



Spatial genetic patterns of *Octopus vulgaris* Mediterranean populations support the hypothesis of a transitional zone across the Siculo-Tunisian Strait

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Abstract Recent research hypothesised that the Siculo-Tunisian Strait might fit, at least for some species, the picture of a genetic transitional zone instead of a sharp genetic break between the Western and Eastern Mediterranean basins. The present study aimed at using the common Octopus, *Octopus vulgaris* as an empirical test-case to evaluate this hypothesis. To accomplish this goal, 458 new sequences of the mitochondrial gene *cytochrome c oxidase* subunit I were used. Combining the new sequences with those

available on public databases, we assembled a dataset containing 920 sequences to investigate the spatial genetic patterns across 34 Mediterranean populations of *O. vulgaris*. The genetic structure of this species was assessed combining analysis of molecular variance and Median-Joining networks. Results supported the hypothesis of a complex spatial genetic pattern across the Sicilian channel. Contemporary factors, such as marine currents, likely affect the species' genetic structuring across this area. Overall, our results highlighted that focusing the attention on the whole transitional area rather than on a unique genetic break might help to detect similar patterns across different species. Finally, acknowledging the occurrence of complex spatial genetic patterns across transitional zones may improve stock identification and management practices for commercially valuable species.

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Introduction

Improving the knowledge on species' spatial genetic patterns is crucial for future conservation and management plans, with an emphasis on those commercial species for which management strategies have not been sufficiently enforced (Leonart & Maynou, 2003). This task is particularly important in the Mediterranean Sea, where anthropogenic pressures and ongoing climate change are likely to have large and increasing impacts on marine biodiversity (Lejeusne et al., 2010; Coll et al., 2012). The Mediterranean Sea represents a natural focus of research interest for marine biodiversity related to other World's regions (Bianchi, 2007; Lejeusne et al., 2010; Coll et al., 2012). In fact, the Mediterranean harbors, on average, 7% of total World marine biodiversity, with high proportions of both species' richness and endemics, despite it represents only 0.8% and 0.3% of the World ocean area and volume, respectively (Coll et al., 2012 and references therein). Moreover, the semi-enclosed and highly compartmentalized nature of the Mediterranean, make it a potential model to study marine ecosystems (Lejeusne et al., 2010; Coll et al., 2012). Because of the complex topography, the variable oceanographic and ecological features, the present-day variety of climatic conditions, and the complex geological history, the Mediterranean is subdivided into distinct sub-basins (Bas, 2009), marine ecoregions (Spalding et al., 2007) and biogeographic sectors (Bianchi, 2007). Thus, the Mediterranean offers the opportunity of investigating how the interplay between abiotic and biological factors shape interspecific as well as intraspecific diversity across transitional zones (Patarnello et al., 2007).

From a biogeographic perspective, increasing attention has been long paid to the boundary between the Western and the Eastern basins of the Mediterranean Sea, which lies between the Siculo-Tunisian strait (STS) and the eastern limit of the Ionian Sea (Villamor et al., 2018). Based on evidence from literature, Bianchi (2007) outlined four main hypotheses for the placement of the boundary between the

Western and the Eastern Mediterranean basins. In the first hypothesis (*i*), the boundary was placed in the mid Ionian Sea, thus including Sicily, Calabria and the Gulf of Taranto in the Western Mediterranean basin. Alternatively (hypothesis *ii*), the boundary was placed to the middle of STS, with the Pantelleria Island included in the Western Mediterranean, whilst the Pelagie and Malta Islands in the Eastern Mediterranean. Another scenario (hypothesis *iii*) placed the boundary so that Western Mediterranean included yet the whole Sicily but not the Ionian coast of Calabria and the Gulf of Taranto. Finally, the location of the boundary was proposed to be at the Peloponnese and the Aegean Sea, rather than at the STS (hypothesis *iv*). From the genetic viewpoint, the picture is much more complex: most of investigations highlighted divergence between Western and Eastern Mediterranean populations, although other subdivisions of the Mediterranean Sea might have been overlooked (*e.g.*, Borrero-Pérez et al., 2011; Mokhtar-Jamai et al., 2011; Cossu et al., 2015). For instance, two genetic breaks (the Almeria-Oran front and the Gulf of Bejaia) delimit a wide mosaic hybrid zone along Algerian coasts, in which deeply divergent lineages of *Mytilus galloprovincialis* Lamarck, 1819 mix (El Ayari et al., 2019). Moreover, apart from the recognition of biogeographic subdivisions, tracing the precise boundary between the two major Mediterranean basins is still a debated issue. In fact, the location of genetic breaks varies among species, ranging from the STS (Borsa et al., 1997; Tarnowska et al., 2010; Souissi et al., 2018) to east off the Sicilian coasts (Sá-Pinto et al., 2010, 2012; Sanna et al., 2013) and even further east in the mid or eastern Ionian Sea (Boissin et al., 2011; Ragionieri & Schubart, 2013; Villamor et al., 2014; Deli et al., 2018). There are also species that do not show genetic structuring across this boundary area, including fishes (Fruciano et al., 2011; Schunter et al., 2011a, b; Barbieri et al., 2014) and marine invertebrates (Maltagliati et al., 2010; Fratini et al., 2016; Pannacciulli et al., 2017). Likely, this variety of outcomes reflects the interplay between the species' evolutionary histories (Patarnello et al., 2007), the life-history traits and environmental factors such as oceanographic features or inadequate sampling (Pascual et al., 2017). In light of the above considerations, the whole area encompassing the south Tyrrhenian Sea, the Sicilian channel and the Ionian Sea could be viewed as a “transitional zone” between

the Western and Eastern Mediterranean basins, which is characterised by heterogeneous levels of admixture in the area, as proposed by Villamor et al. (2018). According to these authors, this picture would fit better than a sharp genetic break, whereby there is an abrupt shift of allele frequencies at a geographic location, to explain the complex genetic patterns reported in this area so far.

In the present study, we used the common octopus, *Octopus vulgaris* Cuvier 1797 (Mollusca: Cephalopoda), as an empirical test-case of the hypothesis advanced by Villamor et al. (2018). The common octopus is a benthic cephalopod of commercial interest distributed in the whole Mediterranean Sea, Atlantic Ocean and North-West Pacific (Warnke et al., 2004). Although *O. vulgaris* is likely a complex of cryptic species rather than a widespread cosmopolitan species (Sales et al., 2013; De Luca et al., 2014), Mediterranean populations of *O. vulgaris* belong unambiguously to a single species (De Luca et al., 2016; Melis et al., 2018). The common octopus lives in a variety of habitats, such as rocky, sandy and muddy bottoms as well as seagrass beds, from the coastline to the outer edge of continental shelf at depths of approximately 200 m (Belcari et al., 2002). The species has a short-life cycle (9–15 months) including a paralarval stage (Smale & Buchan, 1981; Hernández-López et al., 2001). Paralarvae, which are morphologically very similar to adults, hatch from the eggs and play an important role in dispersal as they conduct an active pelagic life for 35–60 days depending on water temperature (Villanueva et al., 1997; Katsanevakis & Verriopoulos, 2006; O'Connor et al., 2007). Therefore, gene flow in *O. vulgaris* is likely to be affected by oceanographic features and biogeographic barriers (Maltagliati et al., 2002). In contrast to paralarvae, adult migrations are of very restricted range, including daily migrations for forage (Mather, 1994) and seasonal migrations of females in relation to reproductive behavior (Smale & Buchan, 1981). Reasonably, the contribution of adult migrations to gene flow can be considered negligible if compared to paralarval dispersal.

As *O. vulgaris* is a commercially valuable species in Mediterranean fisheries, species' patterns of genetic diversity have been widely investigated from regional-to basin-scale level in the Mediterranean region (Casu et al., 2002; Maltagliati et al., 2002; Cabranes et al., 2008; Fadhlouï-Zid et al., 2012; De Luca et al., 2016;

Melis et al., 2018). Overall, the hypothesis of a single panmictic unit within the Mediterranean Sea was rejected. For instance, Melis et al. (2018) found shallow but significant genetic structuring at regional scale along Sardinian coasts by using microsatellites. De Luca et al. (2016) highlighted the strong genetic divergence of a population from the Strait of Messina to the other Mediterranean populations, which might clue into the isolation of the marine biota within this strait (Bianchi, 2007). On the other hand, an isolation-by-distance driven pattern of genetic variation was inferred along Spanish coasts (Cabranes et al., 2008). At larger spatial scales, the presence of a genetic break between the Western and Eastern Mediterranean basins was evidenced using allozymes (Maltagliati et al., 2002), and *cytochrome c oxidase* subunit III (Fadhlouï-Zid et al., 2012) but it was not clearly supported by microsatellites (De Luca et al., 2016).

The objective of the present study was to investigate the spatial genetic structure of *O. vulgaris* across the Mediterranean Sea, focusing our attention on the Siculo-Tunisian Strait and the surrounding areas. We aimed at ascertaining whether spatial genetic variation fits a scenario characterized by a sharp phylogeographic break, taking into account two potential locations of it (namely Bianchi's hypotheses ii and iii) or, alternatively, a transitional area with smoothed genetic change. To achieve this goal, we used the mitochondrial gene *cytochrome c oxidase* subunit I (COI), which is effective to infer both the historical and contemporary processes shaping the spatial genetic patterns in various marine invertebrates (Sanna et al., 2013, 2014; Cordero et al., 2014; Cossu et al., 2015; Pannacciulli et al., 2017; Villamor et al., 2018).

Materials and methods

COI amplification and sequencing

COI fragments of 641 bp were amplified from genomic DNA stored at -20°C using Folmer et al. (1994) primers LCO1490 (5'-GGTCAACAAATCA-TAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). Each 25 μl PCR mixture contained approximately 100 ng of total genomic DNA, 0.40 μM of each primer, 1.75 U of Taq DNA Polymerase (Sigma-

Aldrich®), 1 × reaction buffer and 200 μM of each dNTP. MgCl₂ concentration was set at 2 mM, and 12.5 mg of BSA were added to the reaction mixture. PCRs were performed according to the following steps: 1 cycle of 3 min at 94°C, 35 cycles of 40 s at 94°C, 45 s at 51°C and 1 min and 40 s at 72°C, with a final extension performed at 72°C for 5 min. After that, PCR samples were cooled to 4°C. Negative controls were included for each primer to check for contamination. After electrophoresis on 2% agarose gels, the PCR products were purified using ExoSAP-IT (USB Corporation, Santa Clara, California, USA) and sequenced using an external sequencing core service (Macrogen Europe, Amsterdam, Netherlands). Dual peaks of similar height, which could be interpreted as evidence of mitochondrial pseudogenes in the nucleus (Numts) or heteroplasmy, were not observed in any of the electropherograms. The PCR products did not show any occurrence of aspecificity, excluding the possibility of multiple nuclear mtDNA-like sequences.

Dataset assembling

A dataset containing 920 partial sequences of the mitochondrial gene *cytochrome c oxidase* subunit I (COI) was assembled. Of these, 458 COI fragments (Table 1) were newly sequenced from genomic DNA obtained from previous studies (Casu et al., 2002; Fadhlaoui-Zid et al., 2012), whereas the remaining sequences were retrieved from Genbank (Fadhlaoui-Zid et al., 2013; Amor et al., 2014; Melis et al., 2018; Jassoud et al., unpubl. data), Barcode Of Life Data System (BOLD, Roura et al., 2019) and from supplementary material (De Luca et al., 2016). Only localities with a sample size of at least five sequences were retained in the present study. Overall, the dataset encompassed 34 Mediterranean localities (Online Resource 1: Table S1, Fig. 1).

Data analysis

Sequences were visualised and checked with Chromas Pro 1.7.6 (<http://technelysium.com.au>) and aligned using the CLUSTAL W algorithm (Thompson et al., 1994) implemented in the software BioEdit 7.2.3 (Hall, 1999). Neither insertions nor deletions were found in the final alignment. Only one new haplotype was found in the Mediterranean, which was deposited

in GenBank with the following accession number: MT812944.

The software POWSIM 4.1 (Ryman & Palm, 2006) adjusted for mtDNA data (Larsson et al., 2009) was used for testing the power of detecting population differentiation. For each independent simulation, 1000 pseudo-datasets simulating the same number of populations (N), haplotypes and population sampling size (S) as the real dataset were created. Each simulation tested the power of detecting population differentiation at a different level of genetic differentiation ($F_{ST} = 0$ to 0.2), whose range was chosen based upon observed pairwise F_{ST} values (Fig. 2). Two further simulation sets investigated the power of detecting population differentiation at small and uniform sampling sizes ($S = 5$ and 10). All simulations were run assuming a constant effective population size of 2000 individuals (N_e), no migration across demes, and varying the number of generations of genetic drift (T) in order to obtain the expected level of genetic differentiation at equilibrium. Finally, the effect of N and S on the expected level of statistical type II error (the proportion of simulations in which population differentiation is detected when $F_{ST} = 0$, which is expected to be around 5%) was assessed. Three independent sets of simulations ($N = 10, 20, 30$) were carried out varying the sampling size ($S = 5$ to 50 by steps of five individuals) and keeping generations of genetic drift constant ($T = 0$).

Analysis of molecular variance (AMOVA), as implemented in Arlequin 3.5.2.3 software (Excoffier & Lischer, 2010), was used to partition the genetic variance in the within- and among-sample components. Then, different grouping criteria were adopted depending on the Western-Eastern Mediterranean boundary considered, namely the boundary located at the STS, and off the eastern Sicilian coast (hypotheses *ii* and *iii* in Bianchi, 2007). Estimates of genetic divergence among all groups were calculated using Φ -statistics. Three hierarchical levels of subdivision were obtained: Φ_{CT} (the degree of differentiation among basins), Φ_{SC} (the degree of differentiation among samples within basins), and Φ_{ST} (the degree of differentiation among samples). Φ -statistics were computed using the genetic distances estimated with the Tamura (1992) model of sequence evolution, selected with the Bayesian information criterion (BIC) in MEGA 7.0.14 (Kumar et al., 2016). In addition to the Φ -statistics, which includes information on both

Table 1 Partial sequences of Cytochrome c oxidase subunit I (COI) that were newly sequenced for the present study from genomic DNA obtained in previous studies (Casu et al. 2002; Fadhlouli-Zid et al. 2012)

Population	Label	New sequences/Sample size
Vigo	VIG	16/27
Barcelona	BAR	17/23
Sete	SET	8/10
Alghero	AHO	19/26
Porto Torres	PTO	22/23
Olbia	OLB	27/28
Galite Island	GAI	48/49
Leghorn	LEG	21/28
Porto Santo Stefano	PSS	24/29
Naples	NAP	26/30
Strait of Sicily	SSI	19/20
Malta	MAL	24/24
Portopalo	PPA	15/15
Coastal Kerkennah Islands	CKI	31/31
Deep-water of Kerkennah Islands	DWK	43/45
Zarzis	ZAR	37/37
Tajoura	TAJ	19/20
Ancona	ANC	16/19
Heraklion	CRE	26/29
Total		458

For more details on localities see Table S1

haplotype frequency and genealogy, the Weir & Cockerham (1984) F -statistics, which takes into account only haplotype frequency, was also computed. The latter can be more powerful than Φ -statistics at detecting significant differentiation, when haplotype divergence does not contain any relevant information (Penant et al., 2013). Furthermore, since F -statistics does not depend on the degree of genetic distances among haplotypes, in terms of accumulation of mutations, it can detect population differentiation even after few generations of genetic drift. A non-parametric permutation method with 10000 replicates was used to test whether the derived indices were significantly different from zero.

A spatial analysis of molecular variance (SAMOVA) implemented in SAMOVA 2.0 (Dupanloup et al., 2002) was used to retrieve the likely number of hierarchical groups explaining most of the genetic structure. The method aims at finding the grouping scheme that maximizes variance among groups, without a prior population structure definition. The software was run with 100 random initial conditions for 10000 iterations, with number of tested groups, $K = 2$ to 7, and the most likely genetic structure was inferred either with or without spatial

information on the sampling localities of *Octopus vulgaris*. The best partition obtained by SAMOVA was then used in the AMOVA framework as outlined above.

The relationships among haplotypes were analysed by a Median-Joining Network with default values (Bandelt et al., 1999) using the PopART v.1.7 software (Leigh & Bryant, 2015). Finally, the genetic structuring resulting from SAMOVA and the spatial distribution of haplotypes were superimposed on a map depicting the mean surface marine currents of the Mediterranean Sea over the years from 1998 to 2015. The map was Generated using the European Union (EU) Copernicus Marine Service Information (<http://marine.copernicus.eu/>): monthly mean currents over the period considered here were downloaded by Mediterranean Sea physical reanalysis (MEDREA 1987-2015) dataset (Simoncelli et al., 2014), whose model horizontal grid resolution is $1/16^\circ$ (about 6–7 km). The resulting netCDF file was uploaded within the R 3.5.2 statistical environment (R Core Team, 2015) using the library `ncdf4` 1.17 (Pierce, 2019). The netcdf file was cropped in order to contain only months from July to December, which correspond to the reproductive and recruitment period of *O.*

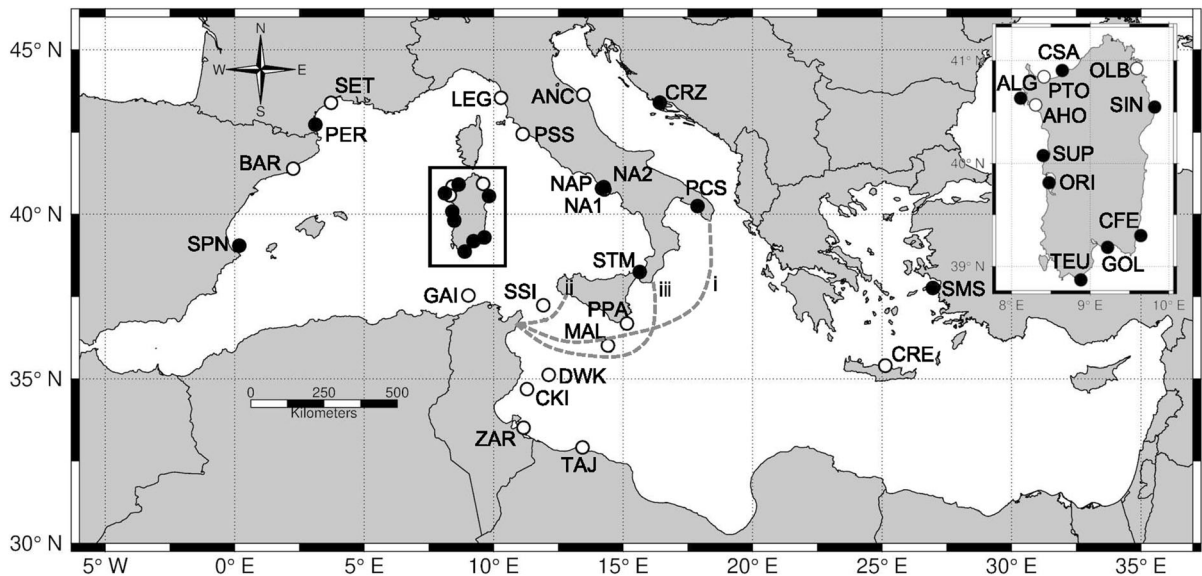


Fig. 1 Sampling locations of *Octopus vulgaris*. Open circles mark localities at which Cytochrome c oxidase subunit I (COI) was newly sequenced, whereas closed circles mark localities at which COI partial sequences were retrieved from Genbank (see Online Resource 1: Table S1 for further details). Gray dotted lines outline three hypotheses advanced for the location of the biogeographic boundary between Western and Eastern Mediterranean (modified from Bianchi 2007). The inset shows the localities sampled in Sardinia. *SPN* Valencia, *BAR* Barcelona, *PER* Perpignan, *SET* Sete, *ALG* Alghero 2010–2012, *AHO*

Alghero 1999, *PTO* Porto Torres, *CSA* Castelsardo, *SUP* Su Pallosu, *ORI* Oristano, *TEU* Teulada, *GOL* Gulf of Cagliari, *OLB* Olbia, *SIN* Siniscola, *CFE* Capo Ferrato, *GAJ* Galite island, *LEG* Livorno, *PSS* Porto Santo Stefano, *NA1* Napoli 2015 station 1, *NA2* Napoli 2015 station 2, *NAP* Napoli 1999, *STM* Strait of Messina, *SSI* Channel of Sicily, *MAL* Malta, *PPA* Porto Palo, *PCS* Porto Cesareo, *CKI* Kerkennah island coastal waters, *DWK* Kerkennah island deep waters, *ZAR* Zarzis, *TAJ* Tajoura, *ANC* Ancona, *CRZ* Split, *CRE* Heraklion, *SMS* Samos

vulgaris in the Mediterranean (Garofalo et al., 2010). The new file was used to compute the resulting direction and mean current velocity over the period 1998–2015, which were plotted on a map using the R packages *oceanmap* 0.1.1 (Bauer, 2018), *OceanView* 1.0.5 (Soetaert, 2016) and *RNetCDF* 2.3.1 (Michna & Woods, 2017). Pie charts showing the genetic makeup of each population were superimposed on the map using the R package *marmap* 1.0.4 (Pante & Simon-Bouhet, 2013).

Results

Two data sets were obtained as the result of the different length of COI sequences that were available on public databases. Since the data set including the shortest sequences (500 bp) resulted in a reduction of both the number of haplotypes and the power of detecting genetic differentiation, only the alignment of 860 sequences that were 621 bp long was used for

further analyses. Overall, 64 haplotypes were recorded, including sequences from eastern Atlantic (Online Resource 1: Table S2).

Based on the simulations, our data had sufficient power (> 95%) to detect weak population differentiation ($F_{ST} \geq 0.01$). However, there was an excess of false positives, as population differentiation was wrongly detected in the 23% of pseudo-datasets (Online Resource 1: Fig. S1). Conversely, the test was too conservative when sample size was small ($S = 5$ and 10 individuals) as the rate of false positives was ten-fold less than its expectation (α -error = 0.004 and 0.007, respectively). Small sample size also decreased the power of detecting population differentiation when genetic divergence was weak. At $S = 10$ individuals, the power of detecting population differentiation reached 95% at $F_{ST} \geq 0.015$. Halving of sample size ($S = 5$) increased up to five times the degree of genetic divergence ($F_{ST} = 0.05$) needed to get a power of detecting population differentiation > 95%. The detection rate of false positives

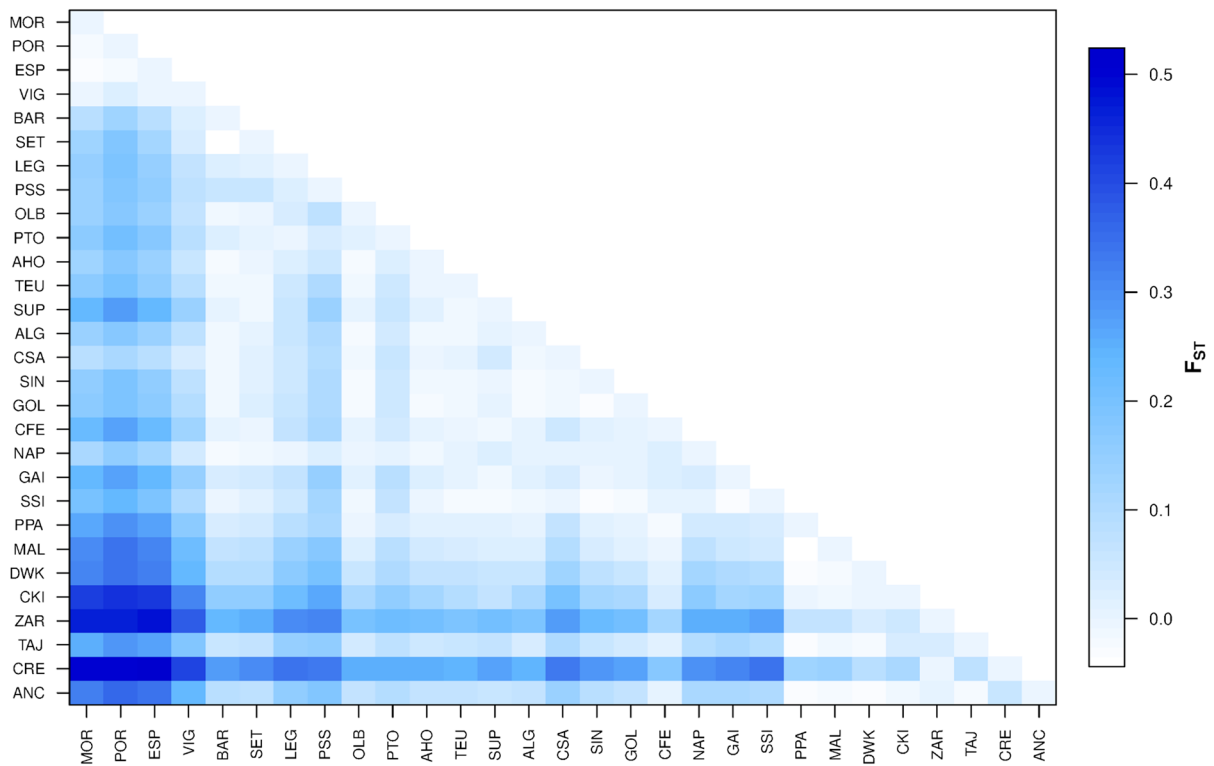


Fig. 2 Heatmap of pairwise F_{ST} values estimated from haplotype frequencies. The magnitude of genetic divergence among population pairs increase from white to blue

depended on the number of populations and sample size (Online Resource 1: Fig. S1). In all cases, such rate did not exceed its expectation (α -error $\leq 5\%$) and tended to increase with the number of populations, whereas the effect of sampling size was less predictable. Therefore, only localities with $S > 10$ were retained for analyses that used summary statistics to estimate genetic divergence: 24 out of 34 populations were thus used within the AMOVA and SAMOVA frameworks. Such removal was enough to barely reduce the α -error rate to the expected level (α -error = 0.051).

Overall, *Octopus vulgaris* showed significant genetic structuring across the study area either based on Φ -statistics (overall $\Phi_{ST} = 0.065$; $p < 0.001$) or F -statistics (overall $F_{ST} = 0.073$; $p < 0.001$). Regardless the metric used, AMOVA analyses highlighted significant genetic structuring between the Eastern and Western Mediterranean, either when the boundary was placed at the STS or shifted east off Sicily (Table 2 and Online Resource 1: Table S4). However, both metrics (Φ_{CT} and F_{CT}) were slightly higher when

populations from Sicilian channel were grouped according to Bianchi's (2007) biogeographical hypothesis "iii" (Fig. 1). The increase of the amount of variance explained by shifting eastward the boundary was greater when metric based only on haplotype frequency was used. Overall, such metric always showed a slightly greater genetic variance than metric accounting for mutations as well (Table 2 and Online Resource 1: Table S4).

Results based on SAMOVA, which searches for the grouping scheme that maximizes variance partitioning, highlighted an even more complex pattern (Table 2; Online resource 1: Table S2, Fig. S2). Populations along the Sicilian channel, DWK (Kerkennah Island Deep Waters, Tunisia) and TAJ (Tajoura, Lybian coast) were included in the Western Mediterranean group. Porto Santo Stefano (PSS, Tyrrhenian Sea) and all the remaining Eastern Mediterranean populations were ranked in the second and the third group, respectively. This grouping scheme showed an among-group variance component accounting for about 12% (Φ -statistics, $\Phi_{CT} = 0.120$;

Table 2 Analysis of molecular variance (AMOVA) based on F -statistics

Source of variation	Degrees of freedom	Sum of squares	Variance components	% of variation	F -statistics
Two-level AMOVA					
Among populations	23	28.262	0.029	7.30	$F_{ST} = 0.073^{***}$
Within populations	698	255.591	0.366	92.70	
Three-level AMOVA based on Hypothesis ii: (BAR, ALG, AHO, PTO, CSA, SUP, TEU, GOL, OLB, SIN, CFE, GAI, LEG, PSS, NAP); (SSI, MAL, PPA, CKI, DWK, ZAR, TAJ, ANC, CRE)					
Among groups	1	13.235	0.039	9.40	$F_{CT} = 0.094^{***}$
Among populations within groups	22	15.028	0.011	2.55	$F_{SC} = 0.028^{***}$
Within populations	698	255.591	0.366	88.04	$F_{ST} = 0.120^{***}$
Three-level AMOVA based on hypothesis iii: (BAR, ALG, AHO, PTO, CSA, SUP, TEU, GOL, OLB, SIN, CFE, GAI, LEG, PSS, NAP, SSI, MAL, PPA); (CKI, DWK, ZAR, TAJ, ANC, CRE)					
Among groups	1	14.279	0.052	11.80	$F_{CT} = 0.118^{***}$
Among populations within groups	22	13.983	0.009	2.13	$F_{SC} = 0.024^{***}$
Within populations	698	255.591	0.366	86.08	$F_{ST} = 0.139^{***}$
Three-level AMOVA based on SAMOVA best grouping scheme: (BAR, ALG, AHO, PTO, CSA, SUP, TEU, GOL, OLB, SIN, CFE, GAI, LEG, NAP, SSI, MAL, PPA, DWK, TAJ); (CKI, ZAR, ANC, CRE); (PSS)					
Among groups	2	14.317	0.054	12.52	$F_{CT} = 0.125^{***}$
Among populations within groups	22	13.945	0.010	2.32	$F_{SC} = 0.026^{***}$
Within populations	698	255.591	0.366	85.16	$F_{ST} = 0.148^{***}$

For three level AMOVA groups were according to: Bianchi's (2007) hypotheses (iii) and (ii) with the phylogeographic boundary placed at the Siculo-Tunisian strait and east off Sicily, respectively; the best grouping scheme retrieved by SAMOVA (Spatial Analysis of Molecular Variance). See Fig. 1 for population abbreviations. NS: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

$p < 0.001$) and 12.5% (F -statistics, $F_{CT} = 0.125$; $p < 0.001$) of total genetic variance.

The median-joining network highlighted a shallow level of divergence among haplotypes as most of them were separated by one mutational step and did not evidence any genetic break between the Western and eastern Mediterranean basins (Fig. 3). The four most common haplotypes (hereafter H1, H2, H10 and H17; Online resource 1: Table S3) were widespread across basins and localities, albeit their spatial distribution was uneven (Fig. 3). The frequency of H1 and H2, which were by far the most common haplotypes in the eastern Atlantic, decreased from west to east; however, the latter spread into the eastern Mediterranean reaching non-negligible frequencies, whereas the former was mainly restricted to the western basin (Fig. 4). Conversely, H10 and H17 showed more complex spatial patterns: H10 mainly spread across the Central Mediterranean, reaching the highest frequencies in a region encompassing the Sardinian and Sicilian channels, as well as the Tunisian coast north

of the Gulf of Gabes (Fig. 4), H17 was the most geographically widespread haplotype in the Mediterranean, with its frequency decreasing from the Aegean Sea (eastern Mediterranean) to the Balearic Sea (western Mediterranean). The remaining haplotypes either occurred in a unique population (private haplotypes) or showed a restricted geographic distribution: for instance, the spatial distribution of H25, H27 and H31 (Fig. 3) encompassed an area delimited by the mainland coast of Italy in the East and the northern coast of Sardinia in the west (Fig. 4).

Discussion

The present study supports the hypothesis suggested by Villamor et al. (2018): the Sicilian channel and the neighbouring areas should be viewed as a transitional area, characterised by complex connectivity patterns between the Western and the Eastern Mediterranean. Consistent with this picture, genetic patterns observed

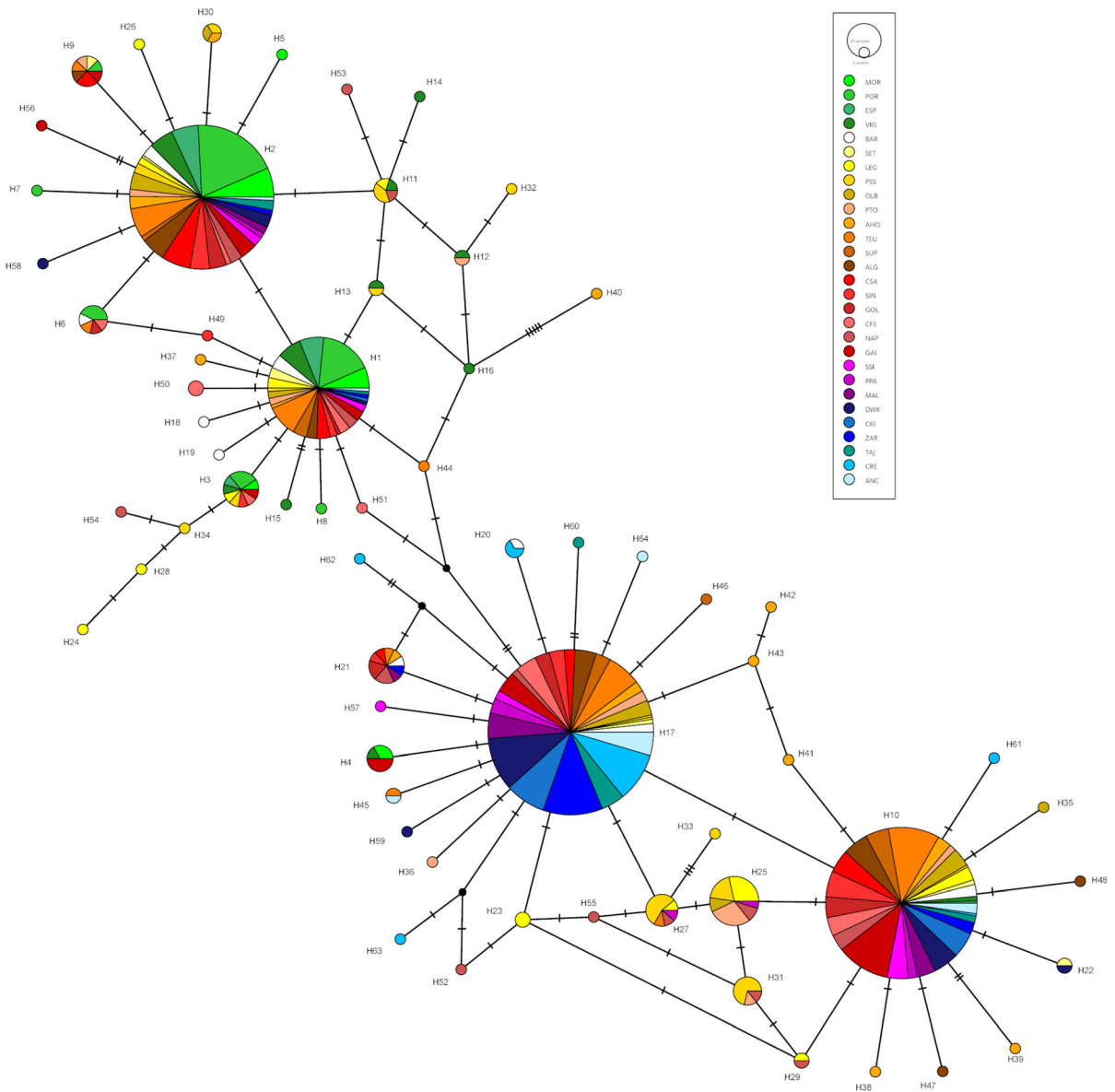


Fig. 3 Median-Joining Network (MJN) showing the relationships among haplotypes of *Octopus vulgaris*. Circles represent haplotypes, whose size is proportional to their frequency. To ease the graphic visualisation, the length of edges connecting haplotypes is not proportional to the number of mutations, which are drawn as hatchmarks. Circles are coloured based on the

frequency of haplotypes in each population. Shades of green are used for Atlantic populations; shades of white, yellow, orange and red for western Mediterranean populations; shades of magenta, purple and blue for eastern Mediterranean populations. Black dots represent median vectors, which may be interpreted as intermediate unsampled haplotypes

in *O. vulgaris* across the STS roughly match those reported for *Patella caerulea* Linnaeus, 1758 in the same area (Villamor et al., 2018), as well as those of other species, regardless their different life-history traits (e.g., Maltagliati et al., 2010; Barbieri et al., 2014; Deli et al., 2018, 2019). Conversely, other

species show a sharp genetic break across the Sicilian channel or east off it such as, for instance, the limpets *P. rustica* Linnaeus, 1758 and *P. ulysiponensis* Gmelin, 1791 (Sá-Pinto et al., 2010, 2012). Such a stark contrast with *P. caerulea* (Villamor et al., 2014, 2018) is not counterintuitive as biogeographic

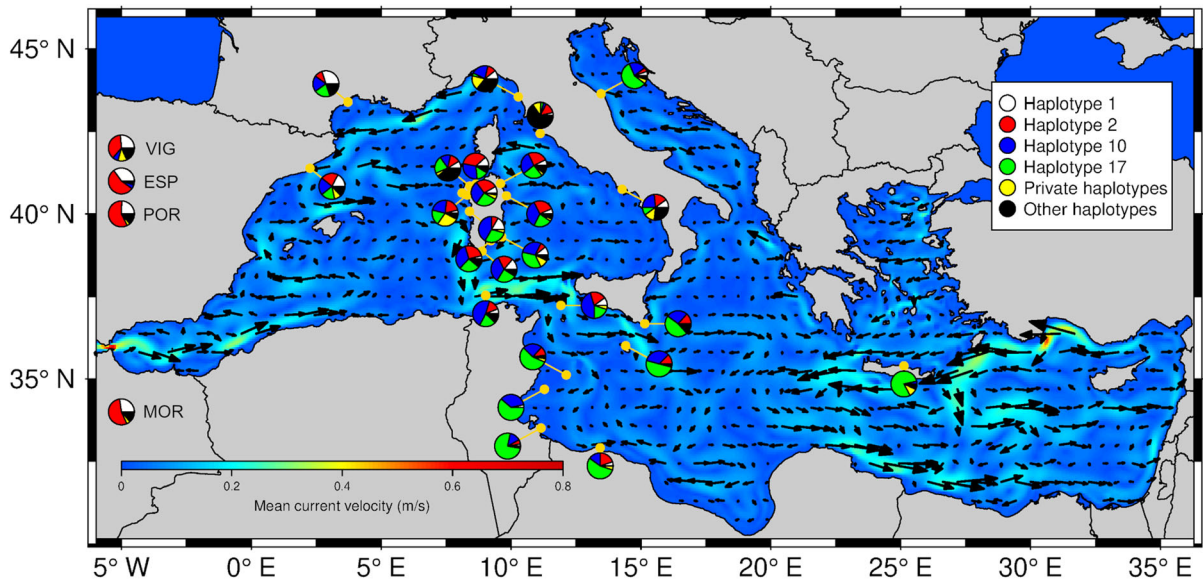


Fig. 4 Haplotype frequencies superimposed on a map of monthly of marine currents. Pie charts show the genetic makeup of each population: slices correspond to the proportion of main haplotypes plus the cumulated frequencies of private and remaining haplotypes in each population. The map of marine currents was assembled from a dataset of monthly mean currents ranging from 1998 to 2015, generated using E.U. Copernicus Marine Service Information (<http://marine.copernicus.eu/>).

boundaries can affect in different ways even closely related species with comparable biological traits (Galarza et al., 2009). Likely, dispersal and connectivity cannot be generalized for a given geographic area, as they depend on each species' life history and biological traits (Fratini et al., 2016; Pascual et al., 2017), as well as the evolutionary history and the possible presence of endogenous genetic barriers among formerly isolated lineages as envisioned by the coupling hypothesis (Bierne et al., 2011). Therefore, focusing the attention on the whole transitional area instead of a unique genetic break may increase the ease of detecting common patterns across species as the need for geographically coincident steep changes of allele frequencies is released in the former case.

Our results do not support an abrupt shift of haplotype frequencies at a precise location as it could be expected for a sharp genetic break. Indeed, either the biogeographic boundary located at the STS or east off Sicily (hypotheses *ii* and *iii* in Bianchi, 2007) are supported by AMOVA analyses, although different metrics yielded slightly different results (Table 2; Online Resource 1: Table S4). The amount of genetic

variation explained by partitioning populations according to the two barriers hypothesized by Bianchi (2007) that were considered in the present study is comparable when accounting for both haplotype frequency and genealogy (Online Resource 1: Table S4). Otherwise, genetic differentiation among groups resulting from the eastward shift of biogeographical boundary (hypothesis *iii*) is greater than that assessed based on the hypothesis *ii* (11.80 and 9.40%, respectively), when only haplotype frequency is considered (Table 2). Reasonably, the spatial genetic pattern observed across this area for populations of *Octopus vulgaris* is more complex than a simple shift of haplotype frequencies in east–west direction. In addition to a north–west south–east gradient of genetic variation, the present study highlights the partial isolation of the Gulf of Gabes from the rest of the Central Mediterranean. According to the best grouping scheme retrieved by SAMOVA, only CKI (Kerkennah Coastal Waters) and ZAR (Zarzis) on the Tunisian coast rank in the same group with ANC (Ancona) and CRE (Heraklion, Crete) in the Adriatic and Aegean Sea, respectively. This result also fits with

the low levels of genetic diversity reported on CKI and ZAR by Fadhlouli-Zid et al. (2012). Conversely, all the other Eastern Mediterranean populations clustered with the Western Mediterranean populations. Such apparently chaotic genetic pattern may reflect the influence of the complex hydrographical regime that characterizes the Sicilian channel and neighboring areas. The pattern of marine currents in the Sicilian channel is seasonally variable and influenced by the presence of eddies and gyres (Poulain & Zambianchi, 2007; Ciappa, 2009 and references therein). Atlantic water, advected toward STS by the Algerian current, split into two branches, one of which flows south-eastward along Sicilian coast (the Atlantic Ionian Stream, AIS) and the other southward over the Tunisian shelf at 13°E, about 160 km east off the Tunisian coast (the Atlantic Tunisian Current, ATC) (Poulain & Zambianchi, 2007; Ciappa, 2009). Whereas the former is stronger in summer and weaker in winter, the opposite trend has been observed for the ATC, which is almost undetectable in summer. The winter pattern of this current is still debated: whereas drifter trajectories indicate intrusion of AW in the Gulf of Gabes along the mainstream of ATC (Poulain & Zambianchi, 2007), patterns of chlorophyll concentration suggest that ATC is deviated eastward at about 35°N by an anticyclonic gyre (the Sydra gyre) and reach Lybian coast flowing along its border (Ciappa, 2009). Atlantic water can still reach Lybian coast in summer by a current that flows southward from the southeastern tip of Sicily and then merge with the eastern arm of the Sydra gyre (Ciappa, 2009).

The spatial distribution of haplotype frequencies fits quite well with the pattern of marine currents, with the most widespread haplotypes showing a gradual rather than a steep change of their frequencies in a northwest-southeast direction (Fig. 4). Not surprisingly, DWK and TAJ (Kerkennah deep waters and Tajoura, respectively) show a genetic makeup more similar to populations on the northern side of the Sicilian channel than to the other Tunisian populations (Kerkennah coastal waters and Zarzis). DWK is located close to the mainstream of the ATC, whereas TAJ is close to a trait of coast that can be reached either by the ATC (Poulain & Zambianchi, 2007) or by the southward current flowing by the southeastern tip of Sicily (Ciappa, 2009). Thus, spatial genetic patterns of *O. vulgaris* observed in the northern sector of the Sicilian Channel, at Kerkennah deep waters and

Tajoura might reflect a stronger advection of paralarvae from the Western Mediterranean related to the southern Tunisian coast and the Gulf of Gabes. The isolation of the latter area as well as that of Adriatic and Aegean seas related to the Mediterranean Sea, might explain why scattered populations as Kerkennah coastal waters, Zarzis, Ancona and Heraklion show similar genetic makeups. If the frequency and geographic distribution of the main haplotypes reflects the species' demographic history in the Mediterranean, we may speculate that this populations still retain the genetic signature of older historical events.

The comparison of measures of genetic differentiation based on Φ - and F -statistics also supports the role of marine currents in shaping the spatial genetic patterns of *O. vulgaris*. The slightly higher value of the latter measure indicates that accumulation of mutations, which is accounted for the estimation of Φ -statistics parameters and reflects the relative contribution of past evolutionary history, has a little effect on genetic structuring (Fadhlouli-Zid et al., 2012). Conversely, F -statistics is more powerful at detecting population differentiation after few generations of genetic drift, as it relies only on haplotype frequencies to estimate genetic divergence (Penant et al., 2013).

A last interesting remark deals with the case of PSS population, which does not group with any other Mediterranean population according to SAMOVA (Online resource 1: Fig. S2). This population displayed a peculiar genetic makeup (Fig. 3): in contrast to other Western Mediterranean populations, the most common haplotypes show a relatively low frequency. In fact, PSS is characterized by the occurrence of high-frequency haplotypes that are rare elsewhere, apart from LEG, NAP and PTO (haplotypes 25, 27 and 31, Fig. 3). Based on the life history traits of *O. vulgaris*, we cannot rule out that this pattern reflect temporal rather than spatial genetic variation, even though neither the present study (Online resource 1: Table S5) nor Melis et al. (2018) evidenced the occurrence of temporal genetic subdivision. Even though it is not geographically widespread in the present study, it may still occur at local scales as in other marine invertebrates in which recruitment and growth are affected by environmental factors. For instance, spatio-temporal genetic variation was found by Calderón et al. (2012) during a five-years genetic monitoring of the sea urchin, *Paracentrotus lividus* (Lamarck, 1816). Notwithstanding an overall genetic homogeneity

across the timeframe of the study, the genetic composition of some studied populations changed markedly in a particular year, with the appearance of many private haplotypes. Such a change in the genetic makeup was related to the unusual circulation pattern in that year, which likely favored an abnormal migration of recruits from genetically heterogeneous source populations (Calderón et al., 2012). The same scenario cannot be discarded for *O. vulgaris*, as marine circulation in the Tyrrhenian Sea is characterized by a high degree of seasonal and inter-annual variability, with the formation of mesoscale eddies and gyres (Iacono et al., 2013). Such oceanographical features coupled with the sea surface temperature variation may influence recruitment and growth in *O. vulgaris*. For instance, the number and extent of spawning and nursery grounds show inter-annual variability associated with environmental conditions in the Central Mediterranean (Garofalo et al., 2010). Genetic monitoring over different years is needed to disentangle these two competing, but not mutually exclusive scenarios in *O. vulgaris*.

Conclusions

The present study does not support the presence of a sharp genetic break across the Siculo-Tunisian strait in Mediterranean populations of *O. vulgaris*. The complex genetic patterns observed in this species are another clue in on the hypothesis that the Central Mediterranean might be a transition zone. Nevertheless, as many species show a sharp genetic break across the STS or east off this channel, more studies realising a dense geographic sampling around this area are needed to assess if and how widespread this genetic pattern is in marine organisms with different life-histories.

Although investigating the drivers that shape spatial genetic structure is beyond the scope of the present study, our results indicate that spatial genetic structure in *O. vulgaris* is influenced by contemporary factors as oceanographic features. However, marine currents might not be the only or the main factor contributing to shape the genetic structure observed in the present study. In addition to exogenous (due to oceanographic and/or environmental factors), endogenous genetic barriers (due to historical isolation followed by secondary contact) are important factors underpinning

complex genetic patterns, as outlined by the coupling hypothesis (Bierne et al., 2011). The use of nuclear markers and joint biophysical-genetic models, which combine larval dispersal and spatially explicit genetic models, may shed light into the spatial distribution of genetic variation expected over ecologically relevant timescales. These models may thus help better define the relative contributions of historical and contemporary factors on species' genetic structuring.

Moreover, the limited connectivity between the Gulf of Gabes and the other regions may have important implications for common octopus fisheries, suggesting that populations located in this area might be regarded as an independent stock, relying mostly on self-recruitment. The results outlined above further stress the need of designing an exhaustive geographical sampling to unravel phylogeographical patterns in areas that are characterized by a complex oceanography as the Central Mediterranean. As evidenced by Fratini et al. (2016), limited sampling of both populations and individuals per population might hamper the detection of genetic structuring in marine invertebrates with larval dispersal stages.

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Data availability The datasets generated and/or analysed during the current study are available in the FIGSHARE repository, <https://doi.org/10.6084/m9.figshare.12957380>.

Declarations

Conflict of interest The authors declare that they have no conflict of interest

Ethical approval The study did not involve endangered or protected species. New DNA sequences were obtained from genomic DNA stored at -20°C that had been isolated from tissue samples collected in previous studies (See materials and methods for further details). Therefore, no specific permissions were required for locations and activities involved in this study.

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