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RESEARCH ARTICLE

Strongly structured populations and reproductive habitat fragmentation increase the vulnerability of the Mediterranean starry ray *Raja asterias* (Elasmobranchii, Rajidae)

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Abstract

1. The Mediterranean starry ray (*Raja asterias*) populations within the Mediterranean Sea are susceptible to high rates of bycatch in the multispecies trawl fisheries. Understanding its population structure and identifying critical habitats are crucial for assessing species vulnerability and setting the groundwork for specific management measures to prevent population decline.

2. To assess the population structure of *R. asterias* in the Mediterranean, the genetic variation in nine population samples at one mitochondrial marker and eight nuclear microsatellite loci was analysed. Moreover, 172 egg cases collected in the Strait of Sicily were identified at species level using integrated molecular and morphological approaches.

3. Genetic analyses revealed that the Mediterranean starry ray comprises three distinct units inhabiting the western, the central-western, and the central-eastern areas of the Mediterranean. An admixture zone occurs in the Strait of Sicily and the Ionian Sea, where individuals of the central-western and central-eastern population units intermingle.

4. The joint morphometric–genetic analyses of rajid egg cases confirmed the presence of more than one species in the admixture area, with a predominance of egg cases laid by *R. asterias*. DNA barcoding revealed that egg cases and embryos of *R. asterias* shared several haplotypes with adult individuals from the central-western and central-eastern Mediterranean Sea, revealing that females of both populations laid numerous eggs in this area.

5. According to these findings, detailed taxonomic determination of egg cases, when combined with seasonal migration studies, could improve the capability to

identify important spawning or nursery areas for the Mediterranean starry ray, particularly in those admixture zones relevant to maintaining genetic diversity.

6. Finally, these new insights should be considered to update the Action Plan for the Conservation of Cartilaginous Fishes in the Mediterranean Sea with effective measures to reduce the impact of skate bycatch in trawling and safeguard egg cases in nursery areas.

KEYWORDS

bycatch, connectivity, microsatellite loci, mitochondrial DNA, nursery areas

1 | INTRODUCTION

The Mediterranean Sea is one of the world's hotspots for marine biodiversity, with a very rich species diversity (Bianchi & Morri, 2000). Unfortunately, in recent decades, severe human impacts, such as overfishing and pollution, have threatened ecosystems and communities and led to rapid alterations in marine fauna and habitats (Bianchi, 2007; Cuttelod et al., 2008; Ferretti et al., 2008; Colloca et al., 2013; Ferretti et al., 2013). Cartilaginous fishes are vulnerable to anthropogenic stressors because of their *K*-selected life-history traits, such as low fecundity rate, slow growth, longevity, large size at hatching, and late maturity age (Cortés, 2000; Stevens et al., 2000; Sims, 2010). The decline in shark and ray landings is closely related to indirect and direct fishing pressures (Ferretti et al., 2008; Ferretti et al., 2013; Davidson, Krawchuk & Dulvy, 2016; Adams et al., 2018). Accordingly, the Mediterranean Sea is included by the International Union for Conservation of Nature among the three global biodiversity hotspots where sharks and rays are severely threatened and where national and international action plans have to be implemented to protect them from bycatch (Dulvy et al., 2016).

Among the 86 Mediterranean elasmobranch species (48 sharks, 38 rays; Otero et al., 2019), the Mediterranean starry ray *Raja asterias*, Delaroche 1809, has been considered an endemic demersal skate. However, its distribution was recently extended beyond the Strait of Gibraltar to northern Morocco and possibly south to Mauritania with a verified record from the Gulf of Cadiz (Ordines et al., 2017; Froese & Pauly, 2020). This species inhabits muddy or sandy bottoms from shallow waters to 150 m depth with age-related abundance (Catalano et al., 2003; Romanelli et al., 2007). In particular, juvenile (total length 80–90 mm) and adult individuals appear to aggregate differently in the north and central Adriatic Sea (Serena, Mancusi & Barone, 2010; Ferrà et al., 2016). *Raja asterias* is an ecologically important predator within the demersal community that feeds on a wide variety of resources, including crustaceans, teleosts, and cephalopods (Capapé & Quignard, 1977; Cuoco, Mancusi & Serena, 2005; Serena et al., 2005; Romanelli et al., 2007; Bradai, Saidi & Enajjar, 2012, Coll, Navarro & Palomera, 2013; Navarro et al., 2013). Although northern, central-western, and eastern Mediterranean populations have appeared demographically stable over time (Capapé, 1977; Minervini, Giannotta & Bianchini, 1985; Massutí & Moranta, 2003; Romanelli et al., 2007; Serena, Mancusi & Barone, 2010; Tserpes et al., 2013; Serena et al., 2015; Follesa et al., 2019), there is evidence that *R. asterias* responded negatively to intense bottom trawl fisheries in several shallow coastal areas; for example, in the north Tyrrhenian Sea (Abella & Serena, 2005; Abella, Mancusi & Serena, 2017), in the north-western Mediterranean (Coll, Navarro & Palomera, 2013; Navarro et al., 2013; Biton-Porsmoguer & Lloret, 2020), and in the Adriatic Sea (Ferrà et al., 2016). Furthermore, the species is a bycatch of 'rapido' vessels targeting flatfish (e.g. *Solea* spp., *Psetta maxima*, and *Scophthalmus rhombus*). This led the International Union for Conservation of Nature to increase the conservation risk of *R. asterias* from Least Concern to Near Threatened (Serena et al., 2015).

As with other Rajidae, the Mediterranean starry ray is oviparous, laying large, benthic egg cases at 30–40 m depth (Barone et al., 2007). A very restricted and shallow zone (5–50 m depth) in the boundary between the Ligurian and Tyrrhenian seas was identified as a nursery and spawning area of *R. asterias* (Serena & Relini, 2006), although more than one elasmobranch species may be using the area to maximize reproductive success and overall fitness (Enajjar, Saidi & Bradai, 2015; Tavares, Rodriguez & Morales, 2016; Martins et al., 2018; Heupel et al., 2019). The identification of such spawning and nursery areas can provide important information on the species reproductive ecology and population structure. Three primary criteria for the general definition of a shark nursery area have helped to identify these habitats: (1) a higher density of individuals than other areas, (2) the tendency to remain or return for extended periods (i.e. site fidelity), and (3) the repeated use of the area over time (Heupel, Carlson & Simpfendorfer, 2007). Specifically for skates, Hoff (2016) recognized more than a single type of nursery habitat and suggested the distinction between 'egg case nursery' (i.e. an area used for depositing eggs in contact with benthic/stationary materials over multiple years) and 'juvenile nursery' (i.e. a habitat distinct from an egg-laying area in which post-hatching juveniles occur in high abundance and that contribute significantly to population recruitment success). Thus, species assessment and consequent identification of nurseries have been possible owing to the species-specific morphology of egg cases (Ebert & Davis, 2007; Ishihara et al., 2012; Porcu et al., 2017; Mancusi et al., 2021).

Although much information concerning the spatial variability and the ecological relationships of Mediterranean elasmobranch species, including *R. asterias*, was obtained from fishery data (Follesa et al., 2019; Mulas et al., 2019), the population structure of the starry ray in the Mediterranean Sea is still unknown. A preliminary study investigated the molecular taxonomy of Mediterranean elasmobranchs by compiling a DNA barcode reference library for 42 species inhabiting the area. The phylogeography and population genetic structure of *R. asterias* was also explored, and the results suggested the occurrence of three different haplotype groups in the Mediterranean Sea, corresponding to the Balearic division, the Sardinia and Ionian divisions, and the Adriatic division (Cariani et al., 2017). Low migration potential of the species, the relatively small size of individuals, and the absence of a pelagic phase (Serena, Mancusi & Barone, 2010) are factors potentially driving low rates of genetic connectivity and non-panmictic populations. A deeper understanding of population structure through a more complete genetic assessment is required as a prerequisite to improving risk analyses and protection strategies of *R. asterias*.

This work deals with the phylogeography and population connectivity of the Mediterranean populations of starry ray, through a genetic variation analysis of nine geographical samples at the mitochondrial gene cytochrome oxidase I (COI) and eight nuclear microsatellites loci (simple sequence repeats (SSRs)) linked to expressed sequence tags (ESTs). In addition, this study addresses the morphological and molecular identification at the species level of rajid egg cases collected in the Strait of Sicily to test the nursery role of this area for *R. asterias* and/or related species.

2 | METHODS

2.1 | Sampling of individuals

To assess the geographical population structure of *R. asterias*, nine coastal sampling locations were identified in the Mediterranean (Table 1; Figure 1). From west to east, the nine sampling sites (with acronyms) were: Moroccan (MOR) and Algerian (ALG) coasts located in the western Mediterranean; Sardinian (SARD) coasts, north Tyrrhenian (NTYR) and central Tyrrhenian (CTYR) Sea in the central-western Mediterranean; south Adriatic (SADR) and north Adriatic (NADR) Sea in the central-eastern Mediterranean; Strait of Sicily (SIC) and western Ionian Sea (WION) located in a transitional zone between central-western and central-eastern Mediterranean.

Individuals of *R. asterias* were mainly collected yearly, in the framework of different research trawl surveys conducted in the Mediterranean Sea from 2000 to 2012 (e.g. the 'Mediterranean international bottom trawl survey' (MEDITS; Spedicato et al., 2019), the 'Italian national trawl surveys' (GRUND; Relini, 2000), and the Adriatic rapido trawl survey SoleMon (Grati et al., 2013). Additional samples were provided by regional institutions (i.e. ARPA Toscana, Italy) during dedicated cruises or were collected at local markets mainly supplied by the artisanal fishery (e.g. individuals from Algerian and Moroccan coasts; Table S1).

Whenever possible, the sex, maturity, and size measures (i.e. weight, total length, and disc width) of individuals were collected. Then, soft tissue samples (finclip or skeletal white muscle slice) were taken from individuals and were stored in 96% ethanol at −20 °C until laboratory analysis. The list of individuals sampled and the associated sampling information are detailed in Table S1.

TABLE 1 Geographical area, sampling period, and sample size for each marker data set of the Mediterranean *Raja asterias* population samples. Temporal replicates are indicated with A and B

Population sample (acronym)		Sampling period	COI	EST-SSR
Moroccan coasts (MOR)		2008–2012	1	1
Algerian coasts (ALG)		2000–2004 (A)	13	10
		2008–2012 (B)	16	16
Sardinia coasts (SARD)		2008–2012	25	25
North Tyrrhenian Sea (NTYR)		2000–2004 (A)	7	7
		2008–2012 (B)	52	52
Central Tyrrhenian Sea (CTYR)		2000–2004 (A)	51	48
		2008–2012 (B)	3	3
Strait of Sicily (SIC)	Adults	2008–2012	12	11
	Eggs/embryos	2008–2016	39	—
Western Ionian Sea (WION)		2000–2004 (A)	2	2
		2008–2012 (B)	5	5
South Adriatic Sea (SADR)		2000–2004	10	8
North Adriatic Sea (NADR)		2000–2004 (A)	40	39
		2008–2012 (B)	28	28
Total			304	255

Abbreviations: COI, partial sequence of the mitochondrial gene cytochrome oxidase subunit I; EST-SSR, panel of eight expressed sequence tag-linked microsatellite loci.
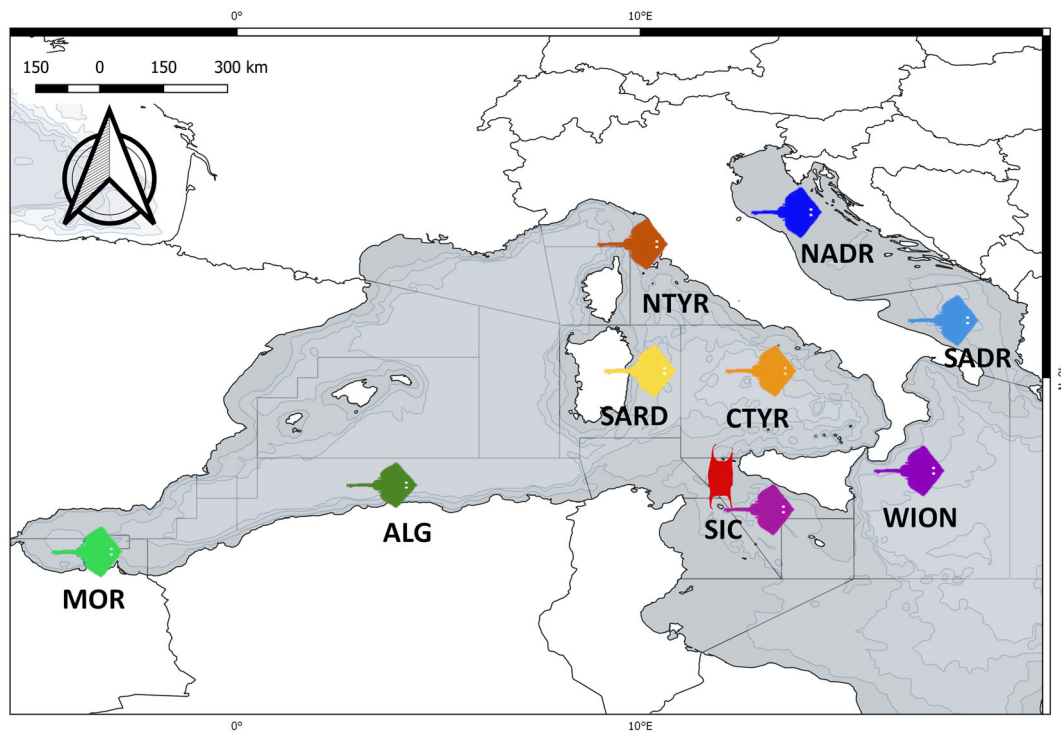
FIGURE 1 Sampling sites of *Raja asterias* in the Mediterranean. Codes of the geographical population samples are given in Table 1. Colours refer to the haplotype network legend of Figure 2

Based on the estimated generation time of *R. asterias* (∼6 years; Serena et al., 2015), individuals from four geographical population samples were grouped in two temporal replicates to assess intergenerational genetic variability (replicate A: from 2000 to 2004; replicate B: from 2008 to 2012; Table 1). The temporal replicates were combined in the COI data analysis because of the irrelevant mutation rate in this temporal framework. On the contrary, the two temporal replicates were analysed separately in the EST-SSR analyses to assess genetic stability over generations.

2.2 | Sampling and morphometric assessment of egg cases

Skate egg cases were collected during different scientific trawl survey programmes (i.e. MEDITS and GRUND) and from surveys of landings from commercial fisheries (i.e. CAMPBIOL; Milisenda et al., 2017) carried out from 2008 to 2016 in the Siculo-Tunisian Strait. All egg cases were sampled and stored in 70% ethanol at −20 °C until laboratory analyses. Before the taxonomic identification, they were rehydrated in water for about 30 minutes.

Following the identification keys described in Mancusi et al. (2021), 233 egg cases were initially identified at genus level (*Raja*). Then, 172 undamaged egg cases were selected for morphometric analyses. The list of egg cases sampled and the associated sampling information are detailed in Table S2.

Eight morphometric linear variables were recorded with a 1 mm resolution calliper (Mancusi & Serena, 2015; Massi et al., 2018). In

detail, measurements were made of egg case length (ECL), central body length (CBL), egg case width (ECW), anterior and posterior apron length (AAL and PAL respectively), keel width (KW), and anterior and posterior horn length (AHL and PHL respectively)—see Mancusi et al. (2021, figure 2). After morphometric measurements, egg cases were stored in 96% ethanol at −20 °C until genetic analyses.

For each egg case, the probability of assignment to a given species was performed based on two morphometric linear co-variables (ECW and AHL) and following the discriminant function tool detailed in Mancusi & Serena (2015) (method provided in Table S2, spreadsheet 'data_analyses'). The two measures were plotted to visualize the resulting species determination.

2.3 | Genetic analyses

Detailed protocols used for DNA extraction for both adults and egg cases are reported in Supporting Information Text S1. In particular, DNA was extracted from embryos when present or from residuals of embryonic tissues scrubbed from the internal side of the case. Then, the molecular identification of egg cases at the species level was carried out with the same polymerase chain reaction (PCR) conditions used for the adult individuals. Details of PCR amplification, DNA sequencing, and genotyping of mitochondrial (Ward et al., 2005) and nuclear markers (El Nagar et al., 2010) are provided in Supporting Information Text S1 and Table S3.

2.3.1 | COI variation and population differentiation

Electropherograms were manually edited, and sequences were aligned using the ClustalW algorithm (Thompson, Higgins & Gibson, 1994) available in the software package MEGA v.7 (Kumar, Stecher & Tamura, 2016). Twenty-nine COI public sequences of $R.$ $asterias$ from Cariani et al. (2017) were retrieved from GenBank and added to the final COI data set (National Center for Biotechnology Information accession numbers: GU805132–GU805135, GU805179–GU805180, GU805199, GU805385–GU805389, GU805423, GU805440–GU805442, GU805813, GU805816, KT307374–KT307384).

The software DnaSP v.6 (Rozas et al., 2017) was used to infer the genetic diversity for each population sample as the number of haplotypes n, haplotype diversity Hd, and nucleotide diversity π. Phylogeographic relationships among haplotypes were reconstructed using a median joining (MJ) tree clustering algorithm (Bandelt, Forster & Rohl, 1999) implemented in the software PopART (Leigh & Bryant, 2015).

Estimates of genetic differentiation were computed as pairwise fixation indexes Φ_{ST} based on the haplotype frequency distribution analysis (equivalent to an F analysis; Cockerham, 1973) corrected for inter-haplotype sequence divergence using ARLEQUIN v.3.5 (Excoffier & Lischer, 2010). The statistical significance of fixation indices was tested by a permutation test (1,000 iterations) and applying the Bonferroni correction (Rice, 1989).

The analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was carried out using ARLEQUIN v.3.5 (Excoffier & Lischer, 2010) grouping the Mediterranean samples on the basis of a geographical structure on three hierarchical levels: among geographical areas, among populations within geographical areas and within populations. The statistical significance of the resulting values of the pairwise fixation index was estimated by comparing the observed distribution with a null distribution generated by 1,000 permutations.

Genetic divergence among population samples was investigated through a Bayesian model-based clustering algorithm as implemented in the software BAPS v.6.0 (Cheng et al., 2013).

The demographic history of $R.$ $asterias$ was reconstructed using different approaches. The distribution of pairwise nucleotide differences between haplotypes was assessed (mismatch distribution; Rogers & Harpending, 1992). The population size fluctuations were estimated using the Bayesian skyline plot method (Drummond et al., 2005) implemented in BEAST v.1.10.4 (Suchard et al., 2018) by using Markov chain Monte Carlo integration under a coalescent model. Chains were run for 100 million generations, and the first 10% were discarded as 'burn-in'. The substitution model HKY (Hasegawa, Kishino & Yano, 1985) was selected following the Bayesian information criterion with jModelTest2 (Darriba et al., 2012). An evolutionary rate of 0.005 per million years was applied, previously detected for the closely related species $Raja$ $clavata$ Linnaeus, 1758 by Chevolot et al. (2006). Demographic history through time was reconstructed using Tracer v.1.5 (Rambaut et al., 2018) with constant growth for the skyline model.

2.3.2 | EST-SSR variation and population differentiation

The EST-SSR allele size was scored using GENEMARKER v.1.8 (SoftGenetics Inc) after setting the proper panels for binning analysis. Micro-Checker v.2.2.3 was used to check the data for genotyping errors and null alleles (Van Oosterhout et al., 2004). Given the presence of null alleles (see Section 3), their frequency for each locus and population were inspected with the program FreeNA (available at http://www.montpellier.inra.fr/URLB/; Chapuis & Estoup, 2007).

Genetic diversity indices as the number of alleles k and the allelic richness A were computed using FSTAT v.2.9.3.2 (Goudet, 2001). The observed heterozygosity H_o and the expected heterozygosity H_e were calculated using GENETIX v.4.05 (Belkhir et al., 1996). Deviation from Hardy–Weinberg equilibrium (was tested using the online software GENEPOP v.4.2 (Rousset, 2008) with a Bonferroni correction for multiple tests (Rice, 1989).

Pairwise F_{ST} values were calculated with ARLEQUIN v.3.5 with 1,000 permutations and alpha value equal to 0.05. In order to correct for the positive bias induced by the presence of null alleles on F_{ST} estimation, pairwise F_{ST} values were also estimated with the program FreeNA, following the Refined Estimation by Excluding Null Alleles (ENA) method described in Chapuis & Estoup (2007). The AMOVA was carried out imposing the same groupings and conditions used in the mitochondrial DNA (mtDNA) data analysis.

Bayesian multi-locus clustering was used to estimate the number of population samples in the Mediterranean $R.$ $asterias$, as implemented in STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000). The Bayesian approach estimates, for each value of the number of populations K, the posterior probability ($\ln(K)$) of the data. However, because the probability of the data does not always provide an accurate estimate of the number of populations, the second-order rate of change of the probability of the data between successive K values (ΔK) was also used when more than one population was likely to be present (Evanno, Regnaut & Goudet, 2005). Simulations were run with 10,000 steps burn-in and 100,000 steps in the Markov chain. To identify a good alignment of clusters obtained with different K values, the results of model-based populations structure analyses was postprocessed with CLUMPAK (Kopelman et al., 2015) and STRUCTURE HARVESTER (Earl & VonHoldt, 2012).

Divergence among population samples was assessed through a discriminant analysis of principal components of multi-locus genotypes (DAPC; Jombart, Devillard & Balloux, 2010) performed in R v.4.0.0 (R Core Team, 2019) within the package adegenet v.2.1.3 (Jombart, 2008).

Furthermore, a mixed-stock analysis for the individuals collected in the Siculo-Tunisian Strait was performed as implemented in the R package rubias (Moran & Anderson, 2018), using mixture simulations and considering as reference reporting units the geographical collections defined by previous analyses.

3 | RESULTS

3.1 | Specific identification of Sicilian egg cases

The discriminant function based on ECW and AHL assigned 155 out of 172 of the egg cases to *R. asterias* with 90–100% probability (Table S2; Figure S1), one egg case to *Raja miraletus* L., 1758 (EC_084; 100% probability), and three to *Raja polystigma* Regan, 1923 (EC_040, 84%; EC_128, 98%; and EC_147, 86%). The remaining 13 egg cases shared the identification probability among more than one species: two to *R. asterias*–*R. miraletus* (EC_171 and EC_064), eight to *R. asterias*–*R. clavata* (EC_015, EC_023, EC_024, EC_065, EC_069, EC_110, EC_118, EC_149), two to *R. polystigma*–*R. asterias*–*R. clavata* (EC_060, EC_088), and one to *R. asterias*–*R. clavata*–*R. polystigma* (EC_124). Values of descriptive statistics for all the eight linear measures are shown in Table S4.

A subsample of 110 egg cases and embryos was COI barcoded. Only 40 of them gave high-quality sequences suitable for identifying maternal species. Coherently with the morphometric identification results, most of the samples were identified as *R. asterias*, and one capsule was identified as *R. miraletus* (EC_084). Only two specimens out of the 13 that shared the morphometric identification probability among different species (EC_088 and EC_171) were barcoded, and both were univocally assigned to *R. asterias*. The presence of embryos, at different stages of development, was recorded in 18 egg cases.

3.2 | Genetic diversity and population structure based on COI variation

The COI data set of Mediterranean *R. asterias* included 265 individual sequences and 23 haplotypes. In addition, 39 COI sequences and 14 haplotypes obtained from egg cases and embryos of *R. asterias* collected in the Siculo-Tunisian Strait were included. All 33 haplotype sequences were deposited in GenBank (accession numbers: MW300433–MW300465). The diversity indexes showed moderate to low levels of variability within most population samples (Table 2).

In the MJ network, individual and egg-case COI haplotypes were subdivided in three main geographical haplogroups, each of them showing a star-like topology (Figure 2). The 'complex-star network' typically involves high-frequency haplotypes and connections preventing the identification of the most ancient haplotypes. Additional haplotypes are linked to the most frequent ones by a single (or a few) mutational steps, as the product of recent diversification. In the present study, the first haplogroup (H_1–H_6) comprised the haplotypes shared by individuals collected from the western Mediterranean area (ALG, MOR); the second haplogroup (H_7–H_11 plus the haplotypes from egg cases/embryos from H_23–H_31) was composed of haplotypes possessed by individuals from the central–western Mediterranean area (NTYR, CTYR, SARD) and by some of the individuals and egg cases/embryos of the transitional area (SIC, WION); the third haplogroup (H_12–H_22 plus the haplotypes from egg cases/embryos H_32 and H_33) included haplotypes shared among individuals from the central-eastern Mediterranean area (NADR, SADR) and the remaining individuals and egg cases/embryos from the transitional area (SIC, WION). Based on the sharing of the H_2 haplotype with most of the Algerian individuals, the single individual from MOR was pooled with the ALG population sample in further genetic analyses.

High and significant Φ_{ST} values indicated genetic differentiation among population samples of the three geographical areas (Table 3). The ALG–MOR population sample showed significant Φ_{ST} values ($P = 0$) with all the other samples. The highest values of pairwise Φ_{ST} were shown by the comparisons between central-western (NTYR, CTYR and SARD) and central-eastern (NADR and SADR) population samples, with values ranged from 0.686 to 0.786, but also with the western area (ALG–MOR) with values between 0.665 and 0.706. Lowest and non-significant values were found among population samples within each geographical area. Both adults and eggs/embryos of the SIC population sample showed medium-level pairwise Φ_{ST} values (0.242–0.508) with all the other population samples, whereas

TABLE 2 Genetic diversity of cytochrome oxidase subunit I sequences of the Mediterranean *Raja asterias* (acronyms are consistent with Table 1)

Population sample	N	n	Hd ± SD	π ± SD
ALG_MOR	30	6	0.559 ± 0.088	0.0186 ± 0.0163
SARD	25	2	0.080 ± 0.072	0.0022 ± 0.0048
NTYR	59	5	0.132 ± 0.060	0.0047 ± 0.0071
CTYR	54	3	0.174 ± 0.067	0.0049 ± 0.0073
SIC, adults	12	4	0.636 ± 0.128	0.0497 ± 0.0347
SIC, eggs+embryos	39	14	0.856 ± 0.034	0.0867 ± 0.0510
WION	7	3	0.524 ± 0.209	0.0423 ± 0.0329
SADR	10	4	0.533 ± 0.180	0.0167 ± 0.0163
NADR	68	8	0.431 ± 0.073	0.0133 ± 0.0130
	265	33	0.668 ± 0.026	0.0721 ± 0.0476

Abbreviations: Hd, haplotype diversity; *N*, number of samples; *n*, number of haplotypes; π, nucleotide diversity.
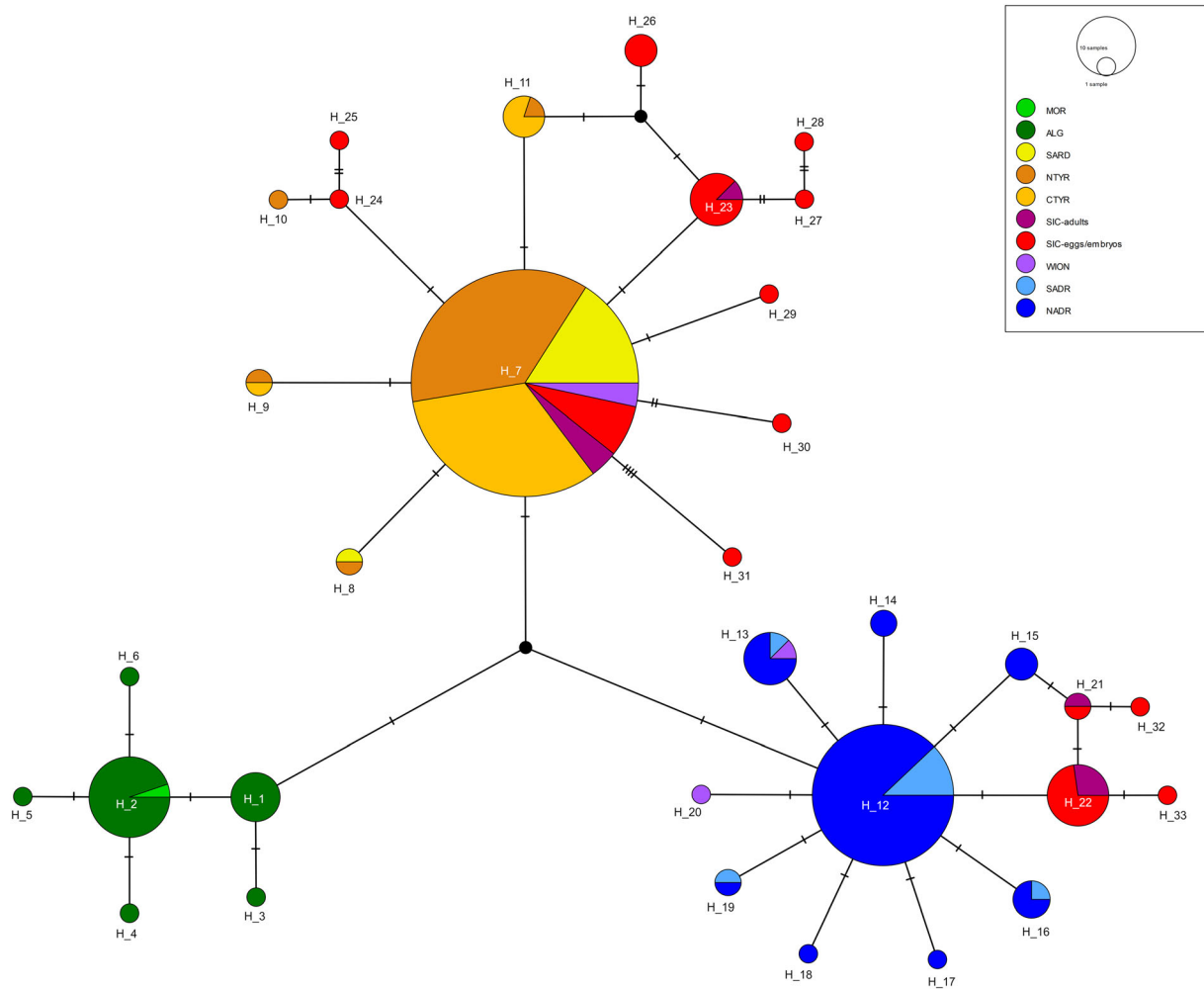
FIGURE 2 Median-joining network of cytochrome oxidase subunit I (COI) haplotypes shown by the Mediterranean *Raja asterias*. The network was built on 265 and 39 COI sequences obtained from skate individuals and from egg cases and embryos respectively. Circles are proportional to haplotype frequencies. Orthogonal bars between branch nodes indicate substitutions. Black circles at network nodes represent unsampled haplotypes. Codes of the geographical population samples are given in Table 1

TABLE 3 Pairwise fixation index (below diagonal) and corresponding *P*-value (above diagonal) for each pair of comparison for the cytochrome oxidase subunit I alignment (acronyms are consistent with Table 1)

	ALG_MOR	SARD	NTYR	CTYR	SIC, adults	SIC, eggs+embryos	WION	SADR	NADR
ALG_MOR		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SARD	**0.665**		0.791	0.318	0.000	0.000	0.100	0.000	0.000
NTYR	**0.706**	−0.020		0.555	0.000	0.000	0.118	0.000	0.000
CTYR	**0.672**	0.006	−0.003		0.000	0.000	0.545	0.000	0.000
SIC-adults	**0.411**	**0.270**	**0.295**	**0.242**		0.127	0.436	0.000	0.000
SIC-eggs/embryos	**0.286**	**0.315**	**0.371**	**0.336**	0.018		0.018	0.000	0.000
WION	**0.453**	0.176	0.156	0.107	−0.001	0.099		0.009	0.000
SADR	**0.451**	**0.776**	**0.786**	**0.745**	**0.413**	**0.270**	**0.463**		0.973
NADR	**0.519**	**0.686**	**0.711**	**0.687**	**0.508**	**0.381**	**0.541**	−0.041	

Note: Bold type indicates significant values after the Bonferroni correction (*P* < 0.001389).

WION showed no significant values except for the comparisons with the ALG–MOR and NADR.

The highest percentage of molecular variation among groups (55.32%) together with the lowest value of molecular variation among populations within a group (0.96%) was observed when WION and SIC population samples were grouped separately, highlighting the subdivision of population samples into four groups (ALG–MOR; SARD, NTYR, CTYR; SIC adults and eggs/embryos, WION; and NADR, SADR) as the one that best fitted the geographical grouping (AMOVA 3, Table 4).

The Bayesian clustering analysis (Figure 3a) revealed three genetic clusters, supported with a high percentage value (99.99%). In particular, the first cluster (in black) included individuals from the ALG–MOR population sample; the second group (in grey) included individuals belonging to the NTYR, CTYR, and SARD population samples; the third one (in white) comprised individuals from NADR and SADR. Individuals from SIC and WION were associated with both the second and the third clusters, suggesting the Siculo-Tunisian area as a potential admixture zone of the two clusters.

The mismatch distribution analysis showed skewed unimodal profiles for each geographical area, as expected for demographically expanding populations (Figure 4a), whereas SIC and WION showed a bimodal pattern. The Bayesian skyline plot analyses carried out on the pooled samples suggested a constant population size increase in the Mediterranean Sea with a sudden expansion between ~50,000 and 35,000 years ago (Figure 4b).

TABLE 4 Analysis of molecular variance (AMOVA) results obtained with three alternative scenarios of population structure of the Mediterranean *Raja asterias* samples. Results are given for both marker types. Codes of the geographical population samples are given as in Table 1

AMOVA groupings	COI			EST-SSR		
	Percentage of variations	Fixation index	P-value	Percentage of variations	Fixation index	P-value
AMOVA 1, three groups: (1) ALG_MOR; (2) SARD_NTYR_CTYR; (3) NADR_SADR_ SIC_WION						
Among groups	39.74	$\Phi_{CT} = 0.39739$	0.0178	8.71	$F_{CT} = 0.0871$	0.0059
Among populations within group	17.33	$\Phi_{SC} = 0.28756$	0.0000	5	$F_{SC} = 0.0548$	0.0000
Within populations	42.93	$\Phi_{ST} = 0.57068$	0.0000	86.29	$F_{ST} = 0.1381$	0.0000
AMOVA 2, three groups: (1) ALG_MOR; (2) SARD_NTYR_CTYR_SIC_WION; (3) NADR_SADR						
Among groups	52.19	$\Phi_{CT} = 0.52188$	0.0059	9.76	$F_{CT} = 0.0975$	0.0000
Among populations within group	9.04	$\Phi_{SC} = 0.18899$	0.0000	4.58	$F_{SC} = 0.0508$	0.0000
Within populations	38.78	$\Phi_{ST} = 0.61224$	0.0000	85.66	$F_{ST} = 0.1433$	0.0000
AMOVA 3, four groups: (1) ALG_MOR; (2) SARD_NTYR_CTYR; (3) SIC_WION; (4) NADR_SADR						
Among groups	55.32	$\Phi_{CT} = 0.55323$	0.0020	9.13	$F_{CT} = 0.0912$	0.0000
Among populations within group	0.96	$\Phi_{SC} = 0.02147$	0.2036	4.34	$F_{SC} = 0.0477$	0.0000
Within populations	43.72	$\Phi_{ST} = 0.56282$	0.0000	86.53	$F_{ST} = 0.1347$	0.0000

Abbreviations: COI, partial sequence of the mitochondrial gene cytochrome oxidase subunit I; EST-SSR, panel of eight expressed sequence tag-linked microsatellite loci.
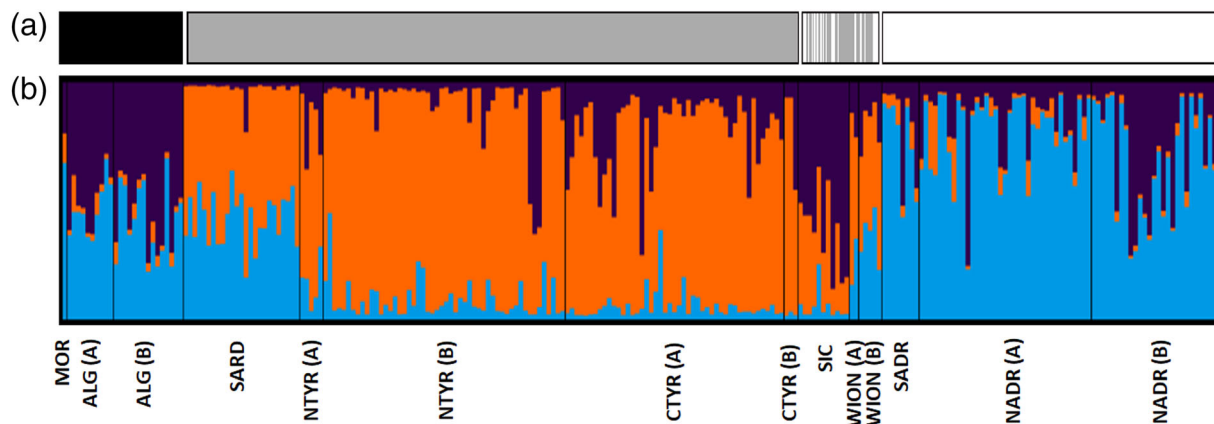

FIGURE 3 Bayesian admixture analysis of *Raja asterias* individuals in the Mediterranean Sea. (a) Distribution of the cytochrome oxidase subunit I clades in the population samples inferred with BAPS; each colour represents one distinct haplogroup (cluster), and each bar represents a different individual. CI: confidence interval. (b) Results of the Bayesian clustering using STRUCTURE with $K = 3$; each vertical bar represents one individual, in which a different colour represents the estimated cluster membership. Codes of the geographical population samples are given in Table 1
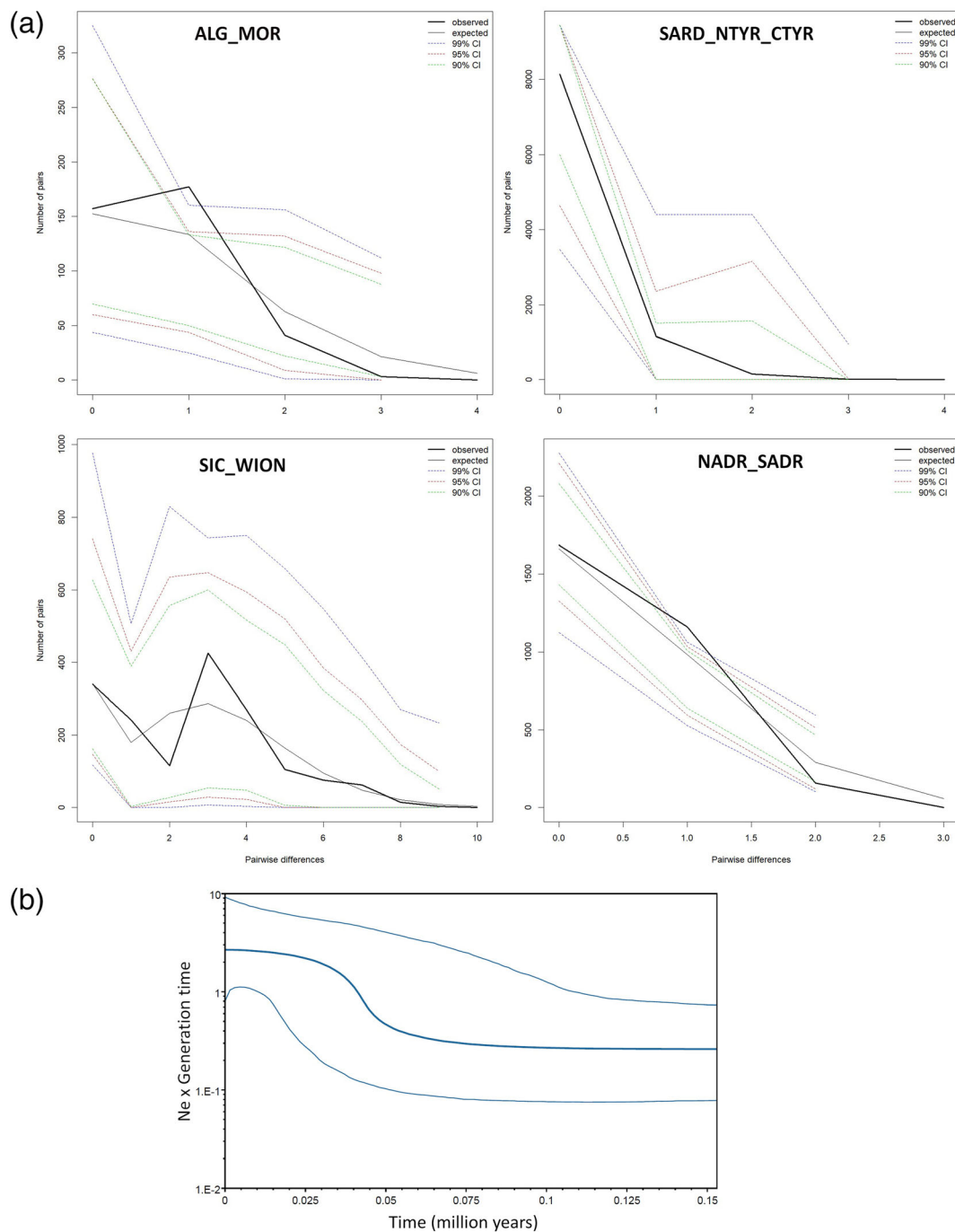
FIGURE 4 Demographic history obtained from the cytochrome oxidase subunit I data set of the Mediterranean *Raja asterias*. (a) Expected and observed mismatch distribution, with confidence intervals, for each distinct geographical area; the horizontal axis is the number of nucleotide pairwise differences between pairs of individuals. Acronyms are consistent with Table 1. (b) Bayesian skyline plot. The *Y*-axis indicates the population size change, expressed as effective population size (Ne) multiplied per Generation time, and the *X*-axis is the mean time in millions of years; the thick solid line represents the mean estimate, and the other blue lines represent 95% highest posterior density intervals

3.3 | Genetic diversity and population structure based on eight EST-SSR loci

The EST-SSR data set included a total of 248 individuals over the 255 originally genotyped to maintain the minimum call rate at 62.5%. Stuttering errors were found only in LERI44 in two population samples (CTYR replicate A and NADR replicate B). The presence of null alleles due possibly to an excess of homozygotes was observed in five loci out of eight for at least one population sample. The estimate of their frequency varied widely among loci and populations (see Table S5).

Summary statistics of population genetic diversity were estimated only in samples with at least five individuals genotyped, whereas all 248 were included in individual-based multi-locus analyses. All eight EST-SSR loci were polymorphic, with the number of alleles ranging from 4 to 20 (Table S6). The highest allelic richness was found in SIC (with an average value of 4.112), whereas a lower value was detected in SARD (with an average value of 2.664). Significant deviations from Hardy–Weinberg equilibrium after the Bonferroni correction were detected sporadically for some population samples (Table S6).

Low values of pairwise F_{ST} were observed among all population samples (not using ENA method, Table 5; using the ENA method, Table S7) and were highly significant ($P < 0.001$) in 21 out of 55 comparisons after correction for multiple testing (Table 5). Non-significant values resulted among population samples within each geographical area except for the comparisons SARD–NTYR replicate B, SARD–CTYR replicate A, and NTYR replicate B–CTYR replicate A. Higher differences were observed in the comparison NTYR replicate B–ALG (both replicates) and between NTYR replicate B and NADR (both replicates). The AMOVA, conducted with different arbitrary groupings of samples, did not reveal substantial differences in the distribution of molecular variance (Table 4).

The individual-based Bayesian clustering revealed more than one genetic population of *R. asterias* in the Mediterranean. The number of genetic clusters associated with the highest probability was $K = 3$, considering as appropriate the value of K next to the reaching of plateau (Figure S2-A). The maximum value of ΔK was verified with $K = 3$, following Evanno, Regnaut & Goudet (2005) (Figure S2-B). The clustering obtained from STRUCTURE and reanalysed with CLUMPAK consistently provided the highest value of mean similarity score for $K = 3$ (Figure 3), in which the three genetic clusters corresponded to the three groups of population samples from the main geographical areas in different proportions.

The DAPC showed a robust separation of distinct geographical groups (Figure 5). Three major groups were formed by individuals from population samples of the western Mediterranean (MOR and ALG), the central-western Mediterranean (with NTYR, CTYR, and SARD), and the central-eastern Mediterranean (NADR, SADR). Most of the individuals from SIC and WION population samples were intermediate between the central-eastern and the central-western population samples while a few intermingled with individuals of the three main groups confirming that the Siculo-Tunisian area acted as admixture zone for the Mediterranean *R. asterias* populations.

Evidence obtained with the mixed-stock analysis showed that, when considering the unique Mixture Collection SIC_WION, the NTYR_CTYR_SARD reporting unit reached the highest proportion of contribution (estimated mixing proportion Pi = 80.60%), with the NTYR collection contributing with $\pi = 66.70\%$ (Table S8). Minor contributions were provided by NADR_SADR ($\pi = 9.84\%$) and ALG_MOR ($\pi = 9.54\%$) in the same inferred mixture. On the other hand, when considering SIC and WION separately, a higher percentage of contribution was supplied by NADR_SADR and ALG_MOR within the SIC mixture collection ($\pi = 14.10\%$ and $\pi = 15.60\%$ respectively; Table S8).

In all population structure analyses the temporal replicates associated with several geographical samples provided consistently congruent results, supporting temporal stability of the inferred pattern of genetic connectivity.

4 | DISCUSSION

4.1 | Highly structured populations

This study addressed the first comprehensive population structure assessment of genetic diversity and spatial connectivity of the

TABLE 5 Pairwise F_{ST} (below diagonal) and associated *P*-values (above diagonal) for microsatellite data conducted on 242 individuals of *Raja asterias* from each geographical area and temporal replicates (A, B) in the Mediterranean Sea (acronyms are consistent with Table 1)

	ALG (A)	ALG (B)	SARD	NTYR (A)	NTYR (B)	CTYR (A)	SIC	WION	SADR	NADR (A)	NADR (B)
ALG (A)		0.873	0.000	0.161	0.000	0.000	0.004	0.028	0.081	0.012	0.050
ALG (B)	−0.017		0.000	0.135	0.000	0.000	0.013	0.002	0.006	0.000	0.009
SARD	**0.081**	**0.095**		0.229	0.000	0.000	0.000	0.013	0.011	0.000	0.000
NTYR (A)	0.041	0.046	0.032		0.382	0.236	0.770	0.809	0.744	0.302	0.305
NTYR (B)	**0.245**	**0.205**	**0.151**	0.014		0.000	0.000	0.043	0.000	0.000	0.000
CTYR (A)	**0.175**	**0.154**	**0.095**	0.023	0.040		0.001	0.174	0.000	0.000	0.000
SIC	0.080	0.052	**0.097**	−0.021	**0.113**	0.074		0.118	0.100	0.000	0.001
WION (B)	0.094	0.119	0.097	−0.044	0.059	0.031	0.042		0.735	0.087	0.032
SADR	0.053	0.089	0.075	−0.013	**0.150**	0.084	0.044	−0.017		0.506	0.161
NADR (A)	0.059	**0.075**	**0.111**	0.015	**0.189**	**0.147**	**0.086**	0.041	−0.002		0.072
NADR (B)	0.037	0.039	**0.120**	0.018	**0.216**	**0.166**	0.088	0.074	0.026	0.014	

Note: Temporal replicate samples were maintained separate.
Bold type indicates significant values after a Bonferroni correction ($P < 0.000909$).
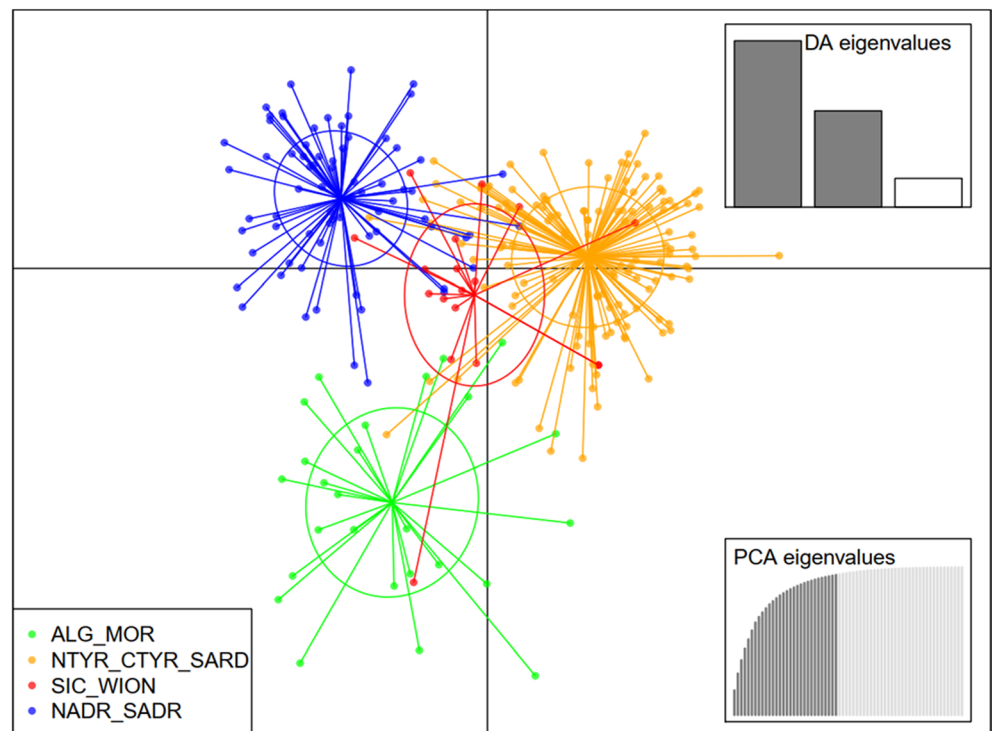
Mediterranean starry ray using a multi-marker approach. Even if the lack in the analysis of samples from the adjacent north-eastern Atlantic (Ordines et al., 2017) prevents the identification of further genetic discontinuity across the Strait of Gibraltar (Patarnello, Volckaert & Castilho, 2007), a strong genetic structuring was revealed in the commonly known distribution area of this skate (i.e. the western and central Mediterranean).

Through a widespread and robust sampling design coupled with the use of high-resolution markers, the results obtained confirm the preliminary evidence of phylogeographic structure of *R. asterias* in the Mediterranean provided by Cariani et al. (2017). Our results support the existence of three differentiated and well-recognized genetic units: one inhabiting the western Mediterranean and including individuals from the Moroccan and Algerian coasts, one in the central-western Mediterranean, with a high number of Tyrrhenian and Sardinian individuals, and one inhabiting the central-eastern Mediterranean and mainly represented by individuals from the Adriatic Sea. The inferred structure appeared robust, as it was obtained with all analyses performed on both typology genetic data (mtDNA: the MJ network and the Bayesian clustering analyses; nuclear DNA: the individual-based Bayesian clustering and the DAPC reconstruction). Three main lineages were identified, each represented by a particular haplogroup dominating specific areas across the range sampled. The only exception was represented by haplotypes observed in the Siculo-Tunisian Strait and western Ionian Sea. In contrast to other rajid species, it was not possible to distinguish which haplotypes could be considered as the most ancient (Chevolot et al., 2006; Pasolini et al., 2011; Jenkins, Castilho & Stevens, 2018). Similar to the findings of phylogeographical patterns, the number of genetic clusters identified by the Bayesian approach

was $K = 3$, with a clear and robust separation of the three distinct geographical groups.

The mixed-stock analysis based on the nuclear data confirmed previous results obtained here with different approaches. In particular, the overall contribution of three main reporting units to the mixture collection SIC_WION appeared congruent with the results described in the DAPC plot, highlighting the close relatedness existing between the individuals collected in the Siculo-Tunisian Strait and Western Ionian Sea and those collected in the north Tyrrhenian Sea. Furthermore, when considering the Sicilian and western Ionian mixture collections separately, the mixing proportions showed high consistency with the STUCTURE bar plot, with the Sicilian collection being also related to the Algerian reference and the western Ionian being mostly represented by central and north Tyrrhenian Sea.

The significant genetic similarity of samples collected from these transitional areas of Siculo-Tunisian Strait and western Ionian Sea with all three of the population units revealed that individuals belonging to the central-western and central-eastern Mediterranean genetic units intermingled in such mixing areas. The admixing and transitional nature of these areas is strongly supported by the consistency of patterns revealed by both mtDNA and nuclear DNA data. Individuals from this area shared haplotypes with those from the surrounding genetic units and displayed an intermediate position in the spatial differentiation analysis. This pattern of differentiation could suggest that Sicilian and western Ionian starry rays are genetically related to individuals from the adjacent areas because of complex, although poorly documented, behaviour. For instance, female philopatry could enhance the starry ray migration to that specific transitional area for mating and egg deposition. The occurrence of philopatry and/or residency has been demonstrated in

31 species of batoids, for which morphological limitations, such as body size and locomotion strategy, could influence seasonal movements and long-distance migrations (Flowers et al., 2016). Similarly, the starry ray exhibits relatively small body size and low dispersal potential (Serena, Mancusi & Barone, 2010; Serena et al., 2015). Population genetic studies have frequently revealed that demersal batoids display population structure over spatial scales of a few hundred kilometres owing to regional philopatry (Li et al., 2013; Li et al., 2015). Fidelity to spawning areas and/or nursery grounds has been reported for *Bathyraja aleutica* (Gilbert, 1896), *Bathyraja interrupta* (Gill & Townsend, 1897), and *Bathyraja parmifera* (Bean, 1881) in the eastern part of the Bering Sea (Hoff, 2010).

The geographical structuring of a few rajid species in the Mediterranean has been revealed by studies combining different genetic approaches (Chevolot et al., 2006; Pasolini et al., 2011; Frodella et al., 2016; Ferrari et al., 2018). All this evidence accounts for patterns of genetic differentiation and potential ecological/environmental factors driving natural history and micro-evolution of Mediterranean skate populations, such as oceanographic fronts and discontinuities or palaeoclimatic changes (Ferrari et al., 2018). In detail, the thornback ray, *R. clavata*, showed phylogeographic structure on a larger scale than the Mediterranean, being characterized by well-defined lineages inhabiting three main macro-areas: the Mediterranean basin, the Azores, and the Atlantic continental shelf (Chevolot et al., 2006). Moreover, *R. clavata* exhibited a weak but detectable structuring in the Levantine Sea (Ferrari et al., 2018). The brown ray *R. miraletus* showed a strongly structured pattern with four geographical clades within the Mediterranean corresponding to the populations off the Algerian coast (western), Tyrrhenian Sea (central-western), Adriatic Sea (central-eastern), and Levantine (eastern) (Ferrari et al., 2018). On the contrary, *R. polystigma* showed a near panmictic population in the central-western Mediterranean, except for the Adriatic individuals, which exhibited a slightly differentiated haplotype from the rest of the Mediterranean population (Frodella et al., 2016). The difference among the patterns of population structure of these closely related species can be explained by different ecological features, especially their bathymetric range (Pennino et al., 2013; Ramírez-Amaro et al., 2018). The two well-structured species *R. miraletus* and *R. asterias* share a narrow bathymetric range with preferences for continental shelf habitats, from 10 to 150 m and 10 to 170 m respectively (Serena, Mancusi & Barone, 2010). The Mediterranean starry ray inhabits the deeper bathymetric range of the continental shelf, preferring muddy and sandy bottoms (Serena et al., 2015) and areas with terrigenous sediments (Navarro et al., 2013; Ferrà et al., 2016). Spatial and ecological constraints could have determined a highly fragmented use of the habitat by the species. The absence of a suboptimal continuum in the ecological conditions in the Mediterranean could lead to genetic structuring of populations. For this reason, the extended deep marine waters in the western and eastern Mediterranean could be considered as dispersal barriers for the starry ray, as already demonstrated for other demersal elasmobranch species (Karl, Castro & Garla, 2012; Kousteni

et al., 2015). For instance, among sharks, *Scyliorhinus canicula* is one of the most interesting cases of strongly differentiated populations across the Mediterranean Sea, likely depending on the complex geomorphology of the basin (i.e. the permanent hydrographic fronts characterizing the Alboran and Balearic sub-basins; Ramírez-Amaro et al., 2018) and on the limited dispersal capability of this species (Barbieri et al., 2014; Gubili et al., 2014; Kousteni et al., 2015).

The initial observed population expansion of *R. asterias* may have occurred in the Mediterranean in the Late Pleistocene with a sudden expansion from ~50,000 to ~35,000 years ago. This trend seems to be supported by low genetic diversity across the sampling locations and a star-like haplotype network. It is reasonable to hypothesize that the Mediterranean Sea has served as a refugium to *R. asterias* during past global climatic changes, as is the case also for other rajids (Chevolot et al., 2006). In particular, marine species may undergo demographic variations, but life history traits such as low fecundity, long generation time, and a slow evolutionary rate would slow down the genetic recovery after a bottleneck event (Domingues, Hilsdorf & Gadig, 2018; Parra et al., 2018). The recent expansion following the latter seems to overlap with the period between 57,000 and 29,000 years ago characterized by sea-level oscillations (Benjamin et al., 2017) that could have influenced the demographic history of the species. Therefore, demographic expansion does not seem congruent with the end of the Last Glacial Maximum (from 26,500 to 20,000 years ago) but would be slightly older. This seems to be in line with other Mediterranean and north-east Atlantic marine fauna, characterized by Early and Late Pleistocene demographic expansions (Aboim et al., 2005; Charrier et al., 2006; Chevolot et al., 2006; Larmuseau et al., 2009; Ferrari et al., 2018).

4.2 | Importance of egg cases in the identification of nursery areas

The huge number of hauls containing egg cases in the Siculo-Tunisian Strait contributed effectively to validating morphological and genetic criteria to assign a given egg case to *R. asterias*. Although no definite species identification was possible for 13 egg cases out of 172, the two morphometric measures selected are a reliable tool for the identification of most of the cases analysed and could accelerate future species identification.

Comparing these results with the few previous studies on *R. asterias* egg cases—12 egg cases from waters off the Languedocian coast (Capapé et al., 2006) and 59 egg cases off Sardinian coasts (Porcu et al., 2017)—the relative smaller size of egg cases in the Siculo-Tunisian Strait could be related to female size. Indeed, positive relationships between egg case length and female total length have been observed in other species of Rajidae and Scyliorhinidae (Ishiyama, 1958; Templeman, 1982; Braccini & Chiaramonte, 2002; Iglesias, Du Buit & Nakaya, 2002; Oddone, Mesa & Ferreira de Amorim, 2006).

The identification of species from egg cases is a useful tool for resolving taxonomic uncertainties and can contribute to improving

knowledge of the spatial distribution of specific zones, such as spawning or nursery areas, which is a priority for the conservation of elasmobranchs (Mancusi et al., 2021). The presence of a large quantity of egg cases in the Siculo-Tunisian Strait and the repeated use of the area over a number of years seem to suggest the existence of an egg-case nursery (Heupel, Carlson & Simpfendorfer, 2007; Hoff, 2016; Martins et al., 2018). However, further quantitative investigations on egg case and adult densities are strongly recommended. Spatial reproductive ecology and site-fidelity behaviour of *R. asterias* need to be improved by studies focusing on spatial and seasonal movement of starry ray individuals using tagging methods, especially in zones where different populations intermingle (e.g. passive acoustic telemetry, mark-and-recapture and fine-scale fishing surveys; Simpson, Humphries & Sims, 2021). Furthermore, extending tagging studies to juvenile individuals could clarify how the starry ray uses the Siculo-Tunisian Strait and whether this area, besides playing a role as an 'egg case nursery area', acts also as a 'juvenile nursery' *sensu* Hoff (2016). If future seasonal migration studies confirm its role as a nursery, the combination with genetic results obtained here could unravel the admixture nature of the area. The Siculo-Tunisian Strait represents a crucial connection between the western and eastern Mediterranean Basin, ensuring the reproductive reservoirs and genetic richness for the entire species.

4.3 | Implications for management and conservation

The overall decline of elasmobranch species at the global level has led to the growing and necessary consideration of the degree of fragmentation of populations in relation to their vulnerability to human activities (e.g. climate change effects, overfishing, bycatch and their relative downstream threats; Ferretti et al., 2005; Elliott et al., 2020). To date, mitigation measures, such as the designation of marine protected areas or shark sanctuaries and fishing effort changes, have been adopted at different geographical scales to help allow the recovery and/or protection of local populations of vulnerable species (Baum et al., 2003; Shephard et al., 2012; Ward-Paige et al., 2012). Nevertheless, the establishment and effectiveness of such actions may depend on different factors, as population dynamics or mating success may not represent a possible solution. For these reasons, now more than ever, the implementation of conservation policies requires an interdisciplinary strategy, incorporating data on genetics and reproduction (Domingues, Hilsdorf & Gadig, 2018; Melis et al., 2020), ecological and life-history traits such as migratory patterns (Neat et al., 2015), and use of habitats and resources (Coll, Navarro & Palomera, 2013).

The Mediterranean starry ray is described as a minor commercial species at the regional and subregional levels (Food and Agriculture Organization of the United Nations, 2020) and appears among the skate species most affected by high rates of bycatch in multispecies trawl fisheries (Biton-Porsmoguer & Lloret, 2020; Geraci et al., 2021). An essential step in the implementation of conservation policies of

biological resources is the knowledge of the population unit boundaries. Thus, effective plans for future species viability should incorporate the intraspecific levels of biodiversity. In this context, evolutionarily significant units (ESUs; sensu Moritz, 1994; Moritz, 2002) are generally defined as populations that warrant separate management or prioritization for conservation because of high genetic and ecological distinctiveness, and they represent ideal targets for conservation because they contain the raw material for future evolutionary adaptations (Pauls et al., 2013). According to Moritz's (1994) criteria, at least three Mediterranean ESUs of *R. asterias* can be identified based on the genetic structure results: one inhabiting the coasts of Morocco and Algeria, a second distributed in the Tyrrhenian coastal waters of Italy and Sardinia, and the third in the whole of the Adriatic Sea. Each proposed ESU is genetically differentiated and reproductively isolated from other population units and represents an important component in the evolutionary legacy of this species (Waples, 1991). These outcomes should be acknowledged in the definition of management plans for each identified unit and used to update the *Action Plan for the Conservation of Cartilaginous Fishes (Chondrichthyans) in the Mediterranean Sea* (Specially Protected Areas Regional Activity Centre & UN Environment/Mediterranean Action Plan, 2020).

To achieve this main goal, each unit should be better characterized for bio-ecological parameters (e.g. from population dynamics to demography, from life-history traits to maturity stages), and the long-term monitoring of bycatch data at the local scale should be implemented. Among the conservation and risk mitigation actions that could be considered to reduce vulnerability to fishery-based stressors, it would be important to assess for each identified unit the size at maturity of both sexes to establish a minimum conservation reference size (Frisk, Miller & Fogarty, 2001; Barausse et al., 2014) by estimating growth through multiple marginal analyses (e.g. dermal thorns, vertebral units) and tagging experiments (Cailliet & Goldman, 2004; Carbonara et al., 2020). In addition, as suggested by Biton-Porsmoguer & Lloret (2020) in the westernmost part of the Mediterranean and in the adjacent Gulf of Cadiz, the release of all skate should be mandatory in the areas where populations are strongly impacted by trawling, given the high survival rates of skates once discarded (Enever et al., 2009; Saygu & Deval, 2014). Such an integrated strategy, recently adopted in the southern Adriatic Sea on the congeneric *R. clavata* L. (Carbonara et al., 2020), shows promising results in assessing the stock status and, consequently, in defining conservation guidelines across the whole Mediterranean for each population unit.

On the other hand, starry ray individuals inhabiting the Siculo-Tunisian Strait and surrounding areas cannot be considered a distinct ESU because the genetic variation shown here is a combination of those of the three aforementioned regional populations. However, this unit is of substantial ecological and reproductive significance to the species (Waples, 1991) and could be considered as a unit for conservation purposes, as demonstrated by both genetic and ecological evidence (Moritz, 1994). Favourable biological and ecological conditions of the area identify the Siculo-Tunisian Strait as a valuable habitat for the reproduction of

several fish species (Bradai, Saidi & Enajjar, 2012). The complex oceanographic conditions enhance the high productivity and fish spawning, making the Siculo-Tunisian Strait an important nursery and aggregation ground for a number of commercially and non-commercially valuable species (Garofalo et al., 2004; Garofalo et al., 2008; Garofalo et al., 2011). Further feeding data likely obtained by morphological and isotope gut content analyses may be useful to verify niche partitions or overlap with other rajid species or intraspecific patterns—see surveys conducted by Sviben et al. (2019) in the Adriatic Sea and Yemışken et al. (2018) in the Levantine Sea.

The identification of critical grounds for the protection and restoration of cartilaginous fish (i.e. mating, spawning, and nursery areas) is a priority of the Specially Protected Areas Regional Activity Centre, especially for those species/populations with restricted and fragmented distribution and for commercially exploited species. Thus, the development of spatial planning approaches in favour of the resource conservation becomes mandatory. The outcomes of the monitoring and the geo-localization of species' egg cases collected in the Siculo-Tunisian Strait suggest this approach to identify critical zones in the rest of the distribution area of *R. asterias*. Once these reproductive areas are defined, protection actions are needed to tackle the negative impact of fisheries. For instance, the restriction of fisheries by seasonal prohibition of trawling in periods when females lay egg cases (summer to early autumn) and the introduction of restrictions on anchoring to protect egg cases (Biton-Porsmoguer & Lloret, 2020) could be effective local measures for the conservation of the Siculo-Tunisian population of starry ray and other skates.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The sequence data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank, reference number GU805132 - GU805135, GU805179 - GU805180, GU805199, GU805385 - GU805389, GU805423, GU805440 - GU805442, GU805813, GU805816, KT307374 -KT307384, MW300433 - MW300465. The EST-SSR data that support the findings of this study are available as genotype matrix from the corresponding author upon request.

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REFERENCES

Abella, A.J., Mancusi, C. & Serena, F. (2017). *Raja asterias*. In: P. Sartor, A. Mannini, R. Carlucci, E. Massaro, S. Queirolo, A. Sabatini, G. Scarcella, R. Simoni (Eds.) *Synthesis of the knowledge on biology, ecology and fishery of the halieutic resources of the Italian seas*, Biologia Marina Mediterranea, vol. 24(1). Genoa, Italy: Società Italiana di Biologia Marina, pp. 144–149.

Abella, A.J. & Serena, F. (2005). Comparison of elasmobranch catches from research trawl surveys and commercial landings at port of Viareggio, Italy, in the last decade. *Journal of Northwest Atlantic Fishery Science*, 35, 345–356. https://doi.org/10.2960/J.v35.m526

Aboim, M.A., Menezes, G.M., Schlitt, T. & Rogers, A.D. (2005). Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence analysis. *Molecular Ecology*, 14(5), 1343–1354. https://doi.org/10.1111/j.1365-294X.2005.02518.x

Adams, K.R., Fetterplace, L.C., Davis, A.R., Taylor, M.D. & Knott, N.A. (2018). Sharks, rays and abortion: The prevalence of capture-induced parturition in elasmobranchs. *Biological Conservation*, 217, 11–27. https://doi.org/10.1016/j.biocon.2017.10.010

Bandelt, H.J., Forster, P. & Rohl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barausse, A., Correale, V., Curkovic, A., Finotto, L., Riginella, E., Visentin, E. et al. (2014). The role of fisheries and the environment in driving the decline of elasmobranchs in the northern Adriatic Sea. *ICES Journal of Marine Science*, 71(7), 1593–1603. https://doi.org/10.1093/icesjms/fst222

Barbieri, M., Maltagliati, F., Roldán, M.I. & Castelli, A. (2014). Molecular contribution to stock identification in the small-spotted catshark, *Scyliorhinus canicula* (Chondrichthyes, Scyliorhinidae). *Fishery Resources*, 154, 1–16. https://doi.org/10.1016/j.fishres.2014.01.021

Barone, M., De Ranieri, S., Fabiani, O., Pirone, A. & Serena, F. (2007). Gametogenesis and maturity stages scale of *Raja asterias* Delaroche, 1809 (Chondrichthyes, Raijdae) from the south Ligurian Sea. In: *Biodiversity in enclosed seas and artificial marine habitats*. Dordrecht, Netherlands: Springer, pp. 245–254. https://doi.org/10.1007/978-1-4020-6156-1_22

Baum, J.K., Myers, R.A., Kehler, D.G., Worm, B., Harley, S.J. & Doherty, P.A. (2003). Collapse and conservation of shark populations in the northwest Atlantic. *Science*, 299(5605), 389–392. https://doi.org/10.1126/science.1079777

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (1996). GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Available at: https://www.scienceopen.com/document?vid=7cfcd230-1958-4cfc-a571-ce0ba003e63f [Accessed 1 April 2020]

Benjamin, J., Rovere, A., Fontana, A., Furlani, S., Vacchi, M., Inglis, R.H. et al. (2017). Late Quaternary sea-level changes

and early human societies in the central and eastern Mediterranean Basin: An interdisciplinary review. *Quaternary International*, 449, 29–57. https://doi.org/10.1016/j.quaint.2017.06.025

Bianchi, C.N. (2007). Biodiversity issues for the forthcoming tropical Mediterranean Sea. *Hydrobiologia*, 580(1), 7–21. https://doi.org/10.1007/s10750-006-0469-5

Bianchi, C.N. & Morri, C. (2000). Marine biodiversity of the Mediterranean Sea: Situation, problems and prospects for future research. *Marine Pollution Bulletin*, 40(5), 367–376. https://doi.org/10.1016/S0025-326X(00)00027-8

Biton-Porsmoguer, S. & Lloret, J. (2020). Potential impacts of bottom trawling on species of skates (Rajiformes: Rajidae): The case of the Gulf of Cádiz and the western Mediterranean. *Cybium*, 44(3), 255–263. https://doi.org/10.26028/cybium/2020-443-006

Braccini, J.M. & Chiaramonte, G.E. (2002). Reproductive biology of *Psammobatis extenta*. *Journal of Fish Biology*, 61(1), 272–288. https://doi.org/10.1111/j.1095-8649.2002.tb01752.x

Bradai, M.N., Saidi, B. & Enajjar, S. (2012). *Elasmobranchs of the Mediterranean and Black Sea: Status, ecology and biology. Bibliographic analysis*, Studies and Reviews. General Fisheries Commission for the Mediterranean, No. 91. Rome, Italy: FAO.

Cailliet, G.M. & Goldman, K.J. (2004). Age determination and validation in chondrichthyan fishes. In: J. Carrier, J.A. Musick, M.R. Heithaus (Eds.) *Biology of sharks and their relatives*. Boca Raton, FL: CRC Press, pp. 399–447. https://doi.org/10.1201/9780203491317.pt3

Capapé, C. (1977). Contribution à la biologie des Rajidae des côtes tunisiennes. IV. *Raja asterias* Delaroche, 1809: Répartition géographique et bathymétrique, sexualité, reproduction et fécondité. *Bulletin du Muséum National d'Histoire Naturelle*, 435(305), 305–326. https://www.biodiversitylibrary.org/part/282042

Capapé, C., Guélorget, O., Vergne, Y., Marquès, A. & Quignard, J.P. (2006). Skates and rays (Chondrichthyes) from waters off the Languedocian coast (southern France, northern Mediterranean): A historical survey and present status. *Annales: Series Historia Naturalis*, 16(2), 165–178.

Capapé, C. & Quignard, J.-P. (1977). Contribution à la biologie des Rajidae des côtes tunisiennes. 6. *Raja asterias* Delaroche, 1809. Régime alimentaire. *Bulletin de l'Institut National Scientifique et Technique d'Océanographie et de Pêche Salammbô*, 4(2–4), 319–332.

Carbonara, P., Bellodi, A., Palmisano, M., Mulas, A., Porcu, C., Zupa, W. et al. (2020). Growth and age validation of the thornback ray (*Raja clavata* Linnaeus, 1758) in the south Adriatic Sea (central Mediterranean). *Frontiers in Marine Science*, 7, 1100. https://doi.org/10.3389/fmars.2020.586094

Cariani, A., Messinetti, S., Ferrari, A., Arculeo, M., Bonello, J.J., Bonnici, L. et al. (2017). Improving the conservation of Mediterranean Chondrichthyans: The ELASMOMED DNA barcode reference library. *PLoS ONE*, 12(1), e0170244. https://doi.org/10.1371/journal.pone.0170244

Catalano, B., Mancusi, C., Clò, S., Dalu, M., Serena, F. & Vacchi, M. (2003). Marcatura e rilascio di esemplari giovanili di razza stellata *Raja asterias* nelle acque toscane: Risultati preliminari e prospettive di lavoro. In: *Atti XXXIII congresso SIBM. Castelsardo (SS), 3–8 giugno 2002*, Biologia Marina Mediterranea vol. 10(2). Genoa, Italy: Società Italiana di Biologia Marina, pp. 789–791.

Chapuis, M.P. & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24(3), 621–631. https://doi.org/10.1093/molbev/msl191

Charrier, G., Chenel, T., Durand, J.D., Girard, M., Quiniou, L. & Laroche, J. (2006). Discrepancies in phylogeographical patterns of two European anglerfishes (*Lophius budegassa* and *Lophius piscatorius*). *Molecular Phylogenetics and Evolution*, 38(3), 742–754. https://doi.org/10.1016/j.ympev.2005.08.002

Cheng, L., Connor, T.R., Sirén, J., Aanensen, D.M. & Corander, J. (2013). Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. *Molecular Biology and Evolution*, 30(5), 1224–1228. https://doi.org/10.1093/molbev/mst028

Chevolot, M., Hoarau, G., Rijnsdorp, A.D., Stam, W.T. & Olsen, J.L. (2006). Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology*, 15(12), 3693–3705. https://doi.org/10.1111/j.1365-294X.2006.03043.x

Cockerham, C.C. (1973). Analyses of gene frequencies. *Genetics*, 74(4), 679–700.

Coll, M., Navarro, J. & Palomera, I. (2013). Ecological role, fishing impact, and management options for the recovery of a Mediterranean endemic skate by means of food web models. *Biological Conservation*, 157, 108–120. https://doi.org/10.1016/j.biocon.2012.06.029

Colloca, F., Cardinale, M., Maynou, F., Giannoulaki, M., Scarcella, G., Jenko, K. et al. (2013). Rebuilding Mediterranean fisheries: A new paradigm for ecological sustainability. *Fish and Fisheries*, 14(1), 89–109. https://doi.org/10.1111/j.1467-2979.2011.00453.x

Cortés, E. (2000). Life history patterns and correlations in sharks. *Reviews in Fisheries Science*, 8(4), 299–344. https://doi.org/10.1080/10408340308951115

Cuoco, C., Mancusi, C. & Serena, F. (2005). Studio sulle abitudini alimentari di *Raja asterias* Delaroche, 1809 (Chondrichthyes, Rajidae). *Biolgia Marina Mediterranea*, 12(1), 504–508.

Cuttelod, A., García, N., Abdul Malak, D., Temple, H. & Katariya, V. (2008). The Mediterranean: A biodiversity hotspot under threat. In: J.C. Vié, C. Hilton-Taylor, S.N. Stuart (Eds.) *The 2008 review of the IUCN Red List of Threatened Species*. Gland, Switzerland: IUCN.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, 9, 772. https://doi.org/10.1038/nmeth.2109

Davidson, L.N.K., Krawchuk, M.A. & Dulvy, N.K. (2016). Why have global shark and ray landings declined: Improved management or overfishing? *Fish and Fisheries*, 17(2), 438–458. https://doi.org/10.1111/faf.12119

Domingues, R.R., Hilsdorf, A.W.S. & Gadig, O.B.F. (2018). The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics*, 19(3), 501–525. https://doi.org/10.1007/s10592-017-1038-3

Drummond, A.J., Rambaut, A., Shapiro, B. & Pybus, O.G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, 22(5), 1185–1192. https://doi.org/10.1093/molbev/msi103

Dulvy, N.K., Allen, D.J., Ralph, G.M. & Walls, R.H.L. (2016). *The conservation status of sharks, rays, and chimaeras in the Mediterranean Sea*. Malaga, Spain: IUCN. https://doi.org/10.13140/RG.2.2.22020.53129

Earl, D.A. & VonHoldt, B.M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Ebert, D.A. & Davis, C.D. (2007). Descriptions of skate egg cases (Chondrichthyes: Rajiformes: Rajoidei) from the eastern North Pacific. *Zootaxa*, 1393(1), 1–18. https://doi.org/10.11646/zootaxa.1393.1.1

El Nagar, A., McHugh, M., Rapp, T., Sims, D.W. & Genner, M.J. (2010). Characterisation of polymorphic microsatellite markers for skates (Elasmobranchii: Rajidae) from expressed sequence tags. *Conservation Genetics*, 11(3), 1203–1206. https://doi.org/10.1007/s10592-009-9919-8

Elliott, S.A., Bearup, D., Carpentier, A., Larivain, A., Trancart, T. & Feunteun, E. (2020). Evaluating the effectiveness of management measures on skates in a changing world. *Biological Conservation*, 248, 108684. https://doi.org/10.1016/j.biocon.2020.108684

Enajjar, S., Saidi, B. & Bradai, M.N. (2015). The Gulf of Gabes (Central Mediterranean Sea): A nursery area for sharks and batoids (Chondrichthyes: Elasmobranchii). *Cahiers de Biologie Marine*, 56, 143–150. https://doi.org/10.21411/CBM.A.4265FA1C

Enever, R., Catchpole, T.L., Ellis, J.R. & Grant, A. (2009). The survival of skates (Rajidae) caught by demersal trawlers fishing in UK waters.

Fisheries Research, 97(1–2), 72–76. https://doi.org/10.1016/j.fishres.2009.01.001

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, 131(2), 479–491. https://doi.org/10.1093/genetics/131.2.479

Ferrà, C., Fabi, G., Polidori, P., Tassetti, A.N., Leoni, S., Pellini, G. et al. (2016). *Raja asterias* population assessment in FAO GFCM GSA17 area. *Mediterranean Marine Science*, 17(3), 651–660. https://doi.org/10.12681/mms.1657

Ferrari, A., Tinti, F., Maresca, V.B., Velonà, A., Cannas, R., Thasitis, I. et al. (2018). Natural history and molecular evolution of demersal Mediterranean sharks and skates inferred by comparative phylogeographic and demographic analyses. *PeerJ*, 6, e5560. https://doi.org/10.7717/peerj.5560

Ferretti, F., Myers, R.A., Sartor, P. & Serena, F. (2005). Long term dynamics of the Chondrichthyan fish community in the upper Tyrrhenian Sea. ICES CM 2005/N:25. Copenhagen, Denmark: International Council for the Exploration of the Sea.

Ferretti, F., Myers, R.A., Serena, F. & Lotze, H.K. (2008). Loss of large predatory sharks from the Mediterranean Sea. *Conservation Biology*, 22(4), 952–964. https://doi.org/10.1111/j.1523-1739.2008.00938.x

Ferretti, F., Osio, G.C., Jenkins, C.J., Rosenberg, A.A. & Lotze, H.K. (2013). Long-term change in a meso-predator community in response to prolonged and heterogeneous human impact. *Scientific Reports*, 3(1), 1057. https://doi.org/10.1038/srep01057

Flowers, K.I., Ajemian, M.J., Bassos-Hull, K., Feldheim, K.A., Hueter, R.E., Papastamatiou, Y.P. et al. (2016). A review of batoid philopatry, with implications for future research and population management. *Marine Ecology Progress Series*, 562, 251–261. https://doi.org/10.3354/meps11963

Follesa, M.C., Marongiu, M.F., Zupa, W., Bellodi, A., Cau, A., Cannas, R. et al. (2019). Spatial variability of Chondrichthyes in the northern Mediterranean. *Scientia Marina*, 83(S1), 81–100. https://doi.org/10.3989/scimar.04998.23A

Food and Agriculture Organization of the United Nations. (2020). *The state of Mediterranean and Black Sea fisheries 2020*. Rome, Italy: General Fisheries Commission for the Mediterranean. https://doi.org/10.4060/cb2429en

Frisk, M.G., Miller, T.J. & Fogarty, M.J. (2001). Estimation and analysis of biological parameters in elasmobranch fishes: A comparative life history study. *Canadian Journal of Fisheries and Aquatic Sciences*, 58(5), 969–981. https://doi.org/10.1139/f01-051

Frodella, N., Cannas, R., Velonà, A., Carbonara, P., Farrell, E., Fiorentino, F. et al. (2016). Population connectivity and phylogeography of the Mediterranean endemic skate *Raja polystigma* and evidence of its hybridization with the parapatric sibling *R. montagui*. *Marine Ecology Progress Series*, 554, 99–113. https://doi.org/10.3354/meps11799

Froese, R. & Pauly, D. (2020). FishBase. World Wide Web electronic publication. Available at: https://www.fishbase.org [Accessed 22 February 2021]

Garofalo, G., Bel Hassen, M., Jarboui, O., Zgozi, S., Gristina, M., Fiorentino, F. et al. (2008). Preliminary results on spatial distribution of abundance indices, nursery and spawning areas of *Merluccius merluccius* and *Mullus barbatus* in the central Mediterranean. GCP/RER/010/ITA/MSM-TD 19. MedSudMed Project: Rome, Italy.

Garofalo, G., Fiorentino, F., Bono, G., Gancitano, S. & Norrito, G. (2004). Localisation of spawning and nursery areas of red mullet (*Mullus barbatus*, Linnaeus) in the Italian side of the Strait of Sicily (central Mediterranean). *GIS/Spatial Analyses in Fishery and Aquatic Sciences*, 2, 101–110.

Garofalo, G., Fortibuoni, T., Gristina, M., Sinopoli, M. & Fiorentino, F. (2011). Persistence and co-occurrence of demersal nurseries in the Strait of Sicily (central Mediterranean): Implications for fishery management. *Journal of Sea Research*, 66(1), 29–38. https://doi.org/10.1016/j.seares.2011.04.008

Geraci, M.L., Ragonese, S., Scannella, D., Falsone, F., Gancitano, V., Mifsud, J. et al. (2021). Batoid abundances, spatial distribution, and life history traits in the Strait of Sicily (central Mediterranean Sea): Bridging a knowledge gap through three decades of survey. *Animals*, 11(8), 2189. https://doi.org/10.3390/ani11082189

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at: http://www2.unil.ch/popgen/softwares/fstat.htm [Accessed 15 March 2020]

Grati, F., Scarcella, G., Polidori, P., Domenichetti, F., Bolognini, L., Gramolini, R. et al. (2013). Multi-annual investigation of the spatial distributions of juvenile and adult sole (*Solea solea* L.) in the Adriatic Sea (northern Mediterranean). *Journal of Sea Research*, 84, 122–132. https://doi.org/10.1016/j.seares.2013.05.001

Gubili, C., Sims, D.W., Veríssimo, A., Domenici, P., Ellis, J., Grigoriou, P. et al. (2014). A tale of two seas: Contrasting patterns of population structure in the small-spotted catshark across Europe. *Royal Society Open Science*, 1(3), 140–175. https://doi.org/10.1098/rsos.140175

Hasegawa, M., Kishino, H. & Yano, T.A. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, 22(2), 160–174. https://doi.org/10.1007/BF02101694

Heupel, M.R., Carlson, J.K. & Simpfendorfer, C.A. (2007). Shark nursery areas: Concepts, definition, characterization and assumptions. *Marine Ecology Progress Series*, 337, 287–297. https://doi.org/10.3354/meps337287

Heupel, M.R., Kanno, S., Martins, A.P.B. & Simpfendorfer, C.A. (2019). Advances in understanding the roles and benefits of nursery areas for elasmobranch populations. *Marine and Freshwater Research*, 70(7), 897–907. https://doi.org/10.1071/MF18081

Hoff, G.R. (2010). Identification of skate nursery habitat in the eastern Bering Sea. *Marine Ecology Progress Series*, 403, 243–254. https://doi.org/10.3354/meps08424

Hoff, G.R. (2016). Identification of multiple nursery habitats of skates in the eastern Bering Sea. *Journal of Fish Biology*, 88(5), 1746–1757. https://doi.org/10.1111/jfb.12939

Iglesias, S.P., Du Buit, M.H. & Nakaya, K. (2002). Egg capsules of deep-sea catsharks from eastern north Atlantic, with first description of the capsule of *Galeus mirinus* and *Apristurus aphyodes* (Chondrichthyes: Scyliorhinidae). *Cybium*, 26(1), 59–63.

Ishihara, H., Treloar, M., Bor, P.H., Senou, H. & Jeong, C.H. (2012). The comparative morphology of skate egg capsules (Chondrichthyes: Elasmobranchii: Rajiformes). *Bulletin of the Kanagawa Prefectural Museum, Natural Science*, 41, 9–25.

Ishiyama, R. (1958). Observations on the egg-capsules of skates of the family Rajidae, found in Japan and its adjacent waters. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 118(1), 1–24.

Jenkins, T.L., Castilho, R. & Stevens, J.R. (2018). Meta-analysis of northeast Atlantic marine taxa shows contrasting phylogeographic patterns following post-LGM expansions. *PeerJ*, 6, e5684. https://doi.org/10.7717/peerj.5684

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Devillard, S. & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically

structured populations. *BMC Genetics*, 11(1), 94. https://doi.org/10.1186/1471-2156-11-94

Karl, S.A., Castro, A.L.F. & Garla, R.C. (2012). Population genetics of the nurse shark (*Ginglymostoma cirratum*) in the western Atlantic. *Marine Biology*, 159(3), 489–498. https://doi.org/10.1007/s00227-011-1828-y

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A. & Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across *K*. *Molecular Ecology Resources*, 15(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Kousteni, V., Kasapidis, P., Kotoulas, G. & Megalofonou, P. (2015). Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (*Scyliorhinus canicula*) in the Mediterranean Sea. *Heredity*, 114(3), 333–343. https://doi.org/10.1038/hdy.2014.107

Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Larmuseau, M.H.D., Houdt, J.K.J.V., Guelinckx, J., Hellemans, B. & Volckaert, F.A.M. (2009). Distributional and demographic consequences of Pleistocene climate fluctuations for a marine demersal fish in the north-eastern Atlantic. *Journal of Biogeography*, 36(6), 1138–1151. https://doi.org/10.1111/j.1365-2699.2008.02072.x

Leigh, J.W. & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Li, N., Chen, X., Sun, D., Song, N., Lin, Q. & Gao, T. (2015). Phylogeography and population structure of the red stingray, *Dasyatis akajei* inferred by mitochondrial control region. *Mitochondrial DNA*, 26(4), 505–513. https://doi.org/10.3109/19401736.2013.861423

Li, N., Song, N., Cheng, G. & Gao, T. (2013). Genetic diversity and population structure of the red stingray, *Dasyatis akajei* inferred by AFLP marker. *Biochemical Systematics and Ecology*, 51, 130–137. https://doi.org/10.1016/j.bse.2013.08.009

Mancusi, C., Massi, D., Baino, R., Cariani, A., Crobe, V., Ebert, D.A. et al. (2021). An identification key for Chondrichthyes egg cases of the Mediterranean and Black Sea. *European Zoological Journal*, 88(1), 436–448. https://doi.org/10.1080/24750263.2020.1862926

Mancusi C. & Serena F. (2015). Diagnosi morfologica delle capsule ovigere dei condroitti mediterranei. Metodiche di studio e chiavi di determinazione specifica. Seminario IAMC-CNR, Mazara del Vallo (TP). Available at: http://eprints.bice.rm.cnr.it/16152/1/Diagnosi%20morfologica%20delle%20capsule%20ovigere%20dei%20condroitti%20mediterranei.pdf

Martins, A., Heupel, M., Chin, A. & Simpfendorfer, C. (2018). Batoid nurseries: Definition, use and importance. *Marine Ecology Progress Series*, 595, 253–267. https://doi.org/10.3354/meps12545

Massi, D., Cariani, A., Mancusi, C., Titone, A., Ferrari, A., Crobe, V. et al. (2018). Biometry and molecular data of egg-cases of *Raja asterias* Delaroche, 1809 (Chondrichthyes, Rajidae) in the Strait of Sicily (central Mediterranean). In: *Atti XLIX congresso SIBM. Cesenatico, 4–8 giugno 2018*, Biologia Marina Mediterranea vol. 25(1). Genoa, Italy: Società Italiana di Biologia Marina, pp. 249–250.

Massutí, E. & Moranta, J. (2003). Demersal assemblages and depth distribution of elasmobranchs from the continental shelf and slope off the Balearic Islands (western Mediterranean). *ICES Journal of Marine Science*, 60(6), 1398–1398. https://doi.org/10.1016/S1054-3139(03)00144-9

Melis, R., Vacca, L., Bellodi, A., Cau, A., Porcu, C., Follesa, M.C. et al. (2020). Insights into population genetics, connectivity and demographic history of the longnosed skate *Dipturus oxyrinchus* (Linnaeus, 1758) in the western Mediterranean Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30(1), 86–103. https://doi.org/10.1002/aqc.3241

Milisenda, G., Vitale, S., Massi, D., Enea, M., Gancitano, V., Giusto, G.B. et al. (2017). Discard composition associated with the deep water rose shrimp fisheries (*Parapenaeus longirostris*, Lucas 1846) in the south-central Mediterranean Sea. *Mediterranean Marine Science*, 18(1), 53–63. https://doi.org/10.12681/mms.1787

Minervini, R., Giannotta, M. & Bianchini, M.L. (1985). Observations on the fishery of Rajiformes in central Tyrrhenian Sea. *Oebalia*, 11(2), 583–591.

Moran, B.M. & Anderson, E.C. (2018). Bayesian inference from the conditional genetic stock identification model. *Canadian Journal of Fisheries and Aquatic Sciences*, 76(4), 551–560. https://doi.org/10.1139/cjfas-2018-0016

Moritz, C. (1994). Defining 'evolutionarily significant units' for conservation. *Trends in Ecology & Evolution*, 9(10), 373–375. https://doi.org/10.1016/0169-5347(94)90057-4

Moritz, C. (2002). Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology*, 51(2), 238–254. https://doi.org/10.1080/10635150252899752

Mulas, A., Bellodi, A., Cannas, R., Carbonara, P., Cau, A., Marongiu, M.F. et al. (2019). Resource partitioning among sympatric elasmobranchs in the central-western Mediterranean continental shelf. *Marine Biology*, 166(12), 153. https://doi.org/10.1007/s00227-019-3607-0

Navarro, J., Coll, M., Preminger, M. & Palomera, I. (2013). Feeding ecology and trophic position of a Mediterranean endemic ray: Consistency between sexes, maturity stages and seasons. *Environmental Biology of Fishes*, 96(12), 1315–1328. https://doi.org/10.1007/s10641-013-0109-7

Neat, F., Pinto, C., Burrett, I., Cowie, L., Travis, J., Thorburn, J. et al. (2015). Site fidelity, survival and conservation options for the threatened flapper skate (*Dipturus* cf. *intermedia*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25(1), 6–20. https://doi.org/10.1002/aqc.2472

Oddone, M.C., Mesa, A. & Ferreira de Amorim, A. (2006). The egg capsule of *Rioraja agassizi* (Müller & Henle) (Elasmobranchii, Rajidae), endemic to the SW Atlantic. *Pan-American Journal of Aquatic Sciences*, 1(2), 43–48.

Ordines, F., Baro, J., Ramírez-Amaro, S., Serena, F. & Sobrino, I. (2017). First substantiated record of *Raja asterias* Delaroche, 1809 (Elasmobranchii: Rajiformes: Rajidae) in the Gulf of Cádiz, north-eastern Atlantic. *Acta Ichthyologica et Piscatoria*, 47(1), 101–106. https://doi.org/10.3750/AIEP/02161

Otero, M., Serena, F., Gerovasileiou, V., Barone, M., Bo, M., Arcos, J.M. et al. (2019). *Identification guide of vulnerable species incidentally caught in Mediterranean fisheries*. Malaga, Spain: IUCN. Available at: https://portals.iucn.org/library/sites/library/files/documents/2019-050-En.pdf

Parra, G.J., Cagnazzi, D., Jedensjö, M., Ackermann, C., Frere, C., Seddon, J. et al. (2018). Low genetic diversity, limited gene flow and widespread genetic bottleneck effects in a threatened dolphin species, the Australian humpback dolphin. *Biological Conservation*, 220, 192–200. https://doi.org/10.1016/j.biocon.2017.12.028

Pasolini, P., Ragazzini, C., Zaccaro, Z., Cariani, A., Ferrara, G., Gonzalez, E.G. et al. (2011). Quaternary geographical sibling speciation and population structuring in the eastern Atlantic skates (suborder Rajoidea) *Raja clavata* and *R. straeleni*. *Marine Biology*, 158(10), 2173–2186. https://doi.org/10.1007/s00227-011-1722-7

Patarnello, T., Volckaert, F.A. & Castilho, R. (2007). Pillars of Hercules: Is the Atlantic–Mediterranean transition a phylogeographical break? *Molecular Ecology*, 16(21), 4426–4444. https://doi.org/10.1111/j.1365-294X.2007.03477.x

Pauls, S.U., Nowak, C., Bálint, M. & Pfenninger, M. (2013). The impact of global climate change on genetic diversity within populations and

species. *Molecular Ecology*, 22(4), 925–946. https://doi.org/10.1111/mec.12152

Pennino, M.G., Muñoz, F., Conesa, D., López-Quílez, A. & Bellido, J.M. (2013). Modeling sensitive elasmobranch habitats. *Journal of Sea Research*, 83(9), 209–218. https://doi.org/10.1016/j.seares.2013.03.005

Porcu, C., Marongiu, M.F., Bellodi, A., Cannas, R., Cau, A., Melis, R. et al. (2017). Morphological descriptions of the eggcases of skates (Rajidae) from the central-western Mediterranean, with notes on their distribution. *Helgoland Marine Research*, 71(1), 10. https://doi.org/10.1186/s10152-017-0490-2

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Ramírez-Amaro, S., Ordines, F., Picornell, A., Castro, J.A., Ramon, C., Massutí, E. et al. (2018). The evolutionary history of Mediterranean Batoidea (Chondrichthyes: Neoselachii). *Zoologica Scripta*, 47(6), 686–698. https://doi.org/10.1111/zsc.12315

Relini, G. (2000). La ricerca sulla pesca: Le risorse demersali. In: *Le ricerche sulla pesca e sull'acquacoltura nell'ambito della L. 41/82. Roma, 15–16 dicembre 1998. Parte IV—Relazioni*, Biologia Marina Mediterranea vol. 7. Genoa, Italy: Società Italiana di Biologia Marina, pp. 13–45.

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution*, 43(1), 223–225. https://doi.org/10.2307/2409177

Rogers, A.R. & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, 9(3), 552–569. https://doi.org/10.1093/oxfordjournals.molbev.a040727

Romanelli, M., Colasante, A., Scacco, U., Consalvo, I., Finoia, M.G. & Vacchi, M. (2007). Commercial catches, reproduction and feeding habits of *Raja asterias* (Chondrichthyes: Rajidae) in a coastal area of the Tyrrhenian Sea (Italy, northern Mediterranean). *Acta Adriatica*, 48(1), 57–71.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E. et al. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34(12), 3299–3302. https://doi.org/10.1093/molbev/msx248

Saygu, İ. & Deval, M.C. (2014). The post-release survival of two skate species discarded by bottom trawl fisheries in Antalya Bay, eastern Mediterranean. *Turkish Journal of Fisheries and Aquatic Sciences*, 14(4), 947–953. https://doi.org/10.4194/1303-2712-v14_4_14

Serena, F., Abella, A., Walls, R. & Dulvy, N.K. (2015). *Raja asterias (starry skate)*. *The IUCN Red List of Threatened Species*, 2015, e.T63120A48913317. https://doi.org/10.2305/IUCN.UK.2015-1.RLTS.T63120A48913317.en

Serena, F., Barone, M., Mancusi, C. & Abella, A.J. (2005). Reproductive biology, growth and feeding habits of Raja asterias (Delaroche, 1809), from the north Tyrrhenian and South Ligurian Sea (Italy), with some notes on trends in landing. In 2005 ICES Annual Science Conference, 20–24 September 2005, Aberdeen, Scotland. Copenhagen, Denmark: International Council for the Exploration of the Sea, CM2005/N:12.

Serena, F., Mancusi, C. & Barone, M. (Eds.) (2010). *Field identification guide to the skates (Rajidae) of the Mediterranean Sea. Guidelines for data collection and analysis*, Biologia Marina Mediterranea, vol. 17(2). Genoa, Italy: Società Italiana di Biologia Marina.

Serena, F. & Relini, G. (2006). Use of scientific campaigns (trawl surveys) for the knowledge of the sensitive habitats. A review of the MEDITS, GRUND and APHIA data with special attention to the Italian seas. In: N. Başusta, C. Keskin, F. Serena, B. Seret (Eds.) *The proceedings of the workshop on Mediterranean cartilaginous fish with emphasis on southern and eastern Mediterranean*. Turkish Marine Research Foundation Publication No. 23. Istanbul, Turkey: Turkish Marine Research Foundation, pp. 135–148.

Shephard, S., Gerritsen, H., Kaiser, M.J. & Reid, D.G. (2012). Spatial heterogeneity in fishing creates de facto refugia for endangered Celtic Sea elasmobranchs. *PLoS ONE*, 7(11), e49307. https://doi.org/10.1371/journal.pone.0049307

Simpson, S.J., Humphries, N.E. & Sims, D.W. (2021). Habitat selection, fine-scale spatial partitioning and sexual segregation in Rajidae, determined using passive acoustic telemetry. *Marine Ecology Progress Series*, 666, 115–134. https://doi.org/10.3354/meps13701

Sims, D. (2010). Sharks of the open ocean: Biology, fisheries and conservation. *Fish and Fisheries*, 11(3), 313–314. https://doi.org/10.1111/j.1467-2979.2010.00367.x

Specially Protected Areas Regional Activity Centre & UN Environment/Mediterranean Action Plan. (2020). In: M.N. Bradai (Ed.) *Action plan for the conservation of cartilaginous fishes (Chondrichthyans) in the Mediterranean Sea*. Tunis, Tunisia: SPA/RAC.

Spedicato, M.T., Massutí, E., Mérigot, B., Tserpes, G., Jadaud, A. & Relini, G. (2019). The MEDITS trawl survey specifications in an ecosystem approach to fishery management. *Scientia Marina*, 83(S1), 9–20. https://doi.org/10.3989/scimar.04915.11X

Stevens, J.D., Bonfil, R., Dulvy, N.K. & Walker Stevens, P.A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science*, 57(3), 476–494. https://doi.org/10.1006/jmsc.2000.0724

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J. & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, 4(1), vey016. https://doi.org/10.1093/ve/vey016

Sviben, S., Trkov, D., Mavrič, B., Kružić, P. & Lipej, L. (2019). Observations on the diet of the starry skate, *Raja asterias* Delaroche, 1809 (Elasmobranchii: Rajidae) in the Adriatic Sea. *Mediterranean Marine Science*, 20(3), 577–584. https://doi.org/10.12681/mms.19338

Tavares, R., Rodriguez, J.P. & Morales, M. (2016). Nursery area and size structure of the lemon shark population, *Negaprion brevirostris* (Poey, 1868), in Los Roques Archipelago National Park, Venezuela. *Universitas Scientiarum*, 21(1), 33–52. https://doi.org/10.11144/Javeriana.SC21-1.naas

Templeman, W. (1982). Development, occurrence and characteristics of egg capsules of the thorny skate, *Raja radiata*, in the northwest Atlantic. *Journal of Northwest Atlantic Fishery Science*, 3(1), 47–56. https://doi.org/10.2960/J.v3.a4

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Tserpes, G., Maravelias, C.D., Pantazi, M. & Peristeraki, P. (2013). Distribution of relatively rare demersal elasmobranchs in the eastern Mediterranean. *Estuarine, Coastal and Shelf Science*, 117, 48–53. https://doi.org/10.1016/j.ecss.2012.09.020

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Waples, R.S. (1991). Pacific salmon, *Oncorhynchus* spp., and the definition of 'species' under the Endangered Species Act. *Marine*

Fisheries Review, 53(3), 11–22. http://spo.nmfs.noaa.gov/mfr533/mfr5332.pdf

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B - Biological Sciences*, 360(1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Ward-Paige, C.A., Keith, D.M., Worm, B. & Lotze, H.K. (2012). Recovery potential and conservation options for elasmobranchs. *Journal of Fish Biology*, 80(5), 1844–1869. https://doi.org/10.1111/j.1095-8649.2012.03246.x

Yemışken, E., Forero, M.G., Megalofonou, P., Eryilmaz, L. & Navarro, J. (2018). Feeding habits of three batoids in the Levantine Sea (north-eastern Mediterranean Sea) based on stomach content and isotopic data. *Journal of the Marine Biological Association of the United Kingdom*, 98(1), 89–96. https://doi.org/10.1017/S002531541700073X

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