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Connectivity in the Deep: Phylogeography of the Velvet Belly Lanternshark

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Abstract

The velvet belly lanternshark, *Etmopterus spinax*, is a deep-sea bioluminescent squaloid shark, found predominantly in the northeast Atlantic and Mediterranean Sea. It has been exposed to relatively high levels of mortality associated with by-catch in some regions. Its late maturity and low fecundity potentially renders it vulnerable to over-exploitation, although little remains known about processes of connectivity between key habitats/regions. This study utilised DNA sequencing of partial regions of the mitochondrial control region and nuclear ribosomal internal transcribed spacer 2 to investigate population structure and phylogeography of this species across the northeast Atlantic and Mediterranean Basin. Despite the inclusion of samples from the range edges or remote locations, no evidence of significant population structure was detected. An important exception was identified using the control region sequence, with much greater (and statistically significant) levels of genetic differentiation between the Mediterranean and Atlantic. This suggests that the Strait of Gibraltar may represent an important bathymetric barrier, separating regions with very low levels of female dispersal. Bayesian estimation of divergence time also places the separation between the Mediterranean and Atlantic lineages within the last 100,000 years, presumably connected with perturbations during the last Glacial Period. These results demonstrate population subdivision at a much smaller geographic distance than has generally been identified in previous work on deep-sea sharks. This highlights a very significant role for shallow bathymetry in promoting genetic differentiation in deepwater taxa. It acts as an important exception to a general paradigm of marine species being connected by high levels of gene-flow, representing single stocks over large scales. It may also have significant implications for the fisheries management of this species.

Keywords: population genetics, mitochondrial DNA, ITS2, fisheries management, seascape genetics

1. Introduction

The over-exploitation of shelf fish stocks has triggered many fisheries to exploit marine resources at ever greater depths of the ocean (Koslow et al., 2000; Morato et al., 2006). Yet, the inaccessibility of the deep ocean means our understanding of the ecology of deep-sea organisms can often lag behind that of their pelagic and continental shelf counterparts. This is especially true for chondrichthyans, as their lower commercial value has sometimes resulted in prioritisation of research for higher-value teleosts. Where investigations into the ecology of members of the group have been conducted, they have often focused on large pelagic sharks that are considered particularly vulnerable to exploitation in high seas fisheries (Dulvy et al., 2008; Dulvy et al., 2014). However, deep-water chondrichthyans (i.e. those occurring below 200 m) are a very diverse group and represent nearly half of the known species of skate, ray, chimera and shark (Kyne and Simpfendorfer, 2010). Furthermore, work on chondrichthyans more widely has highlighted that their life-history strategies, typically consisting of low lifetime fecundities, slow growth rates, late age at maturity and high longevities, render them especially vulnerable to over-exploitation (Stevens et al., 2000; Simpfendorfer and Kyne, 2009). The very limited information on deep-water species suggests they are no exception, with some exploited groups already showing declines and recommendations of zero catch for some species in the northeast Atlantic already in place (Graham et al., 2001; ICES, 2009, 2010; Neat et al., 2015). In the Mediterranean Sea relevant policies are currently in place for managing and protecting vulnerable chondrichthyans, e.g. the GFCM Recommendation GFCM/36/2012/3 (GFCM,

2012) on conservation of sharks and rays. Therefore, there is an urgent need to understand more about the biology, ecology and population structure of deep-water chondrichthyans to make valuable predictions on the long-term effects of fishing and ensure they can be sustainably managed into the future (Cunha et al., 2012).

The velvet belly lanternshark, *Etmopterus spinax*, is a deep-sea bioluminescent squaloid shark that exhibits a distribution across the Mediterranean Sea and Northeast Atlantic Ocean (Compagno et al., 2005; Ebert and Stehmann, 2013). Its typical bathymetric range within these locations is between 70 and 2000 m, mostly captured between 200 and 500 m, living on the outer continental shelves and upper slopes (Compagno et al., 2005; Neat et al., 2015), and on oceanic islands slopes and seamounts (Menezes et al., 2006). This species is considered vulnerable to over-fishing due to its late maturity with estimated maturation times of four to eight years, females also mature much later and to a greater size than males (Coelho and Erzini, 2008). Furthermore, the reproductive cycle in *E. spinax* has been suggested to last two to three years, before viviparous parturition, giving this species a low fecundity (Coelho and Erzini, 2008; Porcu et al., 2014). Despite this, the species remains common, frequently occurring as by-catch in deep-water fisheries landing crustaceans and teleosts, where it has been exposed to relatively high levels of mortality. Lacking any commercial value and commonly discarded in trawl and longline fisheries, it has been poorly studied (Coelho and Erzini, 2008). However, recent work investigating density-dependent mechanisms found that the effects of over-exploitation are already evident in *E. spinax*, with individuals showing a reduction in body size at maturity within the northeast Atlantic due to elevated fishing pressures (Coelho et al., 2010). In this region it has been assessed as Near

Threatened by the IUCN Red List of Threatened Species, as its numbers have declined by almost 20% from 1970 to 1998–2004 (Coelho et al., 2009). Although fishing effort has declined in recent years, numbers from the Rockall Trough area have remained low and stable without any indication of recovery (Neat et al. 2015).

In fisheries management, sustainable exploitation and conservation depend on solid understanding of connectivity and population differentiation, thereby allowing the identification of discrete populations which represent demographically independent stocks (Booke, 1999). This study investigates the phylogeography and population structure of *E. spinax* across the northeast Atlantic and Mediterranean Basin using nuclear and mitochondrial DNA sequencing and joins a relatively small body of work specifically examining connectivity in deep-water sharks, (Veríssimo et al., 2011, 2012; Cunha et al., 2012; Catarino et al., 2015). Previous work has suggested little evidence of population structure in members of this group, with gene-flow occurring at all but the largest oceanic distances assessed, which supports the generally held paradigm of high connectivity and low population structure in marine species (Palumbi, 1992; Ward et al., 1994). This contrasts with increasing indications of significant population structure in some demersal, shelf species (Chevolot et al., 2006; Ovenden et al., 2009, Griffiths et al., 2011; Gubili et al., 2014) and shares more similarity with studies of large, wide-ranging pelagic sharks that have often only revealed genetic differentiation over broad inter- or intra-oceanic scales (e.g. Pardini et al., 2001; Schmidt et al., 2009). The velvet belly lanternshark provides an interesting counter-point to previous work on bathypelagic sharks as it represents a more abundant species with a shallower mesopelagic distribution. Sample collection has also included

relatively geographically proximate regions of the Atlantic Ocean and Mediterranean Basin, which have been shown to harbour distinct populations or phylogeographic lineages in other chondrichthyan fish (Chevolot et al. 2006; Griffiths et al 2011; Gubili et al., 2011; Catarino et al., 2015), with the Strait of Gibraltar (12.9 km wide and 284 m deep) proposed as an important barrier to gene-flow in the region (reviewed in Patarnello, et al., 2007). Therefore, it is hoped that this work will provide further insight into processes of connectivity in the deep-sea and the role of oceanographic barriers to gene-flow i.e. the field of seascape genetics.

2. Materials & Methods

Lanternshark tissue was obtained from individuals caught during research cruises across the Mediterranean Basin and Northeast Atlantic Ocean. Within the Mediterranean, tissue samples were obtained from Cyprus, Greece and Italy. In the North Atlantic Ocean samples were collected from the Azores, Rockall Trough (west of Scotland), West of Ireland, Norway and the North Sea (Fig. 1, Table 1). Fin clips were collected and immediately stored in absolute ethanol or RNAlater. DNA was extracted using Chelex resin (Walsh et al., 1991) or phenol-chloroform extraction protocol (Sambrook et al., 1989). The mitochondrial DNA (mtDNA) control region (CR) was polymerase chain reaction (PCR) amplified using primers ElasmoCR15642: 5'- TTGGCTCCCAAAGCCAARATTCTG-3' and ElasmoCR16638: 5' CCCTCGTTTTWGGGGTTTTTCGAG-3' following published conditions (Stonero et al., 2003). The nuclear ribosomal internal transcribed spacer 2 (ITS2) locus was also amplified using primers FISH5.8SF: 5'-TTAGCGGTGGATCACTCGGCTCGT-3' and FISH28SR: 5'-TCCTCCGCTTAGTAATATG CTTAAATTCAGC-3' (Shivji et al., 2001). This reaction proved

149 difficult to optimise and whilst published PCR conditions were sometimes successful an
150 adapted profile was required: one minute at 94°C, 35 cycles of 94°C for one minute, 64-68°C
151 for one minute, 72°C for one minute and 30 seconds and a final extension at 72°C for ten
152 minutes. PCR products were cleaned and sequenced by Macrogen, Seoul, Korea using
153 primers ElasmoCR15642 and FISH28SR. The resulting sequences were checked in BioEdit
154 7.0.9 (Hall, 1999), and aligned using ClustalW (Thompson et al., 1994).

155

156 Summary statistics including the number of haplotypes (*Hn*), haplotype diversity (*h*),
157 nucleotide diversity (π) and polymorphic sites (*p*) were calculated in Arlequin 3.5.2.2
158 (Excoffier et al., 2005). Genetic differences among localities were also calculated in Arlequin
159 using the frequency-based F_{ST} estimates, using 10,000 permutations. Sequential Bonferroni
160 corrections to the significance level were also applied (Rice, 1989). Additionally, Jost's *D*
161 (Jost, 2008) was calculated using the R (R Development Core Team) package "mmod"
162 (Winter, 2012). Differences between male and female dispersal were examined by
163 comparing pairwise genetic distances between sampling locations; male-biased dispersal
164 would show lower genetic distances in nuclear markers than in mitochondrial markers (as
165 they are maternally inherited). Paired t-tests were used to compare mt F_{ST} and Jost's *D*
166 against nuclear F_{ST} and Jost's *D* in R 3.2.1. Within GenAlEx 6.5 (Peakall and Smouse, 2012),
167 Tamura-Nei's pairwise genetic distances were calculated using the default settings and
168 visualized by principal coordinate analysis (PCoA). Median-joining networks for each locus
169 were generated with Network 5.0 (http://www.fluxus-engineering.com). Signatures of
170 population expansion were investigated by calculating Tajima's *D* (Tajima, 1989) and Fu's *F*s
171 (Fu, 1997) statistics in Arlequin. These test statistics are expected to be significantly negative

172 in cases of recent population expansion. Historical population dynamics were also evaluated
173 by mismatch distribution analysis. Typically, a population of constant size is characterized by
174 a multimodal distribution, whereas one under expansion shows a unimodal distribution
175 (Rogers and Harpending, 1992).

176

177 Levels of migration and divergence time were estimated using a Markov chain Monte Carlo
178 approach in MDIV (Nielsen and Wakeley, 2001), with all samples combined within the
179 Atlantic, against those collected across the Mediterranean (this pooling of samples for each
180 region is supported by the results of the pairwise F_{ST} analysis, PCoA and an addition
181 hierarchical AMOVA that found significant variation amongst these groups; p = 0.019, but
182 remained non-significant within these groups; p = 0.286). Initial runs were performed to
183 obtain an upper limit of the scaled migration rate and divergence time (*M*max and *T*max
184 respectively). The Hasegawa, Kishino and Yano (HKY) finite-sites model of mutation was
185 applied. A total of 5,000,000 cycles in the Markov chain were utilised, incorporating 500,000
186 cycles as a burn-in. Twelve repeat runs were completed and set with maximum values for *T*
187 and *M* of five, using different random seeds to check the consistency of estimation. The
188 migration rate (m) and divergence time (t) were then estimated using the following
189 parameters $\theta=4N_e v$, $M=2N_e m$ and $T=t/2Ne$ (where N_e=effective population size, υ=mutation
190 rate in the region sequenced). A range of divergence rates for the CR (0.5%, 1.0% and 1.5%
191 per MY, reflecting low rates from other elasmobranchs; Duncan et al., 2006; Keeney and
192 Heist 2006; Schultz et al., 2008; Griffiths et al., 2011) and a generation time of eight years
193 (Gennari and Scacco, 2007; Coelho and Erzini, 2008) were applied in the calculations.

194

3. Results & Discussion

Overall, 130 partial CR sequences were aligned, comprising 913 base pairs (bp), 28 haplotypes and 18 variable sites (Accession numbers: KX494599-494728). Comparing across sample collections, the haplotype diversity ranged from 0.500 (Norway) to 0.959 (Ireland), whereas nucleotide diversity values ranged from 0.002 (Cyprus, Norway) to 0.004 (Azores; Table 1). Despite the similar number of sequenced individuals for the ITS2 region, all summary statistic values were lower. The number of haplotypes was 13 and 10 variable sites for 133 aligned sequences of 572 bp length (Accession numbers: KX494729-494861). Haplotype diversities varied from 0.482 (Ireland) to 0.829 (Azores), whereas nucleotide diversity varied from 0.0000 (Norway) to 0.002 (Azores; Table 1).

Pairwise F_{ST} values ranged from −0.031 to 0.283, and -0.175 to 0.106 for the CR and ITS, respectively (Table 2). The majority of pairwise comparisons among Mediterranean and Atlantic populations showed significant differentiation at the corrected 5% level for the CR (the only exception being between Norway and the Mediterranean populations, presumably due to the very small sample size). No significant genetic structure was found among sampling collections for the ITS2 marker. Jost's *D* values were similar to the F_{ST}, with the highest value of genetic divergence found between Italy and Rockall (0.151) for the CR and between Greece and Norway (0.028) for the ITS2 (Table 2). Some suggestions of sex-biased dispersal (male dispersal and female philopatry) were detected as mtDNA values of population differentiation were significantly greater than those reported from the nuclear marker when comparing across the sampling area (F_{ST} paired t-test; t=4.452, p=<0.001 and Jost's *D* paired t-test; t=6.356, p=<0.001).

218

219 The CR haplotype network further highlights evidence of population structure between the
220 Atlantic and Mediterranean (Fig. 2a), with the regions clearly demonstrating different
221 frequencies of haplotypes, including unique haplotypes. This contrasts with the ITS2
222 network, as haplotypes did not assort by location, suggesting a lack of population structure
223 (Fig. 2b). The PCoA also supports this pattern with clear separation of the Mediterranean
224 and Atlantic samples with the CR, but little evidence of regional structure with the ITS2.
225 Demographic analyses showed that most sample collections had negative Tajima's *D* and
226 Fu's *F* values for both markers (Table 1). Only Ireland showed a significantly negative *F* value
227 and a unimodal distribution, suggesting population expansion for the CR. Conversely, only *D*
228 values for North Sea, Rockall, Ireland and Cyprus for the ITS2 were significant, suggesting
229 subtle population expansion. The remaining sample collections produced non-significant
230 results. Given evidence of weak population structure within each geographic region, we
231 combined samples within the Atlantic and Mediterranean basins. In the Atlantic, signs of
232 expansion were present from analysis of the *F* value in the CR and both the *F* and D value
233 and from the ITS2, this contrast with a bi-modal mismatch distribution for both markers. In
234 the Mediterranean, significantly negative values were detected in the Tajima's *D* from the
235 ITS2 and Fu's F from the CR, which also generated a unimodal mismatch analysis.

236

237 However, signs of expansion were detected solely in the Mediterranean group, where a
238 significantly negative *F* value and unimodal mismatch distribution was found.

239

240 The Bayesian estimation of divergence and migration rates with MDIV revealed a relatively
241 recent split and very low levels of migration between the Atlantic and Mediterranean
242 groups (Table 3). Varying the estimates of mutation rate altered divergence time from
243 34,335 and 103,005 years and the produced migration rates between 1.189E-05 and 3.566E-
244 05.

245

246 The results clearly demonstrate high levels of connectivity and gene-flow in *E. spinax* across
247 the northeast Atlantic, even at the most extreme oceanic distances considered in this study,
248 which specifically includes limits to the species range. In particular, the Azores represent the
249 most remote archipelago of the North Atlantic, some 1,300 km from Portugal, suggesting
250 that if simple oceanic distance was to provide a barrier to dispersal, population sub-division
251 may well be observed at this scale (Aboim et al., 2005; Chevolot et al., 2006; Knutsen et al.,
252 2009; Ball et al., 2016). Some hints at the distinctiveness of this group are suggested by the
253 PCoA for both ITS2 and CR, but this is not supported by pairwise F_{ST} analysis. The results
254 from Straube et al. (2011) are highly relevant, whilst predominantly studying cryptic
255 diversity of lantern sharks from the southern hemisphere, weak but significant genetic
256 differentiation was described between Chilean and New Zealand samples of the southern
257 lantern shark (*Etmopterus granulosus*). This remains the most closely related deep-water
258 shark to *E. spinax* that has been analysed to date, and suggests that gene-flow may be
259 limited at extreme intra-oceanic distances, separated by non-continuous continental self.
260 Furthermore, detailed investigations of population structure in the leafscale gulper shark
261 (*Centrophorus squamosus*; Veríssimo et al., 2011), Portuguese dogfish (*Centroscymnus*
262 *coelolepis*; Veríssimo et al., 2012; Catarino et al., 2015) and long-nosed velvet dogfish

(*Centroscymnus crepidater*; Cunha et al., 2012) have not identified evidence of significant differentiation at the intra-oceanic scale, even in more cosmopolitan species than *E. spinax*, where samples can be collected across greater oceanic distances. The consensus appears to be that genetic homogeneity across ocean basins suggest single stocks occur at this scale, and even between ocean basins, levels of differentiation remain relatively low (Cunha et al., 2012; Catarino et al., 2015). Therefore, our results support this general finding of high levels of connectivity in deep-water sharks, but importantly extend these to a species with a much shallower habitat.

There is one important exception to the finding of non-significant population structure between the samples; analysis of the CR demonstrates Jost's *D* and F_{ST} values that were much greater in comparisons between samples from the Atlantic and Mediterranean. The *p*-values associated with these groups, were all below the 5% significance level, with the majority remaining significant even after sequential Bonferroni correction (the Norwegian sample collection is an exception, presumably associated with its small sample size). The PCoA also demonstrates this pattern of regional differentiation, with clear separation of the Atlantic and Mediteranean sample collections. Private haplotypes are associated with the Atlantic and Mediterranean regions, suggesting discrete phylogeographic histories, with Bayesian estimation also supporting little evidence of connectivity. Moreover, divergence of the Atlantic/Mediterranean stocks occurred within the last 100,000 years i.e. during perturbations association with the Last Glacial period, 110,000-12,000y BP (Kukla, 2005). This significant population structure occurs at a smaller scale than has typically been demonstrated in deep-water species, especially deep-sea sharks, and has important

management implications for identifying demographically independent stocks. Whilst Atlantic samples are lacking from Portugal/Morocco region and the far western area of the Mediterranean, it seems likely that reductions in gene-flow are associated with the Strait of Gibraltar (especially given the genetic homogeneity observed across the rest of the Mediterranean). The narrow, and especially shallow, nature of the Strait could conceivably act as a barrier to deep-water species, with the concept of bathymetry as a barrier to gene-flow also supported by work on other deep-sea taxa (e.g. Knutsen et al., 2009, 2012), although the results remain somewhat surprising as the Strait is less likely to remain such a barrier to the shallower dwelling, mesopelagic lanternshark. Our findings share much in common with very recent work on *C. coelolepis* (Catarino et al., 2015), which also identified significant population sub-division between Mediterranean and Atlantic deep-sea shark populations, where even the estimated time-scales of separation between these groups were highly congruent (i.e. within the last 100,000 yrs). More detailed analysis in the western Mediterranean, Strait of Gibraltar and Portugal is needed to determine the exact position of the genetic discontinuity in this case. The Almeria-Oran oceanographic density front, which has been identified as the barrier to gene-flow in many fish species, due to reductions in the passive movement of pelagic eggs and larvae (reviewed in Patarnello et al., 2007), is less likely to affect lanternsharks with viviparous reproduction and active dispersal. Very limited penetration of the western Mediterranean by Atlantic populations cannot be ruled out, as has been observed in other species (Bremer et al., 2005; Gubili et al., 2014), where perhaps west-to-east gradients in temperature and salinity across the Mediterranean Basin, limit the penetration of Atlantic adapted genotypes.

An intriguing dichotomy is observed between the nuclear ITS2 and mtCR sequence data, with significant differentiation observed only in the mtDNA dataset. Perhaps the most likely explanation for this striking difference relates to different evolutionary pressures on these two genetic markers. The reduced effective population size of mtDNA leads to expectations of greater F_{ST} values (Daly-Engel et al., 2012), whereas analysis of ITS2 sequences within elasmobranchs has generally shown them to be conserved within species, rendering this locus an appropriate tool for global identification of shark species (Abercombie et al., 2006; Pinhal et al., 2012). However these differences between markers, especially significant comparisons between estimates of Jost's *D*, which is believed to give a less biased estimate of genetic differentiation (Jost, 2008), suggest this could be due to sex-biased dispersal. There is growing evidence for female philopatry and male-biased dispersal in sharks (e.g. Hueter et al., 2005, Mucientes et al., 2009, Gubili et al., 2014), including deep-sea species (Veríssimo et al., 2012). Whilst being wary of over-interpreting these data, they are consistent with growing recognition of significant differences in behaviour between male and female sharks and could have important implications for conservation and management (e.g. via fisheries targeting nursery grounds or over-harvesting females).

It is important to acknowledge a limitation that may have some bearing on the results; the ITS2 region is known to have multiple copies, which could potentially complicate the interpretation if paralogues are compared. However, the ITS2 is still widely utilised in phylogeography and has even been shown to produce consistent sequences in elasmobranchs (Lieber et al., 2013, Garcia et al., 2014). The fact that few haplotypes were identified at this locus and no significant population structure was detect also means this

issue is unlikely to have affected the conclusions. Additionally, the use of DNA sequencing in the current study may give a more historical focus regarding connectivity and new techniques utilising high through-put sequencing, especially a comprehensive suite of nuclear single nucleotide polymorphisms (SNPs), may provide more power to detect population structure in elasmobranchs.

In summary, this study demonstrates high levels of gene-flow in *E. spinax* across the northeast Atlantic, adding to a relatively limited literature examining the population genetics and phylogeography of potentially vulnerable deep-sea sharks. Furthermore, the significant population sub-division observed between the Atlantic and Mediterranean lanternsharks highlight a potentially significant role for bathymetry to present barriers to connectivity, with important implications for fisheries management. The results contribute to growing recognition that there are important exceptions to a general paradigm of marine species being connected by high levels of gene-flow and representing single stocks over large geographic scales, with oceanographic factors considered to be vital in understanding connectivity in the deep.

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Table 1: Details of sample collections with mitochondrial and nuclear DNA summary statistics. Approximate capture locations, Lon; longitude, Lat; latitude, TL; range in total length (mm), *n*; number of individuals; H_n, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; sd, standard deviation is in brackets; p, polymorphic sites; *D*, Tajima's *D* value; *F*, Fu's F_s value. Values in bold were significant at a 5% level.

Sample Collection	Collection Date	Lat	Lon	TL	CR							ITS2						
					n	H_n	*H* (sd)	π(sd)	p	*D*	*F*	*n*	H_n	*H* (sd)	π (sd)	p	*D*	*F*
Norway	02/02/2013	64.341	11.252	330-420	4	2	0.5000 (0.2652)	0.0016 (0.0015)	3	-0.7544	1.7161	3	2	0.6667 (0.3143)	0.0000	2	0.0000	1.0609
North Sea	12/01/2013	59.907	4.198	145-465	22	8	0.8442 (0.0438)	0.0029 (0.0018)	7	1.1357	-1.07222	16	5	0.7000 (0.0795)	0.0005 (0.0007)	4	**-1.9103**	-0.6284
Rockall Trough	09/09/2011 - 11/09/2011	57.600	-9.580	130-550	19	11	0.9298 (0.0361)	0.0030 (0.0019)	8	0.6868	**-4.6045**	18	6	0.7255 (0.0737)	0.0008 (0.0008)	5	**-2.2117**	-1.2632
Ireland	28/09/2007 - 30/09/2007	53.274	-12.240	230-490	19	14	0.9591 (0.0307)	0.0036 (0.0022)	10	0.5418	**-8.3412**	24	4	0.4819 (0.1106)	0.0004 (0.0005)	3	**-1.3511**	-0.0786
Azores	26/05/2000	38.267	-29.156	310-455	15	10	0.9143 (0.0559)	0.0037 (0.0022)	10	0.2956	**-3.7081**	15	7	0.8286 (0.0823)	0.0015 (0.0013)	5	-1.47221	-2.17792
Atlantic					79	21	0.9130 (0.0130)	0.0032 (0.0019)	16	-0.3099	**-8.5898**	76	11	0.6751 (0.0435)	0.0024 (0.0016)	8	**-1.9018**	**-3.9128**
Italy	27/01/2015	43.453	9.622	110-270	15	6	0.8286 (0.0643)	0.0018 (0.0013)	6	-0.3569	-1.33133	18	3	0.6863 (0.3519)	0.0003 (0.0005)	2	-0.69865	1.52944
Greece	22/05/2015	38.971	26.377	142-283	18	7	0.8235 (0.0609)	0.0019 (0.0013)	7	-0.4400	-1.81077	18	3	0.6993 (0.0435)	0.0004 (0.0006)	2	-0.40199	1.48844
Cyprus	08/07/2011	34.702	33.130	120-315	18	8	0.7974 (0.0885)	0.0016 (0.0012)	7	-0.9196	**-3.6318**	21	6	0.7714 (0.0539)	0.0008 (0.0008)	5	**-1.7268**	-1.05555

Mediterranean					51	14	0.8259 (0.0396)	0.0018 (0.0012)	10	-0.6916	**-7.1195**	57	6	0.7005 (0.0260)	0.0022 (0.0016)	5	**-1.4827**	-0.2252

Table 2. Pairwise measures of population structure. Values, in upper diagonal are relate to the CR and lower diagonal values from ITS. F_{ST} values are described in the upper line of each cell, those that in bold remain significant after sequential Bonferroni correction (initial p-value = 0.05/28), p-values included in brackets. The lower line describes values of Jost's *D*.

	Norway	**North Sea**	**Rockall Trough**	**Ireland**	**Azores**	**Italy**	**Greece**	**Cyprus**
Norway	-	0.131 (0.072) 0.048	0.146 (0.025) 0.026	0.128 (0.020) 0.049	0.181 (0.020) 0.059	0.283 (0.002) 0.016	0.283 (0.005) 0.107	0.280 (0.003) 0.106
North Sea	-0.137 (1.000) 0.000	-	-0.010 (0.591) 0.007	0.047 (0.030) 0.014	0.056 (0.034) 0.014	**0.153 (<0.001)** 0.145	**0.119 (<0.001)** 0.090	**0.144 (<0.001)** 0.118
Rockall Trough	-0.150 (1.000) 0.000	-0.047 (1.000) 0.000	-	-0.003 (0.573) 0.000	-0.007 (0.564) 0.000	**0.107 (<0.001)** 0.151	0.086 (0.003) 0.099	**0.102 (0.001)** 0.126
Ireland	-0.175 (1.000) 0.000	0.106 (0.047) 0.000	0.090 (0.040) 0.000	-	-0.031 (0.974) 0.000	0.076 (0.004) 0.104	0.067 (0.008) 0.064	**0.092 (0.001)** 0.092
Azores	-0.112 (1.000) 0.015	0.030 (0.184) 0.012	0.011 (0.278) 0.006	0.050 (0.106) 0.015	-	**0.129 (<0.001)** 0.147	**0.109 (<0.001)** 0.103	**0.129 (<0.001)** 0.134
Italy	-0.111 (0.783) 0.019	-0.034 (0.686) 0.007	-0.012 (0.497) 0.013	0.093 (0.056) 0.012	0.027 (0.196) 0.007	-	-0.009 (0.495) 0.006	0.058 (0.059) 0.008
Greece	-0.091 (0.764) 0.028	-0.013 (0.496) 0.015	0.009 (0.338) 0.020	0.090 (0.061) 0.017	0.024 (0.218) 0.010	-0.054 (1.000) 0.000	-	0.005 (0.382) 0.024
Cyprus	-0.098 (0.857) 0.015	-0.026 (0.666) 0.006	-0.014 (0.541) 0.009	0.098 (0.027) 0.009	0.013 (0.274) 0.007	-0.044 (1.000) 0.000	-0.041 (0.932) 0.000	-

Table 3. Divergence times (*t*) and migration rates (*m*) estimated between Mediterranean and Atlantic groups based on the control region sequences. Maximum likelihood values of parameters θ, M and T estimated with the non-equilibrium model using the program MDIV. A vector of divergence times was utilised (divergence per million years) and a generation time of eight years.

Divergence rates (per MY)	**Mutation Rate (*v*)**	**Divergence Time (*t*) in years**	**Migration Rate (*m*)**
0.5%	0.00001826	103004.6	1.18879E-05
1%	0.00003652	51502.3	2.37759E-05
1.5%	0.00005478	34334.87	3.56638E-05

Figure 1. Map of sample collections (produced in GeoMapApp v. 3.6.0; http://www.geomapapp.org/).

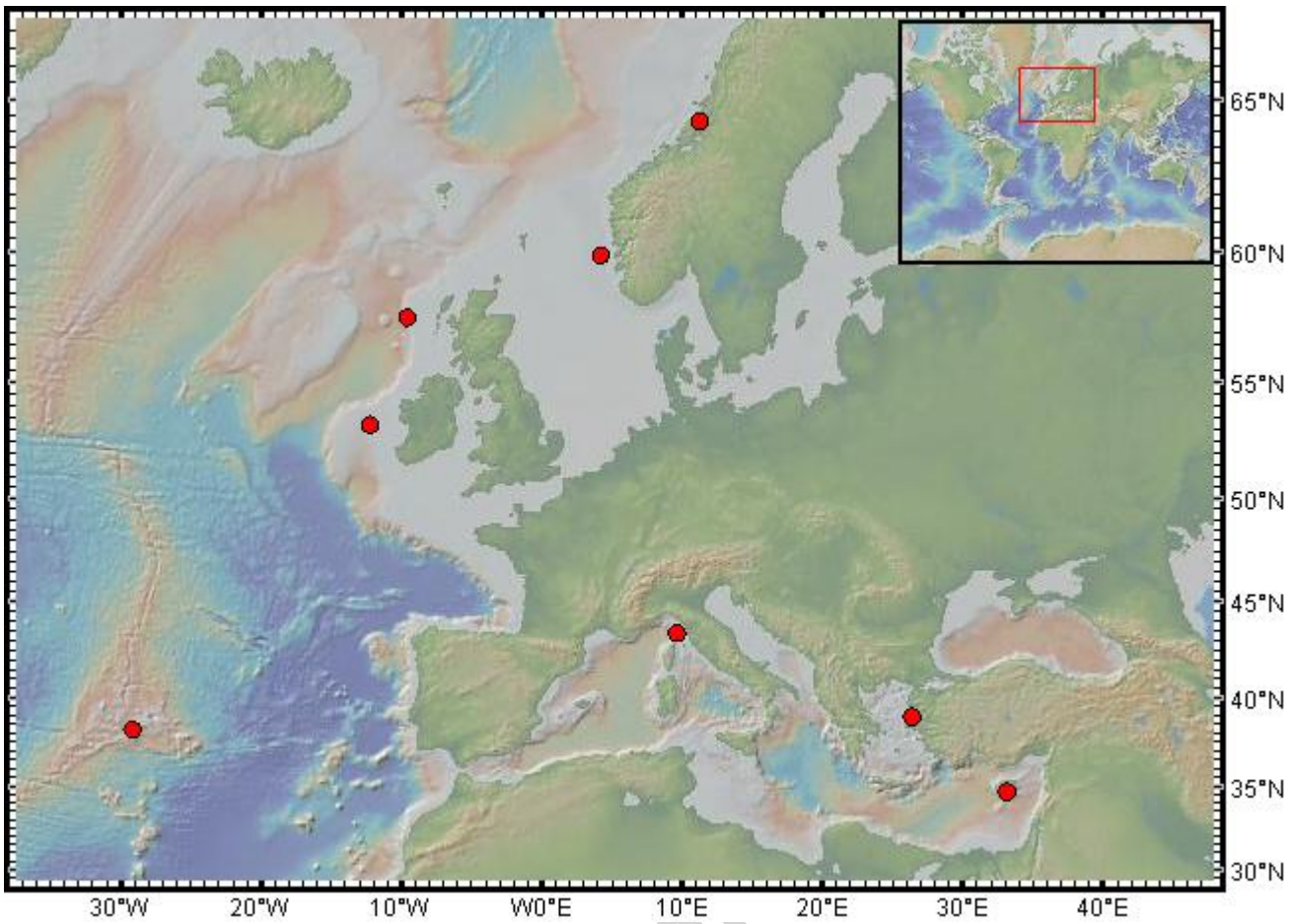


Figure 2. Haplotype networks. Figure a shows network of CR sequences, and b of ITS2 sequences. Relative size of the circles indicates the frequency of the haplotype.

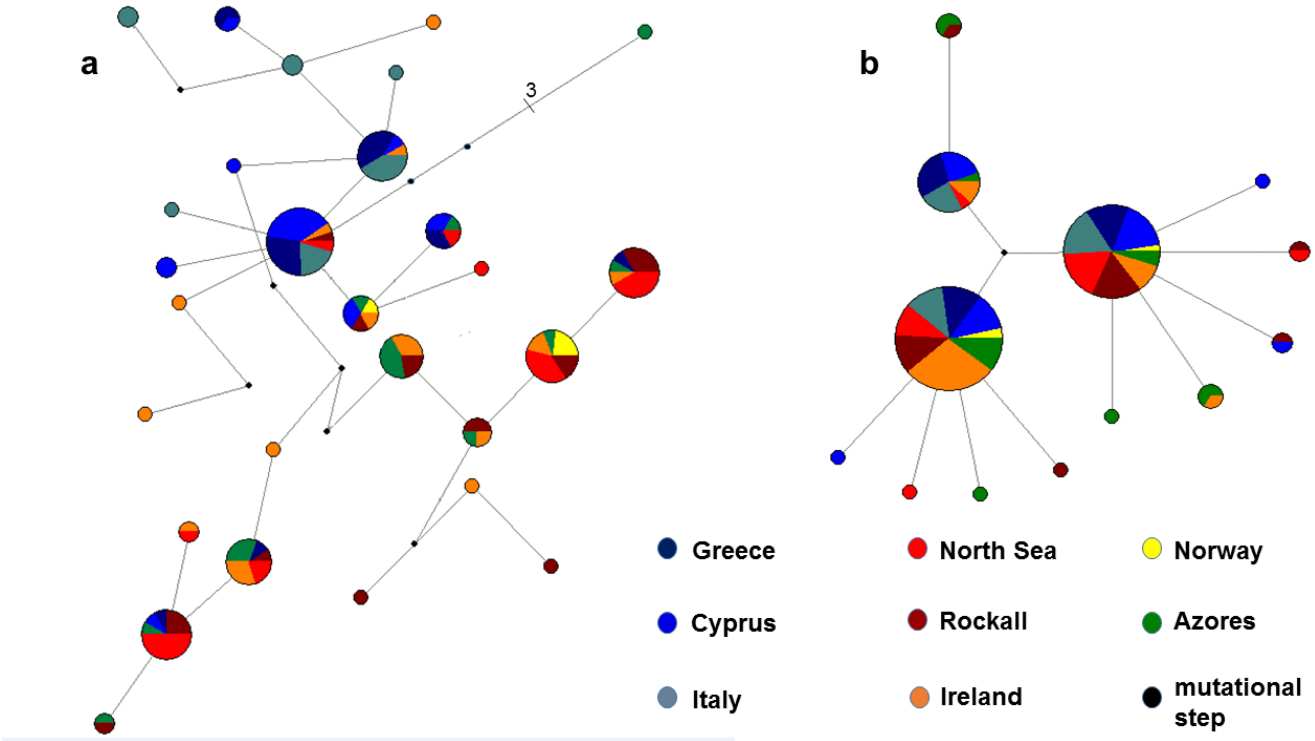
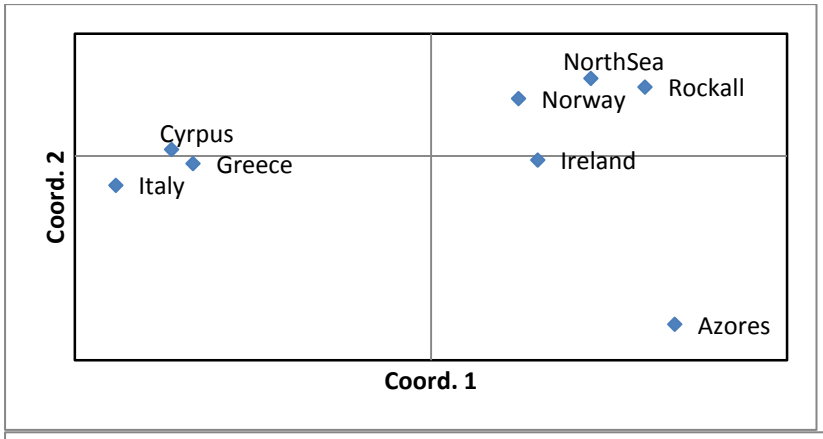


Figure 3. Principle components analysis of Tamura-Nei genetic distances. A is based on CR, where coordinate 1 explains 28.31% and coordinate 2 explains 15.67% of the variation in the data. B is based on the ITS2 sequence, where coordinate 1 explains 30.11% and coordinate 2 explains 20.20% of the variation.

A



B