Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

Mitochondrial D-loop DNA analyses of Norway lobster (*Nephrops norvegicus*) reveals genetic isolation between Atlantic and East Mediterranean populations

Jeanne Gallagher[1], John A. Finarelli[2], Jónas P. Jonasson[3] and Jens Carlsson[1]

[1]Area52 Research Group, School of Biology and Environmental Science/Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland; [2]School of Biology and Environmental Science/Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland and [3]Marine and Freshwater Research Institute, Skúlagata 4, 101 Reykjavík, Iceland

Original Article

Cite this article: Gallagher J, Finarelli JA, Jonasson JP, Carlsson J (2019). Mitochondrial D-loop DNA analyses of Norway lobster (*Nephrops norvegicus*) reveals genetic isolation between Atlantic and East Mediterranean populations. *Journal of the Marine Biological Association of the United Kingdom* **99**, 933–940. https://doi.org/10.1017/S0025315418000929

Received: 2 February 2018
Revised: 24 September 2018
Accepted: 24 September 2018
First published online: 26 October 2018

Key words:
Atlantic; control region; genetic structure; glacial refugia; Mediterranean; mitochondrial DNA; phylogenetics

Author for correspondence:
Jeanne Gallagher, E-mail: jeanne.gallagher@ucdconnect.ie

Abstract

Nephrops norvegicus is a commercially valuable demersal fisheries species. Relatively little is understood about this species' population dynamics across its distribution with previous mitochondrial and microsatellite studies failing to identify significant population-level differentiation. In this study, sequence variation in the mitochondrial (mtDNA) D-loop was analysed from samples across the distribution range, and compared with COI sequences for this species retrieved from GenBank. Analysis of a 375 bp fragment of the D-loop revealed significant genetic differentiation between samples from the North-east Atlantic and the east Mediterranean ($F_{ST} = 0.107$, $P < 0.001$). Tau (τ), theta (θ_0 and θ_1) and Fu's F_S values suggest the species spread between 10,500 to 19,000 ybp and subsequently expanded rapidly across the Atlantic.

Introduction

Nephrops norvegicus (Linnaeus, 1758) is a benthic-dwelling, decapod crustacean that inhabits burrows in patches of soft, muddy sediment between ~4–800 m depth (Holthuis, 1991; Johnson *et al.*, 2013). The species' distribution ranges from Iceland and northern Norway in the North Atlantic, to Morocco and the Mediterranean in the south (Maltagliati *et al.*, 1998; Bell *et al.*, 2006; Johnson *et al.*, 2013). *Nephrops norvegicus* is dioecious, with mating occurring following a brief courtship shortly after females moult (Powell & Eriksson, 2013). Females produce 900–6000 eggs in a brood (Powell & Eriksson, 2013), with dispersal occurring in the larval phase, which can last up to 50 days (Hill, 1990; Dickey-Collas *et al.*, 2000). Survival of the larvae depends on a combination of factors including suitable temperature, food availability and access to suitable substrate (Dickey-Collas *et al.*, 2000; Aguzzi & Sardà, 2008; Pochelon *et al.*, 2009). Upon settling, juveniles occupy or create burrows to avoid predation (Powell & Eriksson, 2013). Adult *N. norvegicus* do not migrate or leave their mud patch at any point (Aguzzi & Sardà, 2008).

Commonly sold as Norway lobster, Dublin Bay prawn, scampi or langoustine, *N. norvegicus* is a commercially important fisheries species (Thorpe *et al.*, 2000), with the most recent global landing estimates at 54,000 tonnes in 2014 (FAO 2017; Thorpe *et al.*, 2000). Within the EU, 2017's *N. norvegicus* landings are estimated to be worth over €278 million (Marine Institute, 2017). For management purposes the species is currently divided into approximately 40 geographic groups, known as functional units (FUs) and geographical survey areas (GSAs), across its distribution (Relini *et al.*, 1999; Ungfors *et al.*, 2013).

Effective management relies on accurate and reliable information on how species are distributed over time and space. Current assessment of *N. norvegicus* is largely based on underwater video surveys (Johnson *et al.*, 2013; Marine Institute, 2016). Although the species has a relatively long larval stage (50 days), the low mobility of adults may increase the vulnerability of stocks to local overfishing relative to other highly mobile commercial species. Commercial fishing has been suggested as the principal driver of population dynamics for the species (Thorpe *et al.*, 2000). Despite the substantial economic value of *N. norvegicus* fisheries, there is limited knowledge of the species' genetic population structure and whether it aligns with existing functional, biological or management units (Stamatis *et al.*, 2006).

Population genetics has proven highly suited for identifying biological populations by quantifying the connectivity (gene flow/isolation) among them. Population genetics can also assess vital demographic parameters, such as effective population size, evolutionary history and recent demographic expansion (Beissinger & McCullough, 2002). Mitochondrial DNA (mtDNA) has several advantages in population genetic studies. For example, as a maternally inherited haploid marker there is a lack of genetic recombination which is ideal for studying deep-historical population dynamics (Held *et al.*, 2016). Zane *et al.* (2000) used single strand conformation polymorphism analyses of mtDNA in populations of Northern krill,

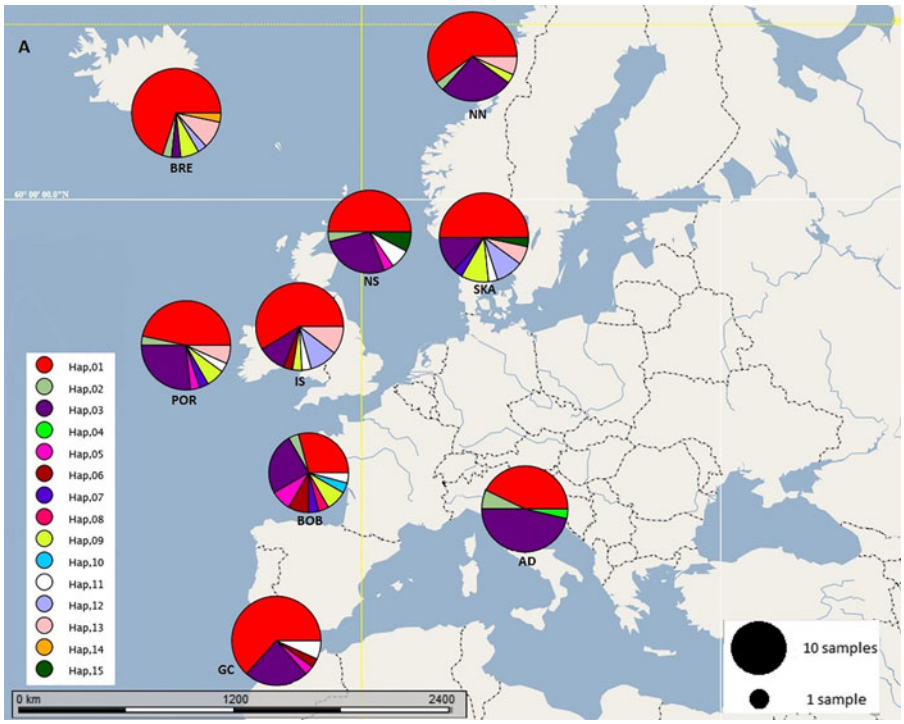

Fig. 1. Map of the geographic distribution and frequency of haplotype groups in nine sample sites of *N. norvegicus*. AD: Adriatic, BOB: Bay of Biscay, BRE: Breiðamerkurdjúp, GC: Gulf of Cadiz, IS: Irish Sea (west), NN: Northern Norway, NS: North Sea, POR: Porcupine, SKA: Skagerrak haplotypes.

Meganyctiphanes norvegica (M. Sars, 1857) to reveal at least three distinct populations in the North-east Atlantic and an Atlantic–Mediterranean divide. Yuhara *et al.* (2014) utilized mtDNA cytochrome c oxidase subunit I (COI) analyses to clarify the genetic diversity and connectivity among local coastal populations of the saltmarsh sesarmid crab, *Clistocoeloma sinense* (Shen, 1933) around the Japanese coastline.

With respect to *N. norvegicus*, allozyme analyses on 110 individuals from one Scottish and two Mediterranean localities (Aegean Sea and Adriatic Sea) failed to reveal genetic differentiation (Passamonti *et al.*, 1997). Maltagliati *et al.* (1998) performed allozyme analyses with 15 enzyme systems in *N. norvegicus*, examining one Atlantic and eight Mediterranean samples, with ~100 individuals from each site. While genetic variability was detected, there was no evident population structure. Stamatis *et al.* (2006) used 10 allozyme systems to investigate samples from the North Sea and the Aegean Sea, finding no significant genetic differentiation among 366 examined individuals. Streiff *et al.* (2001) did not recover evidence for population structure among 40 individuals from two Portuguese locations for five microsatellite loci. Stamatis *et al.* (2004) performed a restriction fragment length polymorphism analysis on mitochondrial COI DNA segments in 370 individuals and reported significant but low levels of genetic differentiation. No structure between the Mediterranean Sea and the Atlantic was discovered. Recent population expansion after the Last Glacial Maximum (LGM) was proposed as an explanation. Similarly, no population genetic structure was found using 12 microsatellite loci on 549 individuals from a small geographic range around Iceland (Pampoulie *et al.*, 2011).

Previous studies have yet to recover population differentiation either across the geographic range of *N. norvegicus*, or at finer scales. The mtDNA D-loop has proven hypervariable in other crustacean species with high levels of polymorphism that can be used to discriminate amongst populations (McMillen-Jackson & Bert, 2003, 2004). The current study explores the efficacy of this region to determine the presence of population structure across a subsample of the species' distribution.

Materials and methods

Sampling

Samples were collected from commercial fishing or research vessels from across the geographic distribution of *N. norvegicus* including Iceland, northern Norway, Skagerrak, North Sea, Irish Sea, Porcupine Bank, Bay of Biscay, Gulf of Cadiz and Ancona in the Adriatic Sea (Figure 1). Sex and length were recorded, and first and second pereiopods were removed from each individual before being stored in 80% EtOH. Whole samples that were collected were stored at −20 °C before tail tissue was removed and stored in 80% EtOH. Both males and females (~2:1) with carapace lengths encompassing an equal number (N = 15) of individuals of two length groups (6–35 & 35–70 mm) were selected to minimize the risk of only including a single cohort that could cause family effects and skew the genetic data (Haynes *et al.*, 2016).

DNA analysis

Total genomic DNA was extracted using a modified chloroform/isoamyl alcohol protocol (Petit *et al.*, 1999). Primers (NN3DF 5′-ACA GCG TTA AGA YAC CAT AG-3′ and NnDR 5′-GCT CTC ATA AAC GGG GTA TGA-3′) were designed initially using Primer-3 as implemented in Geneious® 7 (https://www.geneious.com, Kearse *et al.*, 2012) and the D-loop *N. norvegicus* mitochondrial genome (GenBank accession: LN681403.1). The resulting amplicons were relatively larger (~880 bp) than had been designed for (~600 bp) and were sequenced to discover a ~280 bp fragment of the D-loop area missing from within the GenBank data (Appendix 1). Subsequently, new primers JG2 F 5′-CTA CAG ATT TCG TCT ATC AAC-3′ and NnD R 5′-GCT CTC ATA AAC GGG GTA TGA-3′ were designed on these returning sequences to incorporate the newly discovered ~280 bp for a ~680 bp amplicon. Primer sequences' specificity was confirmed using BLAST (Basic Logical Alignment Search Tool; Zhang *et al.*, 2000). Optimal annealing temperature was determined using a gradient PCR.

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press

Table 1. Mitochondrial sequence variability in the D-loop for *N. norvegicus* from the nine sample sites; number of individuals (N), number of haplotypes (N*h*), haplotype diversity (*h*), nucleotide diversity (π), tau (τ), theta for times 0 and 1 (θ_0 and θ_1), Fu's F_S, Harpending's Raggedness index (*Hri*), and sum of squared differences from mismatch analyses (SSD)

Collection	N	N*h*	*h*	π	τ	θ_0	θ_1	Fu's F_S	*Hri*	SSD
Adriatic	28	4	0.616	0.009	2.5	0	11.055	**−26.691***	0.040	0.006
Bay of Biscay	24	10	0.859	0.019	2.2	0	6829.96	**−26.832***	**0.062**	0.006
Breiðamerkurdjúp	30	7	0.508	0.010	2.8	0	6834.96	**−26.748***	**0.055**	**0.010**
Gulf of Cadiz	30	5	0.556	0.008	1.6	0	3429.40	**−27.522***	**0.079**	0.006
Irish Sea	29	7	0.643	0.018	1.6	0	3424.39	**−27.717***	**0.138**	**0.022**
Northern Norway	30	6	0.582	0.009	1.7	0	6844.37	**−27.165***	**0.090**	**0.010**
North Sea	26	5	0.689	0.012	2	0	6829.96	**−26.775***	**0.091**	**0.011**
Porcupine	30	8	0.722	0.013	2.5	0	6834.96	**−26.647***	**0.046**	0.003
Skagerrak	30	8	0.729	0.015	3	0	3414.98	**−26.344***	**0.050**	**0.007**

Values in bold are significant at $P < 0.05$, $^*P < 0.001$.

Table 2. SAMOVA results table for *N. norvegicus*. K corresponds to the number of populations. Optimal F_{CT} and groupings ($K = 2$) are highlighted in bold

Groups (K)	Structure recovered	F_{CT}
2	**[AD][BOB, BRE,GC,IS,NN,NS,POR,SKA]**	**0.1092**
3	[AD][BOB][BRE,GC,IS,NN,NS,POR,SKA]	0.0799
4	[AD][POR][BRE,GC,IS,NN,SKA][BOB][NS]	0.0717
5	[POR][BRE,IS][BOB][AD][GC,NN,NS,SKA]	0.0661
6	[BRE,IS][AD][GC,NN,NS][SKA][POR][BOB]	0.0618
7	[AD][BRE,IS][POR][NN][GC,NS][SKA][BOB]	0.0620
8	[SKA][AD][NS][GC][BOB][NN][BRE,IS][POR]	0.0637

Table 3. AMOVA table of temporal and spatial genetic variation of *N. norvegicus* from sites sampled

AMOVA Source of variation	% of variance	Fixation indexes	F-statistics	*P*
Among groups	10.00	0.126	FSC	<0.001
Among samples within groups	1.14	0.111	FST	0.785
Within populations	88.86	0.100	FCT	<0.001

PCR amplifications were performed in a Biometra T3000 thermocycler (Biolabo, SA) with lid temperature of 95 °C, using a thermal cycling profile of initial heating of 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 61.2 °C for 30 s, and 72 °C for 1 min, followed by a final extension step of 72 °C for 2 min. Completed reactions were held at 4 °C. PCR products were visualized on 1.5% agarose gels to verify amplifications, and purified using ExoSap-IT (Affymetrix Ltd, Santa Clara, CA) prior to Macrogen sequencing.

COI data

In total, 35 *N. norvegicus* COI sequences were downloaded from GenBank covering four different areas: the North Sea, Paris (unknown), Portugal and Turkey (Appendix 2).

Data and statistical analyses

Forward and reverse sequences were aligned and edited in Geneious® v 7.0, using the K80 substitution model (Kimura, 1980), as determined in JMODELTEST v 2.1.4. (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). Spatial analysis of molecular variance, SAMOVA v 2.0 (Dupanloup *et al.*, 2002) was used to define groups of populations that are geographically homogenous and maximally differentiated from each other. Analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was performed in ARLEQUIN v 3.5 (Excoffier & Lischer, 2010) to generate F-statistics, Φ_{ST}, θ, and (τ), the K80 substitution model and 10,100 replicates. Fu's F_S tests whether mutations are selectively neutral. Theta (θ) is defined as 2 Nμ for haploid mitochondrial DNA, where N is the effective population size and μ is mutation rate per sequence per generation (Fu, 1997). Tau (τ) can measure relative time since a population expansion using $T = \tau/2u$, where u is per-nucleotide rate of mutation multiplied by the number of nucleotides in the sequence (Gaggiotti & Excoffier, 2000). Harpending's raggedness index (Harpending, 1994) and mismatch distributions (*SSD*) were both used to test whether the data deviated significantly from a population expansion model. DNASP v 6.10.01 (Rozas *et al.*, 2017) was used to calculate haplotype (*h*) and nucleotide diversity (π) and to estimate the nearest neighbour statistic S_{nn} (Hudson, 2000) with 10,000 permutations. This statistic uses a symmetric island model on haplotype data to measure sequential 'neighbours' from the same geographic space. In all cases involving multiple comparisons significance levels were adjusted for multiple tests using the sequential Bonferroni correction technique (Rice, 1989). A map of *N. norvegicus* haplotypes was constructed using POPART v 1.7 (Leigh & Bryant, 2015). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrograms of Φ_{ST} pairwise distance values for both D-loop and GenBank retrieved COI sequences (Appendix 2) were created in PAST v 3.20 with 1000 bootstrap replicates (Hammer *et al.*, 2001). All software was used with default setting unless specified otherwise.

Results

DNA analysis

Sequence alignments for the D-loop region were trimmed for maximum length and quality using individual sequence chromatograms. Only regions of the D-loop for which both forward and reverse strands yielded unambiguous sequences were included for a fragment of 375 bp. Of the 270 sequenced samples, 13 were excluded due to poor quality sequence reads. A total of 15 haplotypes were resolved (GenBank accession nos. MG972769–

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press

Table 4. Pairwise Φ_{ST} estimates (below diagonal) for D-loop mtDNA data among *N. norvegicus* samples. *P*-values (upper diagonal) in bold were significant after Sequential Bonferroni correction (initial $\alpha = 0.05/8 = 0.00625$)

Population	Adriatic	Bay of Biscay	Breiðamer-kurdjúp	Gulf of Cadiz	Irish Sea	Northern Norway	North Sea	Porcupine	Skagerrak
Adriatic		**0.019**	**<0.001**	**0.021**	**0.024**	**0.031**	**0.046**	**0.026**	**0.016**
Bay of Biscay	0.111		1.336	1.134	0.270	0.870	1.062	0.273	0.717
Breiðamerkurdjúp	0.152	0.046		0.572	0.615	0.627	0.200	0.060	0.670
Gulf of Cadiz	0.144	0.002	0.019		1.196	1.434	1.356	0.140	0.704
Irish Sea	0.143	0.043	−0.009	0.005		0.403	0.333	0.240	0.604
Northern Norway	0.072	0.018	0.011	−0.006	−0.001		1.248	1.554	0.748
North Sea	0.087	−0.007	0.043	−0.015	0.027	−0.010		0.358	0.625
Porcupine	0.057	0.049	0.061	0.051	0.037	−0.010	0.015		0.129
Skagerrak	0.098	−0.014	0.002	−0.010	−0.008	−0.010	−0.009	0.020	

Table 5. Pairwise Φ_{ST} estimates (below diagonal) for COI mtDNA data among GenBank *N. norvegicus* samples. *P*-values (upper diagonal) in bold were significant after Sequential Bonferroni correction (initial $\alpha = 0.05/8 = 0.016$)

	Turkey	Portugal	North Sea	Paris (unknown)
Turkey		**<0.001**	**<0.001**	0.999
Portugal	0.968		0.999	0.994
North Sea	0.936	−0.111		0.999
Paris (unknown)	0.993	0.630	0.470	

MG972783) with nucleotide diversity (π) ranging from 0.008 in the Gulf of Cadiz to 0.019 in the Bay of Biscay, and haplotype diversity ranging from 0.508 in Breiðamerkurdjúp to 0.859 in the Bay of Biscay (Table 1). Frequency and location of the haplotypes were displayed on a haplotype map (Figure 1).

Data and statistical analyses

Population differentiation

The SAMOVA analysis indicated that the best-supported F_{CT} value ($F_{CT} = 0.109$) was achieved when the samples were clustered into two groups. The first group contained only individuals derived from the east Mediterranean (Adriatic) while the second group comprised of individuals from all other sampled areas (Table 2).

An AMOVA analysis, using the SAMOVA structure and the K80 distance model (Kimura, 1980) revealed significant heterogeneity among the nine samples ($\Phi_{ST} = 0.107$, $P < 0.001$). Within-sample variation accounted for 88.86% of the variance ($F_{CT} = 0.100$, $P < 0.001$; Table 3). Pairwise Φ_{ST} values revealed population structure between the eastern Mediterranean (Adriatic) sample and each of the eight other samples from the North Atlantic (Table 4). Significant pairwise Φ_{ST} values for the D-loop sequences ranged from 0.057 (Adriatic/Porcupine) to 0.152 (Adriatic/Breiðamerkurdjúp; Table 4). The nearest-neighbour statistic (S_{nn}) indicated a significant association between sequence similarity and geographic location ($S_{nn} = 0.1250$, $P = 0.032$). Significant pairwise Φ_{ST} values for COI data ranged from 0.968 (Portugal/Turkey) to 0.936 (North Sea/Turkey; Table 5). A UPGMA dendrogram was constructed using sample pairwise Φ_{ST} values to visualize genetic distances for both D-loop and COI data (Figures 2 and 3).

Population expansion

Demographic analyses in ARLEQUIN showed pronounced differences between θ_0 and θ_1 suggesting rapid population expansion in all samples, with less pronounced differences in the Adriatic sample (Table 1). All Fu's F_S values were negative and deviated significantly from zero. Mismatch distributions differed significantly from the distributions expected under population expansion in four of the nine sample sites (Table 1). Harpending's raggedness index ranged from 0.040 to 0.138 from the Adriatic to the Irish Sea respectively and was significant for all except the Adriatic sample (Table 1).

Using the population expansion formula $T = \tau/2u$ with $u = \mu k$, where μ = per nucleotide substitution rate and k = sequence length, population expansion times were estimated between 10,500 to 19,000 ybp. Due to the uncertainty around the point estimate of τ in the ARLEQUIN analyses, 1000 bootstrap replicates were performed drawing random values for tau from between 2.5 and 97.5 percentiles returned by ARLEQUIN. Population expansion times were estimated using $\mu = 19\%$/My from the penaeid prawn and pink shrimp D-loop mutation rate (McMillen-Jackson & Bert, 2003, 2004). From the 1000 bootstrap iterations the mean estimate and upper and lower two standard deviation confidence intervals were calculated (Table 6).

Discussion

This study recovered a previously undocumented 280 bp segment of the *N. norvegicus* mitochondrial genome (GenBank accession no. MG917720). In addition, genetic structure between the North Atlantic and eastern Mediterranean *N. norvegicus* samples was detected in both the mtDNA D-loop and COI region.

Eleven haplotypes are shared among multiple *N. norvegicus* samples, with four haplotypes unique to a single sample: two unique haplotypes are found in the Bay of Biscay, and one each are found in Breiðamerkurdjúp and the Adriatic. SAMOVA revealed distinct population genetic differences between the Atlantic samples and the eastern Mediterranean sample, and the nearest neighbour statistic (S_{nn}) suggests a significant association between D-loop sequence similarity and geographic location. Average Φ_{ST} estimates are at least twice as large between the Adriatic and each Atlantic sample than Φ_{ST} estimates among all Atlantic samples. This suggests that the eastern Mediterranean sample is genetically differentiated from the Atlantic samples. A UPGMA cluster analysis on the D-loop Φ_{ST} distance matrix demonstrated that the largest genetic differentiation exists between the east Mediterranean and all other samples with 100 bootstrap support. The same result was also achieved using GenBank COI data where samples from Turkey in the eastern Mediterranean were genetically differentiated from Atlantic samples. While an Atlantic–Mediterranean divide has been recorded for many highly mobile species (Bargelloni

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press

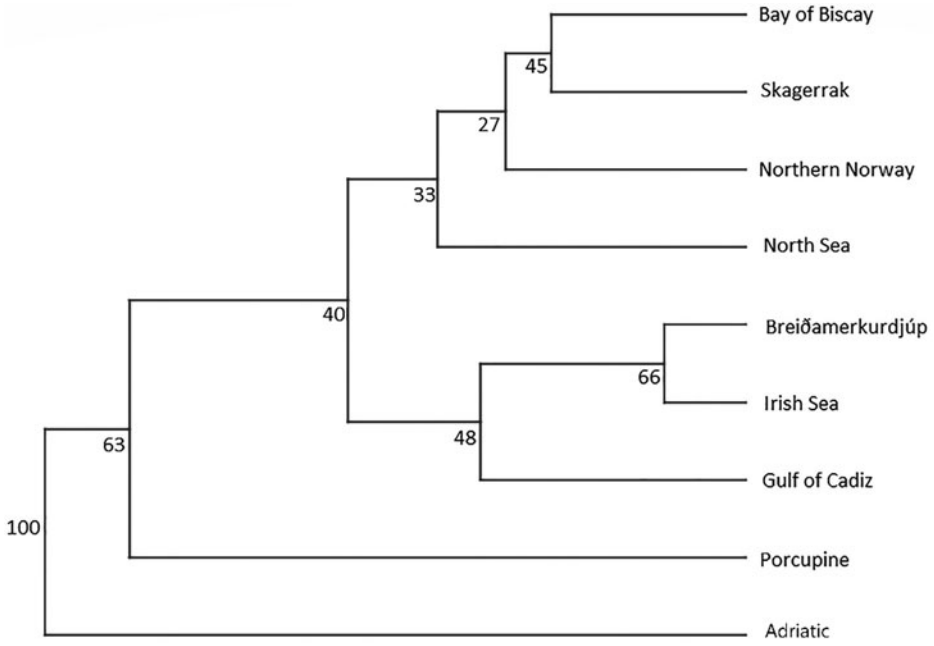

Fig. 2. D-Loop UPGMA dendrogram based on Φ_{ST} pairwise distance values calculated from frequency data among *N. norvegicus* individuals sampled.

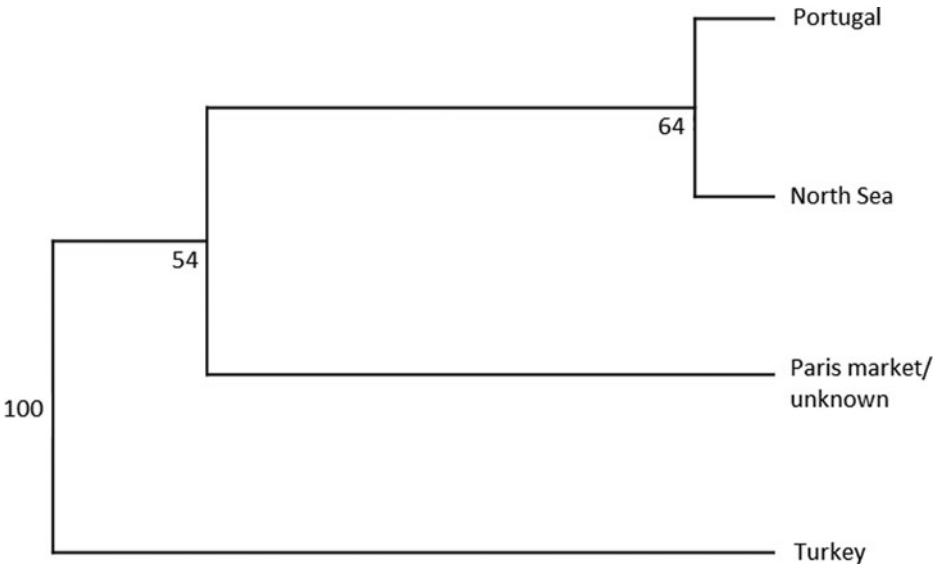

Fig. 3. COI UPGMA dendrogram based on Φ_{ST} pairwise distance values calculated from frequency data of *N. norvegicus* individuals taken from GenBank.

Table 6. Estimated expansion times for *N. norvegicus* with upper and lower two standard deviation confidence intervals

	Adriatic	Bay of Biscay	Breiðamerkurdjúp	Gulf of Cadiz	Irish Sea	Northern Norway	North Sea	Porcupine	Skagerrak
+2 SD	31,875.41	26,006.56	26,329.73	17,736.93	18,207.28	18,834.99	21,034.19	23,777.64	27,923.24
Mean	19,049.10	16,038.80	17,035.32	10,499.87	10,765.61	11,470.40	13,057.05	15,458.56	18,240.16
−2 SD	12,635.90	11,054.92	12,388.11	6881.34	7044.77	7788.11	9068.48	11,299.02	13,398.62

et al., 2003; Carlsson *et al.*, 2004; Farrell *et al.*, 2016), it has not been previously reported for *N. norvegicus*. This could be due to small sample sizes leading to lack of statistical power. Alternatively, certain regions of mtDNA are more variable than others, with D-loop being a hypervariable region never-before explored for this species. The relatively recent expansion time of 10,500 to 19,000 ybp reported in this study could be another reason why differentiation has not been discovered previously in less variable mtDNA regions.

An Atlantic–Mediterranean divide has also been reported for European lobster *Homarus gammarus* (Linnaeus, 1758), which also inhabits a similar distribution range to *N. norvegicus* (Triantafyllidis *et al.*, 2005). Along with this divide, significant substructuring was also found within the Mediterranean. Zane *et al.* (2000) also reported this Atlantic–Mediterranean divide in decapod Northern krill and suggested the Oran-Almeria oceanic front as a barrier to gene flow. A sample of krill east of the straits of Gibraltar was found to be an intermediate genetically between the Atlantic and Ligurian Sea samples. Ladoukakis *et al.* (2002) also discovered an Atlantic–Mediterranean differentiation in blue mussel, *Mytilus galloprovincialis* (Lamarck, 1819) and a further differentiation between Mediterranean and Black Sea

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press

populations. Marine invertebrate chaetognath *Sagitta setosa* (J. Müller, 1847) is also reported as having an Atlantic–Mediterranean divide, with a sharp division within the Mediterranean basin between the Adriatic sea and other areas (Peijnenburg *et al.*, 2006). Sanna *et al.* (2013) also reported a genetic divergence across the Mediterranean in bivalve, *Pinna nobilis* (Linnaeus, 1758). Results revealed genetic divergence among three distinguishable areas: the western Mediterranean/Ionian Sea, the Adriatic Sea, and Aegean Sea/Tunisian coastal areas. Within the Mediterranean the Adriatic, as a semi-closed sea, appears to be genetically divergent from other areas (Patarnello *et al.*, 2007). This is further supported by studies on vertebrates (Stefanni & Thorley, 2003; Rossi *et al.*, 2006; Gaspari *et al.*, 2007; Matić-Skoko *et al.*, 2018).

Negative Fu's F_S values suggest recent demographic expansion (Fu, 1997), and the large difference between θ_0 and θ_1 for all Atlantic sites suggests rapid population expansion. In contrast, the difference in theta values for the Mediterranean sample are two orders of magnitude smaller. When considered with the non-significant Raggedness index, this suggests a less-pronounced population expansion in the Mediterranean.

Estimates of time since expansion ranged from 10,500 to 19,000 ybp. Large confidence intervals around all of the point estimates for the expansion time overlap, indicating that expansion likely occurred within the same time frame for all sampled locations. These time estimates are consistent with those for European lobster, which is believed to have established around 15,000 ybp (Triantafyllidis *et al.*, 2005). These time estimates are also in agreement with those for the LGM in Europe (16,000–31,000 ybp; Ashton *et al.*, 2010), and likely represent population expansion into newly available habitat as the ice retreated.

Observed haplotype diversity was highest in the Bay of Biscay, suggesting this region represents a potential glacial refugium for the Atlantic distribution of the species. The area north of the Bay of Biscay has previously been hypothesized as a refugium for other marine species such as the common mussel *Mytilus edulis* (Linnaeus, 1758) (Śmietanka *et al.*, 2014). These results also support species-distribution models for several other marine invertebrates, including the common starfish *Asterias rubens* (Johnston, 1836), amphipod crustacean *Gammarus duebeni* (Liljeborg, 1852), flat periwinkle *Littorina obtusata* (Linnaeus, 1758), dogwhelk *Nucella lapillus* (Linnaeus, 1758) and barnacle *Semibalanus balanoid* (Linnaeus, 1767) around the LGM (Waltari & Hickerson, 2013).

This study is the first to reveal a significant genetic differentiation between Atlantic and east Mediterranean samples of *N. norvegicus*, which supports a divide found in other marine species. Further divisions within the Mediterranean basin may be found, and future studies should include samples from these areas to examine this. These results support a post-glacial expansion, with Atlantic *N. norvegicus* continuing to expand rapidly. In terms of commercial fisheries management, these results do not support current management practices, as no significant genetic differentiation was found among Atlantic samples that cross several functional units. Utilizing genetic markers (e.g. microsatellites or SNPs) in future studies might provide fisheries management with more information on Atlantic *N. norvegicus*. These results may be important for management within the eastern Mediterranean, as populations experiencing isolation can be more vulnerable to commercial over-exploitation and recovery may be more difficult in the event of population collapse.

Acknowledgements. We would like to thank the scientists and researchers from the Irish Marine Institute, Ifremer, Marine Research Institute Iceland, Spanish Institute of Oceanography, ISMAR Institute of Marine Sciences, Havforskningsinstituttet Norwegian Institute of Marine Research, Marine Scotland and AFBI for the collection and donation of samples involved in this study. We would also like to thank E. Farrell for his assistance and comments on the manuscript.

Financial support. J.G. acknowledges funding from the Irish Research Council (IRC) (GOIPG/2015/2977). This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant agreement No. 678760 (ATLAS). This output reflects only the authors' view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

References

Aguzzi J and Sardà F (2008) A history of recent advancements on *Nephrops norvegicus* behavioral and physiological rhythms. *Reviews in Fish Biology and Fisheries* **18**, 235–248.

Ashton N, Lewis S and Stringer C (2010) *The Ancient Human Occupation of Britain*. London: Elsevier.

Bargelloni L, Alarcon JA, Alvarez MC, Penzo E, Magoulas A, Reis C and Patarnello T (2003) Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic–Mediterranean divide. *Journal of Evolutionary Biology* **16**, 1149–1158.

Beissinger SR and McCullough DR (2002) *Population Viability Analysis*. Chicago, IL: University of Chicago Press.

Bell M, Redant F and Tuck I (2006) *Nephrops* species. In Phillips BF (ed.), *Lobsters: Biology, Management, Aquaculture and Fisheries*. Oxford: Blackwell, pp. 412–461.

Carlsson J, McDowell JR, Díaz-Jaimes P, Carlsson JEL, Boles SB, Gold JR and Graves JE (2004) Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea: population genetics of Mediterranean bluefin tuna. *Molecular Ecology* **13**, 3345–3356.

Darriba D, Taboada GL, Doallo R and Posada D (2012) Jmodeltest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.

Dickey-Collas M, Briggs RP, Armstrong MJ and Milligan SP (2000) Production of *Nephrops norvegicus* larvae in the Irish Sea. *Marine Biology* **137**, 973–981.

Dupanloup I, Schneider S and Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**, 2571–2581.

Excoffier L and Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

FAO (2017) *FishFinder*. Rome: FAO Fisheries and Aquaculture Department. http://www.fao.org/fishery/.

Farrell ED, Carlsson JEL and Carlsson J (2016) Next Gen Pop Gen: implementing a high-throughput approach to population genetics in boarfish (*Capros aper*) for fish stock identification. *Proceedings of the Royal Society Open Science* **3**, 160651.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**, 915–925.

Gaggiotti OE and Excoffier L (2000) A simple method of removing the effect of a bottleneck and unequal population sizes on pairwise genetic distances. *Proceedings of the Royal Society B: Biological Sciences* **267**, 81–87.

Gaspari S, Azzellino A, Airoldi S and Hoelzel AR (2007) Social kin associations and genetic structuring of striped dolphin populations (*Stenella coeruleoalba*) in the Mediterranean Sea. *Molecular Ecology* **16**, 2922–2933.

Guindon S and Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704.

Hammer Ø, Harper DAT and Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **1**, 1–9.

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* **66**, 591–600.

Haynes PS, Browne P, Fullbrook L, Graham CT, Hancox L, Johnson MP, Lauria V and Power AM (2016) Growth in *Nephrops norvegicus* from a tag-recapture experiment. *Scientific Reports* **6**, 35143.

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press

Held C, Koenemann S and Schubart CD (2016) *Phylogeography and Population Genetics in Crustacea*. Boca Raton, FL: CRC Press.

Hill AE (1990) Pelagic dispersal of Norway lobster *Nephrops norvegicus* larvae examined using an advection-diffusion-mortality model. *Marine Ecology Progress Series* **64**, 217–226.

Holthuis LB (1991) *FAO Species Catalogue, Vol. 13. Marine Lobsters of the World. An Annotated and Illustrated Catalogue of Marine Lobsters Known to Date*. FAO Fisheries Synopsis No. 125. Rome: FAO, p. 292.

Hudson RR (2000) A new statistic for detecting genetic differentiation. *Genetics* **155**, 2011–2014.

Johnson MP, Lordan C and Power AM (2013) Habitat and ecology of *Nephrops norvegicus*. *Advances in Marine Biology* **64**, 27–63.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P and Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Ladoukakis E, Saavedra C, Magoulas A and Zouros E (2002) Mitochondrial DNA variation in a species with two mitochondrial genomes: the case of *Mytilus galloprovincialis* from the Atlantic, the Mediterranean and the Black Sea. *Molecular Ecology* **11**, 755–769.

Leigh JW and Bryant D (2015) Popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**, 1110–1116.

Maltagliati F, Camilli L, Biagi F and Abbiati M (1998) Genetic structure of Norway lobster, *Nephrops norvegicus* (L.) (Crustacea: Nephropidae), from the Mediterranean Sea. *Scientia Marina* **62**, 91–99.

Marine Institute (2016) *The Stock Book 2016: Annual Review of Fish Stocks in 2016 with Management Advice for 2017*. Galway: Marine Institute.

Marine Institute (2017) *The Stock Book 2017: Annual Review of Fish Stocks in 2017 with Management Advice for 2018*. Galway: Marine Institute.

Matić-Skoko S, Šegvić-Bubić T, Mandić I, Izquierdo-Gomez D, Arneri E, Carbonara P, Grati F, Ikica Z, Kolitari J, Milone N, Sartor P, Scarcella G, Tokaç A and Tzanatos E (2018) Evidence of subtle genetic structure in the sympatric species *Mullus barbatus* and *Mullus surmuletus* (Linnaeus, 1758) in the Mediterranean Sea. *Scientific Reports* **8**, 676.

McMillen-Jackson AL and Bert TM (2003) Disparate patterns of population genetic structure and population history in two sympatric penaeid shrimp species (*Farfantepenaeus aztecus* and *Litopenaeus setiferus*) in the eastern United States: population genetics of two sympatric shrimp. *Molecular Ecology* **12**, 2895–2905.

McMillen-Jackson AL and Bert TM (2004) Genetic diversity in the mtDNA control region and population structure in the pink shrimp *Farfantepenaeus duorarum*. *Journal of Crustacean Biology* **24**, 101–109.

Pampoulie C, Skirnisdottir S, Hauksdottir S, Olafsson K, Eiriksson H, Chosson V, Hreggvidsson GO, Gunnarsson GH and Hjorleifsdottir S (2011) A pilot genetic study reveals the absence of spatial genetic structure in Norway lobster (*Nephrops norvegicus*) on fishing grounds in Icelandic waters. *ICES Journal of Marine Science* **68**, 20–25.

Passamonti M, Mantovani B, Scali V and Froglia C (1997) Allozymic characterization of Scottish and Aegean populations of *Nephrops norvegicus*. *Journal of the Marine Biological Association of the United Kingdom* **77**, 727–735.

Patarnello T, Volckaert FAMJ and Castilho R (2007) Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break? Phylogeography of the Mediterranean-Atlantic Divide. *Molecular Ecology* **16**, 4426–4444.

Peijnenburg KTCA, Fauvelot C, Breeuwer JAJ and Menken SBJ (2006) Spatial and temporal genetic structure of the planktonic *Sagitta setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. *Molecular Ecology* **15**, 3319–3338.

Petit E, Excoffier L and Mayer F (1999) No evidence of bottleneck in the postglacial recolonization of Europe by the noctule bat (*Nyctalus noctula*). *Evolution* **53**, 1247–1258.

Pochelon PN, Calado R, Dos Santos A and Queiroga H (2009) Feeding ability of early zoeal stages of the Norway lobster *Nephrops norvegicus* (L.). *Biological Bulletin* **216**, 335–343.

Powell A and Eriksson SP (2013) Reproduction: life cycle, larvae and larviculture. *Advances in Marine Biology* **64**, 201–245.

Relini G, Bertrand J and Zamboni A (1999) Sintesi delle conoscenze sulle risorse da pesca dei fondi del Mediterraneo centrale (Italia e Corsica). Synthesis of the knowledge on bottom fishery resources in Central Mediterranean (Italy and Corsica). *Biologia Marina Mediterranea* **6**, 868.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Rossi AR, Perrone E and Sola L (2006) Genetic structure of gilthead seabream, *Sparus aurata*, in the Central Mediterranean Sea. *Central European Journal of Biology* **1**, 636–647.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE and Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* **34**, 3299–3302.

Sanna D, Cossu P, Dedola GL, Scarpa F, Maltagliati F, Castelli A, Franzoi P, Lai T, Cristo B, Curini-Galletti M, Francalacci P and Casu M (2013) Mitochondrial DNA reveals genetic structuring of *Pinna nobilis* across the Mediterranean Sea. *PLoS ONE* **8**, e67372.

Śmietanka B, Burzyński A, Hummel H and Wenne R (2014) Glacial history of the European marine mussels *Mytilus*, inferred from distribution of mitochondrial DNA lineages. *Journal of Heredity* **113**, 250–258.

Stamatis C, Triantafyllidis A, Moutou K and Mamuris Z (2006) Allozymic variation in Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. *ICES Journal of Marine Science* **63**, 875–882.

Stamatis C, Triantafyllidis A, Moutou KA and Mamuris Z (2004) Mitochondrial DNA variation in Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. *Molecular Ecology* **13**, 1377–1390.

Stefanni S and Thorley JL (2003) Mitochondrial DNA phylogeography reveals the existence of an evolutionarily significant unit of the sand goby *Pomatoschistus minutus* in the Adriatic (Eastern Mediterranean). *Molecular Phylogenetics and Evolution* **28**, 601–609.

Streiff R, Guillemaud T, Alberto F, Magalhaes J, Castro M and Cancela ML (2001) Isolation and characterization of microsatellite loci in the Norway lobster (*Nephrops norvegicus*). *Molecular Ecology Notes* **1**, 71–72.

Thorpe SJ, Sole-Cava A and Watts P (2000) Exploited marine invertebrates: genetics and fisheries. *Hydrobiologia* **420**, 165–184.

Triantafyllidis A, Apostolidis AP, Katsares V, Kelly E, Mercer J, Hughes M, Jørstad KE, Tsolou A, Hynes R and Triantaphyllidis C (2005) Mitochondrial DNA variation in the European lobster (*Homarus gammarus*) throughout the range. *Marine Biology* **146**, 223–235.

Ungfors A, Bell E, Johnson ML, Cowing D, Dobson NC, Bublitz R and Sandell J (2013) *Nephrops* fisheries in European waters. *Advances in Marine Biology* **64**, 247–314.

Waltari E and Hickerson MJ (2013) Late Pleistocene species distribution modelling of North Atlantic intertidal invertebrates. *Journal of Biogeography* **40**, 249–260.

Yuhara T, Kawane M and Furota T (2014) Genetic population structure of local populations of the endangered saltmarsh sesarmid crab *Clistocoeloma sinense* in Japan. *PLoS ONE* **9**, e84720.

Zane L, Ostellari L, Maccatrozzo L, Bargelloni L, Cuzin-Roudy J, Buchholz F and Patarnello T (2000) Genetic differentiation in a pelagic crustacean (*Meganyctiphanes norvegica*: Euphausiacea) from the North East Atlantic and the Mediterranean Sea. *Marine Biology* **136**, 191–199.

Zhang Z, Schwartz S, Wagner L and Miller W (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7**, 203–214.

Appendix 1

Nephrops norvegicus D-loop sequence (GenBank accession no. MG917720) with ~280 bp fragment (shown in bold) missing from within the GenBank data.

ATATACACAGATCAGTAAAAATATATTTTTAAGGCTAATCTAAAAA
GTAAACTTATATAATTTCATTGAAATTCATTACARTCTGAAAGTCAATG
ATTTAATTTTATAAATCGACTAAATAAGATCTATAAATAAAA**TCTTACC**
CCTTCAAAAGGTCACTTTCTCCTGAGGGGAGCTCCCTTTTCCCAACG
GGGTAAGATTTCTATTGGGAGAGCAGGATTATAATTATAGAGAGTT
GGGTATAAGGCTTCATTGTTTACACATATATACTATTAAATTAATTAT
ATACATTTATATGTATATATATATATATATATATACTATTTAAATAATA
TTTTCTTAACTTTWTATTTTGTTAACATWTAAATTATTAATAATGTT
TTATAAATTTTATATATTAAAATAAAATACAGTAAAAAAGGTTTTTA
GATAAATTTCTACGAATATTATACTATTATACACAATGGAATTCCACC
AATTCTTTAAAGATCAAAACTTTTCGTGCCGTTTACACTAGTATACAAA
AGAGAAGCTAATTCTAAGCTAATGG

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press

Appendix 2

GenBank accession no.	Location
JQ306231	Portugal, South Coast
JQ306232	Portugal, South Coast
JQ306233	Portugal, South Coast
JQ306236	Portugal, West Coast
JQ306237	Portugal, South Coast
JQ623962	Turkey
KC311407	Turkey
KC789294	Turkey
KC789295	Turkey
KC789296	Turkey
KC789297	Turkey
KC789298	Turkey
KC789299	Turkey
KC789300	Turkey
KC789301	Turkey
KC789302	Turkey
KC789303	Turkey
KC789304	Turkey
KC789305	Turkey
KC789306	Turkey
KC789307	Turkey
KC789308	Turkey
KC789309	Turkey
KC789310	Turkey
KC789311	Turkey
KC789312	Turkey
KC789313	Turkey
KT208521	North Sea
KT208656	North Sea
KT208760	North Sea
KT208840	North Sea
KT208922	North Sea
KT209167	North Sea
KT209472	North Sea
KX420657	Paris market/unknown

Table of *N. norvegicus* COI sequences and their locations accessed on GenBank.

https://doi.org/10.1017/S0025315418000929 Published online by Cambridge University Press