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SNP discovery in albacore and Atlantic bluefin tuna provides insights into world-wide population structure

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SNP discovery in albacore and Atlantic bluefin tuna provides insights into world-wide population structure

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24 ## Summary

25 The optimal management of the commercially important, but over-exploited,
26 pelagic tunas, albacore (*Thunnus alalunga* Bonn., 1978) and Atlantic bluefin
27 tuna (*T. thynnus* L., 1758), requires a better understanding of population
28 structure than has been provided by previous molecular methods. Despite
29 numerous studies of both species, their population structures remain
30 controversial. This study reports the development of single nucleotide
31 polymorphisms (SNPs) in albacore and Atlantic bluefin tuna (BFT) and the
32 application of these SNPs to survey genetic variability across the geographic
33 ranges of these tunas. A total of 616 SNPs were discovered in 35 albacore
34 tuna by comparing sequences of 54 nuclear DNA fragments. A panel of 53
35 SNPs yielded values of F_{ST} ranging from 0.0 to 0.050 between samples after
36 genotyping 460 albacore collected throughout the distribution of this species.
37 No significant heterogeneity was detected within oceans, but between-ocean
38 comparisons (Atlantic, Pacific and Indian oceans along with Mediterranean
39 Sea) were significant. Additionally, a 17 SNPs panel was developed in Atlantic
40 BFT by cross-species amplification in 107 fish. This limited number of SNPs
41 discriminated between samples from the two major spawning areas of Atlantic
42 BFT (F_{ST} = 0.116). The SNP markers developed in this study can be used to
43 genotype large numbers of fish without the need for standardizing alleles
44 among laboratories.

45

46 **Keywords** *Thunnus alalunga*, *Thunnus thynnus*, Single Nucleotide
47 Polymorphism (SNP), SNP discovery, population genetics, fisheries
48 management.

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Introduction

Molecular genetics has led to considerable progress in understanding the ecologies of marine species by providing new insights into the demographic and evolutionary dynamics of wild populations (Hauser & Carvalho 2008). Genetic markers are widely used to identify stocks, to estimate mixed stocks in a fishery, to monitor genetic diversity within populations and to measure connectivity between populations, among many other applications (e.g. Nielsen *et al.* 2009; Waples & Naish 2009). These studies have overturned the classic notion that large marine populations are genetically homogeneous with limited local adaptation by showing extensive genetic population structure in many marine species (reviewed in Hauser & Carvalho 2008). Moreover, genetic structuring has been reported even across small spatial scales (e.g. Knutsen *et al.* 2003; Jørgensen *et al.* 2005; Knutsen *et al.* 2007; Knutsen *et al.* 2011). However, data are still rare for the vast majority of highly exploited species, even though a large number of studies on genetic population structure of marine fish have been published in the past decades. Genetic studies are needed to improve the management of species for which stock structure and migration patterns are still unclear. An improper management of these fishery resources can lead to the extirpation of small independent stocks or to the under-utilization of large stocks.

This study focuses on two widely distributed pelagic tunas, albacore (*Thunnus alalunga* Bonn., 1978) and Atlantic bluefin tuna (*T. thynnus* L., 1758). Albacore is one of the smallest tunas and Atlantic bluefin tuna (BFT) one of the largest in the family Scombridae. While albacore is a widely distributed species, inhabiting both temperate and tropical pelagic waters of

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74 all oceans, the distribution of Atlantic BFT is limited to the North Atlantic
75 Ocean and Mediterranean Sea (e.g. Nakamura 1969; Collette & Nauen 1983;
76 Fromentin & Fonteneau 2001). Both species coexist in the Mediterranean Sea.
77 Harvests of these species are large and of high economical value, especially
78 Atlantic BFT, which is sold for high prices in Japanese fish markets
79 (Magnuson *et al.* 1994). An important Atlantic BFT aquaculture industry,
80 based on the fattening of locally collected fish in floating cages, has
81 developed in the Mediterranean. Moreover, these tunas' life history traits
82 make them susceptible to collapse under continued excessive fishing
83 pressure, as their population growth rates are low (De Roos & Persson 2002).
84 Critical life-history traits include long life spans, large body sizes, late sexual
85 maturity (around 4-5 years but up to 8 years for the Western Atlantic BFT),
86 geographically restricted spawning sites, and relatively short spawning
87 periods of 1 or 2 months (Fromentin & Fonteneau 2001; Fromentin & Powers
88 2005; Rooker *et al.* 2007; Fromentin 2009; Juan-Jordá *et al.* 2011).

89 Since stocks of albacore and Atlantic BFT are currently overexploited, an
90 urgent need exists to improve conservation and management efforts,
91 including the development of alternative methods of population assessment
92 (Juan-Jordá *et al.* 2011; Collette *et al.* 2011). The management of these tunas
93 has to be at the population level, because the extent and dynamics of
94 population structuring underlies the resilience and sustainability of harvested
95 populations. Previous studies of population structure have used four classes
96 of molecular markers: allozymes, DNA blood groups, mitochondrial RFLPs
97 and microsatellite loci (Chow & Ushiama 1995; Yeh *et al.* 2007; Takagi *et al.*
98 1999; Takagi *et al.* 2001; Ely *et al.* 2002; Pujolar *et al.* 2003; Arrizabalaga *et al.*

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3 99 2004; Viñas *et al.* 2004; Nakadate *et al.* 2005; Arrizabalaga *et al.* 2007;
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5 100 Carlsson *et al.* 2007; Boustany *et al.* 2008; Riccioni *et al.* 2010; Davies *et al.*
6
7 101 2011; Viñas *et al.* 2011). Despite these efforts to describe stock structure in
8
9 102 albacore and Atlantic BFT, the population structures of these species remain
10
11 103 controversial (Arrizabalaga *et al.* 2004; Walli *et al.* 2009; Galuardi *et al.* 2010).
12
13 104 Presently, albacore populations are divided into six management units
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15 105 (namely the Mediterranean, North Atlantic, South Atlantic, Indian Ocean,
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17 106 North Pacific and South Pacific stocks) and Atlantic BFT into two units
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19 107 (namely the Western Atlantic stock and the Eastern Atlantic and
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21 108 Mediterranean stock). However, the results of population surveys based on
22
23 109 microsatellite variability illustrate that these management units might not be
24
25 110 consistent with the genetic structures of both species (Riccioni *et al.* 2010;
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27 111 Davies *et al.* 2011; Viñas *et al.* 2011).
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30 112 Two factors explain the current lack of consensus on genetic structure.
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32 113 First, none of the previous genetic studies included samples over the entire
33
34 114 distributional areas of these tunas. Second, none of the previous studies used
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36 115 large numbers of molecular markers, such as multiple single nucleotide
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38 116 polymorphisms (SNPs), which can be assayed rapidly in large numbers of fish
39
40 117 to yield high statistical power to test population-level genetic hypotheses (e.g.
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42 118 Ogden 2011; Helyar *et al.* 2011).
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45 119 The goals of the present study were to develop SNP markers in albacore
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47 120 and Atlantic BFT and to use these markers to make a preliminary survey
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49 121 geographic variability among populations over the geographic ranges of these
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51 122 tunas (Table 1, Fig. 1). The development of SNP markers will aid in the ability
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to conduct collaborative studies among laboratories without the need for standardizing alleles. Further, the development of a large number of markers will increase the power of genetic analysis to detect the small differences among populations that are expected in high gene-flow species, such as the tunas.

Material and methods

Sample collection

Samples of muscle, fin or heart tissue from 460 albacore were collected at 8 locations (representing samples from feeding grounds and including a mixture of juveniles and adults) over the distribution of the species (Table 1; Fig. 1). Additional tissue samples from 107 Atlantic bluefin tuna (BFT) were collected from 3 locations: Western Atlantic, Bay of Biscay and Mediterranean Sea (Table 1). While the Bay of Biscay sample included a mixture of juveniles and adults from a feeding ground, the samples of Western Atlantic and Mediterranean Sea were composed of young-of-the-year (YOY) individuals incapability of trans-oceanic migration and, thus, represented reference samples for both spawning areas (Rooker *et al.* 2008). Samples were either frozen and stored at -20º C, or were preserved in 96% ethanol at 4º C. DNA was extracted from tissues using the DNeasy 96 Blood & Tissue Kit (Qiagen, Hilden, Germany) and quantified using a NanoDrop 1000™ spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) prior to storage at -20ºC for further analysis.

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147 SNP discovery in albacore (*Thunnus alalunga*)

148 Single nucleotide polymorphism (SNP) discovery via comparative sequencing
149 of nuclear DNA fragments was performed on 35 albacore from widely
150 separated areas. Specifically, five fish were selected from each of the six
151 currently hypothesized stocks, Mediterranean, Indian Ocean and Northern
152 and Southern parts of Atlantic and Pacific Oceans, except for the North
153 Atlantic, where 10 fish were used. SNPs were mined from 54 nuclear DNA
154 fragments (Table S1) and were amplified with primers designed with Primer3
155 (Rozen & Skaletsky 2000). In approach I, EPIC primers (Exon-Priming, Intron-
156 Crossing primers; Slate *et al.* 2009) for 19 DNA fragments (average length
157 318 bp) were obtained from the literature (references in Table S1). The
158 primers for the remaining 35 DNA fragments were designed from the
159 alignment of sequences from publically available databases (GenBank and
160 Ensembl). In approach II, 17 pairs of degenerate primers were designed from
161 several teleost sequences (average length 420 bp). In approach III, 18 pairs
162 of primers were designed from *Thunnus* DNA sequences (average length 487
163 bp).

164 Tables S1 and S2 show fragment amplification specifications. Briefly, we
165 used conventional polymerase chain reaction (PCR) to amplify 30 of 54
166 fragments and used touchdown (TD) methodology to amplify the remaining
167 fragments. Reactions were carried out in a thermo-cycler, GeneAmp®PCR
168 System 2700, GeneAmp®PCR System 9700 or Veriti 96 well Thermal Cycler
169 (Applied Biosystems, Foster City, CA), and iCycler (Biorad Laboratories,

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3 170 Hercules, CA). The purified PCR products were sequenced in one direction
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5 171 with either the forward or the reverse PCR primer on an Applied Biosystems
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7 172 (ABI) 3130X capillary electrophoresis Analyzer, with ABI BigDye Terminator
8
9 173 version 3.1 Chemistry (Applied Biosystems). Base-calling from
10
11 174 chromatograms was performed using SeqScape v2.5 (Applied Biosystems).
12
13 175 The BLASTN algorithm was used to verify that the target locus had been
14
15 176 amplified.
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18 177 Nucleotide differences at a site in aligned sequences were considered to
19
20 178 be a SNP, but only when flanking sequences had high quality and the
21
22 179 alternative nucleotide was present in at least two individuals (out of 35; see
23
24 180 above). After filtering for SNPs matching the technical requirements of the
25
26 181 assays, we gave priority to selecting at least one SNP per fragment for a total
27
28 182 of 128 SNPs for genotyping the 460 albacore with TaqMan® OpenArray®
29
30 183 technology (Applied Biosystems). Moreover, a SNP was chosen if it was not
31
32 184 located near the ends of the sequence and if it was more than two bases
33
34 185 away from any other SNP (*Custom TaqMan Genomic Assays Protocol*
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36 186 *Submission Guidelines*).
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42 188 Cross-species amplifications in Atlantic bluefin tuna (*Thunnus thynnus*)
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45 189 SNP discovery in Atlantic BFT was performed by cross-species amplification
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47 190 of the 128 SNPs selected for genotyping in albacore. The 128 SNPs were
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49 191 genotyped with TaqMan ® OpenArray ® technology in 107 Atlantic BFT
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51 192 samples. The same criteria used to validate SNPs in albacore were used to
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53 193 validate individual SNPs in Atlantic BFT.
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6 195 Population analysis
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9 196 Figure 2 outlines the procedures used in this study, starting from SNP
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11 197 discovery in albacore, to the selection of a subset of SNPs (panel) with origin
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13 198 assignment (including loci under selection) or demographic analysis.
14
15 199 Genotyping call rate and minor allele frequencies (MAF) were obtained for
16
17 200 each SNP locus using AutoCaller™ 1.1 (Applied Biosystems). A SNP was
18
19 201 validated if the polymorphism remained in the genotyping results and could be
20
21 202 reliably scored. SNPs with unclear genotypes and those with a call rate below
22
23 203 70% were discarded. Deviation from Hardy-Weinberg expectations (HWE)
24
25 204 was evaluated for each locus and each sampling location (Fisher's exact test
26
27 205 in GENEPOP 4.0; Rousset 2008). The exact test for linkage disequilibrium (LD),
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29 206 as implemented in GENEPOP, was used to detect disequilibria between SNPs
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31 207 on the same DNA fragment and between SNPs on different fragments; $P <$
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33 208 0.001 was used as critical probability for LD tests. SNP loci exhibiting
34
35 209 significant LD were phased into haplotype blocks using the Bayesian
36
37 210 statistical method implemented in PHASE 2.1 for each sample independently
38
39 211 (Stephens et al. 2001).
40
41 212 Expected heterozygosity (H_e), F_{IS} and F_{ST} were estimated with FSTAT 2.9.3
42
43 213 (Goudet 2001). SNPs exhibiting significant departures from HWE ($P < 0.001$)
44
45 214 in one or more samples were deemed unsuitable for estimating population
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47 215 structure and were discarded. When two or more loci occurred on the same
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49 216 DNA fragment, including both haplotype blocks and individual SNP loci, the
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51 217 locus with the largest H_e was selected to ensure independence among the
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218 markers. Loci with large heterozygosities provide more statistical power for
219 population structure analysis than loci with small heterozygosities (Haasl &
220 Payseur 2011; Morin *et al.* 2004; Rosenberg *et al.* 2003; Ryman *et al.* 2006).
221 The SNP panel consisted of these filtered SNPs.

222 We searched for candidate loci under selection (outlier loci) among the
223 remaining loci using the Bayesian likelihood method, as implemented in
224 BAYESCAN 2.0 (Foll & Gaggiotti 2008) and LOSITAN (Beaumont & Nichols
225 1996; Antao *et al.* 2008). Loci identified by BAYESCAN and LOSITAN as outliers
226 were removed from the SNP panel (Richter-Boix 2011). Briefly, two alternative
227 models were defined in BAYESCAN (including and excluding the effect of
228 selection) and their respective posterior odds (PO), the ratio of the posterior
229 probabilities of the two models, were calculated using 20 pilot runs of 5000
230 iterations and an additional burn-in of 50 000 iterations, for a total of 100 000
231 iterations (sample size of 5000 and thinning interval of 10). While only loci
232 with positive $\log_{10}$PO values were considered, strong support for selection
233 was limited to loci with $\log_{10}$PO > 1 ($P < 0.09$; BAYESCAN 2.0 user manual).
234 On the other hand, LOSITAN identifies outlier loci from a plot of heterozygosity
235 versus F_{ST}. To avoid an upward bias in quantiles, LOSITAN was first run for all
236 loci to estimate the mean neutral F_{ST}. Loci that were outside the 0.99
237 confidence interval were removed using only the putative neutral loci to
238 compute again the mean neutral F_{ST}. A second run was then conducted with
239 all loci using the last computed mean.

240 POWSIM 4.0 (Ryman *et al.* 2006) was used to estimate the statistical power
241 required to detect various levels of differentiation with the SNPs developed for

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242 albacore and Atlantic BFT in this study. Burn-in consisted of 1000 steps
243 followed by 100 batches of 1000 steps. X^2 and Fisher's probabilities were
244 used to test the significance of an F_{ST} value for each replicate run. The
245 number of significant F_{ST} values in 1000 replicate simulations provided an
246 estimate of statistical power for a given level of divergence, which was
247 controlled by allowing frequencies to drift for a given number of generations.
248 Simulated effective populations sizes (N_e) equalled 2000 fish. For albacore, all
249 41 SNPs + blocks, 9 blocks only and 32 SNPs only were examined separately
250 for statistical power for divergences ranging from F_{ST} = 0.0005 to 0.016.

251 Population structure was estimated with F_{ST} between samples and with
252 Bayesian individual assignments implemented in STRUCTURE 2.3.3 (Pritchard
253 *et al.* 2000). Pairwise F_{ST} (Weir & Cockerham 1984) values were estimated
254 with FSTAT 2.9.3 (Goudet 2001) and with globally corrected *p*-values. FSTAT
255 combines individual locus *p*-values weighting them according to their
256 polymorphism level (Petit *et al*. 2001). Population groups were defined by
257 non-significant values of mean F_{ST} between samples and by significant values
258 of F_{ST} with other populations (e.g. Waples & Gaggiotti 2006). STRUCTURE
259 uses a Bayesian method to identify the number of clusters (K) of related
260 individuals using HWE and gametic disequilibria among multilocus genotypes.
261 We used the admixture model, independent allele frequencies between
262 populations and the LOCPRIOR option. We compared log-likelihood ratios in 10
263 STRUCTURE runs for values of K = 1 to 10 (Pritchard *et al.* 2000). Each run
264 consisted of 10 000 iterations with a burn-in of 10 000. CLUMPP 1.1.2
265 (Jakobsson & Rosenberg 2007) was used to determine optimal assignments
266 of individual to clusters by maximizing the similarity between pairs of

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267 genotypes in different replicates. These groupings were visualized with
268 DISTRUCT 1.1 (Rosenberg 2004). Outlier loci that were not used to estimate
269 F_{ST}, were added for the STRUCTURE analyses as the latter does not require
270 neutral markers, unlike the F_{ST} analysis.

271

272 Results

273

274 SNP discovery and validation in albacore tuna

275 Fifty-four fragments of nuclear DNA were sequenced in 35 albacore tunas
276 (Table S1). Thirty-five of the 54 fragments showed a high degree of homology
277 with the orthologous sequences in several fishes (BLASTN; E-value < 10^{-5}),
278 including 14 best hits corresponding to other species of *Thunnus* (Table S3).
279 A total of 616 SNPs were discovered, in which an alternative allele was
280 present in at least 2 individuals, with a mean of 11.4 (SD ±10) SNPs per
281 fragment and a ratio of 1/36 bp. At least 1 SNP was present in each DNA
282 fragment, except for a fragment coding for metallothionein (*MT*). A total of 195
283 SNPs were present in fragments amplified with EPIC primers (approach I);
284 182 SNPs were found in fragments amplified with degenerate teleost primers
285 (approach II); and 239 SNPs were present in DNA fragments amplified with
286 *Thunnus* spp. primers (approach III). In addition to SNPs, 19 small indels, 1–5
287 nucleotides in length, were found in 14 fragments with a majority
288 corresponding to mono- or bi-nucleotide indels (84.2%).

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A total of 128 candidate SNPs were selected to genotype the albacore population samples and included 32, 47 and 49 SNPs selected from fragments obtained with approaches I, II and III, respectively. A total of 23 (18%) SNPs failed to amplify for routine genotyping, and 2 SNP loci failed to exceed call rates above 70%. Another 24 loci among the remaining 103 SNPs could not be reliably scored. The remaining 79 validated SNPs (Table S4) showed a mean call rate of 91 ±5% and an average minor allele frequency (MAF) of 0.17 ±0.14 (range 0.001–0.489). Validation success was 72%, 66% and 51% for approaches I, II and III, respectively.

SNP panel for population genetic studies in albacore

Twelve of the 79 validated SNPs departed significantly from HWE in one or more sampling locations and were discarded. The remaining 67 SNPs were tested for linkage disequilibrium (LD). No SNPs were found in LD between DNA fragments; however, 21 SNPs were in LD within fragments and were phased into 9 haplotype blocks (Table S5). After selection of only one independent locus per DNA fragment (see Methods), the final panel of markers included 41 independent markers: 32 individual SNPs, plus 9 haplotype blocks (53 SNPs in total; Table 2). Analysis of these SNPs with BAYESCAN showed no candidate loci influenced by selection. However, LOSITAN detected 3 SNP loci, *HIF1A4, MTF1* and *MYC*, with significantly larger genetic divergences than expected from neutrality. These loci were assumed to be embedded in candidate genes under divergent selection or in DNA fragments linked to genes influenced by selection. In contrast, the low

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313 level of divergence for *PRDX2* suggested that balancing selection may be
314 influencing this locus, or a nearby locus. Interestingly, *MTF1* and *PRDX2*
315 sequences showed the highest homology with orthologs of the teleosts *Fugu*
316 *rubripes* and *Thunnus maccoyii*, respectively (Table S3), but no homologies
317 were found for *HIF1A4* and *MYC* in the teleosts tested. Overall, the average
318 expected heterozygosity over loci was H_e = 0.278 ±0.201 and the average
319 inbreeding coefficient was F_{IS} = 0.032 ±0.085 for the 41 SNP loci in the final
320 panel (Table 2).

321

322 Genetic structure in albacore tuna

323 POWSIM simulations indicated that the 41 independent markers (32 SNPs and
324 9 haplotype blocks) together were able to detect significant differences among
325 samples with F_{ST} = 0.002 in about 84% of the tests and with $F_{ST} \geq 0.004$ in
326 100% of the tests (Table 3). The nine multi-allelic haplotype blocks alone
327 provided about the same amount of statistical power for detecting differences
328 among populations as did the 32 individual SNPs. Each set of markers was
329 able to detect values of $F_{ST} \geq 0.004$ in at least 97% of the tests. Fisher's
330 method for detecting significant differences among samples provided less
331 statistical power than did the X^2 tests.

332 Analyses using the 32 SNPs and nine haplotype blocks together revealed
333 an overall F_{ST} = 0.017 ±0.003 ($P < 0.05$) among the eight albacore sampling
334 locations. Levels of divergence were not significant between sampling
335 locations within oceans, but were significant between oceans (Table 4).
336 Samples from the NE Atlantic (IRE and BIS) were not significantly different

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3 337 from each other or from a sample from the SE Atlantic (SEA). Likewise, no
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5 338 divergence was detected between the three samples from the Pacific (NP,
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7 339 SEP, SWP). However, all comparisons between oceans were significant,
8
9 340 yielding four differentiated genetic entities: 1) NE Atlantic, 2) Mediterranean
10
11 341 Sea, 3) Pacific Ocean and 4) Indian Ocean). Fish from the western
12
13 342 Mediterranean (BAL) showed the highest divergence from the other locations
14
15 343 with an average F_{ST} = 0.034 (range: 0.021–0.050). Fish from the Indian
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17 344 Ocean (IN) were most divergent from the Atlantic and Mediterranean sample
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19 345 locations (mean: F_{ST} = 0.030), but less divergent from Pacific Ocean samples
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21 346 (F_{ST} = 0.010). The individual Bayesian clustering (STRUCTURE) indicated the
22
23 347 largest likelihood of population structure was K = 3, placing samples into three
24
25 348 groups: Mediterranean Sea, Atlantic Ocean, and Indo-Pacific (Fig. 3).
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27 349 Analysis with K = 4 showed that Indian Ocean albacore were differentiated to
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29 350 a small degree from Pacific Ocean albacore, as reflected in the distribution of
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31 351 F_{ST} values between these locations.
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37 353 SNP panel for Atlantic bluefin tuna
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40 354 Primers for the 128 albacore SNPs were used in cross-species reactions to
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42 355 develop SNPs in BFT. Although 32 SNPs successfully amplified, 9 SNPs had
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44 356 low call rates (below 70%), had unclear genotypes, or were not polymorphic,
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46 357 and hence were discarded. This yielded 23 validated SNPs (18%) for BFT.
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48 358 Out of these 23, 18 had been validated also when genotyping albacore while
49
50 359 the other five SNPs (*GNRH3-1-107*, *LDHB-129*, *CYCS-161*, *CS3-118*, and
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52 360 *OPC02*-45; Table S6) were reported as reliably scored and polymorphic only
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3 361 in BFT. The validation success rates in BFT for SNP that had been
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5 362 discovered in albacore by approaches I, II and III were 28% (9 of 32 SNPs),
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7 363 15% (7 of 47 SNPs) and 14% (7 of 49 SNPs), respectively. Additionally, one
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9 364 SNP was discarded from the final panel due to a significant deviation from
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11 365 HWE in at least one sample (Table S6). Tests for LD between the remaining
12
13 366 22 BFT SNPs detected two cases of two linked SNP loci in a single fragment
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15 367 (Table S5). Linked loci were phased into haplotypes. Significant LD was not
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17 368 detected among SNPs on different DNA fragments. A final set of 15
18
19 369 independent markers, 13 individual SNPs and 2 haplotype blocks, were
20
21 370 suitable for surveys of BFT populations (17 SNPs in total; Table 5).
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24 371 Average expected heterozygosity among the 15 loci was H_e = 0.272
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26 372 ±0.178, and the average inbreeding coefficient was F_{IS} = 0.096 ±0.133. The
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28 373 low number of markers tested in BFT precluded the use of outlier detection
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30 374 software, as a larger number of SNPs are required to obtain a reliable
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32 375 estimate of the neutral expectation from which the outliers are detected.
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34 376 Therefore, all 15 loci were used to estimate population structure with F_{ST} and
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36 377 STRUCTURE.
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43 379 Genetic structure in Atlantic bluefin tuna
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46 380 The POWSIM simulations and the average frequencies for the 13 SNPs and 2
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48 381 blocks indicated that statistical power increased from only 0.064 for F_{ST} =
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50 382 0.001 (Fisher's method of determining significance), to 0.579 for F_{ST} = 0.010
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52 383 and to 1.0 for F_{ST} = 0.080 (Table 6). The 15 BFT SNPs showed significant
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54 384 overall differentiation among populations in the NW Atlantic, NE Atlantic and
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Mediterranean (F_{ST} = 0.029 ±0.024, P < 0.05). Populations in the Bay of Biscay and the Mediterranean differed from the populations in the NW Atlantic (BB–NWA, F_{ST} = 0.120 ±0.091, P < 0.01; MED–NWA 0.116 ±0.078, P < 0.01). However, the Bay of Biscay and the Mediterranean did not differ significantly from each other (F_{ST} = 0.004 ±0.007). STRUCTURE indicated that the three samples most likely represented two populations (K = 2), 1) NW Atlantic and 2) Bay of Biscay and Mediterranean (Fig. 4).

Discussion

Our study outlines the development and validation of SNPs in the genomes of albacore and Atlantic bluefin tuna and provides a basis for defining discrete stocks to aid in the commercial harvests of these species. We developed *de novo* 53 SNPs for albacore and 17 cross-species SNPs for Atlantic bluefin tuna. While the focus of the study was on the development of SNP markers, the distributions of our samples allow a preliminary analysis of large-scale population structure. Several variables influence the ability of a set of molecular markers to detect genetic differences between populations. In addition to the well known effects of sample size on power, the geographical extent of a set of samples is crucial to describing population structure.

SNP discovery in Albacore tuna

Our search for variable nucleotide sites yielded 128 SNPs. Of these, 79 (62%) could be validated and were selected for routine genotyping. From

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3 408 these, we selected a final panel of 53 SNPs distributed over 41 loci. The 62%
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5 409 validation success rate is similar to SNP validation success rates in other
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7 410 studies of fishes, including *Gadus morhua* (54%) (Moen *et al.* 2008),
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9 411 *Oncorhynchus nerka* (39%), *O. keta* (54%) and *O. tshawytscha* (64%) (Smith
10
11 412 *et al.* 2005) and *Engraulis encrasicolus* (59%) (Molecular Ecology Resources
12
13 413 Primer Development Consortium *et al.* 2012).
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15
16 414 Our results allow an assessment of levels of polymorphism in the albacore
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18 415 genome. A total of 616 SNPs were discovered with an overall ratio of 1 SNP
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20 416 for each 36 base pairs. This value indicates higher levels of polymorphism in
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22 417 albacore than has been reported, for example, in the salmonids,
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24 418 *Oncorhynchus keta* (1/175 bp), *O. nerka* (1/242 bp) and *O. tshawytscha*
25
26 419 (1/301 bp) (Smith *et al.* 2005), *Salmo salar* (1/586 bp overall, Hayes *et al.*
27
28 420 2004) or in intronic (1/405 bp) and exonic (1/1448 bp) regions (Ryynänen &
29
30 421 Primmer 2006). The large value for albacore, however, is similar to that for
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32 422 European anchovy (*Engraulis encrasicolus*) (1/54 bp, Zarraonaindia 2011).
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38 424 SNP development in Atlantic bluefin tuna
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41 425 We used primers for the 128 SNPs detected in albacore to search for SNPs in
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43 426 Atlantic BFT and achieved an overall validation success rate of 18% (23
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45 427 SNPs). This success rate indicates that the regions flanking these SNPs
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47 428 contain highly conserved sequences so that the SNP primers developed for
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49 429 albacore also work in Atlantic BFT. When successful, cross-species
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51 430 amplifications are a cost-effective method of SNP discovery (Malhi *et al.*
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53 431 2011). Successful cross-species amplifications are thought to be free of
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432 ascertainment bias, especially for SNPs embedded in conserved sequences
433 near or within coding regions (Malhi et al. 2011). Cross-species amplifications
434 have generally not been used to develop SNP markers in fish, because of
435 generally low success rates in other vertebrates (e.g. Seeb *et al.* 2011; Miller
436 *et al.* 2011). For example, only about 1% of the nearly 50 000 SNP loci
437 developed for domestic sheep were polymorphic in two related ungulates
438 (Miller *et al.* 2011). In a panel of a similarly large number of SNPs designed
439 for cattle, only about 2.5 and 3% of the cross-species amplifications were
440 successful in two lines of European bison and two species of antelopes,
441 respectively (Kaminski *et al.* 2012; Ogden *et al.* 2012). However, the species
442 used in these cross-amplification attempts were distantly related to one
443 another, unlike albacore and Atlantic BFT, which are phylogenetically closely
444 related (Chow & Kishino 1995; Chow *et al*. 2006) and are known to hybridize
445 (Viñas & Tudela 2009). Our results are similar to those of Mahli *et al.* (2011),
446 who reported up to 30% cross amplification successes between related
447 species of Old World monkeys.

448

449 Stock structure

450 The statistical power of the SNPs is influenced by several factors. First,
451 the power of the markers to detect population structure increases substantially
452 when locus heterozygosities are larger than $H_e \geq 0.2$ (Haasl & Payseur 2011).
453 In our study, about 60% of the SNP loci in both species showed $H_e > 0.2$.
454 Second, while individual SNPs show less power than multi-allelic
455 microsatellite loci (Ryman *et al*. 2006; Haasl & Payseur 2011), combining

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3 456 physically linked SNPs into haplotype blocks increases statistical power
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5 457 (Gattepaille & Jakobsson 2012). For example, Mesnick *et al.* (2011) retrieved
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7 458 the same population structure for sperm whales (*Physeter macrocephalus*)
8
9 459 with 6 microsatellites, or with 36 SNPs representing 24 independent markers,
10
11 460 including 8 haplotype blocks. In our study, POWSIM simulations showed that
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13 461 haplotype 9 blocks of linked SNPs in albacore had about the same statistical
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15 462 power as 32 individual SNPs. In any case, haplotype blocks and SNPs
16
17 463 together yielded a type II error rate (failure to detect a real difference) for both
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19 464 species of 0% for divergences of F_{ST} = 0.004 or greater.
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21 465
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23 466 Stock structure in Atlantic bluefin tuna
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25 467 The International Commission for the Conservation of Atlantic Tuna (ICCAT)
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27 468 currently manages Atlantic BFT as two stock units that are divided by the mid-
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29 469 ocean longitude at 45°W (Fromentin & Powers 2005). This geographically
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31 470 defined stock concept is supported by two spatially separated spawning areas,
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33 471 one in the Mediterranean and the other in the Gulf of Mexico (Rooker *et al.*
34
35 472 2007). However, tagging and microchemical studies suggest a more complex
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37 473 stock distribution pattern (Rooker *et al.* 2008; Walli *et al*. 2009; Galuardi *et al*.
38
39 474 2010). Foraging aggregations in the NE Atlantic and elsewhere may
40
41 475 potentially originate from both main spawning areas, because trans-Atlantic
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43 476 migrations in both directions have been documented (Magnuson *et al*. 1994;
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45 477 Mather *et al*. 1995; Lutcavage *et al*. 1999; Block *et al*. 2001; Block *et al*. 2005;
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47 478 Rooker *et al*. 2006). However, homing to natal spawning areas (Boustany *et*
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49 479 *al*. 2008; Block *et al*. 2005; Teo *et al*. 2007; Dickhut *et al*. 2009) appear to
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3 480 isolate the two major groups. Recent studies with mtDNA and microsatellite
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5 481 markers show that populations in the Mediterranean Sea may also be
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7 482 structured into partially isolated subpopulations (Riccioni *et al*. 2010; Viñas *et*
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9 483 *al*. 2011).
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13 484 The Atlantic BFT SNP panel developed in the present study, although
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15 485 limited in number due to the SNP discovery approach followed, is the
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17 486 beginning of a valuable tool to improve the management of this overexploited
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19 487 species. The advent of Next Generation Sequencing (NGS) technologies such
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21 488 as Roche´s 454 or Illumina´s HiSeq platforms (see Garvin et al. 2010 and
22
23 489 Seeb et al. 2011 for a review) is making possible to discover hundreds-
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25 490 thousands of SNP type markers in non-model organisms and, further
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27 491 application of these techniques on BFT (and also in albacore) will improve the
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29 492 relevance of the present reported tool. The preliminary analysis in the present
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31 493 study confirms the genetic distinction between the two major spawning areas
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33 494 on the western and eastern margins of the Atlantic (both F_{ST} and STRUCTURE
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35 495 results) and is largely consistent with the ICCAT management plan. While
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37 496 trans oceanic migrations of adults have been documented with tags, finer-
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39 497 scale population structure may also exist. Our SNP analysis showed that the
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41 498 foraging area sample from the Bay of Biscay clustered with the Mediterranean
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43 499 samples indicating the Mediterranean origins of these fish. To our knowledge,
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45 500 this is the first time a mixed age classes of Atlantic BFT in the NE Atlantic
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47 501 have been assigned to a spawning area based on DNA data. Previous
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49 502 insights have come from relatively expensive and laborious tagging
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51 503 experiments (Rooker *et al.* 2007) and from an allozyme study comparing
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samples from Azores (Mid-Atlantic) and the Mediterranean (Pujolar et al. 2003).

The larger levels of differentiation than expected under neutrality may indicate that some SNP loci are embedded in genes under selection. Although the small number of SNPs for Atlantic BFT precluded tests of neutrality, the remarkably large value of F_{ST} = 0.116 indicating a high level of differentiation between populations of Atlantic BFT may result from directional selection on some SNPs. Markers influenced by directional selection often show higher F_{ST} values among populations of fishes than do neutral markers (e.g. André *et al*. 2011; Ackerman *et al*. 2011; Poulsen *et al*. 2011). Additional SNPs for Atlantic BFT are needed to test hypotheses of selection, to estimate population structure and to identify mixed-stock components in fishery areas.

Stock structure in Albacore tuna

ICCAT, the Indian Ocean Tuna Commission (IOTC), the Western and Central Pacific Fisheries Commission (WCPFC) and the Inter-American Tropical Tuna Commission (IATTC) manage albacore with a six-stock model, which includes 1) Mediterranean Sea, 2) North Atlantic, 3) South Atlantic, 4) Indian Ocean, 5) North Pacific Ocean and 6) South Pacific Ocean. These stocks are based on a limited understanding of spawning areas, the geographical distribution of fisheries, life-history variables and the results of tagging studies (Arrizabalaga *et al*. 2004).

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3 526 Our analyses resolved the genetic relationships among oceanic
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5 527 populations. Pairwise F_{ST} values distinguish four albacore groups, 1)
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7 528 Mediterranean Sea, 2) Atlantic Ocean, 3) Pacific Ocean and 4) Indian Ocean,
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9 529 with no within-ocean heterogeneity (Table1, Fig. 1). The tests for
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11 530 heterogeneity did not detect differences between northern and southern
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13 531 populations within the Pacific or within the Atlantic. The Mediterranean group
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15 532 appears to be most differentiated from other global populations, and this is in
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17 533 agreement with previous results for microsatellites (Davies *et al.* 2011). Both
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19 534 SNPs and microsatellites show that Mediterranean and North Atlantic
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21 535 populations are partially isolated from each other, and this genetic separation
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23 536 is consistent with tag-recapture analysis showing limited movement between
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25 537 the North Atlantic and Mediterranean (Arrizabalaga *et al.* 2004). The SNP
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27 538 data also showed that the Indian Ocean populations were genetically closer to
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29 539 Pacific populations than to Atlantic populations. In contrast, a closer
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31 540 relationship between Indian Ocean and Atlantic fish was reported from blood-
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33 541 group frequencies (Arrizabalaga *et al.* 2004).
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36 542 Overall, the genetic results together support the existence of at least 4
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38 543 genetic entities, Mediterranean Sea, Atlantic, Pacific and Indian oceans that
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40 544 are isolated from one another to some degree. These results support
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42 545 management plans in which oceanic populations, including the Mediterranean,
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44 546 are managed separately, but differ from the present use of six management
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46 547 units (stocks). However, due to our relatively limited sampling of locations and
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48 548 individuals within ocean basins, the current six-stock management model
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50 549 represents a conservative approach that reduces the risk of inadvertently
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52 550 overfishing some populations.
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Conclusions

We developed 128 SNP markers *de novo* in albacore tuna and used a final panel 53 SNPs (41 SNPs, including 32 individual SNPs and 9 haplotype blocks) to genotyped over 400 individuals collected over the distributional range of the species. Although the coarse scale of sampling limits our inferences about population structure, the results for albacore are largely consistent with previous molecular studies in indicating the existence of at least four albacore populations: Mediterranean Sea, Atlantic, Pacific and Indian oceans. We then used SNP assays developed for albacore to develop 17 validated SNPs in Atlantic bluefin tuna (15 SNPs, including 13 individual SNPs and 2 haplotype blocks). This small number of SNPs discriminated the two major spawning areas of Atlantic BFT in the Gulf of Mexico and Mediterranean Sea, and identified the Mediterranean origin of juveniles foraging in the Bay of Biscay. The additional development of new SNPs will increase the statistical power needed to resolve the population structures of these two overfished tunas.

SNPs have two advantages over other markers, such as allozymes and microsatellites, for the international management of far ranging tunas. First, a large number of SNPs, individually or in haplotype blocks, provides a large amount of statistical power to detect biologically meaningful genetic differences between stocks of highly mobile tunas. High mobility is expected to produce only small differences between stocks. Second, and most importantly, SNP assays can be used in any laboratory without the need for standardizing allelic variants among research or management groups.

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International cooperation in establishing a universal database is essential for the conservation and management of these vulnerable species with stocks that straddle international boundaries. The SNPs developed for both species can provide valuable tools for population management and can additionally be used as markers to trace fishery products.

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Conflict of interest

The authors have declared no potential conflicts.

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9 902 **Table 1 Sampling details.** Sample code, number of individuals per sample (*N*), sample location, current management stock, FAO
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11 903 major fishing area and geographical coordinates along with year of capture.
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Sample	Abbreviation	*N*	Location	Latitude	Longitude	Year	Current stock	FAO
Albacore tuna								
1	BAL	50	Balearic Sea	40.00	1.58	2005	Mediterranean	37
2	BIS	52	Bay of Biscay	45.10	-4.35	2009	North Atlantic	27
3	IRE	57	Ireland	54.17	-12.89	2008	North Atlantic	27
4	SEA	91	South Africa	-24.25	4.42	2009	South Atlantic	47
5	IN	24	Seychelles	-7.11	54.65	2008–2009	Indian	51
6	NP	101	California	43.50	-127.00	2008	North Pacific	77
7	SWP	30	New Caledonia	-18.53	165.97	2003–2008	South Pacific	71
8	SEP	55	French Polynesia	-19.01	-152.84	2003–2008	South Pacific	71
Atlantic Bluefin tuna								
9	NEA	46	Bay of Biscay	45.10	-4.35	2009	East Atlantic	27
10	MED	46	Balearic Sea	40.58	1.21	2009	East Atlantic	37
11	NWA	15	Northwest Atlantic	36.24	74.49	2008	West Atlantic	21

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3 907 **Table 2 Selected set of SNPs for population genetic studies in *T.***
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5 908 ***alalunga*.** SNP code (in bold), the nuclear DNA fragment were it was
6
7 909 discovered and the discovery approach (I, II and III; see Methods) are shown,
8
9 910 for the panel of 53 SNPs, representing 41 loci including 32 individual SNPs
10
11 911 and 9 haplotype blocks (shaded), along with the number of alleles or
12
13 912 haplotypes and mean values, across samples, for both expected
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15 913 heterozygosity (H_e) and inbreeding coefficient (F_{IS}).
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Fragment	Method	SNP	no. alleles/ haplotypes	Mean H_e (±SE)	Mean F_{IS} (±SE)
ADRB2	I	***ADRB2-97***	2	0.025±0.016	-0.006±0.006
ALDOB1	I	***ALDOB1-47*** ***ALDOB1-95***	4	0.373±0.058	0.150±0.104
CALM4	I	***CALM4-124***	2	0.432±0.040	0.247±0.165
GNRH3-1	I	***GNRH3-1-124***	2	0.365±0.040	0.030±0.047
GNRH3-3	I	***GNRH3-3-219***	2	0.077±0.035	-0.033±0.018
LDHB	I	***LDHB-287***	2	0.362±0.061	0.176±0.205
LYZ	I	***LYZ-128*** ***LYZ-138*** ***LYZ-340***	8	0.579±0.079	-0.005±0.155
MLL25a	I	***MLL25a-144*** ***MLL25a-183***	4	0.526±0.057	0.092±0.076
MYC	I	***MYC-91***	2	0.043±0.082	-0.024±0.050
MYL3	I	***MYL3-97***	2	0.024±0.025	-0.009±0.009
RHO	I	***RHO-111***	2	0.033±0.039	-0.014±0.017
RPS7-2	I	***RPS7-2-69***	2	0.270±0.081	0.180±0.138
APOE	II	***APOE-148***	2	0.269±0.039	0.014±0.116
CYCS	II	***CYCS-132*** ***CYCS-218***	4	0.468±0.062	-0.050±0.096
DAD1	II	***DAD1-444***	2	0.088±0.021	-0.039±0.011
FGB	II	***FGB-257***	2	0.053±0.036	-0.024±0.020
FOS	II	***FOS-107***	2	0.154±0.084	-0.089±0.060
HGF	II	***HGF-375***	2	0.039±0.022	-0.015±0.011
HMOX1	II	***HMOX1-416***	2	0.316±0.102	0.181±0.214
MMP9	II	***MMP9-68*** ***MMP9-111***	4	0.220±0.107	0.051±0.074
MTF1	II	***MTF1-263***	2	0.316±0.110	0.077±0.104

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PSM	II	***PSM-33*** ***PSM-117*** ***PSM-138***	8	0.737±0.037	-0.045±0.102
PTGS2	II	***PTGS2-56*** ***PTGS2-317***	4	0.708±0.022	-0.017±0.154
RASA3	II	***RASA3-188***	2	0.063±0.048	-0.028±0.022
RHOC	II	***RHOC-55***	2	0.054±0.056	-0.021±0.027
RPL12	II	***RPL12-213*** ***RPL12-423***	4	0.444±0.169	0.042±0.042
c-mos	III	***c-mos-242***	2	0.313±0.059	-0.055±0.139
CS1	III	***CS1-197*** ***CS1-442*** ***CS1-512***	6	0.533±0.042	-0.030±0.124
CS3	III	***CS3-394***	2	0.449±0.039	0.006±0.220
CS5	III	***CS5-44***	2	0.494±0.013	0.209±0.161
ELOVL2	III	***ELOVL2-519***	2	0.253±0.052	0.095±0.239
ELOVL3	III	***ELOVL3-365***	2	0.129±0.081	-0.071±0.057
FGG	III	***FGG-242***	2	0.371±0.102	-0.009±0.148
GPX-458	III	***GPX-458***	2	0.495±0.009	0.107±0.115
HIF1A2-3	III	***HIF1A2-3-350***	2	0.449±0.027	0.073±0.083
HIF1A4	III	***HIF1A4-219***	2	0.145±0.122	-0.022±0.043
MB	III	***MB-188***	2	0.174±0.068	0.095±0.225
OPC02	III	***OPC02-249***	2	0.167±0.046	-0.032±0.111
PRDX2	III	***PRDX2-452***	2	0.013±0.023	-0.004±0.009
RAG2	III	***RAG2-114***	2	0.312±0.088	0.147±0.195
Tmo-4C4	III	***Tmo-4C4-188***	2	0.063±0.046	-0.026±0.027

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3 917 **Table 3 Probability of detecting a particular level of differentiation (F_{ST})**
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5 918 **among populations of albacore.** Power analysis conducted with POWSIM
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7 919 (1000 replicates) with mean frequencies and sample sizes used for albacore
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9 920 tuna.
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	41 SNPs and blocks		**9 Blocks**		**32 SNPs**	
F_{ST}	***P*** $\mathbf{X^2}$	***P* Fisher**	***P*** $\mathbf{X^2}$	***P* Fisher**	***P*** $\mathbf{X^2}$	***P* Fisher**
0.0005	0.197	0.186	0.125	0.127	0.156	0.150
0.001	0.439	0.404	0.276	0.278	0.286	0.289
0.002	0.886	0.844	0.662	0.638	0.628	0.625
0.004	1.0	0.999	0.981	0.970	0.980	0.976
0.008	1.0	1.0	1.0	1.0	1.0	1.0
0.016	1.0	1.0	1.0	1.0	1.0	1.0

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3 925 **Table 4. Pairwise F_{ST} values between samples of albacore tuna (*Thunnus*
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5 926 ***alalunga*).** F_{ST} values appear below the diagonal and standard errors above
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7 927 the diagonal. Sample abbreviations as in Table 1, F_{ST} values significantly
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9 928 larger than 0.0 are in bold ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)
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	BAL	BIS	IRE	SEA	IN	NP	SEP	SWP
BAL		0.008	0.008	0.008	0.013	0.010	0.011	0.008
BIS	**0.026*****		0.002	0.005	0.008	0.006	0.007	0.008
IRE	**0.030*****	0.000		0.002	0.007	0.004	0.005	0.008
SEA	**0.033*****	0.004	-0.001		0.008	0.005	0.004	0.007
IN	**0.050*****	**0.020*****	**0.017*****	**0.020*****		0.004	0.005	0.006
NP	**0.033*****	**0.014*****	**0.019*****	**0.016*****	**0.008***		0.002	0.002
SEP	**0.043*****	**0.017*****	**0.016*****	**0.012*****	**0.007***	-0.000		0.003
SWP	**0.021*****	**0.014****	**0.011****	**0.012****	**0.011***	0.001	0.001	

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3 931 **Table 5 Selected set of SNPs for population genetic studies in *T.***
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5 932 ***thynnus.*** SNP code (in bold), the identity of the nuclear DNA fragment where
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7 933 it was discovered in *T. alalunga* along with the applied SNP discovery
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9 934 approach (see Methods) are shown for the panel of 17 SNPs, representing 15
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11 935 independent loci including 13 SNPs and 2 haplotype blocks (shaded), along
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13 936 with the number of alleles or haplotypes and mean values, pooling all
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15 937 sampling locations, for both expected heterozygosity (H_e) and inbreeding
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17 938 coefficient (F_{IS}).

Fragment	Method	SNP	no. alleles/ haplotypes	Mean H_e (±SE)	Mean F_{IS} (±SE)
ADRB2	I	***ADRB2-97***	2	0.046±0.040	-0.016±0.014
GNRH3-1	I	***GNRH3-1-107*** ***GNRH3-1-124***	4	0.676±0.023	-0.056±0.178
LDHB	I	***LDHB-129***	2	0.468±0.068	0.057±0.268
LYZ	I	***LYZ-128***	2	0.062±0.054	0.439±0.380
RPS7-2-313	I	***RPS7-2-313***	2	0.084±0.079	0.083±0.176
TPMA	I	***TPMA-53***	2	0.355±0.100	0.000±0.351
CYCS	II	***CYCS-161***	2	0.175±0.195	0.216±0.240
HGF	II	***HGF-375***	2	0.211±0.090	0.106±0.140
MTF1-263	II	***MTF1-263***	2	0.273±0.055	0.010±0.162
RPL12	II	***RPL12-423***	2	0.486±0.012	0.006±0.157
CS3	III	***CS3-118***	2	0.291±0.253	0.023±0.070
CS5	III	***CS5-395*** ***CS5-425***	4	0.243±0.211	0.137±0.183
FGG	III	***FGG-242***	2	0.401±0.108	0.215±0.198
HIF1A2-3	III	***HIF1A2-3-417***	2	0.145±0.041	0.216±0.500
OPC02	III	***OPC02-45***	2	0.167±0.289	0.000±0.000

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Table 6 Probability of detecting a particular level of differentiation (F_{ST}) among populations of Atlantic bluefin tuna. Power analysis conducted with POWSIM (1000 replicates) with mean frequencies and sample sizes used for Atlantic bluefin tuna.

	13 SNPs and 2 blocks	
F_{ST}	*P* X^2	*P* Fisher
0.001	0.091	0.064
0.0025	0.159	0.104
0.005	0.314	0.240
0.010	0.654	0.579
0.020	0.961	0.931
0.040	1.000	0.998
0.080	1.000	1.000

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Figure legends

Figure 1. Sampling locations and approximate locations of spawning areas in *Thunnus alalunga* and *T. thynnus*. Respectively, black circles and right-oriented hatching for *T. alalunga*, and black squares and left-oriented hatching for *T. thynnus*; both species spawn in Mediterranean waters.

Figure 2: SNP selection design. Design of the filtering steps used to select SNP panels for individual assignment and for genetic population surveys in *T. alalunga* and *T. thynnus* (see Methods for further information)

Figure 3. *Thunnus alalunga* STRUCTURE results. Individual clustering analysis with STRUCTURE (respectively, $K = 2$, $K = 3$ and $K = 4$) of 460 *T. alalunga* individuals for 53 SNPs located in 41 independent fragments. Each vertical bar represents an individual, and sampling locations are separated by vertical black lines. The colour proportions of each bar correspond to the individual's estimated membership fraction to each of the clusters (cluster membership coefficient).

Figure 4. *Thunnus thynnus* STRUCTURE results. Individual clustering analysis with STRUCTURE ($K = 2$) analysis of 107 *T. thynnus* individuals for 17 SNPs on 15 independent DNA fragments. Each vertical bar represents an individual, and sampling locations are separated by vertical black lines. The colour proportions of each bar correspond to the individual's estimated membership fractions to each of the clusters (cluster membership coefficient).

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3 974 ## Supplementary material
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7 975 **Table S1.** Information on the 54 nuclear DNA fragments selected for SNP
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9 976 discovery in *Thunnus alalunga* (see Methods): fragment name, gene region,
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11 977 SNP discovery approach (1, 2 and 3; see Methods), source, forward and
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13 978 reverse primer sequences, PCR annealing temperatures, sequenced
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15 979 fragment lengths and recorded number of SNPs and indels. EPIC primers
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17 980 (Exon-Priming, Intron-Crossing primers; SNP discovery approach I) were
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20 981 obtained from the literature while primers for the remaining DNA fragments
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22 982 were designed from the alignment of sequences from publically available
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24 983 databases (GenBank and Ensembl), respectively, 17 pairs of degenerate
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27 984 primers from several teleost species sequences (approach II) and 18 pairs of
28
29 985 primers from genus *Thunnus* DNA sequences (approach III).
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31 986
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33 987 **Table S2.** Information for the polymerase chain reactions (PCR) and cycling
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36 988 conditions used to amplify the 54 nuclear DNA fragments selected for SNP
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38 989 discovery in *T. alalunga*. While 30 fragments were amplified with a
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40 990 conventional PCR, touchdown (TD) methodology, involving a decreasing
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42 991 annealing Tº, was applied for the remaining fragments. [(1)] Annealing
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44 992 temperature started in the first cycle with that registered in Table S1
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47 993 ("Touchdown temperature" column) for each fragment. Then, annealing
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49 994 temperature decreased 0.5ºC in each subsequent cycle. [(2)] Fixed annealing
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51 995 temperature for each fragment. See "Fixed temperature" column in Table S1.
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53 996
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55 997 **Table S3.** BLASTN homology search results for the *T. alalunga* sequenced
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58 998 fragments. Only *best hits* showing identity ≥ 70% and E-value < $10e^{-5}$ were
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3 999 considered. Out of 54 fragments of nuclear DNA that were sequenced in *T.*
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5 1000 *alalunga*, 35 fulfilled the required conditions.
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10 1002 **Table S4.** Characteristics of the 79 validated SNPs, meaning reliably scored
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12 1003 and polymorphic, in *Thunnus alalunga*. Marker name, SNP discovery
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14 1004 approach (1, 2 and 3; see Methods), call rate (%), alleles and minor allele
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16 1005 frequency (MAF) values are shown along with the global and per location
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18 1006 deviation (*P*) from the Hardy-Weinberg equilibrium (HWE). “SIGN.” means
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21 1007 that at least 1 location was not in HWE for those markers (N.S. corresponds
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23 1008 to not significant); as multiple comparisons were involved, a probability of $P <$
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25 1009 0.001 was considered significant. Last column shows NCBI Assay ID (rs#) for
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27 1010 every individual SNP (NCBI´s dbSNP database).
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30 1011
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32 1012 **Table S5.** Results from tests of Linkage disequilibrium (LD) between all
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34 1013 possible SNP pairs (validated SNPs only) within each DNA fragment, for both
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36 1014 *T. alalunga* and *T. thynnus* (*$P < 0.001$). No SNPs were found in LD when
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38 1015 comparing among DNA fragments. Linked SNPs were phased into haplotypes
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40
41 1016 using the Bayesian statistical method implemented in PHASE 2.1. Haplotypes
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43 1017 were reconstructed by location to avoid biases from population structuring.
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45 1018 Missing genotypes were classified as null genotypes to avoid haplotype
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47 1019 reconstruction errors.
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50 1020
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52 1021 **Table S6.** Characteristics of the 23 validated SNPs in *Thunnus thynnus*.
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54 1022 Marker name, SNP discovery approach (1,2 and 3), call rate (%), alleles and
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56 1023 minor allele frequency (MAF) values are shown along with the global and per
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1024 location deviation (*P*) from the Hardy-Weinberg equilibrium (HWE). "SIGN."
1025 means that at least 1 location was not in HWE for those markers (N.S.
1026 corresponds to not significant); as multiple comparisons were involved, a
1027 probability of $P < 0.001$ was considered significant. Out of the 23 SNPs
1028 validated in BFT by cross-species amplification of 128 SNPs discovered in
1029 albacore (see Methods and Fig. 2), 18 had been validated also when
1030 genotyping albacore while the other five SNPs (*GNRH3-1-107, LDHB-129,*
1031 *CYCS-161, CS3-118,* and *OPC02*-45) were reported as reliably scored and
1032 polymorphic only in BFT (last column shows their individual NCBI Assay IDs
1033 (ss#) in the NCBI´s dbSNP database).

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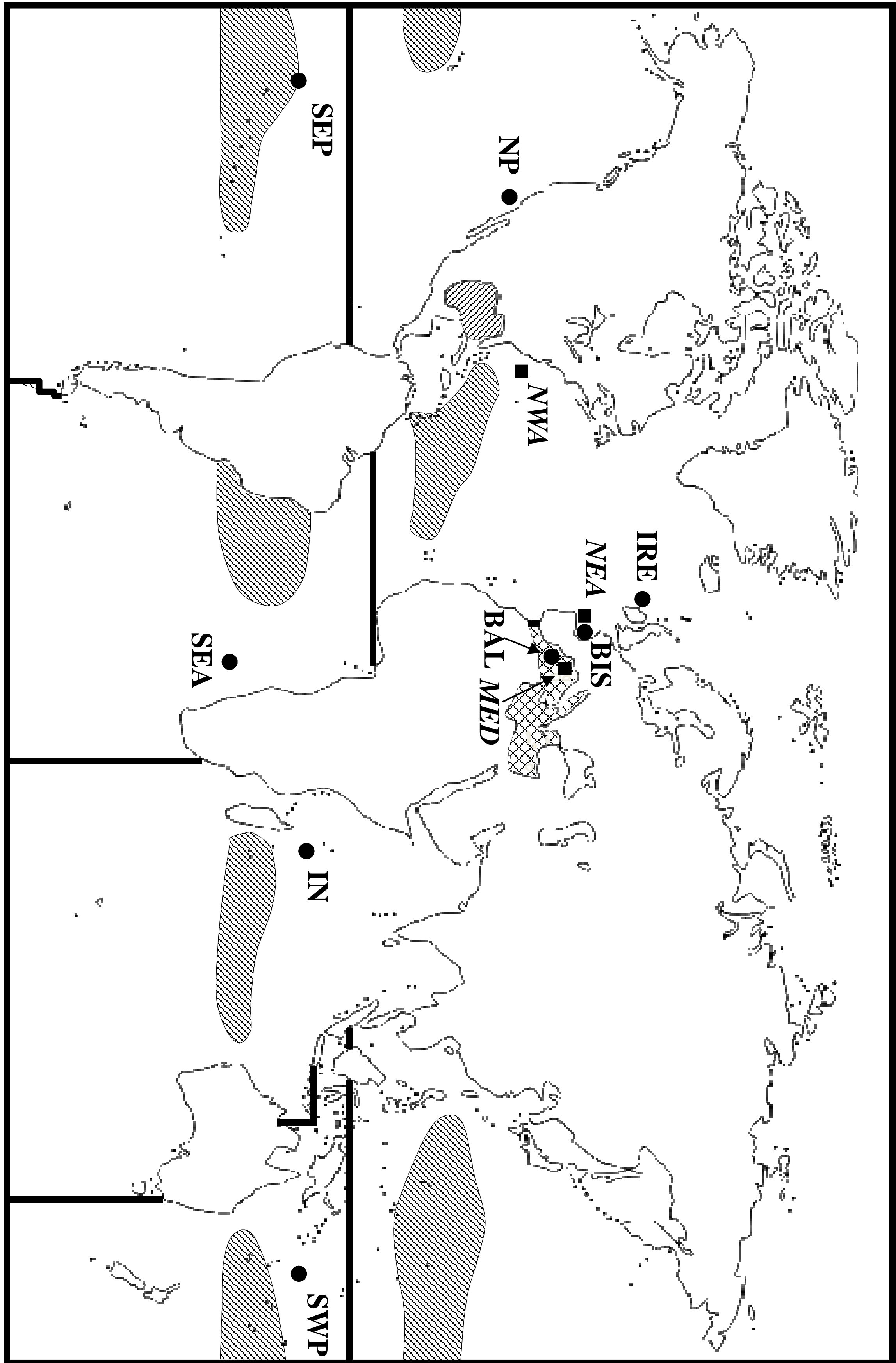
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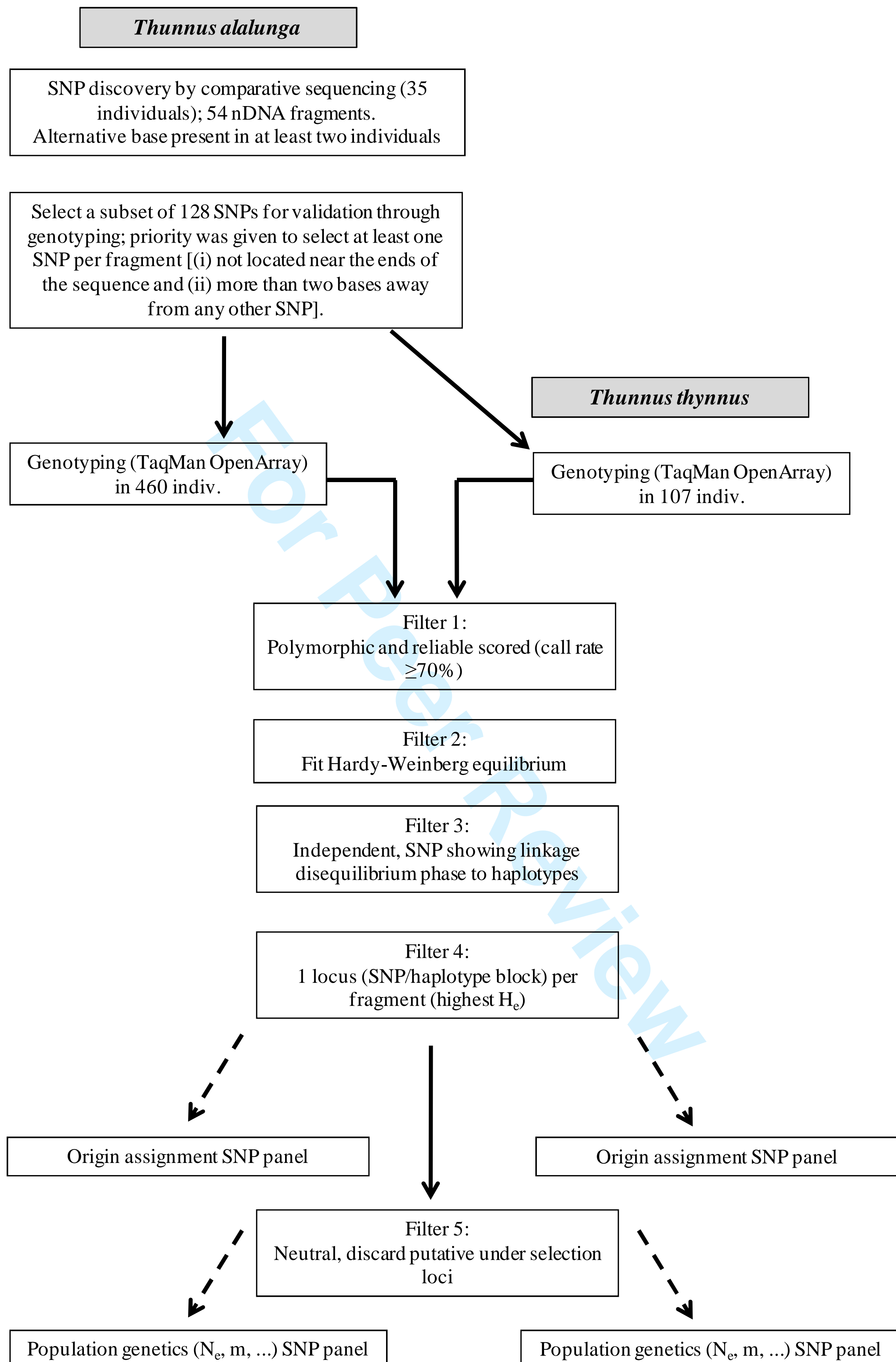

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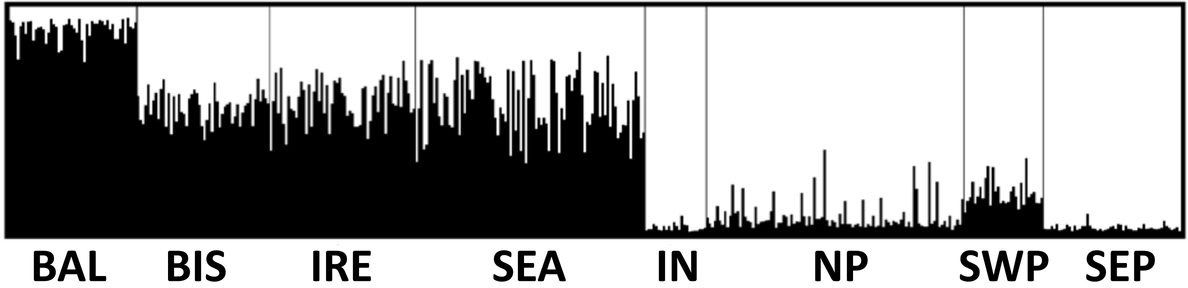

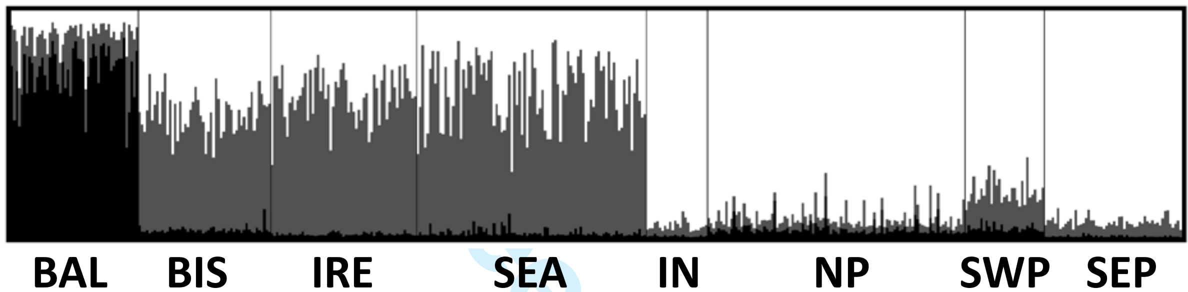

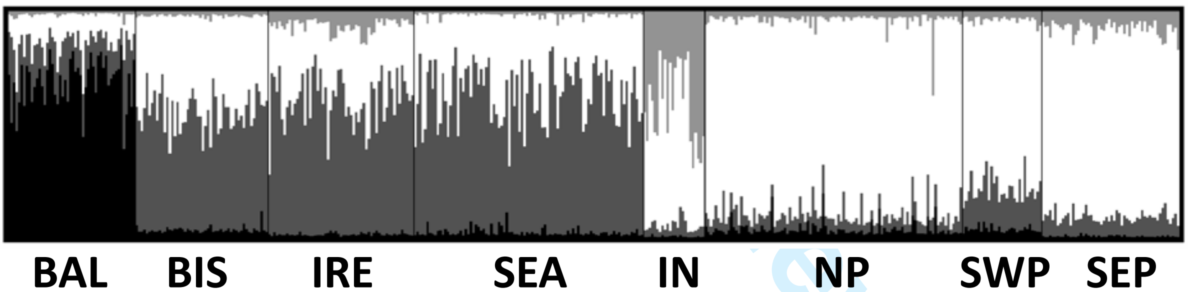

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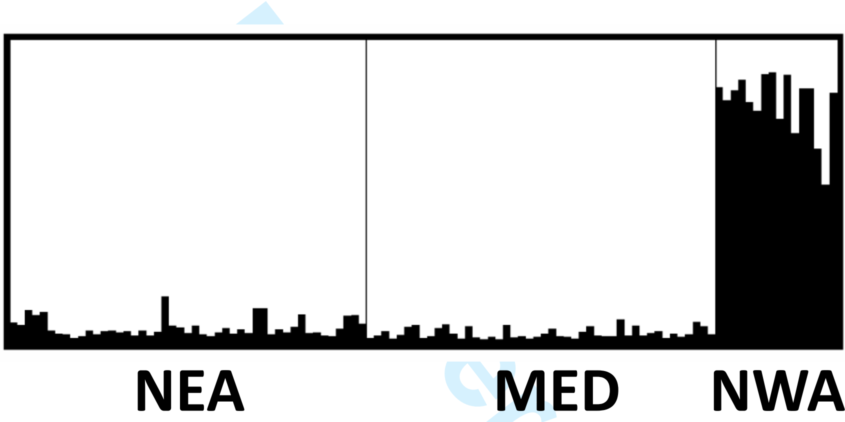

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