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Phylogeography and population structure of European sea bass in the north-east Atlantic

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The European sea bass *Dicentrarchus labrax* represents a historically and commercially valuable species in the north-east Atlantic, although the demographic history and the patterns of geographical structure of the species in the north-east Atlantic remain poorly understood. The present study investigates the population genetic structure of sea bass in north-western European waters, employing different genetic markers [a portion of the mitochondrial (mt)DNA control region and 13 nuclear microsatellites] aiming to unravel demographic history and population connectivity. The results obtained show a previously unrecognized pattern of population divergence at mtDNA, with three strikingly different lineages identified. Extant sea bass populations, including the Mediterranean lineage, derive from an Atlantic ancestor. A much increased number of nuclear microsatellite loci (comparatively to previous studies) still fail to detect biologically meaningful patterns of spatial genetic structuring in the North Atlantic. Past Pleistocene glacial and interglacial events and some degree of female philopatry might be at the basis of the current geographical separation of the Atlantic lineages that has been identified. Signatures of sudden demographic expansions are more evident in the most recent mitochondrial lineages, and their slight, yet significant, geographical segregation leads to the hypothesis that present-day spawning grounds for European sea bass may still to some extent be linked to their most recent glacial refugia. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **104**, 364–377.

ADDITIONAL KEYWORDS: molecular markers – migration – connectivity – sex-biased dispersal – *Dicentrarchus labrax*.

INTRODUCTION

The distribution and structure of populations as we observe them today have been profoundly influenced by Quaternary climatic events (Hewitt, 1996, 1999, 2000). At the beginning of the Pleistocene, approximately 2.5 Mya, the Arctic ice cap had started expanding, followed by cycles of glacial/interglacial ages with an approximately 100 000 years periodicity (Hewitt, 1999). As a result, some species went extinct over large parts of their range, some dispersed to new locations, and some others survived in refugia and then expanded again (Hewitt, 2000). Both the terrestrial and the marine realms were subjected to these species shifts to varying extents.

In the case of the north-east Atlantic, sea level and sea surface temperature underwent important changes as more and more water became trapped in the ice sheets (Hewitt, 1996; Maggs *et al*., 2008). Throughout the last major glacial cycle, which culminated at the Last Glacial Maximum (LGM), 25 000–18 000 years Before Present (BP), most of Britain, and Ireland were covered in ice, although the southern limit of sea ice is still controversial (Maggs *et al*., 2008). From approximately 16 000 years BP, the climate warmed, favouring the expansion of species out of their refugia, interrupted only by the Dryas spell (11 000–10 000 years BP) during which the Atlantic Polar Front moved from Britain to Iberia (Hewitt, 1999), causing a drastic drop (–65 m) of the world sea level. Subsequently, the climate warmed

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again: the sea level rose rapidly, flooding the Dover Strait, reconnecting the English Channel with the southern North Sea by 9000 years BP (Nesbo *et al*., 2000; Streif, 2004).

It is assumed that small northern periglacial refugia (unglaciated zones) may have persisted (Petit *et al*., 2003; Avise, 2004; Provan & Bennett, 2008), although determining their location based on molecular data of cold-temperate species is a difficult task. Despite this, previous studies have proposed the existence of such north-east Atlantic periglacial refugia in the Azores (Chevolot *et al*., 2006), along the Atlantic coast of the Iberian peninsula (Petit *et al*., 2003; Hoarau *et al*., 2007; Larmuseau *et al*., 2009), in the Bay of Biscay (Nesbo *et al*., 2000), along the south-western coast of Ireland (Hoarau *et al*., 2007), in the southern North Sea (Gysels *et al*., 2004) and in the Hurd Deep, a 150 km long depression located in the western English Channel (Coyer *et al*., 2003; Provan, Wattier & Maggs, 2005).

The genetic footprints of Quaternary refugia are evident in the spatial distribution of extant populations, which is typically structured in a way that reflects post-glacial dispersal routes (Bernatchez & Wilson, 1998; Avise, 2004). The study of post-glacial recolonization processes assumes particular importance in the likely scenario of increasing sea surface temperatures as a result of global warming.

The European sea bass *Dicentrarchus labrax* (L., 1758) is a coastal fish distributed throughout the Mediterranean Sea and all along the European Atlantic coast, up to Norway. The British Isles have long represented the north-western limit of its reproductive range, and populations in this area can be expected to be more vulnerable to environmental changes, as has been shown in other taxa (Post *et al*., 2009). This species is characterized by a potentially high dispersal capability, mainly as a result of its long planktonic larval phase (up to 2–3 months) and its ability to migrate across several hundreds of kilometres (Pickett & Pawson, 1994). The migratory behaviour of this species, which is strongly influenced by water temperatures and linked to spawning, has received particular attention (Pawson, Kelley & Pickett, 1987; Pickett, Kelley & Pawson, 2004; Fritsch *et al*., 2007; Pawson *et al*., 2007b). In the north-east Atlantic, the reproductive migration starts in October to December as the water cools down and adult female sea bass move to offshore southward locations (Jennings & Pawson, 1992; Reynolds, Lancaster & Pawson, 2003), mainly in the western English Channel and southern Celtic Sea, to seek water temperatures over 9 °C that will aid gonad maturation (Thompson & Harrop, 1987). After spawning, between February and May, bass return east and north to inshore feeding grounds to which they show a high degree of fidelity (Pawson *et al*., 2008). Post-larvae recruit to nursery areas from June onwards, where juveniles remain until they are 30–40 cm long (Jennings & Pawson, 1992), which corresponds to an age range of 3–6 years. In a tagging study carried out around the British isles, Pickett *et al*. (2004) recorded considerable movement of sub-adult sea bass, in particular from southern to north-western locations along the English coasts. It was hypothesized that this dispersal of adolescent specimens in British waters might preclude the existence of genetically distinct biological units.

Most of the previous population genetic investigations of sea bass have focused on the Mediterranean area (Patarnello *et al*., 1993; Allegrucci, Fortunato & Sbordoni, 1997; Caccone *et al*., 1997; DeLeon, Chikhi & Bonhomme, 1997; Bahri-Sfar, Lemaire, Ben Hassine & Bonhomme, 2000; Lemaire *et al*., 2000). A few other studies have focused on Atlantic populations: Castilho & McAndrew (1998) found low genetic differentiation at five Portuguese populations of juvenile sea bass; Naciri *et al*. (1999) screened three north-east Atlantic adult populations at six microsatellite loci, finding slight genetic differentiation (Portugal versus Belgium); finally, Fritsch *et al*. (2007) screened eight Atlantic populations (both juveniles and adults) at five microsatellite loci, and results obtained suggested the possibility of subtle differentiation of the Irish sample from the rest.

The sea bass is targeted by commercial and recreational fisheries on both winter spawning grounds and summer inshore feeding areas. The commercial fishery developed rapidly in the late 1970s and 1980s, soon showing signs of growth-overfishing (too many young fish being caught), which threatened the sustainability of the stock (Pawson & Pickett, 1987; Pawson, Pickett & Smith, 2005; Pawson, Kupschus & Pickett, 2007a). Both Ireland and the UK (in England and Wales) introduced legislations in 1990 to help the recovery of the stock (Pawson *et al*., 2007a). The introduction of these bass management plans, coupled with warmer winters that favoured years of good recruitment, has led to a recovery of the bass stock around the British Isles (Pawson *et al*., 2007a).

The present study aimed to examine the genetic population structure of sea bass in the north-east Atlantic, using a greater number of more powerful genetic markers than it had previously been used, and link genetic signatures with past climatic events and the current views on migratory behaviour of this species.

MATERIAL AND METHODS

Sampling and DNA extraction

Tissue samples were collected from a total of 398 individuals along the north-east Atlantic coast and

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from one location in the Mediterranean Sea, between 2003 and 2006 (Table 1). Sampling locations were: the south of the Bay of Biscay – France; Wexford – Ireland; Anglesey – Wales; Cornwall – south-west England; Morecambe Bay – north-eastern England; Solent – southern England; Thames estuary – eastern England; Garden City – Belgium; and Masfjord – Norway (Fig. 1). The Mediterranean population was from Sabaudia (central Italy, Tyrrhenian basin). Sample availability was constrained by conservation policies in force mainly in Ireland and the UK. Especially in Ireland, the main limitation was the impossibility of obtaining adult samples because the landing of sea bass from commercial fisheries is illegal. Limitations in act in the UK further limited the availability of samples from the area, and samples from other localities were largely opportunistic and included mature individuals not on their spawning grounds (Table 1).

Sampled tissue consisted of either scales or fin-clips stored in absolute ethanol. DNA was isolated using a modified salt/chloroform extraction protocol (Miller, Dykes & Polesky, 1988) that included an additional chloroform/isoamyl alcohol (24 : 1) step after adding the saturated NaCl solution (Petit, Excoffier & Mayer, 1999).

Mitochondrial (mt)DNA

The *D. labrax* mtDNA is one of the largest mtDNA molecules in fish, approximately 18 kb in length (Cesaroni *et al*., 1997). The Control Region, located between $tRNA^{Pro}$ and $tRNA^{Phe}$ (Venanzetti *et al*., 1994), spans for approximately 2.5 kb (Cecconi, Giorgi & Mariottini, 1995).

Species-specific primers were designed to amplify a 419-bp long fragment of the mtDNA that includes a 283-bp long trait of the 5′ end of the control region. The forward primer was within the $tRNA^{Thr}$ gene (DL tRNA-F: 5′-CGAATGTCGGAGGTTAACT-3′), whereas the reverse was placed to exclude the highly repetitive region (DL CR3-R 5′-ATACACGCGTTTGTG GCACT-3′). A total number of 103 individuals were sequenced, with a mean of ten per location (6–13), which is considered adequate for phylogeographic analysis (Felsenstein, 2006). Details on polymerase chain reaction (PCR) protocols and sequencing are available upon request from the authors. Sequences were assembled in SEQUENCHER, version 4.7 (Gene Codes) and manually aligned in MEGA, version 4.0 (Tamura *et al*., 2007). The final length of the fragment analyzed after alignment was 399 bp.

Microsatellites

Thirteen species-specific dinucleotide microsatellites were chosen among those available in the literature:

Table 1. Sampling location, sample code and genetic diversity parameters inferred from mitochondrial (mt)DNA and microsatellite analyses

				Age	Mitochondrial DNA				Microsatellites						
Location	Code	Capt	N	(years)	Ns	h	H_D	π	H_e	H_o	N_A	A_R	A_P	F_{IS}	N_e (95% CI)
Sabaudia, Italy	MED	2005	48	1	10	4	0.378	0.00155	0.875	0.854	16.85	13.29	6	0.024	1321.3 (*to* ∞)
Solent South Coast, England	SOL	2005	48	1	10	9	0.978	0.01295	0.884	0.873	16.77	13.3	4	0.013	130.5 (*101.7–178.7*)
Thames Estuary, England	TH	2005	48	1	10	7	0.844	0.04196	0.894	0.875	18.23	14.12	3	0.022	52.8 (*46.1–61.2*)
Puffin Island, Wales	WAL	2006	48	*8–10	12	11	0.97	0.02727	0.898	0.888	18	14.06	1	0.011	–1253.2 (*to* ∞)
Wexford, Ireland	WEX	2006	48	1	13	8	0.808	0.00834	0.892	0.881	18.08	14.12	2	0.012	–2113.2 (*to* ∞)
Masfjord, Norway	NOR	2005	48	6–7	10	10	1	0.05062	0.893	0.88	17.77	14.03	4	0.015	6017.1 (*to* ∞)
Garden City, Belgium	BE	2006	42	*8–10	10	6	0.778	0.02936	0.901	0.894	17.85	14.49	2	0.008	596.9 (*to* ∞)
Bay of Biscay (south)	BB	2007	21	*6–8	13	13	1	0.06907	0.889	0.883	13.77	13.77	2	0.007	–210.4 (*to* ∞)
Morecambe Bay, England	MOR	2003	47	5–7	10	9	0.972	0.07056	0.888	0.869	17.08	13.82	1	0.021	392.3 (*227–1290.3*)
Cornwall, England	CNW	2005	6	–	6	3	0.733	0.00606	–	–	–	–	–	–	–

Capt., year in which the correspondent sample was collected; N, sample size for the studied populations; N_s, number of individuals sequenced from each population; h, number of haplotypes; H_D, haplotype diversity; π nucleotide diversity. For the microsatellite data: H_e, expected heterozygosity; H_o, observed heterozygosity; N_A, mean number of alleles; A_R, allelic richness; A_P, number of private alleles; F_{IS}, coefficient of inbreeding; estimated effective population size (N_e), with associated 95% confidence intervals [negative estimates of N_e are usually interpreted as infinite estimates (LDNe user's manual)]. Age is averaged over all individuals of the corresponding population, *Sampled populations for which age was back calculated from length data (Pickett & Pawson, 1994).

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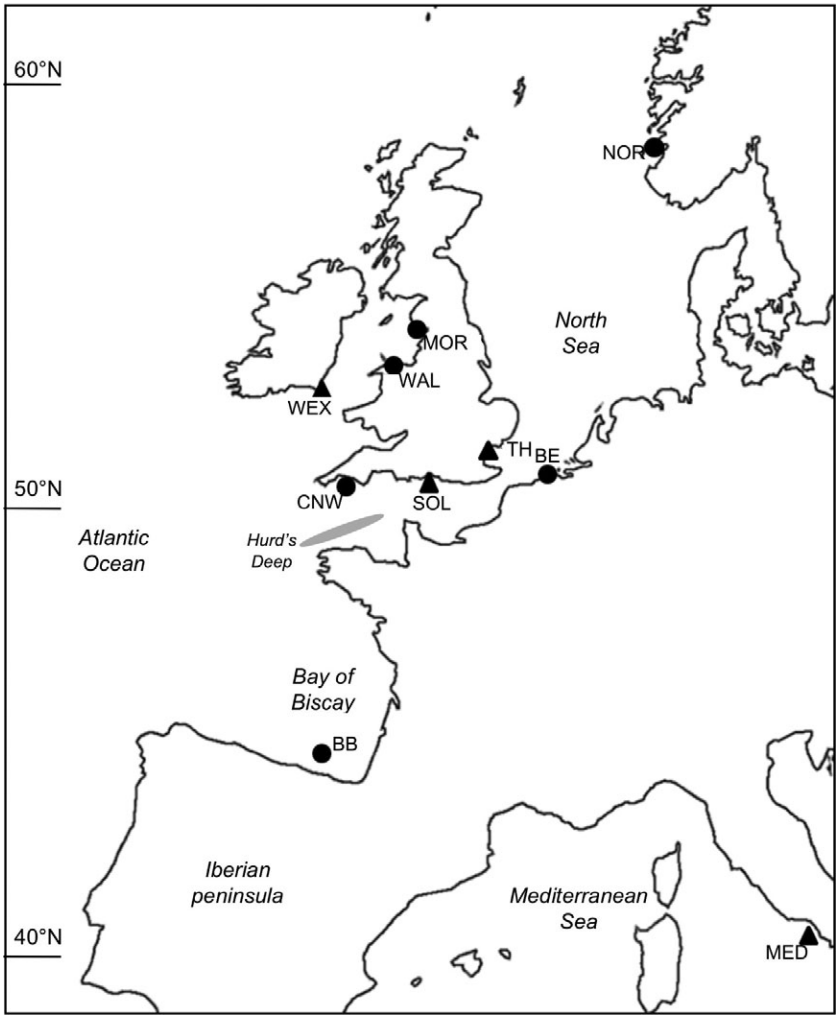

Figure 1. Map showing the study area. Sampling locations are shown by their code (Table 1). Circles indicate adult populations; triangles indicate juveniles (Table 1).

DLA0018, DLA0020, DLA0110, and DLA0119 (Chistiakov *et al*., 2004); Dla-12 (Ciftci, Castilho & McAndrew, 2002); Labrax-8, Labrax-13, Labrax-17, and Labrax-29 (De Leon *et al*., 1995); and DLA0004, DLA0009, DLA0010 and DLA0011 (Tsigenopoulos *et al*., 2003). Loci with optimal polymorphism were selected from different linkage groups (Chistiakov *et al*., 2005, 2008; Volckaert *et al*., 2007) to ensure the greatest possible statistical power and minimize potential sources of bias (e.g. physical linkage in recombination, null alleles). Loci were arranged in three different multiplex reactions and grouped on the basis of their annealing temperature and after testing for the potential formation of primer-dimers and hairpin structures, as implemented in AutoDimer (Vallone & Butler, 2004).

Details on PCR conditions and fragment analysis are available upon request from the authors.

Statistical analysis

mtDNA

Haplotype diversity (H_D) and nucleotide diversity (π) were calculated in DNASP, version 4.50.3 (Rozas *et al*., 2003). MODELTEST (Posada & Crandall, 1998) was used to identify the most appropriate model of evolution (TIM+G) for the data set via the Akaike information criteria (Burnham & Anderson, 1998). A median-joining network was constructed using NETWORK, version 4.5 (Bandelt, Forster & Rohl, 1999; Fluxus-Engineering, 2009). Net divergence (D_a) between the haplogroups identified in the network was calculated in MEGA, version 4 (Tamura *et al*., 2007). Maximum composite likelihood, defined as the sum of the log-likelihoods for all pairwise distances in a distance matrix (Tamura, Nei & Kumar, 2004) and a Gamma distribution of $\alpha = 0.7227$ (as given by MODELTEST) were used and

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10 000 bootstrap replicates were employed to calculate the Standard Error (SE) of D_a. Estimates of divergence time (T) among main control region lineages were then calculated using the formula $T = D_a/2\mu$ (Kimura, 1980), where T represents generation time and μ is the mutation rate, which can be defined as the frequency of new mutations per generation. ARLEQUIN, version 3.11 (Excoffier, Laval & Schneider, 2005) was employed to calculate Φ_{ST} and produce mismatch distributions of pairwise nucleotide differences for each lineage. The pairwise Φ_{ST} matrix was then tested for correlation with geographic distance in a Mantel test (1000 permutations) to check for the presence of isolation-by-distance (IBD) using the software R with the ECODIST package (Goslee & Urban, 2007).

Each clade was tested for both sudden demographic and spatial expansions (Rogers & Harpending, 1992) using tests of goodness-of-fit, generated using parametric bootstrapping with 10 000 replicates. Times since expansion were estimated from the mismatch distributions parameters (τ) using the formula $\tau = 2ut$, where τ is the time since expansion in units of $1/2ut$ generations using a generalized least squared approach (Rogers, 1995; Schneider & Excoffier, 1999); u is the mutation rate for the whole haplotypic sequence, hence given by μ multiplied by the number of base pairs included. The mismatch analysis allows only accepting or rejecting the hypothesis of expansion but does not allow any other inference regarding historical demography of the populations. For this reason, demographic expansion was further investigated by Tajima's D and Fu's F_S tests of neutrality as implemented in ARLEQUIN, version 3.11. For selectively neutral markers, significant negative values of D and F_S are expected in case of haplogroups/populations that underwent a sudden demographic expansion but, although Tajima's D can be sensitive to other evolutionary forces (i.e. selection), Fu's F_S is particularly sensitive to population expansion (Tajima, 1989a, b; Fu, 1997). For demographic analyses, we assumed an evolutionary mutation rate of 11%/site/Myr, considered to be appropriate for the control region in teleosts (Patarnello, Volckaert & Castilho, 2007), and a generation time of 3 years, (*sensu* Lemaire, Versini & Bonhomme, 2005).

Microsatellites

All the sampled individuals were genotyped at the 13 microsatellites, apart from Cornwall (CNW), whose limited sample size ($N = 6$) would impede meaningful statistical treatment (Kalinowski, 2004).

MICROCHECKER, version 2.2.3 (Van Oosterhout *et al*., 2004) was employed to check for the presence of null alleles, large allele drop-out, and scoring errors. Genetic variation among and within the nine populations was assessed calculating expected unbiased (H_e) (Nei, 1978) and observed (H_o) heterozygosities using GENALEX, version 6 (Peakall & Smouse, 2006). FSTAT, version 2.9.3 (Goudet, 1995) was employed to calculate allelic richness (A_R), using the rarefaction method (El Mousadik & Petit, 1996), as well as F_{IS}, to determine deviations from Hardy–Weinberg equilibrium (HWE), and overall and pairwise F_{ST}, using the θ estimator (Weir & Cockerham, 1984), to calculate the variance in allelic frequencies as a result of population substructure. Significance values were tested through 10 000 randomizations and adjusted for multiple comparisons using a sequential Bonferroni method (Rice, 1989).

Recently, concerns have been raised over the reliability of F_{ST} in describing genetic differentiation between subpopulations because of its negative dependence on within-groups heterozygosity, causing estimates to tend to zero when diversity is very high. Accordingly, a new measure of genetic differentiation was proposed to overcome this limit, Jost's D (Jost, 2008). We performed D calculations using SPADE (Chao & Shen, 2003), which implements an estimator of D that is not biased if sample size varies (Jost, 2008). Finally, a Mantel test (Mantel, 1967; Smouse, Long & Sokal, 1986) was performed in R, based on both D and F_{ST} after 1000 permutations, aiming to test for the presence of IBD. The same test was also performed to investigate the correlation between pairwise estimates of genetic estimators (F_{ST} and D calculated from microsatellites).

STRUCTURE, version 2.2 (Pritchard, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2003, 2007) was used to perform a Monte Carlo Markov chain (MCMC) Bayesian assignment test, applying the default settings and 200 000 iterations following a burn-in period of 20 000. Given the growing amount of clustering software now available and the difficulty set by this in selecting the right method (Manel, Gaggiotti & Waples, 2005), the results obtained from STRUCTURE were compared with those obtained from other two clustering programs that implement different approaches: GENELAND (Guillot, Mortier & Estoup, 2005) and FASTRUCT (Chen, Forbes & Francois, 2006). Compared to STRUCTURE, GENELAND performs a MCMC Bayesian assignment incorporating geographical coordinates, and hence is able to spatially define subpopulations, whereas FASTRUCT carries out a maximum likelihood analysis with a no admixture/uncorrelated allele frequencies model through an expectation maximization (EM) algorithm (Dempster, Laird & Rubin, 1977), instead of MCMC. The analysis is faster and delivers results that are

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comparable to those produced by Bayesian clustering software (Chen *et al*., 2007), and reliable also at low levels of population differentiation, as shown for STRUCTURE (Latch *et al*., 2006). Both GENELAND and FASTRUCT assume no deviation from HWE and no linkage disequilibrium.

GENELAND was run with 200 000 MCMC and 20 000 burn-in, and the most likely number of clusters, *K*, was inferred from ten independent runs. Allele frequencies were inferred from independent Dirichlet distributions (Pritchard *et al*., 2000).

FASTRUCT was run with 500 iterations, which was considered to be sufficiently large. In all three cases, the number of clusters was inferred by averaging the log-likelihood values from each independent run over each value of *K* assumed: the highest likelihood corresponds to the best result.

Effective population size (N_e), one of the main factors shaping population structure and determining the nature and rate of genetic drift across generations (Waples, 2002), was estimated for each locality using the method based on gametic disequilibrium implemented in LDNe, version 3.1 (Waples & Do, 2008).

RESULTS

MTDNA

Out of 399 bp sequenced in 103 individuals across ten localities (nine Atlantic and one Mediterranean outgroup), 123 polymorphic sites were identified. A total of 66 haplotypes (GenBank accession numbers JF922607–JF922672) and overall haplotype diversity (H_D) and nucleotide diversity (π) values of 0.940 and 0.06150 were found respectively. When calculated for each Atlantic location, haplotype diversity was in the range 1–0.733, with the highest values recorded in Norway (NOR) and the Bay of Biscay (BB), and the lowest ones in CNW. Highest values of nucleotide diversity were observed in Morecambe Bay (MOR), BB and NOR; the lowest ones in CNW (Table 1). The Mediterranean (MED) fish exhibited the lowest haplotype and nucleotide diversities: 0.378 and 0.00155 respectively.

The median-joining network revealed the presence of four, well separated, haplogroups (Fig. 2A), three of which in the Atlantic: '*Atlantic 1*', which includes seven haplotypes (five from BB and two from MOR) and is separated from the '*Mediterranean*' haplogroup by 34 mutations; '*Atlantic 2*', which is mainly

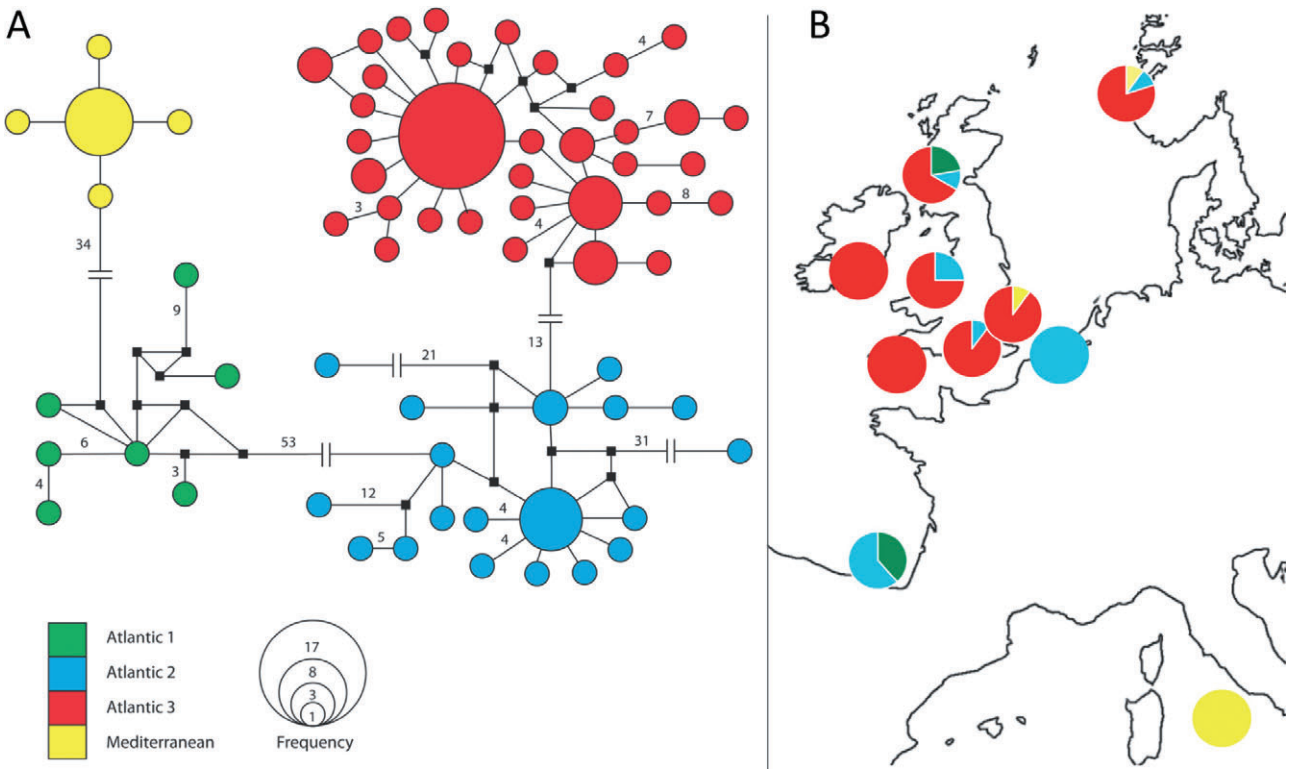

Figure 2. A, median-joining network of Atlantic and Mediterranean samples. Numbers on branches indicates the number of mutations occurring between the major haplogroups. Black squares represent missing haplotypes. B, geographical distribution of haplotypes divided by haplogroups.

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Table 2. F_{ST} pairwise comparisons among localities

	MED	SOL	TH	WAL	WEX	NOR	BE	BB	MOR	CNW
MED	0	0.95788*	0.87523*	0.90059*	0.96354*	0.83846*	0.92017*	0.70075*	0.78433*	0.98329*
SOL	0.031*	0	–0.0339	–0.0048	–0.0165	–0.0195	0.54933*	0.41147*	0.09908	–0.0588
TH	0.0322*	0.0062*	0	0.01474	0.00806	–0.0822	0.43819*	0.3206*	0.01726	–0.0448
WAL	0.0276*	0.0018	0.0024	0	0.05309	–0.032	0.37021*	0.32896*	0.04261	0.05138
WEX	0.0321*	0.002	0.0019	0.0009	0	0.01859	0.64166*	0.48508*	0.15801	0.00366
NOR	0.0271*	0.0006	0.0035	0.0003	0.0014	0	0.33955*	0.25275	–0.0278	–0.013
BE	0.0251*	0.004*	0.0016	–0.0002	0.0017	–0.0009	0	0.22163	0.27229*	0.61978*
BB	0.0284*	0.0018	0.0052	–0.0005	0.0053	0.0008	0.0024	0	0.11027	0.41329
MOR	0.0294*	0.003*	0.0049	0.0014	0.0004	–0.0011	0.0022	0.004	0	0.10034
CNW	–	–	–	–	–	–	–	–	–	0

In the upper diagonal, values are inferred from mitochondrial DNA; in the lower diagonal, values are inferred from microsatellites.

*Significant values after Bonferroni's correction. For location codes, see Table 1.

found along the continental coast [BB and Belgium (BE)] (Fig. 2B), comprising 19 haplotypes (all the individuals from BE, eight from BB, one from NOR, one from Solent, one from MOR, and three from Wales), and is separated from '*Atlantic 1*' by 53 mutational steps. Finally, '*Atlantic 3*' consists of 37 haplotypes predominant in the samples around the British Isles and Norway and separated from '*Atlantic 3*' by only 13 mutational steps. Interestingly, the *Mediterranean* haplogroup (five haplotypes in total) includes two individuals caught in Atlantic locations, one from the Thames estuary (TH) and one from Norway (Fig. 2B).

The net divergence (D_a) between *Atlantic 1* and *Atlantic 2* was 15.4% [95% confidenence interval (CI) = 6.2–24.6%], which approximately corresponds to a time since divergence between groups of 1 400 000 years BP (95% CI = 562 545–2 237 454 years BP); net divergence between *Mediterranean* and *Atlantic 1* was 8.5% (95% CI = 3–14%) yielding an approximate estimate of time since divergence of 770 000 years BP (95% CI = 273 818–1 271 636 years BP). Finally, the distance between *Atlantic 2* and *Atlantic 3* (3%; 95% CI = 0.8–5.1%) dates the split between groups at approximately 270 000 years BP (95% CI = 76 727–468 727 years bp).

Overall Φ_{ST} was equal to 0.31 and was highly significant. Pairwise comparisons among locations were also calculated (Table 2): among the Atlantic locations, BE and BB resulted the most significantly differentiated ones (Fig. 3). No IBD was found based on mtDNA differentiation among Atlantic locations ($r = -0.1139955$, $P = 0.5942759$).

Mismatch distribution analysis (Fig. 4) revealed that all three Atlantic haplogroups conformed to both the sudden demographic and spatial expansion models (*Atlantic 1*: $P_{Dem} = 0.5159$, $P_{Spa} = 0.3232$; *Atlantic 2*: $P_{Dem} = 0.904$, $P_{Spa} = 0.9269$; *Atlantic 3*: $P_{Dem} = 0.6562$, $P_{Spa} = 0.7348$). Time since expansion for the three Atlantic lineages was estimated from the mismatch distributions parameters (τ) based on the sudden demographic expansion analysis results. The 95% CI for τ included zero for *Atlantic 2*. The time since expansion was estimated for *Atlantic 1* at 358 510 years BP (95% CI = 88 106–676 725 years BP) and *Atlantic 3* at 72 146 years BP (95% CI = 14 969–338 482 years BP). Tajima's *D*-value was negative and significant for *Atlantic 2* ($D = -1.82$, $P = 0.019$), whereas both *D* and F_S were highly significant for *Atlantic 3* ($D = -1.84$, $P = 0.0128$; $F_S = -25.60$, $P = 0.000$).

Microsatellite analysis

No evidence of null alleles was found with MICRO-CHECKER. Mean numbers of alleles per locus ranged between 11 for Dla-20 to 22.11 for Labrax-29. Expected heterozygosity (H_e) ranged from 0.875 in the Mediterranean population to 0.901 of the Belgian sample, BE. TH had the highest number of alleles across loci and BB the lowest, probably as a result of its smaller sample size. BE showed the highest allelic richness. None of the populations showed significant F_{IS} values, indicating no significant departures from HWE (Table 1).

Overall F_{ST} was low but significant, both with (0.009; 95% CI = 0.006–0.012) and without (0.002; 95% CI = 0.0009–0.003) the MED population. Apart from the expected obvious divergence of the Mediterranean sample (F_{ST} of at least one order of magnitude larger compared to the others), significant values were only found in comparisons involving SOL (Table 2). Jost's *D* pairwise comparisons, although correlated with those obtained with the F_{ST} index ($r = 0.999$, $P << 0.001$), also detected a few more significant differences among Atlantic populations

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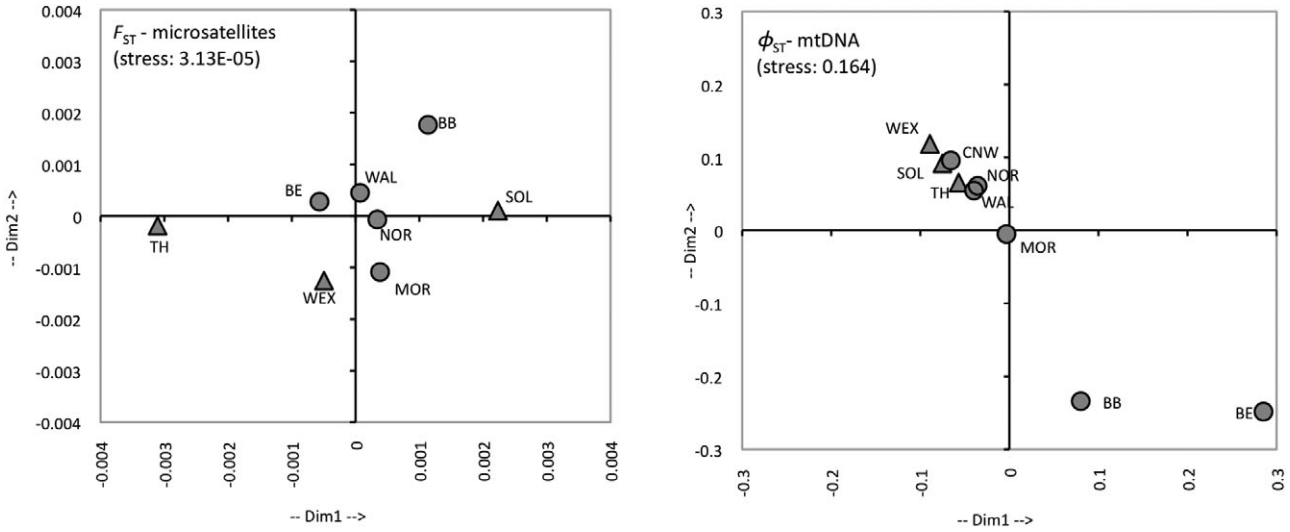

Figure 3. Multidimensional scaling plots based on the matrices of F_{ST} pairwise comparisons calculated from microsatellites (left), and from mitochondrial DNA (right). Triangles indicate populations of juveniles.

(Table 3). F_{ST}-based population structure inferred from the microsatellite and mitochondrial markers did not show any correlation ($r = -0.0266$, $P = 0.519$).

Microsatellite variation in the Atlantic also proved to be uncorrelated with geographic distance, both using F_{ST} ($r = 0.014$, $P = 0.47$) or D ($r = 0.15$, $P = 0.25$).

When the Mediterranean sample was included in the analysis, the modal log-likelihood of the data, Ln$P(D)$, found by STRUCTURE, yielded higher values at $K = 2$ (data not shown), whereas it peaked at $K = 1$ when MED was removed. GENELAND also did not identify any substructure within the Atlantic populations, whereas FASTRUCT (i.e. the only one assuming uncorrelated allele frequencies and no admixture) found the presence of two clusters within the Atlantic populations (data are not shown). Based on these results and on their assumptions, $K = 1$ was considered to be the most likely scenario.

Estimates of effective population size (N_e) (Table 1) indicate that most of the sampled populations were very large (their confidence interval included infinity). The only populations showing a finite (and relatively small) effective size were TH (52.8), SOL (130.5), and MOR (392.3).

DISCUSSION

The sea bass has long been one of the most valuable fish species in the Mediterranean Sea. Over the last few decades, interest from commercial and recreational fisheries has grown greatly also in northwestern Europe, parallelled by an expanding popularity and increased availability of farmed sea bass from the Mediterranean, as assessed by the European Commission (EC, 2004). Yet, the current understanding of sea bass population genetic structuring in the Atlantic remains relatively poor. Very few genetic studies have been conducted and have found low but significant differences when employing allozymes (Castilho & McAndrew, 1998; Naciri *et al*., 1999; Fritsch *et al*., 2007), and a lack of genetic differentiation with microsatellite markers (Tobin, 2003; Naciri *et al*., 1999; Fritsch *et al*., 2007).

The inclusion of many more samples and genetic markers arguably places the present study in a position to address aspects of sea bass population structure with greater power and accuracy compared to previous studies.

Phylogeographic analysis revealed the presence of three very distinct lineages in the Atlantic (Fig. 2). These three groups have diverged at different times during the glacial/interglacial Pleistocene events. The first split likely happened within Atlantic populations approximately 1.4 Mya, during the Early Pleistocene, and resulting in the formation of the two clades *Atlantic 1* and *Atlantic 2*. Later, approximately 770 000 years ago, the Mediterranean lineage begun to diverge from the *Atlantic 1* lineage (currently mainly found in the Bay of Biscay), confirming previous suggestions about the Atlantic origin of Mediterranean sea bass (Lemaire *et al*., 2005).

The most recent split likely occurred approximately 270 000 years ago, separating *Atlantic 3* from *Atlantic 2*. These data confirm earlier findings, according to which in the north-east Atlantic species appear to follow a pre-LGM expansion model, especially for highly mobile species, which were likely to cope better with the climatic changes (Chevolot *et al*., 2007).

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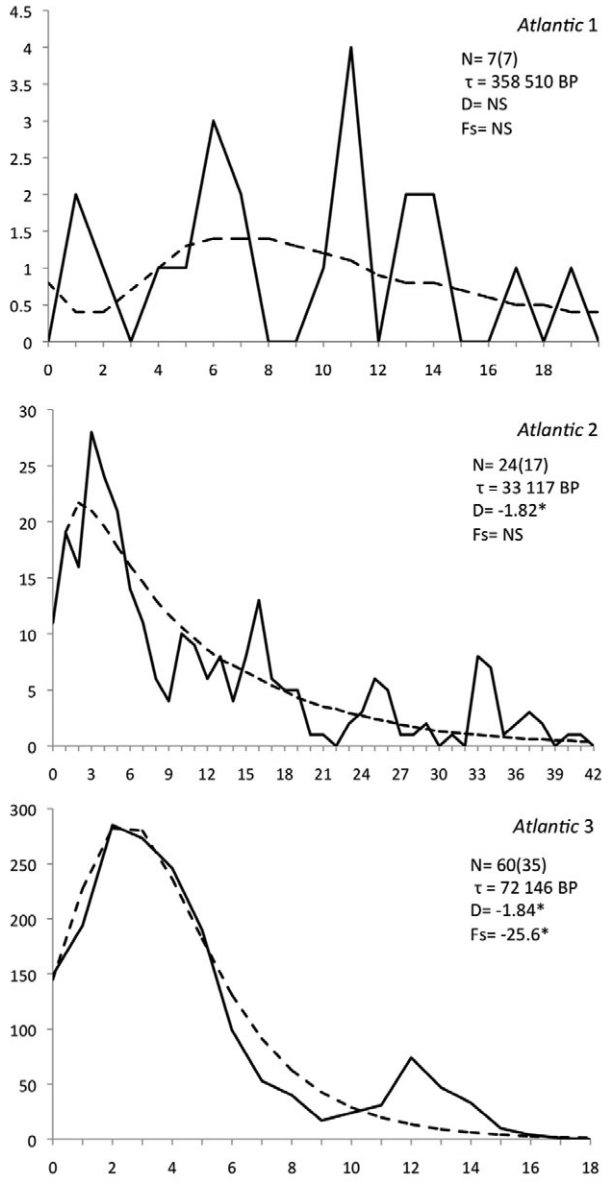

Figure 4. Mismatch distributions of the three haplogroups. Observed values are the full lines, expected ones are indicated by a dashed line. *N*, number of individuals (in brackets number of haplotypes); τ, time since expansion; *D*, Tajima's *D*; F_s, Fu's F_s.
*Statistically significant. NS, not significant.

Table 3. Jost's *D* pairwise comparisons and correspondent 95% confidence interval

	SOL	TH	WAL	WEX	NOR	BE	BB	MOR
MED	**0.258** (0.147, 0.368)	**0.269** (0.175, 0.362)	**0.225** (0.108, 0.340)	**0.283** (0.179, 0.386)	**0.225** (0.131, 0.318)	**0.216** (0.124, 0.307)	**0.228** (0.106, 0.348)	**0.248** (0.110, 0.348)
SOL		**0.051** (0.016, 0.085)	0.016 (0, 0.035)	0.021 (0, 0.044)	0.015 (0, 0.042)	0.046 (0, 0.095)	0.007 (0, 0.034)	**0.038** (0.004, 0.071)
TH			0.026 (0, 0.073)	0.017 (0, 0.044)	0.044 (0, 0.093)	0.028 (0, 0.075)	**0.049** (0.003, 0.094)	**0.049** (0.011, 0.086)
WAL				0.016 (0, 0.046)	0.011 (0, 0.0435)	0.004 (0, 0.0434)	−0.012 (0, 0.0270)	0.015 (0, 0.036)
WEX					0.023 (0, 0.0586)	0.029 (0, 0.0728)	**0.043** (0.004, 0.083)	0.010 (0, 0.011)
NOR						0.004 (0, 0.032)	0.008 (0, 0.028)	−0.008 (0, 0.011)
BE							0.024 (0, 0.061)	0.019 (0, 0.052)
BB								**0.038** (0.002, 0.074)

Values shown in bold are significantly different from zero. For location codes, see Table 1.

The network analysis (Fig. 2) and the mismatch distributions (Fig. 4) indicate that *Atlantic 1*, mainly found in the Bay of Biscay, is probably the most ancient group, from which *Atlantic 2* and *Mediterranean*, respectively, originated. This, together with the high genetic diversity recorded, indicates the possible presence of a glacial refugia in the Bay of Biscay, as detected in a previous study (Nesbo *et al*., 2000), or in a southern location along the Atlantic coasts of the

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Iberian peninsula (Hoarau *et al*., 2007). In addition, the *Atlantic 1* multimodal mismatch distribution is a corroborating reflection of the more stable status of this group, whereas, for example, *Atlantic 3* had a clear unimodal pattern, as would be expected in populations that have undergone a recent demographic expansion (Rogers & Harpending, 1992). On the other hand, *Atlantic 2* showed the typical signs of a population that has undergone a sudden expansion, although its raggedness (Fig. 4) suggests relative stabilization over more recent times.

The results obtained with microsatellites do not support evidence from mtDNA, suggesting that different processes affect the patterns of diversity at the two markers. Multilocus individual-based methods failed to identify a meaningful pattern of structure, possibly largely as a result of the very low levels of differentiation observed: STRUCTURE and other assignment software have been shown to be less effective when the level of genetic diversity is low ($F_{ST} < 0.02$) (Latch *et al*., 2006). In any case, the geographical patterns do not correspond to the three mitochondrial lineages (Fig. 3B).

The discordant structuring patterns inferred by these two markers might have several explanations. First, it may be a result of their characteristics: microsatellites are nuclear, rapidly-evolving and noncoding markers, and hence are suitable for untangling contemporary population structure (Hewitt, 2004), whereas the mtDNA control region typically probes more ancient events, as well as tracks the matrilinear history and demography (Avise, 2000). Thus, one way to look at these results is that mitochondrial DNA might reflect past isolation into three different groups, which would have come into secondary contact, partly erasing the historical signal, a process that has been invoked in other comparable contexts (Sala-Bozano, Ketmaier & Mariani, 2009). Another important aspect is the presence of sex-biased dispersal of sea bass, paired with philopatric behaviour. In particular, we can hypothesize female philopatry, coupled with opposite male behaviour (nonphilopatric): female sea bass migrate to different offshore spawning grounds and go back to the same inshore locations with high fidelity, hence potentially maintaining separated matrilineages. If this is not paired with similar male behaviour, such separation would not be visible at nuclear loci, especially if coupled with the 'random' transportation of larvae from these hypothesized spawning grounds into different inshore grounds by marine currents, and the dispersal of adolescent specimens along the coast between feeding grounds (Pickett *et al*., 2004). This model, and especially the possibility of high influence of marine currents on the recruitment, would also explain some mixing of mitochondrial haplotypes from different putative spawning areas (Fig. 2).

Naturally, the two hypotheses, secondary contact after past isolation and female philopatry, are not mutually exclusive. Sex-biased dispersal could represent the force maintaining a separation that originated during glacial events, and present-day spawning grounds (the location of which is still somewhat speculative) might represent the 'memory' of glacial refugia once occupied by diverging lineages, which are now spatially connected. In line with this view, the two haplogroups *Atlantic 2* and *Atlantic 3* (i.e. the most recently diverged) are also the best separated geographically. Although *Atlantic 2* is mainly located along the European continental coast, *Atlantic 3* is distributed around the British Isles and Norway: this pattern of population structuring revealed by mtDNA analysis might reflect the presence of two different spawning grounds whose location may be linked to that of ancient glacial refugia. It is reasonable to expect that, if spawning grounds are at least in part geographically associated with past refugia, the most recently split lineages would exhibit a more identifiable geographical structuring patterns than earlier, more ancient evolutionary splits, such as the one between *Atlantic 1* and *Atlantic 2*, whose longer history over glaciations and periodical habitat reshaping, would inevitably result in the removal of any trace of spatial structure. For example, *Atlantic 1* and *Atlantic 2* are currently equally represented in the same area: the Bay of Biscay (Fig. 3).

The presence of two Mediterranean haplotypes in Norway and in the Thames estuary (Fig. 2B) could partly be attributable to the establishment of escapees from sea bass farms present along the northern coast of France, which commonly use Mediterranean hatchery strains (EC, 2004), rather than an unlikely migration from the Mediterranean Sea. Furthermore, their typically Atlantic nuclear pattern (as confirmed by STRUCTURE) suggests that the naturalization of such escapees may have been happening for the last few generations. The potential for the establishment of farmed sea bass in wild populations is still largely unexplored. Toledo-Guedes *et al*. (2009) showed that sea bass escapees are able to exploit natural resources, and hence easily survive in the wild, although they found no evidence for the possible future establishment of a self-sustaining population. Nevertheless, our findings raise concerns about the possibility that farm escapees might represent a threat to the genetic integrity of wild populations and certainly highlight the need for further research in this direction.

With the exception of some subtle signal of differentiation between juveniles in SOL and TH (as

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well as the fish from BB, as inferred by Jost's *D*), microsatellite analysis fails to reject convincingly the hypothesis of panmixia. Yet, the nature of the sampling and data from mtDNA prevents us from drawing drastic conclusions in this sense. One important factor that can shape population structure is the effective population size, N_e, defined as the size of an 'ideal' population that shows the same rate of inbreeding or genetic drift as the actual population observed. Despite the typically big census size, a relatively small effective population size is not uncommon in marine species (Hauser & Carvalho, 2008). Most of the sampled populations showed an 'infinite', and hence very large, N_e. Yet, two of the three juvenile populations sampled in the present study (TH, SOL, and Wexford) showed instead reduced estimates of effective size (Table 1), suggesting temporal fluctuations of the effective number of breeders, N_b (Waples, 2005); however, these findings require more in-depth validation because estimates can be greatly affected by incomplete or nonrandom sampling.

The results obtained in the present study unveil a striking and previously unrecognized pattern of population divergence at mtDNA in Atlantic sea bass. This might mainly reflect past Pleistocene events, although some degree of female philopatry might be at the basis of the current geographical separation between two of the three Atlantic lineages identified. An increased number of nuclear microsatellite loci (compared to previous studies) still fail to detect biologically meaningful spatial genetic structuring in the North Atlantic. Although this could indicate high levels of population connectivity over evolutionary time scales, it might also partly be a result of the opportunistic nature of sampling and emphasizes the need for future studies that can target spawning fish on their spawning grounds across their distribution range.

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