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High-resolution genetic analysis reveals extensive gene flow within the jellyfish *Pelagia noctiluca* (Scyphozoa) in the North Atlantic and Mediterranean Sea

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Despite the importance of gelatinous zooplankton as components of marine ecosystems, both ecologically and socio-economically, relatively little information is known about population persistence or connectivity in jellyfish. In the present study, we employed a combination of nuclear microsatellite markers and sequence data from the mitochondrial cytochrome oxidase I (COI) gene to determine levels and patterns of population genetic structuring in the holoplanktonic jellyfish *Pelagia noctiluca* across the northeast Atlantic Ocean and Mediterranean Sea. Our results indicate a high degree of connectivity in *P. noctiluca*, with little evidence of geographical structuring of genetic variation. A small but significant differentiation of Atlantic Ocean and Mediterranean stocks was detected based on the microsatellite data, but no evidence of differentiation was observed with the mtDNA, probably due to the higher power of the microsatellites to detect low levels of genetic structuring. Two clearly distinct groups of genotypes were observed within the mtDNA COI, which probably diverged in the early Pleistocene, but with no evidence of geographical structuring. Palaeodistribution modelling of *P. noctiluca* at the Last Glacial Maximum (LGM; *c.* 21 Kya) indicated large areas of suitable habitat south of the species' current-day distribution, with little reduction in area. The congruent evidence for minimal genetic differentiation from the nuclear microsatellites and the mtDNA, coupled with the results of the palaeodistribution modelling, supports the idea of long-term population stability and connectivity, thus providing key insights into the population dynamics and demography of this important species. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **117**, 252–263.

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Jellyfish (i.e. Phylum Cnidaria, Class Scyphozoa) exhibit a range of life history strategies. Most are metagenic, with an asexually reproducing, life stage which is benthic (the polyp) and a free-swimming or planktonic life stage (the medusa) among other, intermediate, stages (Arai, 1997). Such species are often constrained spatially by the need for accessible substratum for the settlement of polyps, skewing the distribution of resultant blooms towards near-shore waters (Boero *et al.*, 2008). In turn, metagenic

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jellyfish tend to exhibit population structure at modest scales (e.g. Lee *et al.*, 2013), predictable geographical distribution (e.g. Houghton *et al.*, 2006a) and relatively predictable, seasonal blooms (e.g. Houghton *et al.*, 2006b). Some jellyfish species, however, lack this benthic life stage enabling individuals to reproduce more readily in deeper off-shore waters (Boero *et al.*, 2008). *Pelagia noctiluca* is one such species with an apparently vast geographical range spanning the Atlantic, Pacific and Indian Oceans as well as their adjacent seas (Kramp, 1961; Mariottini, Giacco & Pane, 2008). Unlike blooms of metagenic jellyfish which arise from asexual strobilation at the seabed, the free-swimming medusae of *P. noctiluca* arise solely from sexual reproduction in the water column (Rottini Sandrini & Avian, 1983) which may convey a competitive advantage in deep-water habitats. At times, they can be brought onto continental shelves by oceanic water overflow, as is the case on the Irish Continental Shelf (Fraser, 1955; Bastian *et al.*, 2011). Indeed, in this region the species has been known to form aggregations > 4° of latitude (Doyle *et al.*, 2008) and to strand along hundreds of kilometres of coastline numerous times in recent years (Fleming, Harrod & Houghton, 2013).

Understanding the population connectivity of jellyfish has relevance far beyond the prediction of socio-economic impacts (Doyle *et al.*, 2014) with Pauly *et al.* (2008) describing them as 'arguably the most important predators or the sea'. As one of the most venomous species in UK/Irish waters (Mariottini *et al.*, 2008), *P. noctiluca* is certainly *a* noteworthy predator yet, like many gelatinous species, is given scant consideration in fisheries or ecosystem models (Pauly *et al.*, 2008; Sabatés *et al.*, 2010; Doyle *et al.*, 2014; Purcell *et al.*, 2014). On a regional scale, the species first gained notoriety in the Northeast Atlantic following a major fish kill at salmon farms in Northern Ireland in November 2007 causing > £1M in damages in a single day (Doyle *et al.*, 2008). At first this mass incursion of this species in Irish/UK coastal waters in 2007 was reported as unprecedented, yet subsequent desktop studies revealed that *P. noctiluca* was reported in Irish/UK waters in 21 out of a possible 95 years (i.e. 1890–1985; Doyle *et al.*, 2014). More recent studies using beach strandings (Fleming *et al.*, 2013), fisheries by-catch data (Bastian *et al.*, 2011) and continuous plankton recorder records (Licandro *et al.*, 2010) have confirmed that the species is a longstanding feature of Irish/UK shelf waters. Given the ecological implications of these reoccurring blooms (Doyle *et al.*, 2014) and the economic threat they pose to the Irish/UK aquaculture industry (Doyle *et al.*, 2008; Fleming *et al.*, 2013) there is a pressing need to understand the demographic processes that underpin them better.

Within this context, molecular genetics provides the opportunity to explore patterns of connectivity and recruitment underpinning blooms of *P. noctiluca*. Such concepts are pertinent following Licandro *et al.* (2010), who suggested that the prevalence of *P. noctiluca* in the northeast Atlantic (NEA) during 2007 and 2008 may reflect recent hydrographic changes in the region. More specifically, the authors suggested that outbreaks of *P. noctiluca* may follow the progression of the North Atlantic Current (NAC) and the continental slope current (CSC), a northward branch of the Azores Current that flows along the eastern slope boundary of the European basin (Garcia-Soto, Pingree & Valdes, 2002; Pingree, 2002). It was Fraser (1955) who first proposed that a subsurface current carries the 'Lusitanian fauna' from the outflow of the Gulf of Gibraltar to the NEA. The Lusitanian fauna contains zooplankton species more typically of the Mediterranean, such as *P. noctiluca.*

From a molecular perspective most studies of population structure in *P. noctiluca* to date, and indeed jellyfish in general (reviewed in Glynn, Houghton & Provan, 2015), have relied heavily on the mitochondrial cytochrome oxidase I (COI) gene, occasionally with the addition of ribosomal markers such as the internal transcribed spacers ITS1 and ITS2 (e.g. Stopar *et al.*, 2010). While variable, the uniparental mode of inheritance and small effective population size of the mitochondrial genome (relative to that of the nuclear genome) means that the COI may not be an ideal candidate marker for such studies, particularly where levels of genetic structuring are low. Indeed, previous studies have provided somewhat conflicting findings with respect to connectivity in *P. noctiluca*. Using a combination of COI and ITS, Stopar *et al.* (2010) observed a lack of genetic or geographic structuring across the Eastern Atlantic and Mediterranean Sea whilst Miller, von der Heyden & Gibbons (2012) proposed significant structuring between North and South Atlantic populations.

The application of high-resolution microsatellite markers has been effective in uncovering cryptic population structure across the ranges of several marine species that had been thought previously to be panmictic, such as eels (Wirth & Bernatchez, 2001) and microalgae (Provan, 2010). The sole population genetics of *P. noctiluca* to date that employed multiple, unlinked, microsatellite markers focused on smaller-scale population structuring within the Eastern Mediterranean and the Adriatic Seas (Aglieri *et al.*, 2014). Consequently, in the present study we employed the same microsatellites to analyse large-scale patterns of variation over a similar area studied by Stopar *et al.* (2010), but with more extensive sampling of the Northeast Atlantic, since population structuring as a result of historical processes have

been documented in the region for several marine species (reviewed in Provan, 2013). We wanted to determine whether there was any significant differentiation between *P. noctiluca* from the North Atlantic and populations from the Mediterranean Sea following the suggestions of Licandro *et al.* (2010), the historical observation of Fraser (1955), and given that the Strait of Gibraltar has been proposed to be a biogeographic barrier (reviewed in Patarnello, Volckaert & Castilho, 2007), and also whether there was any finer-scale structuring within regions.

MATERIAL AND METHODS

Sampling and DNA extraction

Samples were obtained from live-caught or fresh shore-stranded aggregations of *P. noctiluca* (locations are listed in Table 1 and shown in Fig. 1). Specimens were washed in sea water before whole individuals in some cases, or umbrella/gonadal flesh samples in most cases, were preserved in ethanol. All samples were stored in a 1:3 flesh to ethanol ratio, then stored at −20 °C until extraction. Immediately prior to extraction, flesh was removed from the ethanol and dried using sterile paper towels, rinsed in double-distilled water and dried again on sterile paper towels to remove traces of ethanol. Genomic DNA was extracted using a modified version of the Porebski, Bailey & Baum (1997) CTAB phenol/chloroform protocol whereby extracted DNA which had been subjected to phenol and chloroform wash was stored in a 1:1 supernatant:isopropanol state at −20 °C until needed for PCR, then pelleting and the alcohol wash were carried out before elution. Long-term storage of eluted DNA resulted in loss of high molecular weight (genomic) DNA and reduced amplification success.

Microsatellite genotyping

We utilised eight of the nine microsatellite loci reported for *P. noctiluca* by Aglieri *et al.* (2014), with the exception of locus Pelnoc_40199, which could not be consistently amplified. Forward primers included a 19 bp M13 tail (CACGACGTTGTAAAACGAC) and reverse primers included a 7 bp tail (GTGTCTT). PCR was carried out in a total volume of 10 μL containing 100 ng genomic DNA, 10 pmol of 6-FAM-, PET- or HEX-labelled M13 primer, 1 pmol of tailed forward primer, 10 pmol reverse primer, 1× PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega). PCR was carried out on a MWG Primus thermal cycler using the following parameters: initial denaturation at 94 °C for 5 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system (Life Technologies; Carlsbad, California, USA). Allele

Table 1. *Pelagia noctiluca* sampling locations and summary diversity statistics

			Nuclear					Mitochondrial			
Population	Latitude (N)	Longitude (W)	N	H_O	H_E	F_{IS}	r	N	h	H	π
Shetland Islands	60.457	0.973	24	0.540	0.647	0.172**	0.008^{NS}	22†	17	0.965	0.009
Rathlin Island	55.290	6.197	22	0.426	0.554	0.235**	0.131***	24	14	0.833	0.008
North Atlantic	55.687	8.224	20	0.514	0.635	0.194**	0.035^{NS}	21	14	0.919	0.006
Malinbeg	54.664	8.785	23	0.537	0.647	0.173**	0.019^{NS}	23†	14	0.913	0.015
Lehinch	52.934	9.350	23	0.455	0.615	0.266**	0.043^{NS}	22	16	0.948	0.011
Dingle	52.193	10.478	9	0.500	0.614	0.198**	0.012^{NS}	6	5	0.933	0.006
Sole Bank	48.750	8.167	23	0.591	0.615	0.040^{NS}	0.041^{NS}	20	17	0.979	0.011
Roscoff	48.727	3.983	15	0.455	0.704	0.364**	-0.091^{NS}	11	9	0.946	0.011
Armoricain Shelf	46.879	4.749	16	0.519	0.662	0.222**	-0.011^{NS}	15	11	0.933	0.014
Bay of Biscay	46.446	2.552	9	0.540	0.648	0.177**	0.014^{NS}	6	4	0.800	0.012
Galicia	43.398	8.398	10	0.473	0.659	0.295**	-0.029^{NS}	5	3	0.700	0.013
Cadaques	42.286	−3.280	23	0.555	0.643	0.139**	0.025^{NS}	20	11	0.874	0.008
Villefranche-Sur-Mer	43.702	−7.324	24	0.439	0.648	0.249**	0.002^{NS}	24	19	0.960	0.011
Portofino	44.303	−9.211	24	0.622	0.608	-0.024^{NS}	0.136***	23	17	0.949	0.008

N, number of individuals studied; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; r, relatedness coefficient; h, number of haplotypes detected; H, gene diversity; π, nucleotide diversity; NS, non-significant.

Significance of F_{IS}/r – *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

†Includes one heteroplasmic individual (not analysed).

sizes were scored using LIZ size standards and were checked by comparison with previously sized control samples.

mtDNA sequencing

A 532-bp region of the *P. noctiluca* mtDNA COI gene was amplified using the sequences of primers Pn-COI-F 5′-CCAGGGTCAATGCTTGGAG-3′ and Pn-COI-R 5′-CGAAGAAAGAGGTGTTAAAGTT-3′ designed from GenBank sequence GQ376003. PCR was carried out on a MWG Primus thermal cycler using the following parameters: initial denaturation at 94 °C for 3 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 20 μL containing 200 ng genomic DNA, 10 pmol of each primer, 1× PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$ and 0.5 U Go*Taq* Flexi DNA polymerase (Promega). PCR products (5 μL) were resolved on 1.5% agarose gels and visualised by ethidium bromide staining, and the remaining 15 μL were EXO-SAP purified and sequenced in both directions using the BigDye sequencing kit (V3.1; Applied Biosystems) and run on an AB 3730XL DNA analyser (Life Technologies; Carlsbad, CA, USA).

Data analysis

Tests for linkage disequilibrium between pairs of microsatellite loci in each population were carried out in the program FSTAT (V2.9.3.2; Goudet, 2002). Levels of polymorphism measured as observed (H_O) and expected (H_E) heterozygosity averaged over loci for nuclear microsatellites, and as haplotype (H) and nucleotide (π) diversity for mtDNA, were calculated using the ARLEQUIN Software package (V3.5.1.2; Excoffier & Lischer, 2010). Inbreeding coefficients (F_{IS}) were estimated using FSTAT. To determine the mean levels of relatedness between sampled individuals within populations, the relatedness coefficient (r) of Queller & Goodnight (1989) was calculated using the GenAlEx Software package (V6.1; Peakall & Smouse, 2006), and significance calculated using 999 permutations.

Levels of overall interpopulation differentiation as well as differentiation between Atlantic and Mediterranean populations and population-pairwise differentiation were estimated from allele (microsatellite) and haplotype (mtDNA) frequencies using Φ-statistics, which give an analogue of F-statistics (Weir & Cockerham, 1984) calculated within the analysis of molecular variance (AMOVA) framework (Excoffier, Smouse & Quattro, 1992), also using the ARLEQUIN Software package. A median-joining network showing the relationships between the mtDNA haplotypes was constructed using the NETWORK Software package (V4.5.1.6; www.fluxus-engineering.com). The divergence time (T) between the two observed groups of mtDNA haplotypes was estimated by calculating Nei's genetic distance (D_A) using the DNAsp software package (Librado & Rozas, 2009), and by using the formula $T = D_A/2\mu$ (Nei & Kumar, 2000), where μ, the mutation rate per site per year, was 6.54×10^{-9}, the rate estimated previously for the Cnidarian *Obelia geniculata* (Govindarajan, Halanych & Cunningham, 2005). In addition, tests for population expansion based on Tajima's D and Fu and Li's F and a mismatch distribution analysis, which identifies characteristic 'waves' in the shape of the distribution resulting from expansion (Rogers & Harpending, 1992), were carried out for both the large and the small clades in DNAsp.

To identify possible spatial patterns of gene flow, the software package BAPS (V5; Corander, Waldmann & Sillanpää, 2003) was used to identify clusters of genetically similar populations using a Bayesian approach. Ten replicates were run for all possible values of the maximum number of clusters (K) up to $K = 14$, the number of populations sampled in the study, with a burn-in period of 10 000 iterations followed by 50 000 iterations. Multiple independent runs always gave the same outcome. To further identify possible spatial patterns of gene flow, a principal coordinate analysis (PCA) was carried out in GenAlEx. Inter-individual genetic distances were calculated as described in Smouse & Peakall (1999), and the PCA was carried out using the standard covariance approach.

Because of the genetic homogeneity revealed by the microsatellite loci studied, and to compare the relative power of microsatellites and the mtDNA to detect low levels of population differentiation, simulations were carried out using the POWSIM software package (V4.0; Ryman & Palm, 2006). Simulations were carried out for an effective population size of $N_e = 1000$ to yield F_{ST} values of 0.001–0.020. In all cases, 1000 replicates were run and the power of the analysis was indicated by the proportion of tests that were significant at $P < 0.05$ using the observed allele frequencies for both the four microsatellite loci and the single mtDNA COI region studied [for $F_{ST} = 0$ this corresponds to the Type I (α) error]. For the mtDNA, sample sizes were adjusted as recommended by Larsson *et al.* (2009).

Palaeodistribution modelling

Palaeodistribution modelling was carried out to determine the potential suitable range for *P. noctiluca* at the Last Glacial Maximum (LGM; *c.* 21 KYA) using the maximum entropy approach implemented

in the MaxEnt Software package (V3.3.3; Phillips, Anderson & Schapire, 2006). Species occurrence data between 1950 and 2000 were downloaded from the Global Biodiversity Information Facility data portal (www.gbif.org) and from the Ocean Biogeographic Information System (www.iobis.org), and supplemented with our own population data (188 occurrences in total). Current-day bioclimatic data (MARSPEC; Sbrocco & Barber, 2013) were obtained at 5 min resolution and models were generated using cross-validation of ten replicate runs under the default MaxEnt parameters. Model performance was assessed based on the area under the receiver operating characteristic curve (AUC). Models were projected onto reconstructed bioclimatic data for the LGM (ensemble of five models: CNRM, ECBILTCLIO, FGOALS, HadCM and MIROC-322; Sbrocco, 2014).

RESULTS

Genetic analyses

No evidence of linkage disequilibrium was detected between any of the eight nuclear microsatellite loci analysed. Between six (Pelnoc_40622 and Pelnoc_44003) and 36 (Pelnoc_46263) alleles were detected, with a total of 136 (mean = 17 per locus). Within-population levels of observed (H_O) and

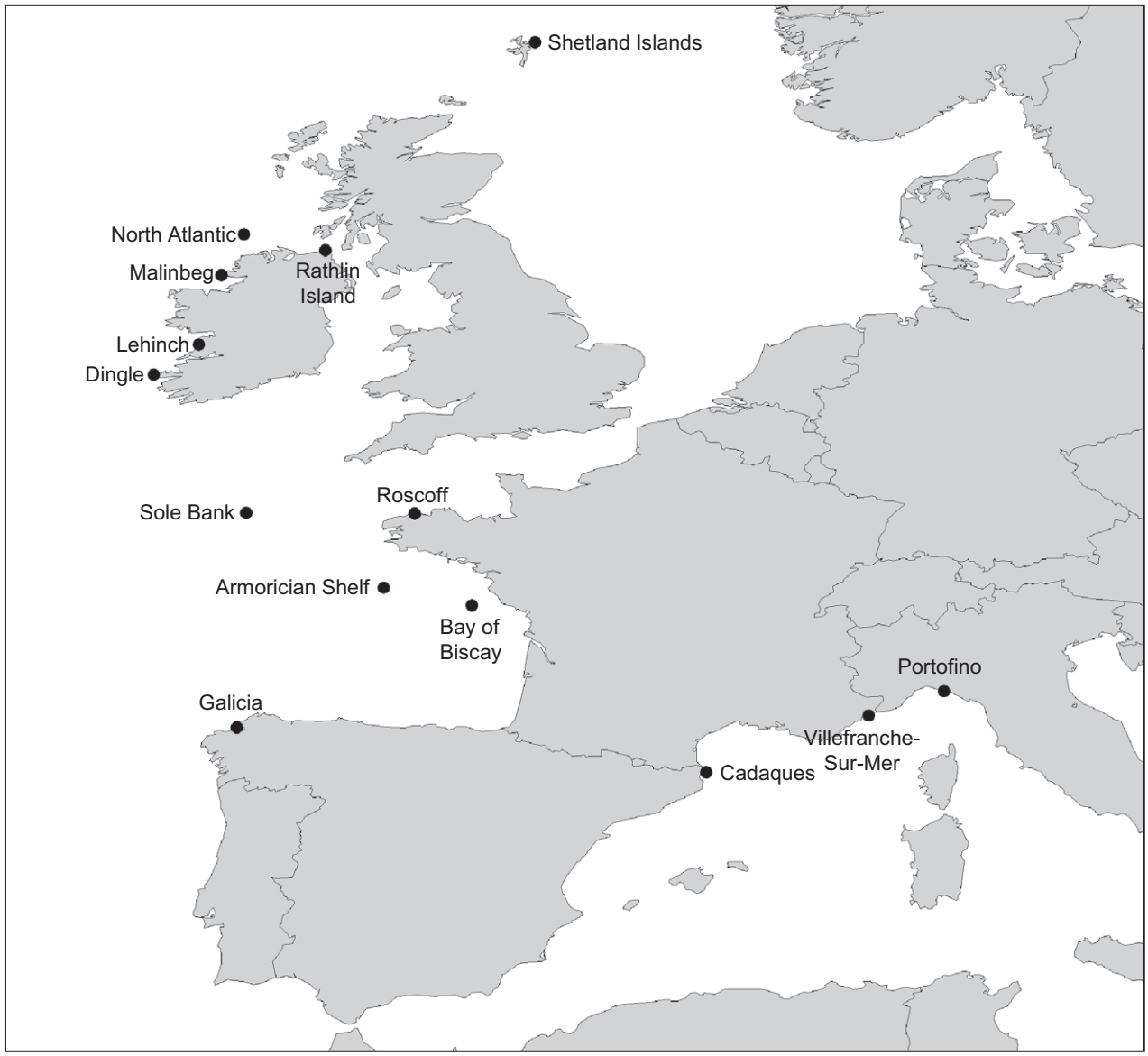

Figure 1. Locations of sites sampled in this study.

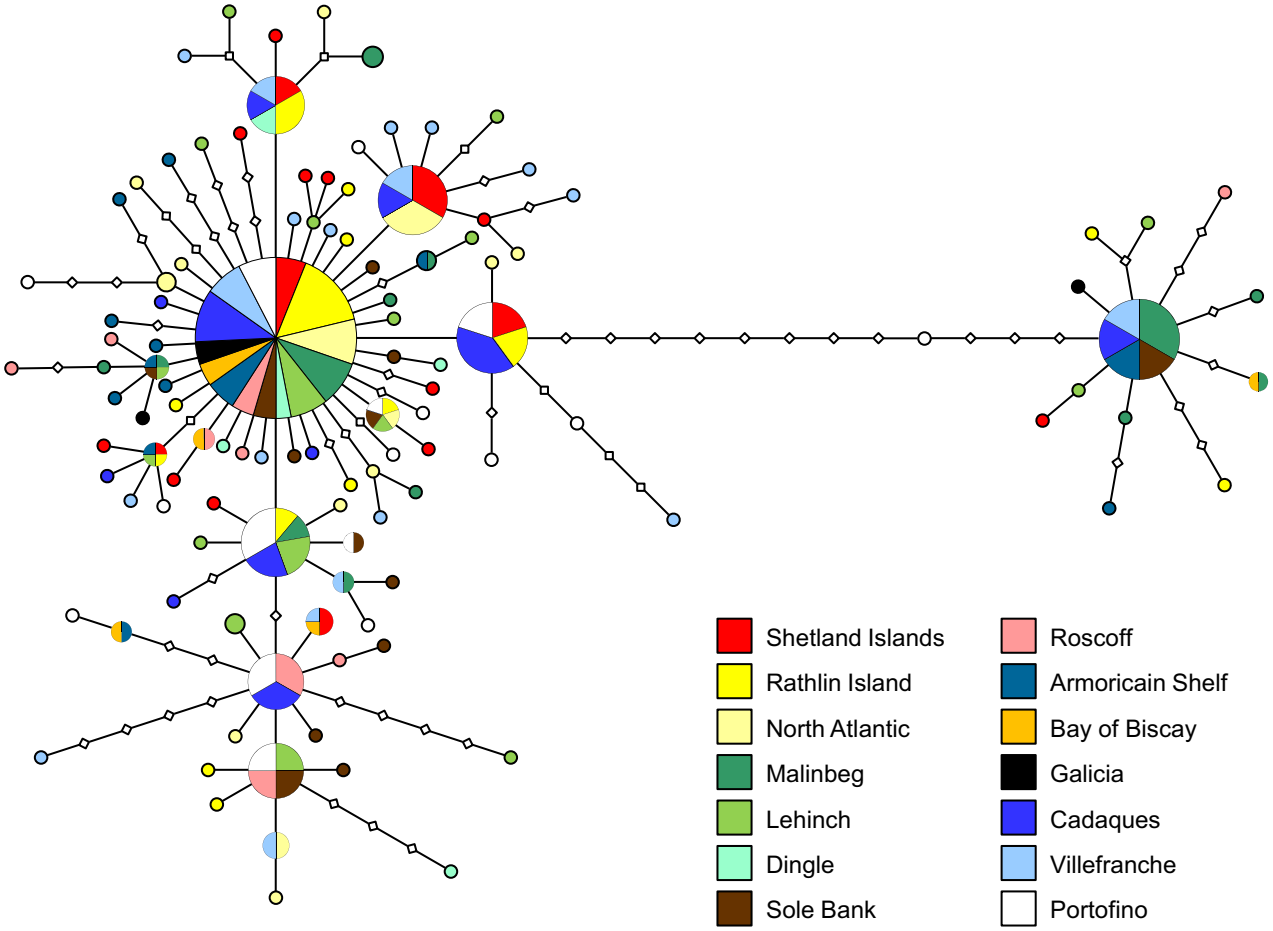

Figure 2. Median-joining network showing relationships between the 116 haplotypes detected by sequencing the mtDNA COI region. Circle sizes are approximately proportional to haplotype frequency: smallest circle represents a single individual, largest circle represents 66 individuals. Each connection represents a single mutation and small open diamonds represent missing intermediate haplotypes.

Table 2. Analysis of molecular variance (AMOVA)

	Nuclear				Mitochondrial			
Source of variation	d.f.	Sum of squares	Variance	%	d.f.	Sum of squares	Variance	%
Among populations (overall)	13	53.939	0.054	2.47***	13	5.877	−0.001	-0.11^{NS}
Within populations	516	1097.42	2.127	97.53	228	104.979	0.46	100.11
Atlantic vs. Mediterranean	1	12.983	0.045	2.02***	1	0.379	−0.001	-0.18^{NS}
Among populations within regions	12	40.957	0.035	1.58***	12	5.497	−0.001	-0.03^{NS}
Within populations	516	1097.42	2.127	96.40***	228	104.979	0.46	100.21^{NS}

NS, non-significant.
***$P < 0.001$.

expected (H_E) heterozygosity ranged from 0.426 (Rathlin Island) to 0.622 (Portofino; mean = 0.512) and from 0.554 (Rathlin Island) to 0.704 (Roscoff; mean = 0.636) respectively (Table 1). Levels of F_{IS} were significantly different from zero in twelve of the 14 populations, and ranged from 0.040 (Sole Bank)

to 0.364 (Roscoff; mean = 0.193). Only two populations (Rathlin Island and Portofino) exhibited significant levels of relatedness between individuals (r = 0.131 and 0.136 respectively). Summary statistics by locus are given in Supporting Information (Supporting Information, Table S1).

Mitochondrial COI sequences were obtained from 242 individuals. Two individuals were found to be heteroplasmic i.e. they displayed double peaks at multiple sites within the sequence, and were discarded from subsequent analyses. A total of 116 mitochondrial COI haplotypes were identified (Fig. 2). These were structured into two groups (103 and 13 haplotypes respectively) separated by nine mutations. Only the most common haplotype was found in all 14 populations analysed, and 94 were found in a single individual. Within populations, between three (Galicia) and 19 (Villefranche-Sur-Mer) haplotypes were detected (mean = 12.21). Levels of haplotype (H) and nucleotide (π) diversity ranged from 0.700 (Galicia) to 0.979 (Sole Bank; mean = 0.904), and from 0.006 (North Atlantic and Dingle) to 0.015 (Malinbeg) respectively (Table 1). The divergence time between the two mtDNA groups was calculated as 1.529 Mya. The mismatch distribution analyses for the large (103 haplotypes) and small (13 haplotypes) clades indicated past population expansion (Supporting Information, Fig. S1), as did the values for Tajima's D (large clade $D = -2.366$, $P < 0.01$; small clade $D = -1.783$, $P < 0.05$) and Fu and Li's F for the large clade – ($F = -5.062$, $P < 0.05$), but not for the small clade ($F = -1.964$, NS).

The analysis of molecular variance (AMOVA) revealed a small but significant overall differentiation based on nuclear microsatellites ($\Phi_{ST[NUC]}$ = 0.025; $P < 0.001$), but no significant structuring based on the mtDNA COI ($\Phi_{ST[MT]} = -0.01$; NS; Table 2). Likewise, the nuclear microsatellites indicated minimal but significant structuring between Atlantic and Mediterranean populations ($\Phi_{CT[NUC]}$ = 0.020; $P < 0.001$), but no significant structuring based on the mtDNA COI ($\Phi_{CT[MT]} = -0.02$; NS; Table 2). Population-pairwise $\Phi_{ST[NUC]}$ values ranged from −0.021 (Shetland Islands/Armoricain Shelf) to 0.081 (Armoricain Shelf/Portofino), whilst pairwise $\Phi_{ST[MT]}$ values ranged from −0.074 (Bay of Biscay/Galicia) to 0.038 (Shetland Islands/Galicia; Table 3). The BAPS analysis indicated that all the individuals analysed were grouped into a single genetic cluster (100% probability). This was reflected in the PCA, which showed no evidence of geographical structuring of individual multilocus genotypes (Fig. 3).

The simulation studies suggested that the nuclear microsatellite data were able to detect F_{ST} values of

Table 3. Population-pairwise Φ_{ST} values. Lower diagonal matrix – nuclear; Upper diagonal matrix – mitochondrial

	SI	RI	NA	MA	LE	DI	SB	RO	AS	BB	GA	CA	VM	PO
SI	–	0.019	−0.003	0.000	0.014	−0.021	−0.002	−0.006	0.002	0.001	0.038	0.006	−0.011	0.002
RI	0.025	–	0.003	0.008	0.018	−0.036	0.030	0.000	0.008	−0.034	−0.035	−0.014	0.014	0.011
NA	0.011	0.019	–	−0.001	0.010	−0.022	0.006	−0.010	−0.002	−0.011	0.002	−0.001	−0.006	0.002
MA	0.002	0.038	0.007	–	0.005	−0.019	−0.007	−0.014	−0.005	0.003	0.021	0.000	−0.002	−0.015
LE	0.014	**0.035**	**0.032**	0.021	–	−0.010	−0.005	0.000	−0.024	−0.002	0.022	−0.007	−0.008	0.007
DI	0.025	0.051	**0.033**	0.030	−0.009	–	−0.009	−0.034	−0.024	−0.040	−0.026	−0.029	−0.027	−0.015
SB	−0.021	**0.030**	0.002	−0.018	−0.001	0.016	–	−0.009	−0.018	0.026	0.050	0.012	−0.009	−0.003
RO	−0.021	0.025	−0.001	−0.011	0.029	0.025	0.024	–	−0.013	−0.035	−0.001	−0.009	−0.011	−0.015
AS	−0.012	0.004	0.001	0.009	0.029	0.025	0.010	−0.001	–	−0.008	0.008	−0.011	−0.015	0.001
BB	−0.004	0.008	0.002	0.009	0.039	0.054	0.022	0.018	0.009	–	−0.074	−0.019	−0.002	0.008
GA	0.032	0.033	0.023	0.032	0.025	0.055	0.018	0.038	0.025	0.015	–	−0.013	0.025	0.027
CA	**0.039**	0.020	0.019	**0.044**	**0.037**	0.035	**0.037**	0.017	0.030	0.003	0.028	–	−0.003	−0.008
VM	0.019	0.013	0.008	0.022	0.026	0.021	0.005	0.003	0.014	−0.003	0.005	0.005	–	0.000
PO	**0.074**	**0.071**	**0.074**	**0.071**	**0.052**	**0.065**	**0.068**	**0.062**	**0.081**	**0.052**	0.041	**0.024**	**0.039**	–

SI, Shetland Islands; RI, Rathlin Island; NA, North Atlantic; MA, Malinbeg; LE, Lehinch; DI, Dingle; SB, Sole Bank; RO, Roscoff; AS, Armoricain Shelf; BB, Bay of Biscay; GA, Galicia; CA, Cadaques; VM, Villefranche-Sur-Mer; PO, Portofino.
Values significantly different from zero are shown in bold.

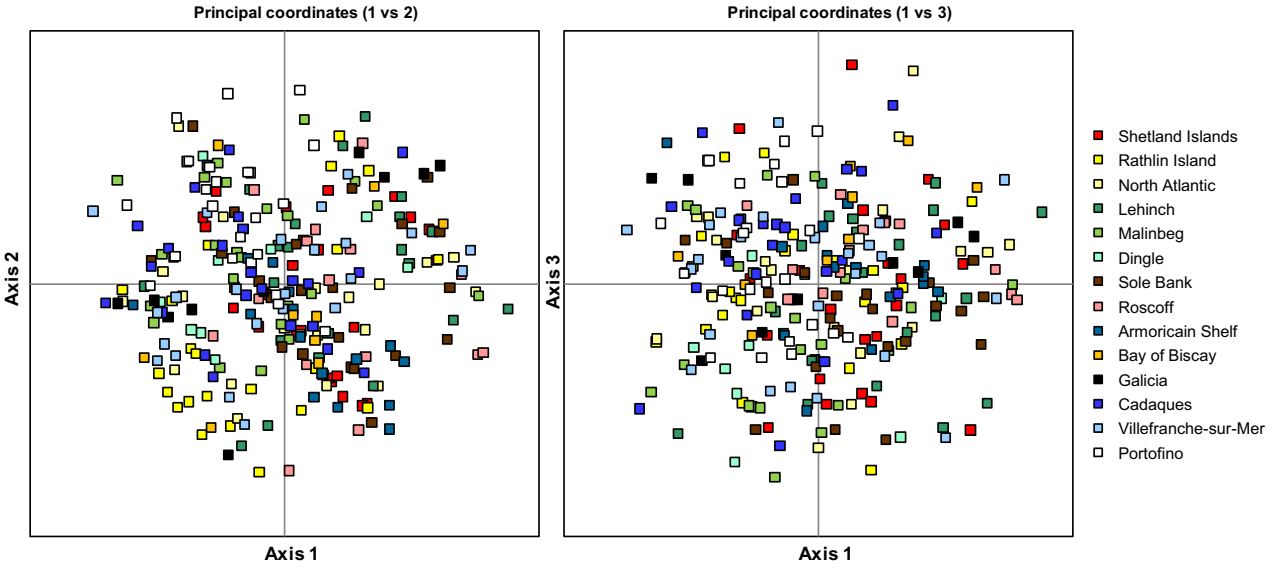

Figure 3. Results of the PCA. The first three axes accounted for 21.71%, 18.12% and 17.29% respectively of the total variation (57.13%).

as low as 0.005 at least 95% of the time (Fig. 4). The mtDNA COI locus had much lower power, only 38% for $F_{ST} = 0.005$, and could only detect $F_{ST} > 0.018$ with a power of above 95%.

Palaeodistribution modelling

For all models, AUC values were high (mean AUC = 0.908; SD = 0.040). The current-day model indicated the presence of suitable habitat for *P. noctiluca* along western Europe between 40°N and 70°N, including both the continental shelf and deeper waters off the Bay of Biscay/northwest Iberia and the Norwegian Sea (Fig. 5A). The palaeodistribution model indicated a southward shift in suitable habitat, with the maximum northern limit off the palaeocoastline around 50°N, as well as more extensive habitat in the Mediterranean Sea (Fig. 5B).

DISCUSSION

The findings of the present study based on high-resolution nuclear and mitochondrial markers indicate a high degree of connectivity in *Pelagia noctiluca* across the Northeast Atlantic and the Mediterranean. There was little overall evidence of geographical structuring of genetic variation, and only a small but significant differentiation of Atlantic Ocean and Mediterranean stocks based on the microsatellite data. No evidence of differentiation was observed with the mtDNA, reflecting the higher power of the microsatellites to detect low levels of genetic

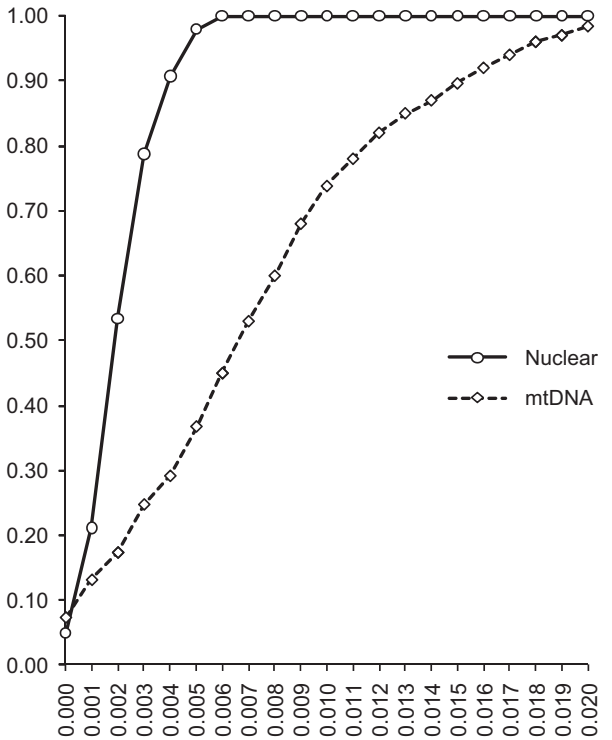

Figure 4. Results of the POWSIM analysis. The *Y*-axis represents the power of the markers to successfully recover the value of F_{ST} indicated on the *X*-axis, expressed as the proportion of 1000 simulations (see text for details). For $F_{ST} = 0$, this is the Type I (α) value.

structuring as indicated by the POWSIM analysis (Larsson *et al.*, 2009). The observed high levels of

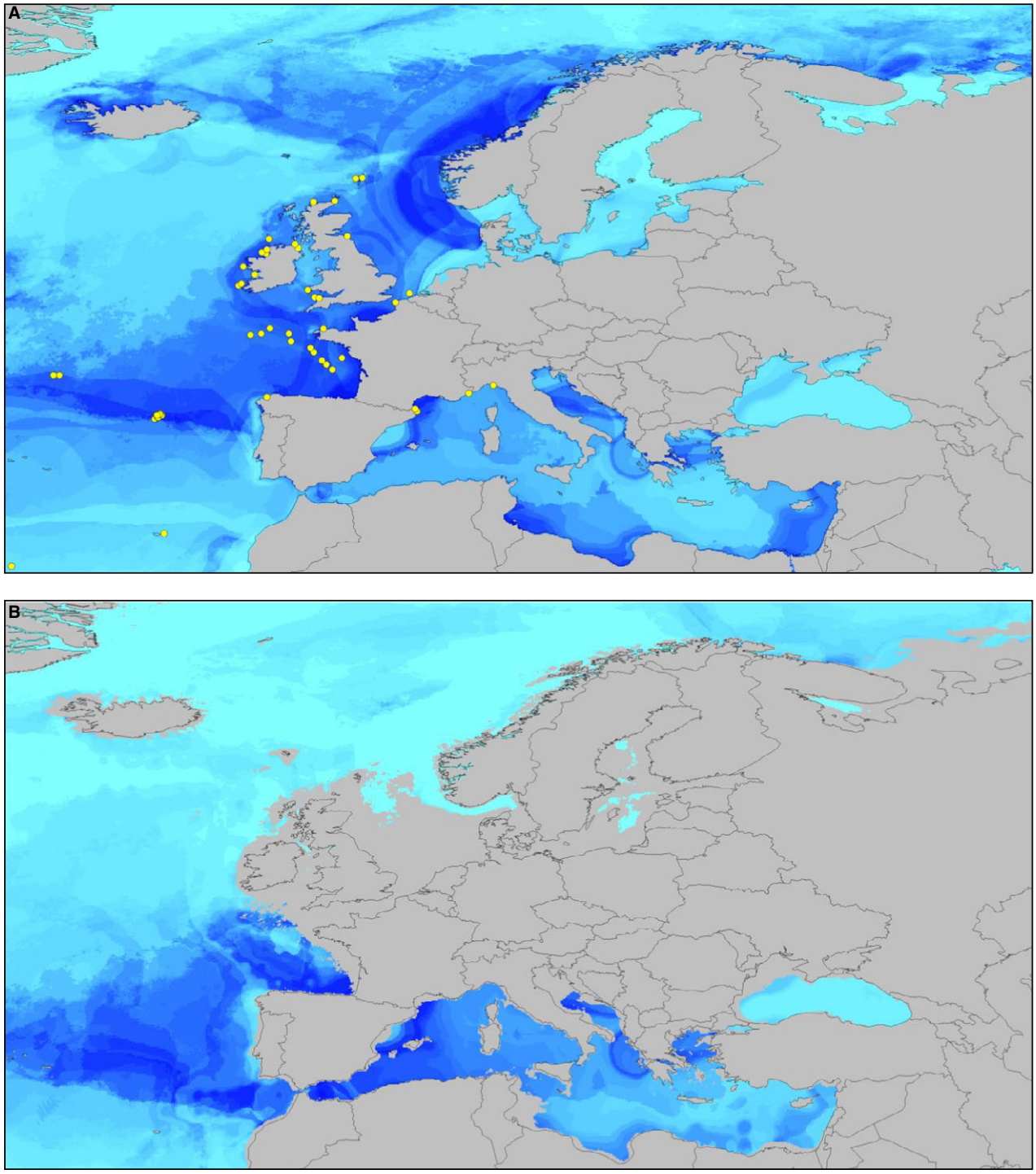

Figure 5. Results of the species distribution modelling: (A) current-day model; (B) palaeodistribution model for the Last Glacial Maximum (LGM *c.* 21 KYA). Darker blue areas indicate those more suitable for *P. noctiluca*. Yellow circles in (A) indicate occurrence data used to generate the models.

genetic diversity across the entire range of the study, as well as the Atlantic-wide distribution of the species (Miller *et al.*, 2012) and, indeed, the pan-global distribution of what is at least a species complex (Kramp, 1961; Mariottini *et al.*, 2008), would appear to be inconsistent with the concept of a Gulf of

Gibraltar source of recurring aggregations in the Northeast Atlantic Ocean and Western Mediterranean Sea as proposed previously by Licandro *et al.* (2010).

Despite the lack of any geographical structuring of genetic variation, two clearly distinct groups of genotypes were observed within the mtDNA COI, a feature also observed by Stopar *et al.* (2010). Such divergences tend to result from periods of isolation, usually associated with the climatic fluctuations that have occurred throughout the Pleistocene (Provan & Bennett, 2008; Provan, 2013). The timing of the divergence, however, places it in the early Pleistocene (*c.* 1.5 Mya), thus ruling out recent episodes of glaciation as the causal factor in promoting divergence. Furthermore, the palaeodistribution model suggests the persistence of a large, continuous population of *P. noctiluca* during the LGM, similar to the scenario observed in the zooplankton *Calanus finmarchicus* (Provan *et al.*, 2009), but in contrast to our earlier findings in the metagenic jellyfish *Rhizostoma octopus* (Glynn *et al.*, 2015). The fact that individuals from both the Atlantic and the Mediterranean are represented by haplotypes from each clade, coupled with the observed lack of any structuring in the microsatellite data set, further suggests extensive admixture since the divergence of the two clades. If this mitochondrial structure were representative of contemporary, ongoing, sympatric divergence, a commensurate divergence in microsatellite lineages would be seen. As this is not the case, mitochondrial clades are likely vestigial remnants of allopatric divergence, subjected to subsequent secondary contact, range overlap and admixture. It is not obvious what factors would have promoted such a divergence *c.* 1.5 Mya, but this period saw the start of a decrease in the North Atlantic Deep Water (NADW) formation, among a range of other oceanic and climatic changes at the same time, prior to the onset of the full glacial periods *c.* 0.9 Mya (Raymo *et al.*, 1990; McClymont & Rosell-Melé, 2005). Phylogenetic divergence dating to around the same time period (*c.* 1.2–1.8 Mya) has been reported for the fish species *Dentex dentex* and *Lithognathus mormyrus* (Bargelloni *et al.*, 2003), but in these cases this divergence has resulted in separate Atlantic and Mediterranean clades.

Significant F_{IS} values were observed in all but two of the populations sampled, which could at first sight be attributed to intra-aggregation inbreeding, as it has been suggested previously that reproduction generally occurs within persistent aggregations of *P. noctiluca* (Russell, 1967; Zavodnik, 1987; Malej, 1989). This scenario, however, is not supported by the analyses of within-population relatedness. Furthermore, the high levels of genetic diversity observed across populations are inconsistent with long-term inbreeding. The Portofino population was one of the two that exhibited significant within-population relatedness between individuals, as well as being the most genetically distinct based on the nuclear pairwise Φ_{ST} estimates. This finding might be seen as evidence for intra-aggregation recruitment, but the same population did not exhibit a significant F_{IS} value. These apparent discrepancies might be symptomatic of complex patterns of recruitment, including the occurrence of Wahlund effects as a result of sampling distinct cohorts within a specific geographical area that may have arisen through sweepstakes recruitment processes (Christie *et al.*, 2010), but set against a long-term backdrop of high levels of broad-scale gene flow over relatively long timescales. Nevertheless, the use of multiple, unlinked markers, and particularly of markers which exhibit dissimilar mutation rates and patterns of inheritance in the present study has proven useful in differentiating contemporary and historical signals of population structure. Our findings point to the long-term persistence of a single, contiguous European population of *P. noctiluca*, with minimal geographical structure. These results thus provide key insights into the population dynamics and demography of this ecologically and socio-economically important species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Results of the mismatch distribution analyses.

Table S1. Diversity statistics for each locus by population. H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; NS, non-significant. Significance of F_{IS} – $*P < 0.05$; $**P < 0.01$.