porpoises.

313 Aside from the two porpoises carrying the haplotype 10, our results indicate that both
314 cohorts sampled at different times along the Iberian coasts belong to the same mtDNA gene
315 pool. We detected no genetic differentiation between the two Iberian cohorts suggesting no
316 significant differences in haplotype frequencies between them (F_{ST} and ϕ_{ST} values = 0; p -value
317 > 0.54, **Table S4**). This result reflects the high prevalence of haplotype 4 which dominates in
318 both cohorts (**Figure 3, S1 and S2**). However, once we accounted for differences in sample
319 size between the two cohorts using a rarefaction method, we observed a significant reduction
320 in genetic diversity in the new cohort compared to the old one as shown by the lower number
321 of haplotypes (H ; **Figure 4a**), and in the two measures of nucleotide diversity (π and θ_w ; **Figure**
322 **4b and 4c**, respectively). Rarefaction curves provided striking evidence of this reduction in
323 genetic diversity (**Figure 4**). The non-overlapping standard errors from a standardized sample
324 size beyond eight sequences suggest that the decline of genetic variation is likely highly
325 significant. For example, the average number of haplotypes (H) observed from 19 sampled
326 sequences in each cohort decreases from 10 to 6 when comparing the old vs. new cohorts. This
327 suggests that the proportion of rare haplotypes has dropped substantially over the 25-years
328 sampling period of this study. Consistent with this observation is the rapid loss in nucleotide
329 genetic diversity as measured by π and θ_w (**Figure 4b and 4c**). Such a substantial loss of genetic
330 variation over 2.5 generations is often attributed to an enhanced effect of genetic drift rather
331 than other evolutionary forces like selection (Hartl, 2020). This could suggest a dramatic
332 decline of the Iberian population in term of effective population size, possibly reflecting a
333 substantial decline in the number of reproducing individuals.

334 Tajima's D (Tajima, 1989) and Achaz's Y (Achaz, 2008) statistics estimated for the two
335 cohorts sampled at different times along the Iberian coast also displayed negative values
336 (Figure 5). At first glance, such negative values would be indicative of a population that
337 underwent a "recent" expansion at evolutionary time-scales (Epps & Keyghobadi, 2015; Smith
338 et al., 2011), most likely due to the foundation of the Iberian population during the period that
339 followed the Last Glacial Maximum (19kyr ago; Fontaine et al. 2014; Fontaine 2016).
340 However, the values estimated for the new cohort for both statistics were significantly less
341 negative (based on non-overlapped standard error estimates) than those from the old cohort.
342 For example, the Tajima's D values obtained from 19 randomly sampled sequences increased
343 from -1.5 for the old cohort to -1.25 for the new (Figure 5a). The change in Achaz's Y values,
344 which account for possible sequencing errors (Achaz, 2008), show an even stronger increase
345 from -1.75 to -0.95 between the old and new cohorts (Figure 5b). It is remarkable to see how
346 both values have become dramatically less negative in the time lapse of only 2.5 generations
347 between the two cohorts. This increase in D and Y values also provides a strong indication of
348 a population losing rare haplotypes as it would be expected for a small and isolated population
349 strongly subject to genetic drift that could be declining.

350 Such a loss of genetic diversity may reflect different demographic scenarios, for example
351 a small population that did not experience any population size changes, but still experiencing
352 strong genetic drift. Alternatively, a scenario of ancient expansion and a very recent decline
353 could be also possible. We used a simulation-based Approximate Bayesian computation
354 analysis relying on the Random-Forest classifier (ABC-RF) to explored statistically six
355 plausible demographic scenarios (Figure 2) describing the evolutionary history of the Iberian
356 population since it diverged from the Mauritanian population within the last 150 generations
357 (Fontaine et al., 2014). Out of the six scenarios compared, the analysis strongly supported the
358 SC6 scenario assuming an "old" expansion (>150 generations) followed by recent decline with

359 the past 3 generations (SC6; **Figure 2, S3 to S5**). Ten replicates of the ABC-RF analysis
360 supported strongly and unanimously this result (**Figure S3**). Scenario SC6 gathered in average
361 (\pm SD) over the ten runs $56.6 \pm 1.3\%$ of the RF votes (**Figure 2 and S3**), with a posterior
362 probability of $66.3 \pm 2.5\%$, a *prior* error rate of $9.5 \pm 0.0\%$, and type II errors $\leq 3.1\% \pm 0.0\%$.
363 Furthermore, the confusion matrix (**Figure S5**) depicting the overall performance of the RF
364 classification analysis indicated high power to distinguish between the competing scenarios
365 ($>90\%$). Such a high-resolution power to discriminate among the different demographic
366 scenarios likely comes from the multiple sampling time points included in the ABC analysis.
367 The goodness-of-fit analysis provided additional support to the demographic model identified
368 by showing that scenario SC6 was the only scenario that produced simulated values that closely
369 matched the observed values for each summary statistics used in this analysis (**Table S5,**
370 **Figure S4 and S6**). This scenario SC6 (Figure 2) suggests that the effective size (N_e) of the
371 Iberian population expanded at least ~ 150 generations ago, thus ~ 1500 years ago, assuming a
372 rough generation time of 10 years (Fontaine et al., 2014). Then, under this scenario, N_e values
373 of the Iberian population would have declined by a factor ≥ 10 within the last three generations
374 (thus 30 years). Thus, this ABC analysis, together with the rarefaction results, adds an
375 additional line of evidence suggesting a rapid loss of genetic variation over the last three
376 generations due to a very recent decline in effective population size. The reader should note
377 however that the effective population size (N_e) is a population genetic concept that differs from
378 and is usually smaller than the *census* population size. It does not represent the number of
379 individuals actually alive at any given time point. Following Frankham (2019), N_e is the
380 number of individuals that would result in the same loss of genetic diversity, inbreeding,
381 genetic drift, etc., if they behaved in the manner of an idealized population. Such idealized
382 population would assume non-overlapping generations, random mating, and no migration,
383 mutation or selection. N_e would thus roughly represent the number of breeding individuals in

384 this idealized population, or more precisely the number of breeding females since we analyzed
385 here maternally inherited fragments of the mitogenome that behave as a single non-
386 recombining haploid unit. Therefore, our results are not intended to estimate exactly how many
387 Iberian porpoises there were at different points in history, but rather they suggest that the
388 number of reproducing females may have been substantially declining in the Iberian population
389 over the last 2.5 generations. However, such interpretation must be taken with caution
390 considering all the assumptions related to the N_e concept.

391 In this study, we also confirmed previous evidence of gene flow from the Iberian
392 population to more northern populations with Iberian haplotypes present in the Bay of Biscay
393 and in the English Channel where admixture between the two lineages, IBMA (or *P. p.*
394 *meridionalis*) and *P. p. phocoena*, has been reported (see Figure 3, S1 and S2; Table S4, and
395 also Ben Chehida et al., 2021; Fontaine et al., 2014; Fontaine et al., 2017). Additionally, we
396 did not find any evidence of gene flow from the populations located further North into the
397 Iberian population, as previously reported (Ben Chehida et al., 2021; Fontaine et al., 2007;
398 Fontaine et al., 2014; Fontaine et al., 2017; Fontaine et al., 2010). Indeed, Fontaine et al. (2014)
399 estimated very asymmetric values of gene flow using the coalescent approach of MIGRATE
400 (Beerli, 2006; Beerli, 2009) based on microsatellite data. While no evidence of immigration
401 was detected toward the Iberian porpoises from the neighboring populations, *ca.* 3 individuals
402 per generation were estimated migrating from the Iberian to the Mauritanian populations, and
403 70 per generation from the Iberian to the northern Bay of Biscay populations (see table 2 in
404 Fontaine et al., 2014). In the northern Bay of Biscay and adjacent waters, a geographically
405 restricted admixture zone was reported forming a well-delimited tension zone (Fontaine et al.,
406 2017). Because the population size of *P. p. phocoena* in the North Sea and adjacent waters is
407 much larger than that of the Iberian population (census N estimates from the SCANS-III survey
408 (Hammond et al., 2017): $\sim 400,000$ vs. $\leq 3,000$ individuals respectively), the observed

409 northward migration may possibly lead to the assimilation of the Iberian gene pool by *P. p.*
410 *phocoena*. In the long term, this process could be an additional cause of concern as it could
411 result in the disappearance of the unique Iberian genetic pool. This scenario is advanced by
412 Macleod et al. (2009), which used habitat modeling to show that species with a similar range
413 as the Iberian harbor porpoises (Figure 1C in MacLeod, 2009) may shift their range northward
414 in response to a 5°C increase in water temperature due to global warming (Figure 1D in
415 MacLeod, 2009). This model suggests that the future range of IBMA could completely overlap
416 with the current range of *P. p. phocoena* (Figure 1D in MacLeod, 2009). It is worth mentioning
417 that Ben Chehida et al. (2021) recently modeled the future distribution of the harbor porpoises
418 in the North Atlantic under the most aggressive scenario of the Intergovernmental Panel on
419 Climate Change (IPCC) in 2050 (RCP8.5; Schwalm et al., 2020) and did not capture this strong
420 northward range shift. The discrepancy between the two models may be related to differences
421 in assumed temperature increase, 2°C and 5°C, in Ben Chehida et al. (2021) and MacLeod
422 (2009), respectively. In addition, the environmental niche modeling in Ben Chehida et al.
423 (2021) did not account for any biotic factors in the simulations. However, environmental niche
424 modelling such as those conducted by MacLeod (2009) and Ben Chehida et al. (2021) needs
425 to be taken with some caution since they do not account for local population adaptation. In the
426 case of IBMA porpoises, their morphological and genetic distinctiveness together with the
427 peculiar upwelling habitat they inhabit suggest that they are adapted to the local conditions.
428 Future genomic studies could assess whether selective processes have impacted the genome of
429 IBMA porpoises and whether there is genetic evidence of such local adaptation process
430 compared to other populations.

431 The results of this study support the hypothesis that the Iberian population is both small
432 and isolated. Furthermore, both the sharp decline in mtDNA genetic diversity over the past 25
433 years and northward migration may represent reasons for concern for the long-term persistence

434 of the Iberian porpoises. One plausible explanation is that the decay of mtDNA genetic
435 variation we reported here could simply reflect the fact that Iberian porpoises constitute a stable
436 population with a very low effective population size (N_e) (SC2 in Figure 2). This scenario SC2
437 was not the best model according to our simulation-based ABC-RF analysis, but the third best
438 one, receiving only 16% of the total RF classification votes, but it remains plausible (**Figure**
439 **2, S3 to S6, Table S5**). In such a condition, genetic drift would have a disproportionate
440 influence on the genetic make-up of this population and could lead to the observed loss of
441 genetic variation over a few generations. Several previous studies showed that the Iberian
442 population has indeed a very low N_e at nuclear microsatellite loci ($N_e < 100$ individuals; Ben
443 Chehida et al., 2021; Fontaine et al., 2014; Fontaine et al., 2010). The mtDNA employed in
444 this study has an effective size four times smaller compared with nuclear autosomal loci. This
445 means that the stochastic loss of genetic variation due to genetic drift would be drastically
446 increased for mtDNA compared to nuclear loci. Thus, the Iberian population might be at a
447 stable census size losing rare haplotypes through the enhanced effect of genetic drift at the
448 mtDNA. Another more plausible explanation receiving the highest support in our ABC-RF
449 analysis (scenario SC6, **Figure 2, S2 to S6, Table S5**) is that the decay of mtDNA genetic
450 variation might be associated with a rapid and recent decline of the number of reproductive
451 females in addition to the overall low N_e (Lande, 1988), as depicted by the scenarios SC6 in
452 **Figure 2**. This scenario SC6 support a severe population decline in the last three generations,
453 leaving at most 10% of the population pre-decline N_e . This hypothesis is even more plausible
454 since there is accumulating evidence that the Iberian population is sending more migrants to
455 adjacent populations than the reverse (Ben Chehida et al., 2021; Fontaine et al., 2014; Fontaine
456 et al., 2010). Thus, in addition to being small and isolated, the recent and strong population
457 decline envisioned under SC6 of the ABC-RF analysis would imply that the Iberian porpoises
458 may become increasingly subject to demographic stochasticity (Allendorf et al., 2012).

459 Because this population is also known to be subject to strong genetic drift and environmental
460 stochasticity (Casabella et al., 2014; Pires et al., 2013), all these effects can accumulate and
461 reinforce each other, potentially precipitating the decline of the Iberian porpoises. This so-
462 called extinction vortex (Allendorf, et al., 2012) can be particularly severe for a long-lived K
463 strategist species, inhabiting a marginal habitat highly subject to environmental variation like
464 the Iberian harbor porpoises (Casabella et al., 2014; Pires et al., 2013) and could put them at a
465 high risk of extinction.

466 The present study relies on a single molecular marker (mtDNA). Therefore, future studies
467 with additional samples and data from the nuclear genome are highly needed to confirm the
468 trends highlighted here. Indeed, genome-wide data can provide further insights into the
469 temporal dynamics of the Iberian population. For example, the reconstruction of historical
470 demography based on genome-wide data (Liu & Fu, 2020; Schiffels & Wang, 2020; Terhorst
471 et al., 2017) can be used to test whether these harbor porpoises maintained a long-term low N_e
472 (Morin et al., 2020; Robinson et al., 2022) since their post-glacial emergence (Fontaine, 2016;
473 Fontaine et al., 2014; Fontaine et al., 2010), or if on the contrary, the population declined
474 recently (Hu et al., 2020). A simulation framework, similar to the one used by Robinson et al.
475 (2022), could be applied to assess whether these porpoises are already facing deleterious effects
476 of the mutation load, despite their current low N_e , or if purging processes have been ongoing
477 similar to those recently reported for the vaquita (*Phocoena sinus*) (Morin et al., 2020;
478 Robinson et al., 2022).

479 Additional data sources also point to large fluctuations in population size in the Iberian
480 porpoises and require careful considerations: the SCANS-III survey in summer 2016 estimated
481 a census size as low as 2,898 animals (CV=0.32) in Iberian waters and Bay of Biscay
482 (Hammond et al., 2017). Annual aerial surveys in Portuguese continental waters between 2011
483 and 2015 estimated an overall average abundance of 2,254 porpoises and corresponding

484 density of 0.09 ind/km² (CV=21.99%) with large interannual fluctuations. The highest annual
485 abundance estimated for that period was 3,207 individuals (CV=38.14%) in 2013, followed by
486 a sharp decrease in 2014, when only 1,653 individuals (CV=43.27%) were estimated (Torres-
487 Pereira et al., 2022). López et al. (2013) estimated an abundance of 683 (CV=0.63, 95%CI:
488 345-951) animals for the Spanish part of the IBMA range (Galicia and Bay of Biscay).
489 Globally, these low census sizes were associated with particularly high bycatches rate (always
490 $\geq 8\%$) with the best estimates varying from 90-197 deaths per year (Pierce et al., 2020) to 130-
491 330 deaths per year (Read et al., 2020). However, it is important to mention that these estimates
492 are mainly based on “on-board observer” and aerial surveys data that deliver wide confidence
493 limits. This is due to low observer coverage of fishing activities in the region, low density of
494 porpoises, and estimates from various sources (observer data, strandings, aerial survey, and
495 interviews). Furthermore, Iberian harbor porpoises are regularly found stranded, often with
496 signs of bycatch mortality, potentially indicating unsustainable mortality rates (IMR-
497 NAMMCO, 2019; López et al., 2002; Pierce et al., 2020; Read et al., 2020; Torres-Pereira et
498 al., 2022; Torres-Pereira et al., 2023a; Vingada & Eira, 2018). In fact, based on Portuguese
499 stranding data, an average of 9.19% (CI=5.25-16.10%) of the porpoise population was
500 estimated to be removed annually by fisheries between 2011 and 2015, corresponding to an
501 average of 207 porpoises removed per year, which largely surpasses the Potential Biological
502 Removal rate conservatively estimated for the same period (22 porpoises, CI: 12-43) (Torres-
503 Pereira et al., 2023a). An additional cause for concern is related to potential overexploitation
504 of the feeding resources (Méndez-Fernandez et al., 2013; Santos & Pierce, 2003). A scarcity
505 in prey availability can be harmful because it enhances interspecific competition, as already
506 reported between harbor porpoises and other marine mammals, for example with the bottlenose
507 dolphin *Tursiops truncatus* whose ecological niche largely overlaps with the harbor porpoise
508 in some portions of Iberian waters (Méndez-Fernandez et al., 2013; Spitz et al., 2006). Hostile

509 interactions between the two species have been documented in Iberian waters and hypothesized
510 to cause harbor porpoises to disperse to less productive narrow shelf areas (Alonso et al., 2000;
511 Spitz et al., 2006).

512

513 **Conclusions**

514 The Afro-Iberian (IBMA) lineage of harbour porpoises was previously recognized as a
515 unique Evolutionarily Significant Unit (Crandall et al., 2000; Moritz, 2002) distinct from the
516 other sub-species devribed in the N. Atlantic and in the Black Sea. IBMA display unique
517 features in terms of genetics, morphology, ecology and evolutionary history following Fontaine
518 et al. (2014) (see also the review of Fontaine, 2016). Nevertheless, they are currently not
519 recognized as a distinct subspecies (Pierce et al., 2022; Pierce et al., 2020). There is also
520 currently no mention of these porpoises in the IUCN Red List of Threatened Species despite
521 the multiple threats faced by this vulnerable lineage. Given the results of this study, the
522 previous findings on their genetic distinctiveness from other populations at both mtDNA and
523 nuclear markers (Ben Chehida et al., 2021; Fontaine, 2016; Fontaine et al., 2007; Fontaine et
524 al., 2014; Fontaine et al., 2017; Fontaine et al., 2010), and the previous concerns raised about
525 their viability (IMR-NAMMCO, 2019; Pierce et al., 2022; Pierce et al., 2020), we recommend
526 immediate actions and further research from the community in the following areas: (1) a formal
527 taxonomic description is needed for these IBMA porpoises to be officially recognized as a
528 distinct subspecies, following the guidelines of the *International Commission on Zoological*
529 *Nomenclature* as recommended by the Taxonomic Committee of the Society for Marine
530 Mammalogy; and (2) a better assessment for the Iberian porpoises in term of their life history
531 traits, ecology, behavior, genetics and genomics, and the threats to the viability for this unique
532 group. This knowledge will help designing better conservation measures and
533 recommendations. It is indeed paramount to ensure that all measures are taken to gain

534 additional knowledge and to apply effective conservation efforts to protect the Iberian
535 population, as recently underline by the OSPAR Marine Mammal Expert Group (OMMEG)
536 (ICES-WGMM, 2021; Pierce et al., 2022). Recently, both countries (Portugal and Spain)
537 updated the conservation status of the harbor porpoises inhabiting Iberian waters: from
538 “Vulnerable” to “Endangered” in Spain (Boletin oficial del estado, 2020) and to “Critically
539 Endangered” in Portugal (Torres-Pereira et al., 2023b). In Portugal, a new Marine Protected
540 Area (PTCON0063) in the northwestern coast was recently designated whereas another one
541 was enlarged in the southwestern coast (PTCON00012) in order to incur higher protection for
542 harbor porpoise hotspots in the country (Vingada & Eira, 2018). Nonetheless, none of the
543 conservation and mitigation measures within the areas’ management plan (Portaria 201/2019)
544 is currently in place and bycatch mortality remains effectively unaddressed. In the future,
545 improving our understanding of the demographic trends and the threats these porpoises are
546 facing is required to devise suitable and tailored larger scale conservation plans for the whole
547 Iberian population. Such measures are timely needed to mitigate the decline of this unique
548 lineage potentially on the brink of extinction.

549

550 **Acknowledgements**

551 We thank all the people and networks involved in the sampling collection of harbor porpoises
552 over the years across the distribution range of the harbour porpoises, including KA Tolley, T
553 Jauniaux, J Haelters, AA Llavona, B and AA Ozturk, V Ridoux, F Caurant, W Dabin, E Rogan,
554 M Sequeira, U Siebert, GA Vikingsson, A Borrell, AA Aguilar, and the national stranding
555 networks: Réseau National d'Échouage and PELAGIS in France; Royal Belgian Institute of
556 Natural Sciences - MUMM, and Dept. Pathology of the U. Liège, Belgium; CEMMA -
557 Coordinadora para o Estudio dos Mamíferos Mariños, Spain; the Portuguese Marine Animal
558 Tissue Bank-MTAB and Regional Stranding Networks coordinated by SPVS and U. of Aveiro,
559 Portugal; the Dutch Stranding Network, SOS Dolfijn, and Dept. Pathology of the U. Utrecht in
560 the Netherlands; and the Dept. pathology of the U. Kiel, Germany. All samples were collected
561 under appropriate permits following the relevant guidelines and regulations, and transferred
562 internationally under CITES permits. We also thank Graham J. Pierce for his feedbacks on the
563 first draft of the manuscript and the participants of the IMR-NAMMCO workshop in Tromsø
564 (Norway, December 2018) for their significant inputs during the development of this study.
565 We are also grateful to the Centre for Information Technology of the University of Groningen,
566 and in particular Fokke Dijkstra and Bob Dröge, for their continuous support and for providing
567 access to the Peregrine high-performance computing cluster. This study was funded by the
568 University of Groningen (The Netherlands). YBC was supported by a PhD fellowship from the
569 University of Groningen.

570 **Conflict of interest**

571 The authors declare that they have no conflict of interest.

572

573

574 **Data Availability**

575 The data sets and scripts supporting this study are available in the IRD Porpoises Genetics and
576 Genomics Dataverse Repository (https://dataverse.ird.fr/dataverse/porpoise_genomics) at
577 <https://doi.org/10.23708/GOPRGV>. Mitochondrial haplotypes were also deposited on the
578 NCBI–GenBank under the Accession Numbers (Accession numbers are listed in Table S1).

579

580 **Author Contributions**

581 Conceptualization: MCF. Data curation: MF, ATP, LN, CE, MCF. Formal analysis: YBC,
582 TS, MCF. Funding acquisition: MCF. Investigation: JT, TS, JPAH, MCF. Methodology: JT,
583 TS, JPAH, MCF. Project administration: MCF. Resources: JT, TS, JPAH, MCF. Software:
584 YBC. Supervision: MCF. Validation: MCF, YBC, JT. Visualization: TS, YBC, MCF.
585 Writing – original draft: YBC, TS, MCF. Writing – review & editing: YBC, MCF with inputs
586 from all the co-authors.

587

588 **REFERENCES**

- 589 Achaz, G. (2008). Testing for neutrality in samples with sequencing errors. *Genetics*, 179(3), 1409-1424.
590 doi:10.1534/genetics.107.082198
- 591 Allendorf, F. W., Luikart, G. H., & Aitken, S. N. (2012). *Conservation and the Genetics of Populations*: John
592 Wiley & Sons.
- 593 Alonso, J., Lopez, A., González, A., & Santos, M. (2000). Evidence of violent interactions between bottlenose
594 dolphin (*Tursiops truncatus*) and other cetacean species in NW Spain. *European Research on Cetaceans*, 14,
595 105-106.
- 596 Álvarez-Salgado, X. A., Figueiras, F. G., Pérez, F. F., Groom, S., Nogueira, E., Borges, A. V., . . . Wollast, R.
597 (2003). The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability.
598 *Progress in Oceanography*, 56(2), 281-321. doi:10.1016/s0079-6611(03)00007-7
- 599 Beerli, P. (2006). Comparison of Bayesian and maximum-likelihood inference of population genetic parameters.
600 *Bioinformatics*, 22(3), 341-345. doi:10.1093/bioinformatics/bti803
- 601 Beerli, P. (2009). How to use MIGRATE or why are Markov chain Monte Carlo programs difficult to use? In A.
602 Rizzoli, C. Vernesi, G. Bertorelle, H. C. Hauffe, & M. W. Bruford (Eds.), *Population Genetics for Animal*
603 *Conservation* (pp. 42-79). Cambridge: Cambridge University Press.
- 604 Ben Chehida, Y., Loughnane, R., Thumloup, J., Kaschner, K., Garilao, C., Rosel, P. E., & Fontaine, M. C.
605 (2021). No leading-edge effect in North Atlantic harbor porpoises: Evolutionary and conservation implications.
606 *Evolutionary Applications*, 14(6), 1588-1611. doi:<https://doi.org/10.1111/eva.13227>
- 607 Bennett, S., Wernberg, T., Arackal Joy, B., de Bettignies, T., & Campbell, A. H. (2015). Central and rear-edge
608 populations can be equally vulnerable to warming. *Nature Communications*, 6(1), 10280.
609 doi:10.1038/ncomms10280
- 610 Boisseau, O., Lacey, C., Lewis, T., Moscrop, A., Danbolt, M., & McLanaghan, R. (2010). Encounter rates of
611 cetaceans in the Mediterranean Sea and contiguous Atlantic area. *Marine Biological Association of the United*
612 *Kingdom. Journal of the Marine Biological Association of the United Kingdom*, 90(8), 1589.
- 613 Boletín oficial del estado. (2020). «BOE» núm. 314, de 1 de diciembre de 2020. 108167-108171.
- 614 Bowen, W. D. (1997). Role of marine mammals in aquatic ecosystems. *Marine Ecology Progress Series*, 158,
615 267-274. doi:10.3354/meps158267
- 616 Breiman, L. (2001). Random Forests. *Machine Learning*, 45(1), 5-32.
617 doi:papers3://publication/doi/10.1023/A:1010933404324
- 618 Carlén, I., Nunny, L., & Simmonds, M. P. (2021). Out of sight, out of mind: how conservation is failing
619 European porpoises. *Frontiers in Marine Science*, 8, 13. doi:10.3389/fmars.2021.617478
- 620 Casabella, N., Lorenzo, M. N., & Taboada, J. J. (2014). Trends of the Galician upwelling in the context of
621 climate change. *Journal of Sea Research*, 93, 23-27. doi:10.1016/j.seares.2014.01.013
- 622 Crandall, K. A., Bininda-Emonds, O. R., Mace, G. M., & Wayne, R. K. (2000). Considering evolutionary
623 processes in conservation biology. *Trends Ecol Evol*, 15(7), 290-295. doi:10.1016/s0169-5347(00)01876-0
- 624 Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and
625 parallel computing. *Nature Methods*, 9(8), 772. doi:10.1038/nmeth.2109
- 626 Donovan, G. P., & Bjørge, A. (1995). Harbour porpoises in the North Atlantic: edited extract from the report of
627 the IWC Scientific Committee. In A. Bjørge & G. P. Donovan (Eds.), *Biology of the phocoenids* (Vol. Special
628 Issue n°16, pp. 3-26). Dublin: Rep. Int. Whal. Commn.

- 629 Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic*
630 *Acids Research*, 32(5), 1792-1797. doi:10.1093/nar/gkh340
- 631 Epps, C. W., & Keyghobadi, N. (2015). Landscape genetics in a changing world: disentangling historical and
632 contemporary influences and inferring change. *Molecular Ecology*, 24(24), 6021-6040. doi:10.1111/mec.13454
- 633 Excoffier, L., & Foll, M. (2011). fastsimcoal: a continuous-time coalescent simulator of genomic diversity
634 under arbitrarily complex evolutionary scenarios. *Bioinformatics*, 27(9), 1332-1334.
635 doi:10.1093/bioinformatics/btr124
- 636 Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population
637 genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564-567.
638 doi:10.1111/j.1755-0998.2010.02847.x
- 639 Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric
640 distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131(2),
641 479-491.
- 642 Fontaine, M. C. (2016). Harbour porpoises, *Phocoena phocoena*, in the Mediterranean sea and adjacent regions:
643 biogeographic relicts of the last glacial period. *Advances in Marine Biology*, 75, 333-358.
644 doi:10.1016/bs.amb.2016.08.006
- 645 Fontaine, M. C., Baird, S. J. E., Piry, S., Ray, N., Tolley, K. A., Duke, S., . . . Michaux, J. R. (2007). Rise of
646 oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in
647 Old World waters. *BMC Biology*, 5(1), 30. doi:10.1186/1741-7007-5-30
- 648 Fontaine, M. C., Roland, K., Calves, I., Austerlitz, F., Palstra, F. P., Tolley, K. A., . . . Aguilar, A. (2014).
649 Postglacial climate changes and rise of three ecotypes of harbour porpoises, *Phocoena phocoena*, in western
650 Palearctic waters. *Molecular Ecology*, 23(13), 3306-3321. doi:10.1111/mec.12817
- 651 Fontaine, M. C., Thatcher, O., Ray, N., Piry, S., Brownlow, A., Davison, N. J., . . . Goodman, S. J. (2017).
652 Mixing of porpoise ecotypes in southwestern UK waters revealed by genetic profiling. *Royal Society Open*
653 *Science*, 4(3), 160992. doi:10.1098/rsos.160992
- 654 Fontaine, M. C., Tolley, K. A., Michaux, J. R., Birkun, A., Ferreira, M., Jauniaux, T., . . . Baird, S. J. E. (2010).
655 Genetic and historic evidence for climate-driven population fragmentation in a top cetacean predator: the
656 harbour porpoises in European water. *Proceedings of the Royal Society. Biology*, 277(1695), 2829-2837.
657 doi:10.1098/rspb.2010.0412
- 658 Fraga, F. (1981). Upwelling off the Galacian Coast, northwest Spain. In *Coastal Upwelling* (pp. 176-182).
- 659 Fraimout, A., Debat, V., Fellous, S., Hufbauer, R. A., Foucaud, J., Pudlo, P., . . . Estoup, A. (2017). Deciphering
660 the Routes of invasion of *Drosophila suzukii* by Means of ABC Random Forest. *Molecular biology and*
661 *evolution*, 34(4), 980-996. doi:10.1093/molbev/msx050
- 662 Frankham, R. (2019). Conservation Genetics. In *Encyclopedia of Ecology* (pp. 382-390).
- 663 Galatius, A., & Gol'din, P. E. (2011). Geographic variation of skeletal ontogeny and skull shape in the harbour
664 porpoise (*Phocoena phocoena*). *Canadian Journal of Zoology*, 89(9), 869-879. doi:10.1139/z11-059
- 665 Gaskin, D. E. (1984). The harbour porpoise *Phocoena phocoena* (L.): regional populations, status, and
666 information on direct and indirect catches. *Rep. int. Whal. Commn.*, 34, 569-586.
- 667 Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Vehtari, A., & Rubin, D. B. (2004). *Bayesian Data*
668 *Analysis, Second Edition*: CRC Press.
- 669 Goodall-Copestake, W. P., Tarling, G. A., & Murphy, E. J. (2012). On the comparison of population-level
670 estimates of haplotype and nucleotide diversity: a case study using the gene *cox1* in animals. *Heredity*, 109(1),
671 50-56. doi:10.1038/hdy.2012.12

- 672 Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms
673 and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0.
674 *Systematic Biology*, 59(3), 307-321. doi:10.1093/sysbio/syq010
- 675 Hammond, P., Lacey, C., Gilles, A., Viquerat, S., Börjesson, P., Herr, H., . . . Oien, N. (2017). *Estimates of*
676 *cetacean abundance in European Atlantic waters in summer 2016 from the SCANS-III aerial and shipboard*
677 *surveys*. Retrieved from [https://scans3.wp.st-andrews.ac.uk/files/2021/06/SCANS-III_design-](https://scans3.wp.st-andrews.ac.uk/files/2021/06/SCANS-III_design-based_estimates_final_report_revised_June_2021.pdf)
678 [based_estimates_final_report_revised_June_2021.pdf](https://scans3.wp.st-andrews.ac.uk/files/2021/06/SCANS-III_design-based_estimates_final_report_revised_June_2021.pdf)
- 679 Hartl, D. L. (2020). *A Primer of Population Genetics and Genomics* (4 ed.). Oxford: Oxford University Press.
- 680 Heide-Jørgensen, M. P., Iversen, M., Nielsen, N. H., Lockyer, C., Stern, H., & Ribergaard, M. H. (2011).
681 Harbour porpoises respond to climate change. *Ecology and Evolution*, 1(4), 579-585. doi:10.1002/ece3.51
- 682 Hoekendijk, J. P. A., Spitz, J., Read, A. J., Leopold, M. F., & Fontaine, M. C. (2018). Resilience of harbor
683 porpoises to anthropogenic disturbance: Must they really feed continuously? *Marine Mammal Science*, 34(1),
684 258-264. doi:10.1111/mms.12446
- 685 Hu, J.-Y., Hao, Z.-Q., Frantz, L., Wu, S.-F., Chen, W., Jiang, Y.-F., . . . Yu, L. (2020). Genomic consequences
686 of population decline in critically endangered pangolins and their demographic histories. *National Science*
687 *Review*, 7(4), 798-814. doi:10.1093/nsr/nwaa031
- 688 Hudson, R. R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence
689 data. *Genetics*, 132(2), 583-589.
- 690 ICES-WGMM. (2021). Workshop on estimation of MORTality of Marine MAMmals due to Bycatch
691 (WKMOMA). *ICES Scientific Reports*, 3(106), 95. doi:<https://doi.org/10.17895/ices.pub.9257>
- 692 IMR-NAMMCO. (2019). *Report of Joint IMR/NAMMCO International Workshop on the Status of Harbour*
693 *Porpoises in the North Atlantic*. Retrieved from Tromsø, Norway: Retrieved from NAMMCO, Tromsø,
694 Norway: https://nammco.no/wp-content/uploads/2020/03/final-report_hpws_2018_rev2020.pdf
- 695 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Drummond, A. (2012).
696 Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of
697 sequence data. *Bioinformatics*, 28(12), 1647-1649. doi:10.1093/bioinformatics/bts199
- 698 Lambert, E., MacLeod, C. D., Hall, K., Brereton, T., Dunn, T. E., Wall, D., . . . Pierce, G. J. (2011). Quantifying
699 likely cetacean range shifts in response to global climatic change: implications for conservation strategies in a
700 changing world. *Endangered Species Research*, 15(3), 205-222. doi:10.3354/esr00376
- 701 Lande, R. (1988). Genetics and demography in biological conservation. *Science*, 241(4872), 1455-1460.
702 doi:10.1126/science.3420403
- 703 Learmonth, J. A., MacLeod, C. D., Santos, M. B., Pierce, G. J., Crick, H., & Robinson, R. (2006). Potential
704 effects of climate change on marine mammals. *Oceanography and Marine Biology*, 44, 431.
705 doi:10.1016/j.envint.2009.10.008
- 706 Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data.
707 *Bioinformatics*, 25(11), 1451-1452. doi:10.1093/bioinformatics/btp187
- 708 Liu, X., & Fu, Y.-X. (2020). Stairway Plot 2: demographic history inference with folded SNP frequency spectra.
709 *Genome Biology*, 21(1), 280. doi:10.1186/s13059-020-02196-9
- 710 Lopes, J. S., & Beaumont, M. A. (2010). ABC: A useful Bayesian tool for the analysis of population data.
711 *Infection, Genetics and Evolution*, 10(6), 825-832. doi:10.1016/j.meegid.2009.10.010
- 712 López, A., Santos, M. B., Pierce, G. J., González, A. F., Valeiras, X., & Guerra, A. (2002). Trends in strandings
713 and by-catch of marine mammals in north-west Spain during the 1990s. *Journal of the Marine Biological*
714 *Association of the UK*, 82(3), 513-521. doi:10.1017/s0025315402005805

- 715 López, A., Vázquez, J. A., Martínez Cedeira, J. A., Cañadas, A., Marcos, E., Maestre, I., . . . Evans, P. (2013).
716 *Abundance estimates for harbour porpoise (Phocoena phocoena) in the Spanish area of the Iberian Peninsula*
717 *Management Unit. (SC/65a/SM20)*. Retrieved from Jeju Island, Republic of Korea:
- 718 MacLeod, C. D. (2009). Global climate change, range changes and potential implications for the conservation of
719 marine cetaceans: a review and synthesis. *Endangered Species Research*, 7(2), 125-136. doi:10.3354/esr00197
- 720 Méndez-Fernandez, P., Pierce, G. J., Bustamante, P., Chouvelon, T., Ferreira, M., González, A. F., . . . Caurant,
721 F. (2013). Ecological niche segregation among five toothed whale species off the NW Iberian Peninsula using
722 ecological tracers as multi-approach. *Marine Biology*, 160(11), 2825-2840. doi:10.1007/s00227-013-2274-9
- 723 Morin, P. A., Archer, F. I., Avila, C. D., Balacco, J. R., Bukhman, Y. V., Chow, W., . . . Jarvis, E. D. (2020).
724 Reference genome and demographic history of the most endangered marine mammal, the vaquita. *Molecular*
725 *Ecology Resources*, 1-13. doi:10.1111/1755-0998.13284
- 726 Moritz, C. (2002). Strategies to protect biological diversity and the evolutionary processes that sustain it. *Syst*
727 *Biol*, 51(2), 238-254. doi:10.1080/10635150252899752
- 728 Moustahfid, H., Hendrickson, L. C., Arkhipkin, A., Pierce, G. J., Gangopadhyay, A., Kidokoro, H., . . .
729 Laptikhovskiy, V. (2021). Ecological-Fishery Forecasting of Squid Stock Dynamics under Climate Variability
730 and Change: Review, Challenges, and Recommendations. *Reviews in Fisheries Science & Aquaculture*, 29(4),
731 682-705. doi:10.1080/23308249.2020.1864720
- 732 Paradis, E. (2010). pegas: an R package for population genetics with an integrated-modular approach.
733 *Bioinformatics*, 26(3), 419-420. doi:10.1093/bioinformatics/btp696
- 734 Pierce, G. J., Petitguyot, M. A. C., Gutierrez-Muñoz, P., Fariñas-Bermejo, A., Fernández-Fernández, D.,
735 Dolman, S., . . . Weir, C. R. (accepted). An endangered population of harbour porpoise *Phocoena phocoena*
736 hidden in plain sight: biology, ecology and conservation of the Iberian porpoise. *Oceanography and Marine*
737 *Biology - An Annual Review*.
- 738 Pierce, G. J., Petitguyot, M. A. C., Gutiérrez-Muñoz, P., Fernández-Fernández, D., Fariñas-Bermejo, A., Read,
739 F. L., . . . Martínez-Cedeira, J. (2022). *Iberian harbour porpoise - an update on fishery bycatch mortality.*
740 *Report N°SC/68D/HIM/10 submitted by the Sub-committees/working HIM to the International Whaling*
741 *Commission (SC/68D/HIM/10; DOI: 10.13140/RG.2.2.35061.14565)*. Retrieved from
- 742 Pierce, G. J., Weir, C. R., Gutierrez, P., Verutes, G., Fontaine, M. C., Hernández-González, A., . . . Covelo, P.
743 (2020). Is Iberian harbour porpoise (*Phocoena phocoena*) threatened by interactions with fisheries?
- 744 Pinela, A. M., Borrell, A., Cardona, L., & Aguilar, A. (2010). Stable isotope analysis reveals habitat partitioning
745 among marine mammals off the NW African coast and unique trophic niches for two globally threatened
746 species. *Marine Ecology Progress Series*, 416, 295-306. doi:10.3354/meps08790
- 747 Pires, A. C., Nolasco, R., Rocha, A., & Dubert, J. (2013). Assessing future climate change in the Iberian
748 Upwelling System. *Journal of Coastal Research*, 165(sp2), 1909-1914. doi:10.2112/si65-323.1
- 749 Portaria 201/2019 of 28 of June. Diário da República no 122/19 - I Série. Ministério Ambiente e Transição
750 Energética e Mar. Lisboa.
- 751 Pudlo, P., Marin, J.-M., Estoup, A., Cornuet, J.-M., Gautier, M., & Robert, C. P. (2016). Reliable ABC model
752 choice via random forests. *Bioinformatics*, 32(6), 859-866. doi:10.1093/bioinformatics/btv684
- 753 R Core Team. (2016). R: A language and environment for statistical computing. Vienna, Austria.
- 754 Read, A. J. (1999). Harbour porpoise *Phocoena phocoena* (Linnaeus, 1758). In R. J. Harrison & S. H. Ridgway
755 (Eds.), *Handbook of marine mammals. Second book of dolphins* (Vol. 6, pp. 323-350). London: Academic Press
756 San Diego.
- 757 Read, F., Santos, M., Ferreira, M., Lopez, A., & Pierce, G. (2020). *Harbour porpoise (Phocoena phocoena) and*
758 *fisheries interactions in the north-west Iberian Peninsula. (SC/68B/SM/06 Rev1)*. Retrieved from

- 759 Robinson, J. A., Kyriazis, C. C., Nigenda-Morales, S. F., Beichman, A. C., Rojas-Bracho, L., Robertson, K. M.,
760 . . . Morin, P. A. (2022). The critically endangered vaquita is not doomed to extinction by inbreeding
761 depression. *Science*, 376(6593), 635-639. doi:10.1126/science.abm1742
- 762 Rojo-Nieto, E., Álvarez-Díaz, P., Morote, E., Burgos-Martín, M., Montoto-Martínez, T., Sáez-Jiménez, J., &
763 Toledano, F. (2011). Strandings of cetaceans and sea turtles in the Alboran Sea and Strait of Gibraltar: a long-
764 term glimpse at the north coast (Spain) and the south coast (Morocco). *Anim. Biodivers. Conserv.*, 34(1), 151-
765 163.
- 766 Rosel, P. E., Dizon, A. E., & Haygood, M. G. (1995). Variability of the mitochondrial control region in
767 populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. *Canadian Journal*
768 *of Fisheries and Aquatic Sciences*, 52(6), 1210-1219. doi:10.1139/f95-118
- 769 Sanders, H. L. (1968). Marine benthic diversity: a comparative study. *The American Naturalist*, 102(925), 243-
770 282. doi:10.1086/282541
- 771 Santos, M. B., & Pierce, G. J. (2003). The diet of harbour porpoise (*Phocoena phocoena*) in the Northeast
772 Atlantic. *Oceanography and Marine Biology, An Annual Review*, 41, 355-390.
- 773 Schiffels, S., & Wang, K. (2020). MSMC and MSMC2: The Multiple Sequentially Markovian Coalescent. In J.
774 Y. Dutheil (Ed.), *Statistical Population Genomics* (pp. 147-166). New York, NY: Springer US.
- 775 Schrider, D. R., & Kern, A. D. (2018). Supervised Machine Learning for Population Genetics: A New
776 Paradigm. *Trends in Genetics*, 34(4), 301-312. doi:10.1016/j.tig.2017.12.005
- 777 Schwalm, C. R., Glendon, S., & Duffy, P. B. (2020). RCP8.5 tracks cumulative CO2 emissions. *Proceedings of*
778 *the National Academy of Sciences*, 117(33), 19656-19657. doi:10.1073/pnas.2007117117
- 779 Sequeira, M. (1996). Harbour porpoise, *Phocoena phocoena*, in Portuguese waters. . *Rep. Int. Whal. Comm.*, 46,
780 583-586.
- 781 Smeenk, C., Leopold, M. F., & Addink, M. J. (1992). Note on the harbour porpoise *Phocoena phocoena* in
782 Mauritania, West Africa. 35, 98-104. doi:papers3://publication/uuid/BD7ABA30-C511-4019-9B79-
783 6D91136299E1
- 784 Smith, B. T., Escalante, P., Hernández Baños, B. E., Navarro-Sigüenza, A. G., Rohwer, S., & Klicka, J. (2011).
785 The role of historical and contemporary processes on phylogeographic structure and genetic diversity in the
786 Northern Cardinal, *Cardinalis cardinalis*. *BMC evolutionary biology*, 11(1), 4261-4212. doi:10.1186/1471-2148-
787 11-136
- 788 Spitz, J., Rousseau, Y., & Ridoux, V. (2006). Diet overlap between harbour porpoise and bottlenose dolphin: An
789 argument in favour of interference competition for food? *Estuarine, Coastal and Shelf Science*, 70(1-2), 259-
790 270. doi:10.1016/j.ecss.2006.04.020
- 791 Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism.
792 *Genetics*, 123(3), 585-595. doi:10.1093/genetics/123.3.585
- 793 Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of
794 mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*, 10(3), 512-526.
795 doi:10.1093/oxfordjournals.molbev.a040023
- 796 Terhorst, J., Kamm, J. A., & Song, Y. S. (2017). Robust and scalable inference of population history from
797 hundreds of unphased whole genomes. *Nature Genetics*, 49(2), 303-309. doi:10.1038/ng.3748
- 798 Torres-Pereira, A., Araújo, H., Matos, F. L., Bastos-Santos, J., Sá, S., Ferreira, M., . . . Eira, C. (2022). Harbour
799 Porpoise Abundance in Portugal over a 5-Year Period and Estimates of Potential Distribution. *Animals*, 12(15).
800 doi:10.3390/ani12151935

- 801 Torres-Pereira, A., Araújo, H., Monteiro, S. S., Ferreira, M., Bastos-Santos, J., Sá, S., . . . Eira, C. (2023a).
802 Assessment of Harbour Porpoise Bycatch along the Portuguese and Galician Coast: Insights from Strandings
803 over Two Decades. *Animals*, 13(16). doi:10.3390/ani13162632
- 804 Torres-Pereira, A., Ferreira, M., Eira, C., López, A., & Sequeira, M. (2023b). *Phocoena phocoena* boto. In M.
805 L. Mathias, C. Fonseca, L. Rodrigues, C. Grilo, M. Lopes-Fernandes, J. M. Palmeirim, M. Santos-Reis, P. C.
806 Alves, J. A. Cabral, M. Ferreira, A. Mira, C. Eira, N. Negrões, J. Paupério, R. Pita, A. Rainho, L. M. Rosalino,
807 J. T. Tapisso, & J. Vingada (Eds.), *Livro Vermelho dos Mamíferos de Portugal Continental* (pp. 190-191).
808 Lisboa, Portugal: Associação para a Investigação e Desenvolvimento de Ciências & Instituto da Conservação da
809 Natureza e das Florestas.
- 810 Vilà-Cabrera, A., Premoli, A. C., & Jump, A. S. (2019). Refining predictions of population decline at species'
811 rear edges. *Global Change Biology*, 25(5), 1549-1560. doi:10.1111/gcb.14597
- 812 Vingada, J., & Eira, C. (2018). *Conservação de Cetáceos e Aves Marinhas em Portugal Continental. O projeto*
813 *LIFE+ MarPro. Conservation of Cetaceans and Seabirds in Continental Portugal. Relatório final do projecto*
814 *NAT/PT/00038*. Retrieved from
- 815 Williamson, M. J., ten Doeschate, M. T. I., Deaville, R., Brownlow, A. C., & Taylor, N. L. (2021). Cetaceans as
816 sentinels for informing climate change policy in UK waters. *Marine Policy*, 131, 104634.
817 doi:<https://doi.org/10.1016/j.marpol.2021.104634>
- 818 Wisniewska, D. M., Johnson, M., Teilmann, J., Rojano-Doñate, L., Shearer, J., Sveegaard, S., . . . Madsen, P. T.
819 (2016). Ultra-high foraging rates of harbor porpoises make them vulnerable to anthropogenic disturbance.
820 *Current Biology*, 26(11), 1441-1446. doi:10.1016/j.cub.2016.03.069
821

Tables and Figures

Table 1: Mitochondrial genetic diversity. The statistics display per groups and overall include the mtDNA sample sizes (N), the number of segregating sites (S), singleton mutations (*Singl.*); sites informative in parsimony (*Parsim.*), number of haplotypes ($\# Hap$), haplotype diversity (Hd), the nucleotide diversity (π), Watterson's theta (θ_w), Tajima's D , Y , Achaz's Y .

Population codes are as follow: BS=Black Sea; NAT=North Atlantic; BB=Bay of Biscay; MA=Mauritania; IB_O=Iberian Old 1990's cohort; IB_N= Iberian new 2010's cohort.

Superscript next to each population code indicate the origin of the mtDNA sequence :
¹Fontaine et al. (2014); ² Fontaine et al. (2014) + this study; 3. This study. See Table S1 for more details on the samples.

	N	S	<i>Singl.</i>	<i>Parsim.</i>	$\# Hap$	Hd	π (%)	θ_w (%)	D	Y
BS¹	12	23	21	2	10	0.96	0.10	0.18	-2.04	-1.56
NAT¹	23	99	57	42	18	0.97	0.41	0.64	-1.46	-0.83
BB¹	14	66	19	47	10	0.95	0.52	0.50	0.22	0.05
MA¹	14	11	1	10	7	0.90	0.07	0.08	-0.66	-1.93
All IB²	71	27	7	20	17	0.72	0.05	0.13	-1.85	-2.12
IB_O¹	19	12	6	6	10	0.74	0.05	0.08	-1.53	-1.77
IB_N³	52	24	5	19	11	0.70	0.06	0.13	-1.77	-2.19
IB_N*³	50	14	7	7	10	0.73	0.04	0.08	-1.58	-1.26
All	134	170	65	105	60	0.91	0.40	0.74	-	-

*Excluding haplotype 10

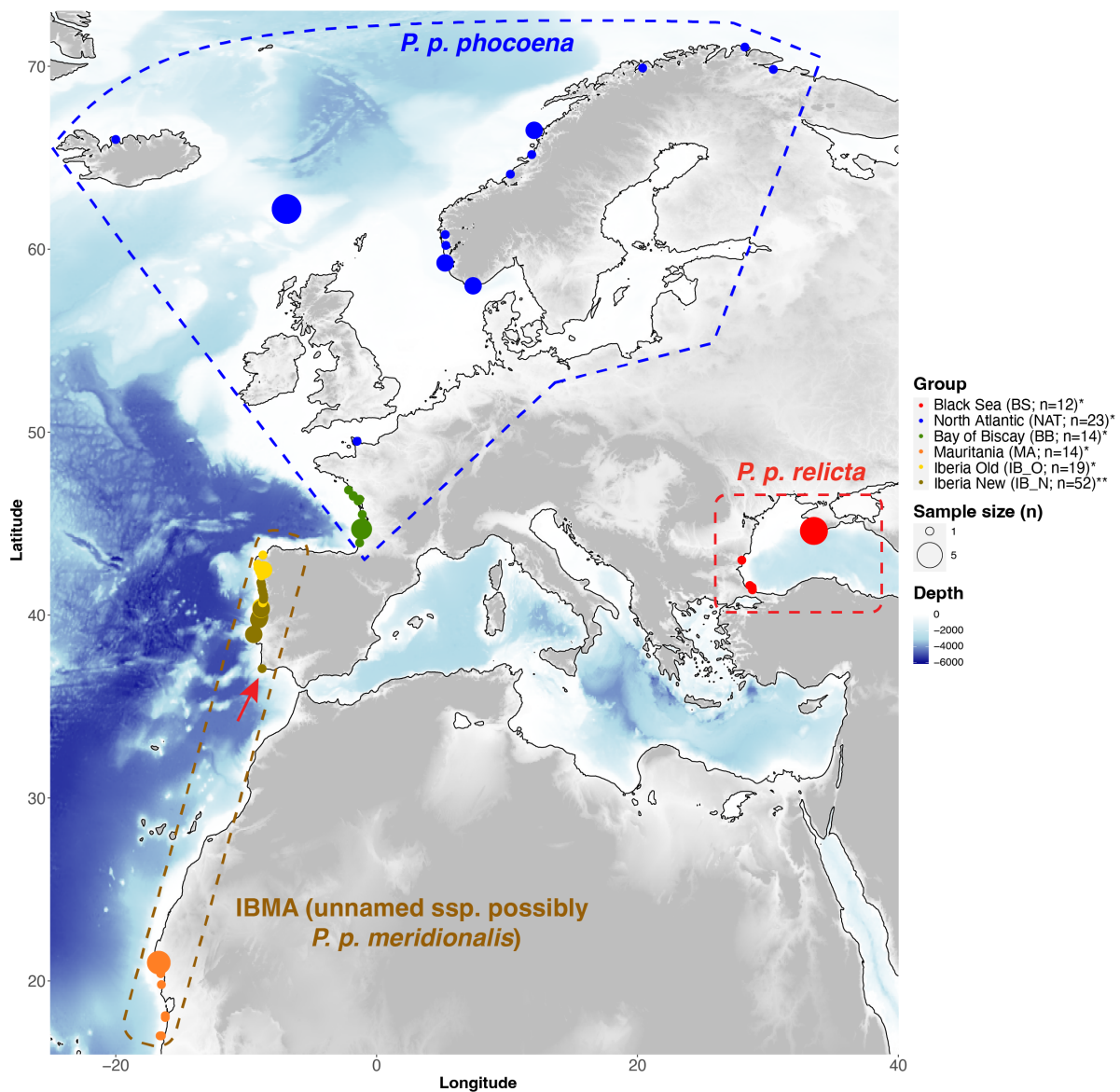


Figure 1. Map showing the sampling locations and sample sizes. Geographic locations are based on approximate GPS coordinates or reported discovery locations. The red arrow indicates the geographical location of the two porpoises carrying Haplotype 10. (* Fontaine et al. (2014); ** This study).

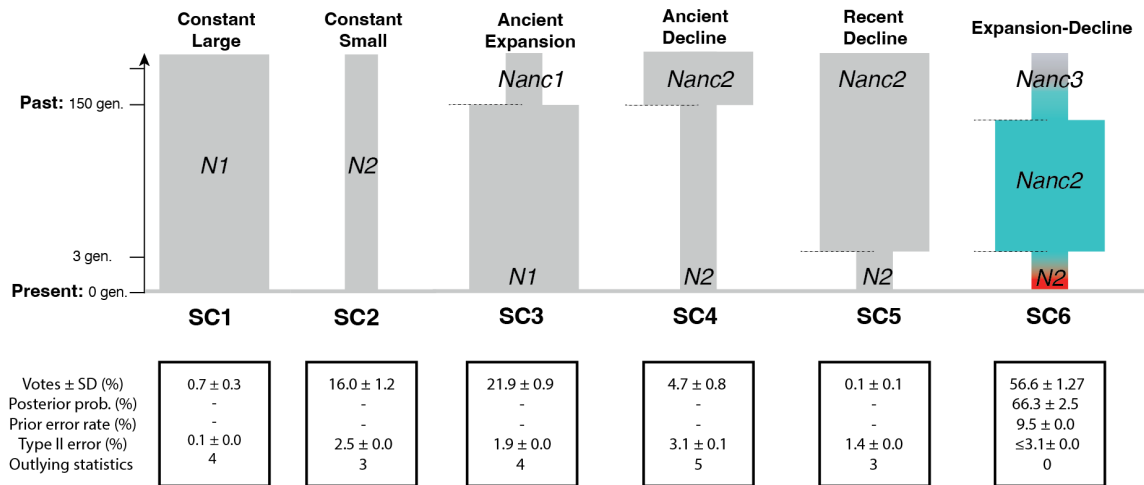


Figure 2. Alternative scenarios tested in the ABC-RF procedure. See [Table S2](#) for further details on the model parameters. Below each scenario are provided the main results including the number of Random Forest (RF) votes (in percent), the posterior probability of the best scenario, the prior-error rate, the type-II error rate, and the number of outlying statistics in the goodness-of-fit testing (see [Table S5](#)). The colored scenario, SC6, was the scenario gathering the highest number of Random Forest votes. The time scale is indicated by the arrow on the left, in generations before present (gen.).

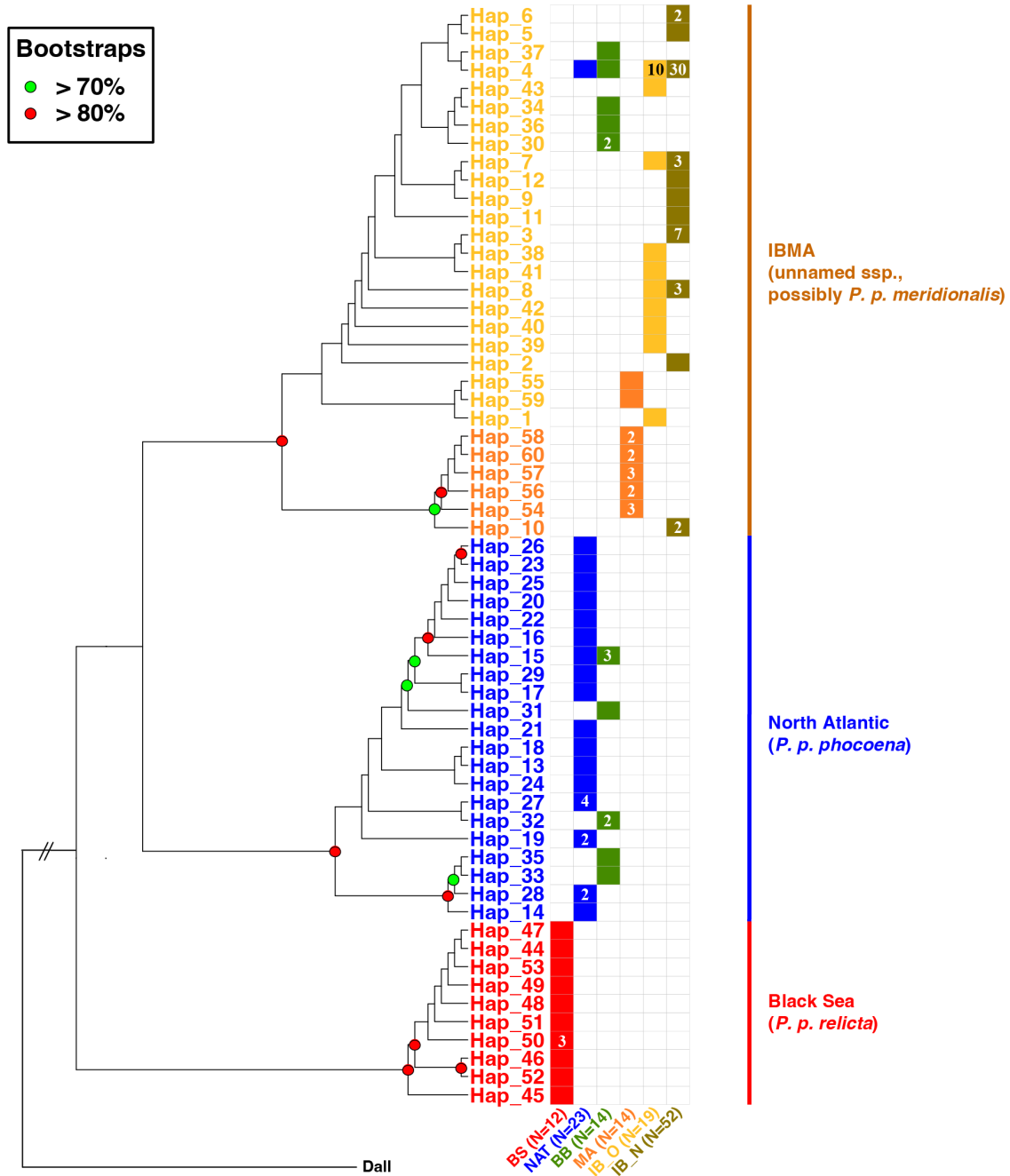


Figure 3. Cladogram derived from the maximum-likelihood mitochondrial phylogeny among unique mitochondrial haplotypes. A branch length transformation proportional to the number of tips under the node (as implemented in *FigTree*) was applied to facilitate the visualization of the branching patterns (see Figure S1 for the actual phylogenetic tree with branch lengths proportional to sequence divergence). The color-coded labels show the geographic origin of the haplotype. The numbers within the boxes refer to the number of individuals carrying the haplotype. No number in a box means that the haplotype was observed only once. Population code are as follow: BS=Black Sea (N=12)*; NAT=North Atlantic (N=23)*; BB=Bay of Biscay (N=14)*; MA=Mauritania (N=14)*; IB_O=Iberian Old (N=19)*; IB_N= Iberian new (N=52)**. [* Fontaine et al. 2014; ** This study].

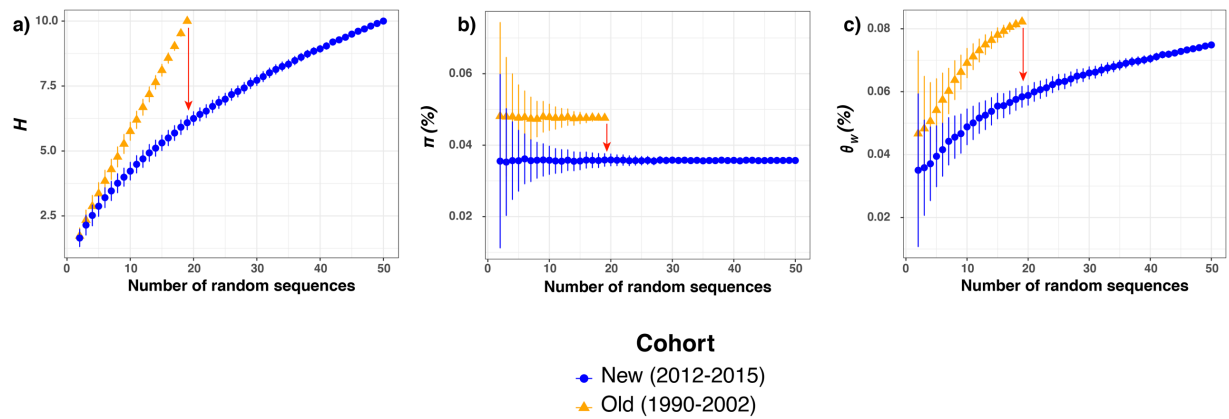


Figure 4. Rarefaction curves showing three statistics describing mitochondrial genetic diversity including (a) number of haplotypes (H), (b) the nucleotide diversity (π), and (c) Watterson's theta (θ_w). The red arrow in each panel shows the direction of change between the old and new cohorts. The X-axis shows the number of sequences randomly resampled in the rarefaction procedure to estimate the statistical values. The mean and standard error are provided at each sample size increment (k) for each statistic and each group, calculated from 5000 sampling with replacement of the k sequences available in each group, with k ranging from 3 to k_{max} up to 50.

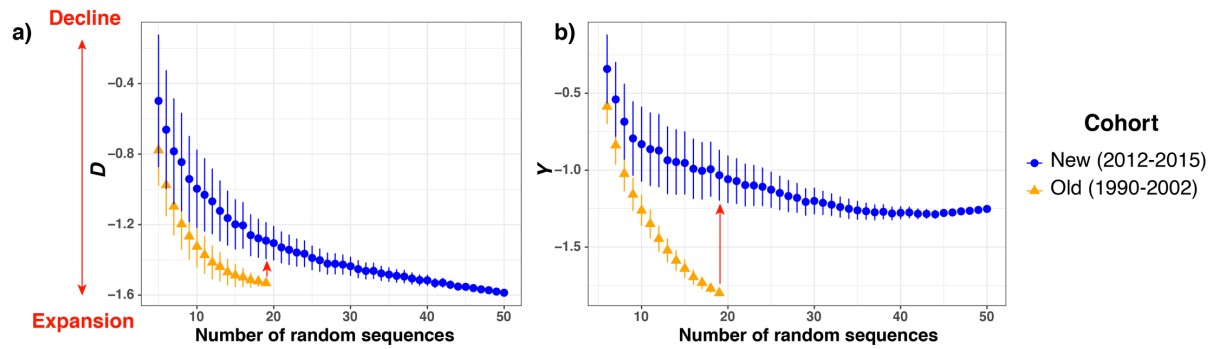


Figure 5. Rarefaction curves showing two neutrality indexes indicative of demographic changes including (a) Tajima's D and (b) Achaz's Y . The red arrow inside each panel shows the direction of the change between the old and new cohorts. The bidirectional arrow shows the interpretation of D and Y in terms of demography. The X-axis shows the number of sequences randomly resampled in the rarefaction procedure to estimate the statistical values.