

Genetic structure and demographic history of the endemic Mediterranean scallop *Pecten jacobaeus* inferred from mitochondrial 16s DNA sequence analysis

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Abstract

Genetic structure and demographic history of the endemic Mediterranean scallop Pecten jacobaeus inferred from mitochondrial 16s DNA sequence analysis. Understanding the genetic population structure of species going through population decline is primordial in implementing a management plan. In the case of *Pecten jacobaeus*, previous genetic studies have been limited to populations in the western Mediterranean (Spain) and the Adriatic Sea (Italy). To check the presence of phylogeographic breaks between the two Mediterranean basins, we scored the variability of the mitochondrial 16S rRNA gene in two populations from the eastern basin (Tunisia and Greece) and pooled them with those cited above. The two newly analyzed populations shared the most frequent haplotypes with the other populations and showed no evidence of phylogeographic breaks. We found lower levels of genetic variability in the Adriatic and the Aegean populations, but not in Tunisia, with respect to the Western Mediterranean. Significant differences in pooled haplotype frequencies indicated some genetic differentiation between the pooled Chioggia and Vouliagmeni populations and the other pooled populations.

Key words: Scallop, Sequence, Genetic diversity, Population structure

Resumen

Estructura genética e historia demográfica de la vieira endémica del Mediterráneo Pecten jacobaeus inferidas a partir del análisis de la secuencia del ADN mitocondrial que codifica la subunidad 16S del ARNr. Con vistas a implementar un plan de gestión para las especies cuyas poblaciones están menguando, es fundamental comprender la estructura genética de dichas poblaciones. En el caso de *Pecten jacobaeus*, los estudios genéticos previos se han limitado a analizar poblaciones situadas en el Mediterráneo occidental (España) y en el mar Adriático (Italia). Para comprobar la presencia de discontinuidades filogeográficas entre las dos cuencas del Mediterráneo, hemos estudiado la variabilidad del gen mitocondrial del ARNr 16S en dos poblaciones de la cuenca oriental (Túnez y Grecia) y la hemos analizado junto con la de las mencionadas anteriormente. Las dos poblaciones estudiadas recientemente compartieron los haplotipos más frecuentes con las otras y no se encontraron indicios de que exista una discontinuidad filogeográfica. Se observó un grado menor de variabilidad genética en relación con el Mediterráneo occidental en las poblaciones del Adriático y el Egeo, pero no en Túnez. Las diferencias significativas observadas cuando se agruparon los datos sobre las frecuencias haplotípicas indicaron la existencia de una cierta diferenciación genética entre las poblaciones de Chioggia (Italia) y Vouliagmeni (Grecia) y las de las otras poblaciones.

Palabras clave: Vieira, Secuencia, Diversidad genética, Estructura de la población

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Introduction

Scallops (family Pectinidae) are filter-feeding bivalve molluscs that live mainly on sandy gravel or gravel seabed and colonize all waters from the northern to the southern hemispheres (Gosling, 2003). Due to its high nutritional values, this group is highly prized as a food source (Caers et al., 1999; Palacios et al., 2005; Telahigue et al., 2010). Currently, more than 40 commercial species of scallop are exploited worldwide. The global production from fishing and aquaculture combined reached 2.5 million tonnes (FAO, 2012).

Among pectinids, the scallops of the genus *Pecten* are the most appreciated in Europe. Two *Pecten* taxa can be found on European coasts. The king scallop *Pecten maximus* (Linnaeus, 1758) occurs in the Atlantic, reaching the coasts of northern Africa. It enters the western end of the Mediterranean Sea, but not further than the Almeria–Oran Oceanographic front, which appears to be a barrier to dispersal of this and many other species in the region (Wilding et al., 1999; Ríos et al., 2002; Saavedra and Peña, 2004, 2005; Morvezen et al., 2016). The king scallop is replaced in the remaining part of the Mediterranean Sea by the great Mediterranean scallop *Pecten jacobaeus* (Linnaeus, 1758) (Rombouts, 1991). The two taxa can be easily distinguished by the shell morphology. However, genetic studies carried out with different types of genetic markers, such as allozymes, mitochondrial DNA restriction fragment–length polymorphism, mitochondrial sequences and microsatellites, suggest that the two scallops could be races or subspecies (Wilding et al., 1999; Ríos et al., 2002; Saavedra and Peña, 2004, 2005; Morvezen et al., 2016). For clarity we will use *P. jacobaeus* to refer to the Mediterranean populations throughout the paper.

The great Mediterranean scallop *P. jacobaeus* occurs in exploitable quantities only in the northern Adriatic Sea. Fishing activities using dredges and trawls came into use in the early 1980s, and the total annual landing has since declined. After 30 years of exploiting the natural beds of this bivalve (particularly in the Adriatic Sea), stocks are now severely depleted (Pranovi et al., 2001; Katsanevakis, 2005). To manage and conserve the species, a better understanding of the genetic variability and genetic population structure is essential (Moritz, 1994; Mahidol et al., 2007).

Several studies have shown that Mediterranean marine species often exhibit important genetic differentiation between western and eastern Mediterranean populations. Such differentiation includes phylogeographic breaks, attributed to the isolation of stocks due to sea level changes during the Pleistocene glaciations (Patarnello et al., 2007; Borrero–Pérez et al., 2011; Bowen et al., 2016). Quite often, genetically differentiated populations are restricted to specific regions such as the Adriatic, the Aegean, or the Gulf of Gabès (Rabaoui et al., 2011; Cordero et al., 2014).

In the case of *P. jacobaeus*, the data available about the genetic population structure of the species are very limited. Previous studies in this species showed relatively little genetic differentiation between populations and an absence of phylogeographic subdivision

(Wilding et al., 1999; Ríos et al., 2002; Saavedra and Peña, 2004, 2005; Morvezen et al., 2016). However, most studies were based on only a few populations from the west Mediterranean Sea. Only two genetic studies in this species have considered samples from the eastern Mediterranean Sea, and these were limited to a single population from the northern Adriatic (Ríos et al., 2002; Saavedra and Peña, 2005). This population showed less genetic variability than other *P. jacobaeus* populations from the western Mediterranean, but it was not possible to state if this was a local effect or a feature shared by all eastern Mediterranean populations. Moreover, no statistically significant genetic differentiation between all populations was found. But since a large part of the distribution area of the species in the Eastern Mediterranean was not sampled, a conclusion of no genetic subdivision in the species would be premature. Clearly, a genetic study based on a wider sampling of *P. jacobaeus* in the eastern Mediterranean is desirable to clarify the genetic structure of this taxon.

Here we present a study of the genetic structure of *P. jacobaeus* over a broader geographical scale than previous studies. We sampled two further populations in the eastern Mediterranean, one in the Aegean Sea (Greece) and the other in the Gulf of Tunis (Tunisia). These new samples expand the geographic area covered to the south and the east. We analyzed these populations together with those studied previously by Saavedra and Peña (2005), thus covering the majority of the range of *P. jacobaeus*. We used partial sequences of the same mitochondrial 16S ribosomal RNA gene studied by the previous authors. This genetic marker is a powerful tool for measuring genetic variation and gene flow among populations (Duran et al., 2009).

Material and methods

Sample collection

A total of 35 *P. jacobaeus* adults of different sizes were sampled from the eastern basin (fig. 1); 22 individuals were dredged from the open sea near Kelibia in the northeastern coast of Tunisia, and 13 specimens were caught in the marine Lake Vouliagmeni, a lagoon located in the Korinthiakos Gulf, on the Aegean coast of Greece. Tissue samples (adductor muscle) from each individual were soaked in 90% ethanol and sent to the IATS–CSIC laboratory (Castellón, Spain) where the molecular analyses procedures were carried out. Samples with ID numbers 'KEL 1 to 22' and 'VOU 1 to 13' are kept at the IATS–CSIC laboratory and are available upon request to the corresponding author.

DNA extraction, amplification and sequencing

Total DNA was extracted by a salt extraction protocol (Miller et al., 1988). We used primer Pec16S–F1 (5'GTTTTAAGGTCGGGAAAG–3') (Saavedra and Peña, 2005) designed from the complete *P. maximus* 16S rRNA sequence deposited in GenBank (accession no. X92688) and reverse primer 16Sbr of Palumbi

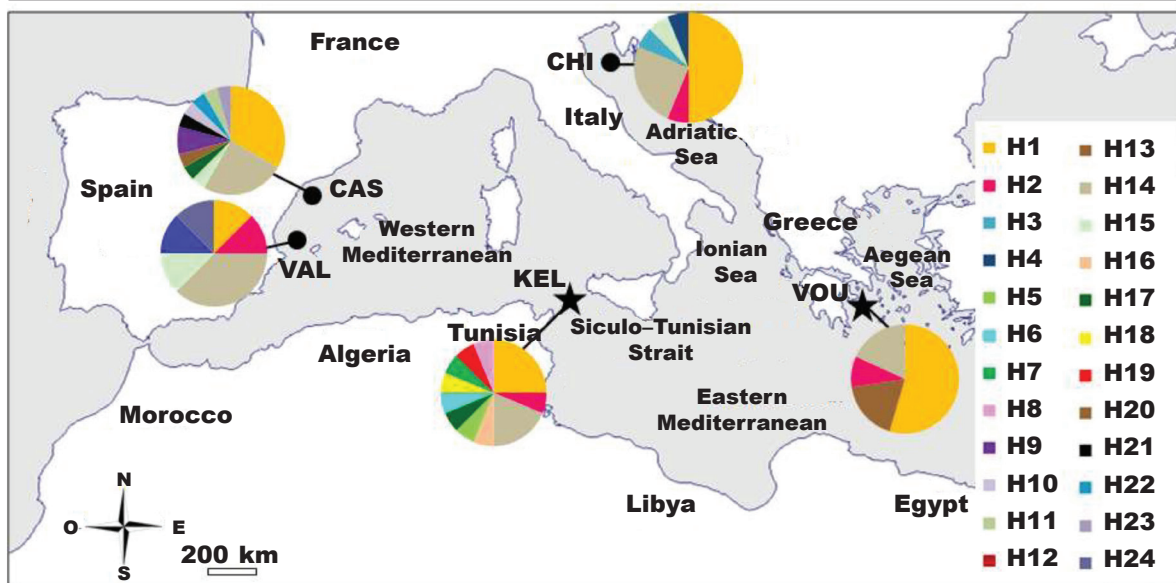


Fig. 1. Geographic location of the two populations of *Pecten jacobaeus* sampled for this study (stars), and of the three previously populations sequenced by Saavedra and Peña (2005) that were included in the analysis (dots): VAL, Valencia; CAS, Castellón; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni. Pie charts show the frequencies of the 16S rRNA haplotypes detected.

Fig. 1. Localización geográfica de las dos poblaciones de *Pecten jacobaeus* muestreadas para este estudio (estrellas), y de las tres poblaciones secuenciadas previamente por Saavedra y Peña (2005) que se han incluido en el análisis (puntos): VAL, Valencia; CAS, Castellón; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni. Los gráficos circulares muestran las frecuencias de los haplotipos detectados en el gen del ARNr 16S.

(1996) (5'CCGGTCTGAACTCAGATCACGT-3') to amplify a fragment of 512 base pairs (bp) of the mitochondrial 16S rRNA gene.

Amplifications were done in 20 μ L reaction volume containing 1 μ L template DNA (~ 500 ng), 0.2 mM of each dNTP, 0.8 μ M of each primer, 1.5 mM of $MgCl_2$, and 0.15 U Taq polymerase (GIBCO–Life Technologies) in the buffer supplied by the manufacturer. PCR was performed using the following parameters: after an initial denaturation at 95°C for 4 min, the PCR mix was subjected to 35 cycles consisting of a 1 min step at 95°C, a 30 s step at 55°C followed by a 30 s step at 72°C, and a final extension of 3 min at 72°C. PCR products were purified with the QiaQuick PCR kit (QIAGEN), and sequenced in an ABI 377 automatic sequencer by using the Big Dye Terminator chemistry in the DNA Sequencing Service of the University of Valencia (Spain). Sequences were edited in BioEdit 7.2.5 (Hall, 1999) and aligned with Clustal W (Thompson et al., 1994), as implemented in BioEdit.

Genetic analyses

Our data were analyzed together with sequences from three other populations, two from the western Mediterranean (CAS and VAL) and one from the Adriatic Sea (CHI) (Saavedra and Peña 2005). Genetic diversity

was estimated for each population with the software DnaSP 5.10.01 (Librado and Rozas, 2009) using several indices, such as the number of haplotypes (h), haplotype diversity (H), number of segregating sites (SS), and nucleotide diversity (θ_{π}). A median-joining (MJ) network was constructed to visualize genetic relationships between mtDNA haplotypes with Network 5.0 software (Bandelt et al., 1999).

Differences in haplotype frequencies between populations were tested using the χ^2 -test (Preacher, 2001). For population structure analysis, we used the Arlequin version 3.5.2.2 program (Fu, 1997) to estimate pairwise F_{ST} -values (Ramos–Onsins and Rozas, 2002) for each population pair using haplotype frequencies. Significance of all pairwise values was measured using 10,000 permutations and assessed using correction for multiple tests under Arlequin. We also applied the same software to examine the distribution of genetic variability into hierarchical levels through Analysis of Molecular Variance (AMOVA) within and between *P. jacobaeus* populations. Nei's genetic distances (Nei, 1972, 1979) were computed from haplotype frequencies and used for constructing a tree showing the similarities between population samples by the Neighbor Joining Method (Saitou and Nei, 1987).

To infer the historical demography, DnaSP was used to conduct neutrality tests of Tajima's D (Tajima,

1989), Fu's F_S (Fu, 1997) and Ramos–Onsins and Rozas' R_2 (Ramos–Onsins and Rozas, 2002) to detect whether there was any deviation from the assumption of neutrality, which would indicate a recent population expansion. Tajima's D is widely used in neutrality tests. It is known that ' D ' is based on the comparison of two different estimates of genetic diversity (Θ and π). ' D ' is expected to be equal to 0 under the neutral equilibrium model. A significant negative value indicates an excess of rare variants as expected under positive selection. It can also reflect a demographic event such as population expansion. A significant positive ' D ' value, in contrast, indicates an excess of intermediate frequency variants reflecting balancing selection or diversifying selection. It can also reflect population structure or bottleneck events (Oleksyk et al., 2010; Camus–Kulandaivelu et al., 2014). Fu's F_S and the Ramos–Onsins and Rozas R_2 -test were confirmed to be the most powerful tools in examining population growth (Ramos–Onsins and Rozas, 2002). The latter was proven to be particularly sensitive for limited sample sizes (Ramos–Onsins and Rozas, 2002). P -values for neutrality statistics were obtained by coalescent simulations with 10,000 replicates.

A goodness of fit test was performed to test the validity of the sudden expansion model using a parametric bootstrap approach based on the sum of square deviations (SSD) between the observed and expected mismatch distributions. The raggedness index, which measures the smoothness of the mismatch distribution, was calculated for each distribution. Small raggedness values represent a population that has experienced sudden expansion whereas higher values of the raggedness index suggest a stationary population or a population that has experienced a bottleneck.

The demographic expansion parameters tau (τ), Θ_0 and Θ_t based on the mismatch distribution outputs from Arlequin were estimated under a demographic expansion hypothesis by a generalized non-linear least-square approach (Schneider and Excoffier, 1999). The time since the expansion (t) was estimated using the Li's formula (Li, 1977): $t = \tau/2u$, where u is the mutation rate per sequence per generation (Schneider and Excoffier, 1999; Rogers, 1995). We adopted the mutation rate of 1.22×10^{-6} proposed by Saavedra and Peña (2005) for the same species and same gene fragment, which is based on fossil calibration.

Finally, mismatch distributions of *P. jacobaeus* populations were performed using DnaSP to test whether demographic processes were consistent with the mismatch distribution test statistics. The distribution is usually multimodal in samples drawn from populations at demographic equilibrium, whereas populations that have gone through a recent demographic expansion are expected to be unimodal (Harpending, 1994).

Results

Sequence variation and haplotype diversity

Sequences of a 512 base pair fragment of the 16S rRNA gene were obtained for 27 individuals from VOU and KEL. The obtained sequences were pooled

with 48 sequences from the populations VAL, CAS and CHI from the study of Saavedra and Peña (2005). Polymorphism was detected at 25 sites: 24 substitutions and two indels (insertion/deletion). Among the substitutions, we identified four transversions and 21 transitions, two sites showed multiple substitutions that allowed detection in twenty-four haplotypes (table 1). Table 2 shows haplotype frequencies. Sixteen haplotypes were previously found by Saavedra and Peña (2005), and eight are new sequences found in KEL and VOU. The sequences of the new haplotypes have been deposited in GenBank under accessions MF183948–MF183955. Among the scored haplotypes, only two (H1 and H4) were common to all studied populations, together representing 60% of the total number of obtained sequences. Among these common haplotypes, haplotype H1 was the most frequent. The remaining haplotypes (71% of total number of haplotypes) were present in a single population (private), with the exception of haplotypes H3, H6 and H10, which appeared in four (VAL, KEL, CHI, VOU), three (VAL, CAS, CHI) and two (CAS, KEL) populations, respectively. Figure 1 shows the geographical distribution of all recorded haplotypes and their respective frequencies per site. The median joining haplotype network (fig. 2) showed two central haplotypes (H1 and H4) linked by four low frequency haplotypes and 17 other haplotypes differing from the central ones by 1–4 mutations. No obvious pattern of haplotype distribution across geographical locations is apparent.

Table 3 shows estimates of gene diversity. Overall, all populations displayed high values of haplotype diversity and low values of nucleotide diversity, as is typical in this species (Saavedra and Peña, 2005). The two populations from the eastern Mediterranean (CHI and VOU) presented the lowest values of haplotype diversity (0.717–0.691) and nucleotide diversity (0.00223–0.00164), while the highest values were observed in KEL (0.925 and 0.0044).

Population genetic differentiation

We quantified the inter-population genetic divergence in haplotype frequencies using the pairwise population differentiation statistic (F_{ST}). The estimated F_{ST} -values varied between 0.006 and 0.117, and were not significant (even with correction for multiple tests), except for the comparison between VOU and VAL (table 4). Since low sample sizes and high haplotype numbers could interfere with statistical detection of genetic differentiation, we performed 2×2 contingency χ^2 -test after pooling the less frequent haplotypes all together, and pooling populations according to their location on the Western or on the Eastern Mediterranean. We carried out two tests that differed in pooling of the Tunisian population of KEL alternatively with the western or with the eastern Mediterranean populations. The rationale for this is that this population is geographically located in the Siculo–Tunisian Strait, just in the area of separation of the two Mediterranean basins. In studies of other species the populations from this region turned out to

Table 1. Variant sites of 16S rRNA haplotypes in *Pecten jacobaeus* scallop: H, haplotype

Tabla 1. Sitios variables de los haplotipos de ARNr 16S en la vieira *Pecten jacobaeus*: H, haplotipo.

	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3				
H	6	7	9	8	9	2	6	7	0	1	3	6	8	1	6	7	0	3	4	5	6	3	3	4	0														
H1	C	-	A	G	A	T	A	A	C	T	-	G	G	A	C	A	A	A	C	T	T	T	C	C	A														
H2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-														
H3	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-														
H4	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-														
H5	T	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-														
H6	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-														
H7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	C	-	-	-													
H8	-	-	G	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-														
H9	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-														
H10	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-														
H11	-	-	-	-	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-	-	-	-	-	-														
H12	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-														
H13	-	-	-	-	-	-	G	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-														
H14	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	G	-	-	-	-	-	-	-	-														
H15	-	C	-	-	-	-	-	G	-	-	-	-	A	-	-	-	G	-	-	-	-	-	-	-	-														
H16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-														
H17	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	G	-	-	-	-	-	-	-	-														
H18	-	-	G	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-														
H19	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	G	-	-	-	-	-	-	-	-	-														
H20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-														
H21	-	-	-	-	-	-	-	-	-	C	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-														
H22	-	-	-	-	-	C	-	-	-	-	-	-	A	-	-	-	-	-	T	-	-	-	-	-	-														
H23	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-														
H24	-	-	-	-	-	-	-	-	T	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-														

be genetically more similar to eastern Mediterranean or to western Mediterranean populations (Patarnello et al., 2007), but in our case data are not conclusive and therefore the two possibilities should be considered. When KEL was pooled with the eastern Mediterranean populations (CHI and VOU) a non-significant result was obtained ($\chi^2 = 1.5$, $df = 2$, $P = 0.22$). In the test in which KEL was pooled with the two western Mediterranean populations (CAS and VAL) we obtained a significant χ^2 of 4.6 ($P = 0.032$, $df = 2$). Moreover, we performed a test between western and eastern populations, excluding KEL. This test was significant ($\chi^2 = 8.11$, $df = 2$, $P = 0.004$). To conclude our analysis of genetic differentiation we performed a hierarchical analysis of molecular variance (AMOVA) taking nucleotide variability into account in addition to

haplotype frequencies. This analysis did not find any significant genetic divergence either among ($P = 0.10$) or within ($P = 0.84$) the western (VAL, CAS and KEL) and eastern (CHI and VOU) Mediterranean basins.

Genetic distances between pairs of populations varied between 0.139 and 0.767. Figure 3 shows the NJ tree obtained from the distance matrix. It reflects the close similarity of CHI and VOU, and the higher divergence of VAL and KEL.

Demographic history of the populations

Results from neutrality tests showed negative values for Tajima's D and Fu's F_s in all studied populations (table 5). Fu's test resulted in highly significant negative F_s values only for KEL (-6.8 ; $P < 0.001$)

Table 2. The haplotype frequencies of 16S rRNA gene in five *Pecten jacobaeus* populations: h, haplotype; VAL, Valencia; CAS, Castellon; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni.

Tabla 2. Frecuencias haplotípicas del gen del ARNr 16S en cinco poblaciones de *Pecten jacobaeus*: h, haplotipo; VAL, Valencia; CAS, Castellón; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni.

h	Haplotype frequency					Total
	VAL	CAS	KEL	CHI	VOU	
H1	1	8	4	8	6	27
H2	–	–	–	–	2	2
H3	1	–	1	1	1	4
H4	3	6	3	4	2	18
H5	–	–	–	1	–	1
H6	1	1	–	1	–	3
H7	–	–	–	1	–	1
H8	–	–	1	–	–	1
H9	–	–	1	–	–	1
H10	–	1	1	–	–	2
H11	–	–	1	–	–	1
H12	–	–	1	–	–	1
H13	–	–	1	–	–	1
H14	–	–	1	–	–	1
H15	–	–	1	–	–	1
H16	–	1	–	–	–	1
H17	–	2	–	–	–	2
H18	–	1	–	–	–	1
H19	–	1	–	–	–	1
H20	–	1	–	–	–	1
H21	–	1	–	–	–	1
H22	–	1	–	–	–	1
H23	1	–	–	–	–	1
H24	1	–	–	–	–	1

and CAS (-5.9 ; $P < 0.001$) populations. However, Tajima's test showed no significant negative D values for all populations except for the KEL sample (1.8 ; $P < 0.001$). The overall negative values resulting from both tests pointed towards an excess of low frequency polymorphisms in relative to expectation. Since the western Mediterranean populations were genetically homogeneous, we carried out the same tests pooling the three samples from this region. The results show a clear deviation from neutrality (D and F_s) and demographic instability (R_2). Potential explanations include population size expansion, positive selection

(Tajima, 1989), purifying selection, and a recruitment of rare alleles from the western or eastern basins.

Figure 4 shows the mismatch frequency spectra for the two populations (KEL, VOU). The studied populations showed a positive skewed unimodal distribution and supported the hypothesis of the sudden expansion model.

None of the sums of squared deviations (SSD) of mismatch distribution (table 6) was significant, indicating that the curves fit the sudden expansion model tested. The significant fit between the observed and the expected distributions was also confirmed by the low and not significant raggedness index values for all studied populations. We noted that the VOU population was distinguished from the other populations with a higher but non-significant R_g value (0.23702). These results were further confirmed by the Ramos-Onsins and Rozas test R_2 . This test gave significant results, rejecting the null hypothesis of constant size and supporting a recent demographic expansion.

The tau value (τ), which reflects the location of the mismatch distribution crest, provided a rough estimation of the time when rapid population expansion started. The observed values of the age expansion parameter (τ) were very close between KEL and VAL (2.250 and 2.695 respectively) suggesting a similar timing of demographic events. In comparison, CHI and VOU showed the lowest τ (0.71). Estimates of Θ_0 and Θ_1 indicated that all studied populations expanded from a very small (close to 0 in almost cases) to a very large size (table 6). The results for the pooled western Mediterranean samples are in the same line. Assuming the mutation rate of 1.22×10^{-6} for 16S gene and the equation $\tau = 2ut$, the time of expansion for *P. jacobaeus* populations likely occurred approximately between 1.10 myr and 0.29 myr before present for VAL and CHI respectively.

Discussion

By sampling two populations of *P. jacobaeus* from Tunisia and Greece, we have increased the number of populations available for the genetic study in the eastern Mediterranean from one to three, and we have also extended the sampled area by $\sim 1,000$ km. We studied a total of five populations, two in the west Mediterranean and three in the eastern Mediterranean. This allowed a comparison between the two basins with acceptable rigor.

The first interesting result of our study is that the 16S haplotype network did not show new clades, in contrast with the network described by Saavedra and Peña (2005), after adding the new populations. This confirms the absence of a phylogeographic break between the two basins that was suggested in the previous study by Saavedra and Peña (2005); however, we note that this does not completely eliminate the possibility of a phylogeographic subdivision in the Mediterranean scallop. For example, in the carpet-shell clam, Cordero et al. (2014) found two mitochondrial clades, but the less frequent clade was restricted to the northern Aegean

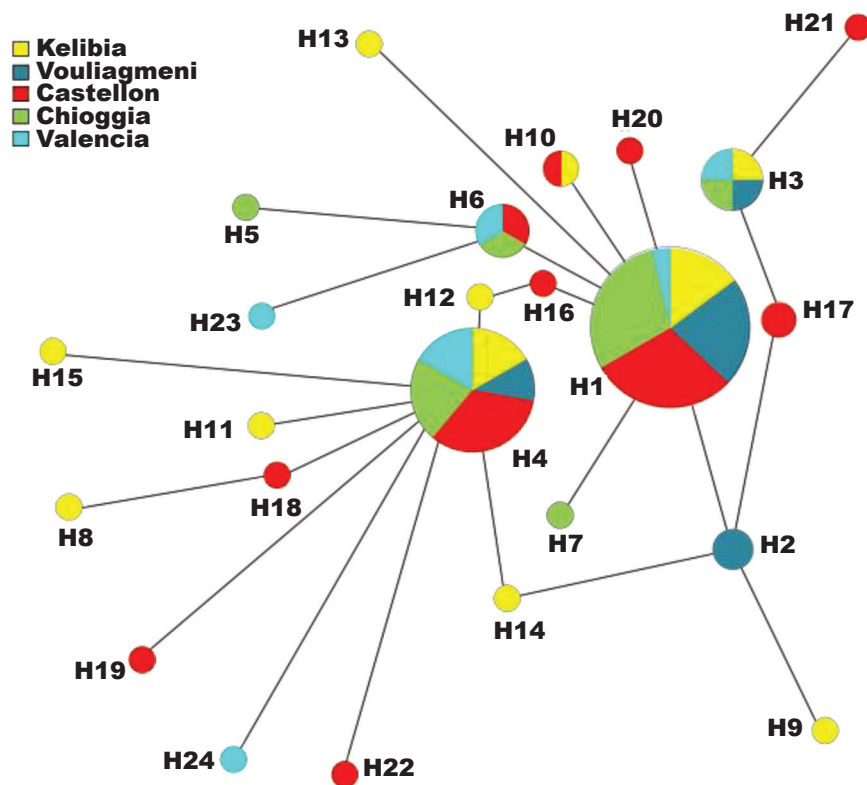


Fig. 2. Median joining haplotype network of the 24 haplotypes determined in *Pecten jacobaeus* specimens at the 16S mitochondrial gene. The circle areas are proportional to haplotype frequency and the lines connecting haplotypes represent one step mutation. The locations of the haplotypes are indicated in color according to the codes in the legend.

Fig. 2. Red de unión de medianas de los 24 haplotipos determinados en los especímenes de *Pecten jacobaeus* en el gen mitocondrial del ARNr 16S. La superficie de los círculos es proporcional a la frecuencia de los haplotipos y las líneas que conectan los haplotipos representan una mutación. La localización de los haplotipos se indica con un código de colores (véase la leyenda).

and Turkey. Therefore, sampling of the easternmost coasts of the Mediterranean, especially the Aegean Sea, would be necessary to be conclusive in the case of the scallop.

Saavedra and Peña (2005) also found no significant differentiation in haplotype frequencies between the three *P. jacobaeus* populations that they studied. We found essentially the same result after adding two eastern Mediterranean populations, as indicated by the non-significant pairwise F_{ST} estimates. However, there was an exception in the comparison of VOU with respect to VAL, which gave a significant F_{ST} value of 0.11. This is a relatively high value for a marine species with a planktonic larval stage that lasts for several weeks. Genetic divergence in this case could be the result of the long geographic distance (3,000 km) that separates these two localities, but the lack of significant differentiation between CAS (which is very close to VAL) and VOU casts

doubt on this explanation, and other explanations should be sought. The VOU sample was taken from Lake Vouliagmeni, which originated ca. 2,000 years ago (Papapetrou–Zamanis, 1969). The lake was originally brackish, as indicated by the fossil fauna recovered, but a channel was open some 100 years ago to connect the lake with the sea and the fauna of the lake was replaced by typically marine species (Vardala–Theodorou and Nicolaidu, 2007). It is at that time when the origin of the VOU scallop population can be established. Since the area of the lake is small (1.8 km long), it is most likely that the population size is small. Consequently, genetic drift should be an important factor acting on the genetic pool of this population and could increase its genetic differentiation over levels typical of open sea populations. On the other hand, the VOU and VAL samples were the smallest in our study, and therefore another possibility is that these samples were

Table 3. Sample size (N), number of haplotypes (h), haplotype diversity (H), segregating sites (SS) and nucleotide diversity based on the pairwise difference between sequences (π) of 16S mitochondrial gene for five *Pecten jacobaeus* populations: VAL, Valencia; CAS, Castellon; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni.

Tabla 3. Tamaños de muestra (N), número de haplotipos (h), diversidad haplotípica (H), sitios segregantes (SS) y diversidad nucleotídica basada en las diferencias entre pares de secuencias (π) del gen del ARNr 16S en cinco poblaciones de *Pecten jacobaeus*: VAL, Valencia; CAS, Castellón; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni.

Population	N	h	$H \pm SD$	SS	$\pi \pm SD$
VAL	8	6	0.893 \pm 0.111	6	0.00394 \pm 0.00088
CAS	24	11	0.841 \pm 0.056	13	0.00336 \pm 0.00057
KEL	16	11	0.925 \pm 0.050	13	0.00444 \pm 0.00080
CHI	16	6	0.717 \pm 0.099	6	0.00223 \pm 0.00056
VOU	11	4	0.691 \pm 0.128	3	0.00164 \pm 0.00042
Total	75	24	0.814 \pm 0.035	23	0.00310 \pm 0.00033

Table 4. Pairwise genetic differentiation statistics (F_{ST}) (below diagonal) and their associated probabilities (P) for the null hypothesis of $F_{ST} = 0$ (above diagonal) among the studied populations of *Pecten jacobaeus*, based on 16S sequences. The bold P -value remained significant after multitest correction: VAL, Valencia; CAS, Castellon; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni; * significant at $p = 0.05$.

Tabla 4. Parámetros estadísticos de diferenciación genética (F_{ST}) (debajo de la diagonal) y sus probabilidades asociadas (P) para la hipótesis nula de $F_{ST} = 0$ (encima de la diagonal), entre parejas de las poblaciones estudiadas de *Pecten jacobaeus*, basados en secuencias parciales del gen del ARNr 16S. El valor de P en negrita se mantuvo significativo tras la corrección multitest: VAL, Valencia; CAS, Castellón; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni; * valores significativos para $p = 0,05$.

	VAL	CAS	KEL	CHI	VOU
VAL	–	0.47273	0.58182	0.19091	0.02727
CAS	0.01195	–	0.94545	0.47273	0.60909
KEL	0.01193	0.01998	–	0.17273	0.23636
CHI	0.04851	0.00766	0.02316	–	0.40909
VOU	0.1174*	0.02933	0.00989	0.00617	–

affected by larger sampling variances of haplotype frequencies, which could have resulted in biased, significant F_{ST} estimates (Kitada et al., 2007). Alternatively, we could be experiencing type II errors in the pair-wise comparisons due to the lack of power to detect small genetic differences among populations. This is suggested by the results of the chi-squared tests with pooled samples and haplotypes. Pooling samples can help to overcome the power problem due to small sample sizes. In this case, the results of the χ^2 -tests after pooling samples gave statistical

support to a west-east differentiation when the KEL population was considered genetically similar to the western Mediterranean, and when KEL was excluded from the tests. Therefore, these tests suggest that the western and eastern basins could exhibit a lower connectivity than the populations located within each of the two basins. A number of studies have shown a decrease in gene flow across the Siculo-Tunisian Strait, which has been recognized as a barrier to gene flow between eastern and western groups for several benthic species with long pelagic larval

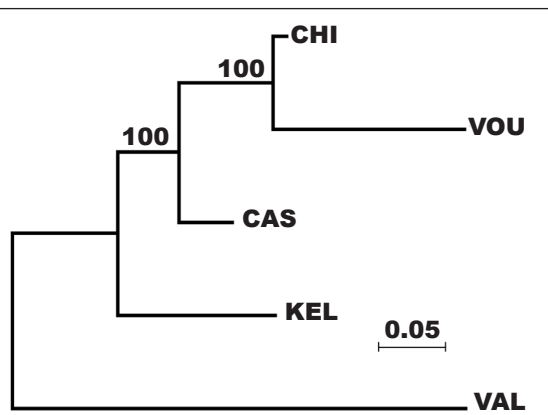


Fig. 3. Unrooted neighbor joining tree showing the genetic differences between populations, based on Nei's genetic distances computed from haplotype frequencies. Significant bootstrap values are also indicated.

Fig. 3. Árbol de unión de vecinos sin raíz que muestra las diferencias genéticas entre poblaciones, basado en las distancias genéticas de Nei calculadas a partir de las frecuencias haplotípicas. Se indican también los valores de bootstrap significativos.

This leads to the hypothesis that the influence of the Atlantic populations contributes to the observed patterns. The Atlantic influence can result from gene flow facilitated by the current that enters the western Mediterranean from the Atlantic and flows eastward along the northern coasts for Africa, past the Almeria–Oran front (Millot and Taupier–Letage, 2005).

The second interesting result of our study regards the levels of intra-population genetic variability. Saavedra and Peña (2005) described lower levels of genetic variability in the Adriatic population (CHI) than in the western Mediterranean populations (VAL and CAS). We found that the Aegean population (VOU) shares this feature with CHI, but that the Tunisian population (KEL) does not, and that it currently shows the highest levels of variability. The high level of genetic variability observed in KEL contrasts with the other two eastern Mediterranean populations, which show only half or a quarter of the nucleotide variability found in the Tunisian population. A number of factors could contribute to this result. One factor could be a gene diversity input from the Atlantic and western Mediterranean favored by the North African current, which flows from the Atlantic to the eastern Mediterranean along the North African coast (Patarnello et al., 2007). This possibility is supported by the χ^2 -test of genetic differentiation with pooled populations and haplotypes, which suggest a higher similarity of KEL to the western Mediterranean populations. In other species, the populations of the northern Tunisian coasts often show similarities to the western Mediterranean populations (Bahri–Sfar et al., 2000; Cordero et al., 2014). Furthermore, the apparent west–east cline for the two most common haplotypes, the highest amount of private haplotypes and genetic variability recorded for the western populations may suggest that the western populations

stages (Nikula and Vainola, 2003; Zitari–Chatti et al., 2009; Tir et al., 2014). However, the NJ tree based on Nei distances shows that the genetic differentiation increases on approaching the Strait of Gibraltar.

Table 5. Statistical tests of neutrality and estimates of demographic parameters in populations of *Pecten jacobaeus*, based on partial sequences of the 16S rRNA gene: VAL, Valencia; CAS, Castellon; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni; WM, West Mediterranean (pooled VAL, CAS, KEL) (* $P < 0.05$ ** $P < 0.001$).

Tabla 5. Pruebas estadísticas de neutralidad y estimaciones de los parámetros demográficos en poblaciones de *Pecten jacobaeus*, basados en secuencias parciales del gen del ARNr 16S: VAL, Valencia; CAS, Castellon; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni; WM, Mediterráneo occidental (agrupación VAL, CAS, KEL) (* $P < 0,05$ ** $P < 0,001$).

	Mismatch	SSD (P)	Tajima's D	Fs	R _g (P)	R ₂
VAL	2.500	0.033 (0.35)	-0.63262	-2.050 (0.057)	0.03444 (0.940)	0.177**
CAS	1.834	0.002 (0.65)	-1.76498	-5.866 (0.001)**	0.04513 (0.280)	0.123**
KEL	2.258	0.001 (0.97)	-1.80726**	-6.830 (0.001)**	0.05201 (0.230)	0.141**
CHI	1.367	0.006 (0.53)	-1.27752	-1.742 (0.089)	0.04611 (1.000)	0.137**
VOU	0.836	0.035 (0.21)	-0.62785	-1.116 (0.079)	0.23702 (0.440)	0.155**
WM	1.151	0.000 (0.70)	-1.92370*	-16.942 (0.00)**	0.03651 (0.550)	0.106**

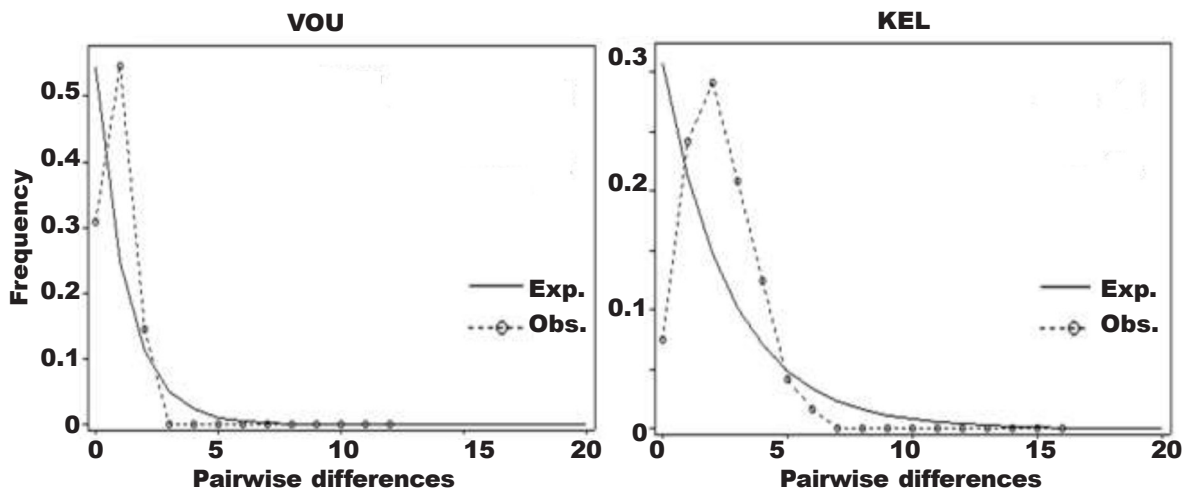


Fig. 4. Observed and expected pairwise mismatch distributions in the two *Pecten jacobaeus* populations (VOU and KEL) under the sudden population expansion model. The number of pairwise nucleotide differences between haplotypes is represented on the abscissa whereas their frequencies are represented on the ordinate axis: KEL, Kelibia; VOU, Vouliagmeni.

Fig. 4. Distribuciones de desajuste observadas y esperadas en las dos poblaciones de *Pecten jacobaeus* muestreadas (VOU y KEL), utilizando el modelo de expansión súbita. El número de diferencias nucleotídicas entre pares de haplotipos se representa en el eje de abscisas mientras que sus frecuencias se representan en el eje de ordenadas: KEL, Kelibia; VOU, Vouliagmeni.

are ancestral and are the source of the eastern populations, with KEL representing an intermediate transition sample between the two basins. Another factor could be that the Adriatic and Aegean populations have been affected by population-specific events that resulted in lower effective population size (N_e) and subsequently in the loss of intrapopulation diversity. The smaller N_e in the Adriatic could be caused by a restriction of available habitat during the glacial epoch, present-day geographic isolation, or historical demographic instability. The Adriatic Sea disappeared almost completely during the last glacial maximum (ca. 18,000 years BP) (Lambeck and Purcell, 2005), and the scallop populations now living in the northern Adriatic certainly derive from a recolonization process which might have resulted in lower genetic variability. Finally, fishing could affect variability levels through its effect on the effective population size. The Adriatic scallop population is the most exploited in the Mediterranean Sea. Several authors have reported large fluctuations of populations and landings of this scallop over the past 50 years (Orel et al., 1993; Mattei and Pellizzato, 1996). Overexploitation activities could cause bottleneck effects and alter genetic diversity within populations, as recorded in several commercially exploited scallops (Saavedra and Peña, 2004; Gaffney et al., 2010; Bert et al., 2011).

Low variability in the VOU population could have resulted from a founder effect and a bottleneck occur-

ring at the moment the population became established a century ago, and could have subsequently reduced N_e . Several studies have recorded low intrapopulation diversity in bivalves that have experienced severe genetic drift due to bottlenecks and/or founder events (Jordaens et al., 2000; Tarnowska et al., 2010; Barbieri et al., 2015). According to Hanzawa et al. (2012), the founder events when the marine lakes were formed must have affected the genetic diversity of the present population. The higher value of R_2 and lower t showed by VOU in the demographic analysis support this view.

In conclusion, our study has clearly determined the absence of a phylogeographic subdivision between the western and the eastern Mediterranean populations of *P. jacobaeus*, and has shown that the populations from the northern coasts of the eastern Mediterranean have lower genetic variability levels due to local historical events that would have affected their N_e . The possibility of a phylogeographic subdivision in the easternmost populations (Aegean Sea, Levantine Sea) remains to be explored. The use of 16S rRNA or other mitochondrial marker could be a nice tool for this. However, the increasing availability of nuclear markers in this species will be of great help for this purpose. In their study on the carpet-shell clam, Cordero et al. (2014) observed a high homogeneity across the Atlantic, the western Mediterranean and the eastern Mediterranean at the mitochondrial marker. However, the study of

Table 6. Parameters for *Pecten jacobaeus* populations obtained from mismatch distribution analyses: age of expansion in units of mutational time (τ), population size before (Θ_0) and after (Θ_1) the expansion in units of mutational time and time since expansion expressed in million years t : VAL, Valencia; CAS, Castellon; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni; WM, West Mediterranean (pooled VAL, CAS, KEL).

Tabla 6. Parámetros de los análisis de la distribución del desajuste de secuencias entre poblaciones de *Pecten jacobaeus*: edad de la expansión en unidades de tiempo mutacional (τ), tamaño de la población antes (Θ_0) y después (Θ_1) de la expansión, en unidades de tiempo mutacional, y tiempo desde la expansión expresados en millones de años t : VAL, Valencia; CAS, Castellón; KEL, Kelibia; CHI, Chioggia; VOU, Vouliagmeni; WM, Mediterráneo occidental (agrupación VAL, CAS, KEL).

Populations	τ	Θ_0	Θ_1	t
VOU	1.000	0.000	3407	0.41
CAS	1.875	0.000	3444	0.77
KEL	2.250	0.000	3469	0.92
VAL	2.695	0.000	27	1.10
CHI	0.7111	0.072	7400	0.29
WM	1.956	0.083	65042	0.78

six nuclear intronic markers revealed that three of these showed clear differentiation between the three basins. In the case of scallops, the microsatellite data reported by Morvezen et al. (2016) suggest that there is greater genetic differentiation for nuclear markers than for mitochondrial DNA between the Atlantic and the western Mediterranean populations, so it is possible that the same could be happening to the genetic differentiation between the western and the eastern basins, or even among other smaller regions within the Mediterranean Sea. Finally, the use of nuclear markers and more extensive sampling will be necessary to determine whether there is a reduced genetic connectivity between scallop populations living in the eastern and western Mediterranean basins.

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