Vol. 49: 87–102, 2022
https://doi.org/10.3354/esr01207

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published October 13

Inferred family structure and dispersal patterns of a Critically Endangered species, *Pinna nobilis*, using molecular analyses: implications for conservation

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ABSTRACT: Knowledge of the genetic structure and dispersal patterns for keystone and vulnerable species is essential to the establishment of conservation strategies. Since autumn 2016, the Critically Endangered *Pinna nobilis* bivalve has suffered mass mortality events throughout the Mediterranean Sea, causing mortality of up to 100% in affected populations. To propose appropriate reintroduction programs for its recovery, the present study sought to determine the genetic structure and local dispersal patterns from a well-documented population of *P. nobilis*. Using 19 microsatellite markers, we obtained genotypic information for 771 individuals from 9 localities within Cabrera National Park (CNP). The CNP population of *P. nobilis* was a single and homogeneous population, with nearly half of the sampled individuals being related through 333 half-sib and 14 full-sib relationships. The siblings belonged to 126 different family clusters composed of 2–8 individuals recruited during several recruitment events from up to 4 different localities. No evidence was found to suggest that the population was self-sustaining, since no parent–offspring dyad was found. However, the fine-scale dispersal patterns observed in Santa Maria Bay highlight the importance of this locality for the sustainment of the population as a whole. These findings suggest that the CNP could be a good choice for future reintroduction programs. Future studies that compile data from this and other studies conducted in CNP should be considered when modeling for reintroduction.

KEY WORDS: Bivalve · Connectivity · Parentage analysis · Marine invertebrates · Conservation actions · Reintroduction

1. INTRODUCTION

For decades, anthropogenic impacts have threatened marine biodiversity, driving marine populations and species to threatened levels or to the brink of extinction (Dulvy et al. 2003, McCauley et al. 2015, Webb & Mindel 2015). This situation has increased the need to implement conservation measures to restore populations and species to their pre-disturbance levels. Considering all the conservation measures available, the translocation of individuals — either through the release of individuals into an existing population (reinforcement) or into an extinct population (reintroduction) — has increasingly been used during the last decades (Seddon et al. 2007, 2014, Swan et al. 2016) and has proven valuable in saving species from extinction and restoring viable populations (Cullingham & Moehrenschlager 2013, e.g. case studies in Soorae 2008, 2010, 2011, 2013, 2016, 2018, 2021). Despite enormous efforts, not all conservation translocations have proven successful, as many have failed to establish self-sustaining pop-

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ulations (Fischer & Lindenmayer 2000). Inadequate planning and management, habitat-related issues, natural history factors, and disease are some of the main reasons for failure (e.g. case studies in Soorae 2008, 2010, 2011, 2013, 2016, 2018, 2021). To avoid a negative outcome, research in reintroduction biology has emphasized the importance of developing and using thorough scientific approaches when planning and conducting translocations (Armstrong & Seddon 2008). It has recently been stated that effective planning often requires more than empirical field data. It should combine empirical data and ecological models to provide useful predictions. When modeling reintroduction, 4 key factors can be considered: habitat suitability, dispersal processes, population dynamics, and interspecific interactions as well as other factors such as diseases (Hunter-Ayad et al. 2020).

In conservation biology, understanding dispersal patterns is necessary to understand how local populations are naturally replenished by the arrival of new recruits from source populations. Empirical data of dispersal processes can be estimated using molecular analyses that aim to determine the genetic structure and connectivity of a population on different scales. Recent studies have demonstrated that an assessment of genetic relatedness and parental analysis may provide information about local and fine-scale connectivity in genetically homogeneous populations with high levels of gene flow (Schunter et al. 2014, Couvray & Coupé 2018). Parentage analyses are helpful in the assessment of local and fine-scale dispersal patterns in marine species in which reproductive characteristics and early life stages make direct observation of dispersal events impossible (Jones et al. 2010). As such, molecular analyses aimed at genetic parentage data are an increasingly common source of empirical dispersal information (Planes et al. 2009, Almany et al. 2017, Dubé et al. 2020). However, despite the increasing number of published articles that attempt to elucidate genetic structure and dispersal patterns for a number of species, the applicability of such data to management and conservation decisions has been relatively poor (Beger et al. 2014, Cook & Sgrò 2019).

The incorporation of genetic information into the design of reintroduction projects in the terrestrial realm is a clear example of its usefulness in proposing more adequate conservation actions. For instance, genetic diversity and structure data were used as the first step to determine the appropriate source population for conservation programs and reintroductions of crocodiles in Thailand (Lapbenjakul et al. 2017). Genetic data was also used to make recommendations for the successful reintroduction of an endangered plant, helping to avoid the negative effects of inbreeding and outbreeding (Kaulfuß & Reisch 2017). Similarly, other authors found that survival rates of reintroduced plant species were much higher when information about the genetic diversity of the target species was included in the project design (Godefroid et al. 2011). In the marine realm, fewer studies have applied genetic data to conservation decision-making. An example is the recommendations made for the sea urchin *Diadema antillarum*, a species that has suffered 2 mass mortality events (MMEs) since the 1980s. Little genetic differentiation was detected between natural locations and between natural and broodstock populations, suggesting the possibility of using captive-bred individuals for reintroduction (Chandler et al. 2017). In a case involving the boring giant clam *Tridacna crocea*, 8 genetic systems were incorporated into spatial planning tools to preserve biodiversity and maintain ecological function (Beger et al. 2014). More recently, the advantages and disadvantages of reintroducing *Pinna nobilis*, a Critically Endangered species, within a shallow bay were evaluated after conducting parentage analyses on the species (Peyran et al. 2022).

Since 2016, there has been an exponential increase in interest in the genetics of *P. nobilis*, which has revealed high population connectivity on large scales (Wesselmann et al. 2018, Peyran et al. 2021) and genetic differentiation on smaller scales (González-Wangüemert et al. 2019). This interest has been motivated by the need to acquire new information about the species in order to find conservation solutions to improve its status, as it is currently classified as a Critically Endangered species on the IUCN Red List (Kersting et al. 2019). Its dramatic population decrease is the result of a new protozoan parasite, *Haplosporidium pinnae* (Catanese et al. 2018, Grau et al. 2022), which has been responsible for multiple MMEs in *P. nobilis* populations since autumn 2016. The first mortalities were reported in early autumn 2016 in populations in southern Spain — the first of several MMEs that resulted in extremely high levels of mortality, reaching up to 100% in all monitored populations (Vázquez-Luis et al. 2017). Next, *H. pinnae* spread through the western Mediterranean Sea (García-March et al. 2020b) and later reached the eastern Mediterranean Sea (Katsanevakis et al. 2019, Lattos et al. 2020, Zotou et al. 2020, Çinar et al. 2021). Today, it is possible to find only few isolated resistant individuals in the open sea (https://www.observadoresdelmar.es/Projects/View/14; M. Vázquez-Luis pers.

obs.). Some unaffected or partially affected populations still remain alive and are primarily located in isolated coastal lagoons from Spain, France, Italy, and Greece (García-March et al. 2020b, Zotou et al. 2020, Donato et al. 2021, Nebot-Colomer et al. 2022). Within this context, natural recolonization of *P. nobilis* from the remaining isolated populations is highly unlikely due to the barrier created by the presence of the parasite in open waters. Furthermore, the few isolated resistant living individuals from open waters may be unable to reproduce due to their low numbers and isolation, thus dismissing them as a source for the repopulation of decimated populations (García-March et al. 2020b, Kersting et al. 2020). Consequently, *P. nobilis* could face total extinction in the medium term unless manipulative conservation actions are taken (García-March et al. 2020b).

When planning future species-reintroduction programs, the selection of the source population and release location are fundamental to the program's success. For *P. nobilis*, the source population would need to be composed of resistant individuals, since eradicating the diseases from the environment is essentially impossible. The selection of the release location is also relevant for the success of the reintroduced population. To correctly choose a release location, it must meet the following criteria: (1) meet all biotic and abiotic requirements of the species, (2) be appropriate habitat for all stages of life of the species, (3) have adequate connectivity to suitable habitat, and (4) the reintroduction must be conducted in an indigenous range capable of supporting an established population (IUCN/SSC 2013).

Given the urgent need to study and select future release locations for resistant individuals of *P. nobilis*, we studied the family genetic structure and dispersal patterns of a highly dense population located within Cabrera National Park (CNP) (Mallorca, Spain), and propose possible localities that may be candidates for reintroduction based on the results obtained. The *P. nobilis* population in CNP is considered one of the most dense, healthy, and aged populations in the Mediterranean Sea (Vázquez-Luis et al. 2014, Basso et al. 2015, Deudero et al. 2015, 2017, García-March et al. 2020a). To reach such a conservation status, we hypothesize that its legal protection, high population connectivity, and a particular specific dispersal pattern allowed the settlement and growth of a dense and healthy population. Therefore, the specific objectives of the present study were to (1) determine population genetic structure, (2) determine the family genetic structure of the population, (3) identify and better understand dispersal processes at local and fine scales, and (4) identify the location best suited for a reintroduction program within CNP based on the genetic results.

2. MATERIALS AND METHODS

2.1. Study area and sampling

The study was carried out in CNP, located in the south of Mallorca (Spain). The CNP was established in 1991. Given the low degree of human activity, it ensures the evolution of species in a natural and pristine environment, as previously demonstrated by Vázquez-Luis et al. (2014) and Deudero et al. (2015). A total of 856 individuals of *Pinna nobilis* were sampled from 25 different sites between 2011 and 2014, mainly in *Posidonia oceanica* seagrass meadows 2–25 m in depth. Multiple sampling sites were present in both Santa Maria Bay (STM) and Port Bay (PORT). Therefore, after checking that there were no genetic differences among them, the sampling sites within a given bay were agglomerated and considered to be the same locality. In addition, given the low number of individuals sampled from the 2 northern sites (Freus and Na Pobra), both sites were also grouped as one locality (POBR) after checking that there was no genetic difference. A total of 9 localities (STM, PORT, Dimoni [DIM], Rates [RAT], Es Colador des Estells [ECE], Estells de Dins [EDD], Es Colador des Imperial [ECI], Olla [OLLA], and POBR) were defined as spatial units (Fig. 1, see Table 1). Moreover, the maximum shell width of all sampled individuals was measured *in situ* using an L-shaped ruler to ±1 mm accuracy (Álvarez et al. 2017), as it has been shown that shell width is closely related to the age of the individuals (García-March et al. 2020a). A biopsy of approximately 1 cm^3 of mantle tissue was collected with tweezers by SCUBA divers. During the dives, tissues were stored in small plastic bags and then transferred to 2 ml vials and preserved in 96% ethanol stored at room temperature. Before sampling in CNP, a test was carried out in a control area in Mallorca (Andratx) to prove sample collection was not lethal and to verify the methodology. The test involved collection of a biopsy from 16 *P. nobilis* individuals followed by periodic monitoring over a period of 2 mo. After 2 mo, 81.3% were alive without any signs of damage, and 18.7% had died due to predators (mainly *Octopus vulgaris*) and boating activities, since the individuals were found broken. In both cases, sam-

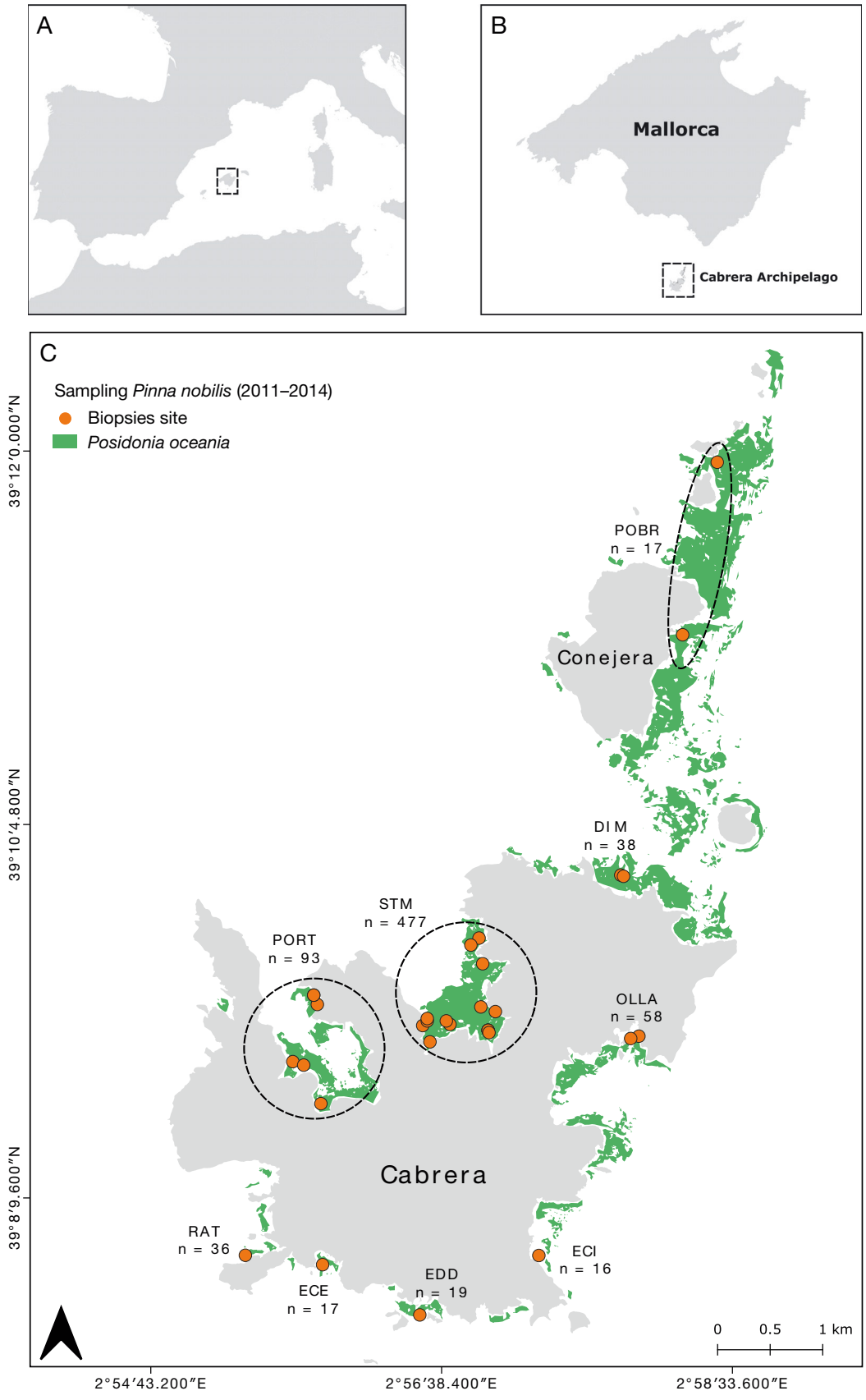

Fig. 1. Study area within the limits of Cabrera National Park (Mallorca, Spain); orange dots: sampling sites for *Pinna nobilis* (n = 25); dashed circles: sampled sites grouped in one locality. STM, PORT, DIM, RAT, ECE, EDD, ECI, OLLA, POBR are the final sampled localities (names coded as in Table 1) with their corresponding number of sampled individuals

pling was carried out with permission from competent authorities, including the 'Servei de Protecció d'Espècies' and 'Cabrera National Park,' both of 'Conselleria de Medi Ambient, Agricultura i Pesca' (Govern de les Illes Balears).

2.2. Genotyping and genetic analyses

A small part of the sampled tissue (2 mm) from each individual was incubated at 55°C for 2–3 h in 200 µl of digest buffer VXL with Proteinase K (QIAGEN). After digestion, total genomic DNA was extracted using a QIAcube HT automated genomic DNA extraction instrument (QIAGEN), according to the manufacturer's instructions. Among all possible markers available, microsatellite markers were chosen for our study, as they are the most adapted marker when performing parentage analyses. Accordingly, the Qiagen Multiplex PCR Kit was used to screen *P. nobilis* samples at 23 highly polymorphic microsatellite loci, previously developed by González-Wangüemert et al. (2014) and Peyran et al. (2020). The same protocol as described by Peyran et al. (2021) was used in the present study. The main features of these loci, such as the number of alleles, the frequencies of the null alleles, and the inbreeding coefficient (F_{IS}), are described in Table S1 in the Supplement at www.int-res.com/articles/suppl/n049p087_supp.pdf. Genotyping was carried out at GenoScreen facilities (Lille, France) on an ABI3730XL Sequencer (Applied Biosystems). All alleles were manually scored and verified using GENEMAPPER v.5.0 (Applied Biosystems).

2.3. Data analysis

2.3.1. Microsatellite loci screening

Null allele frequencies and putative scoring errors were checked for each locus using MICRO-CHECKER v.2.2.3 (van Oosterhout et al. 2004). In total, 6 of the 23 loci exhibited significant null allele frequencies; 4 loci that exhibited values higher than 0.10 were removed from further analyses, but 2 loci that exhibited a value of 0.05 were retained (Table S1), as the results of further genetic structure analyses were similar with and without these 2 loci. The remaining 19 loci did not show any evidence of other technical artefacts, such as large allele dropout or scoring errors due to stuttering. The missing data per locus ranged from 0–18.55% (Table S1). Regarding the screening of individuals, 85 of the 856 screened individuals were removed from the data set after presenting at least 4 missing loci, leading to a final data set composed of 19 loci and 771 individuals.

2.3.2. Genetic diversity

Deviations from Hardy-Weinberg equilibrium were estimated from the F_{IS} (Weir & Cockerham 1984) for each locus and each location using GENETIX v.4.05 (Belkhir et al. 2004) and were tested using an exact test in GENEPOP v.4.5.1 (Rousset 2008) with a Markov chain with default parameters. The total number of alleles and allele frequencies was calculated for each locus in GENALEX v.6.5 (Peakall & Smouse 2012). Genetic diversity was investigated using GENALEX v.6.5, to determine the total (A), mean (N_A), and private (A_p) number of alleles, and the observed (H_o) and expected heterozygosity (gene diversity, H_e) per sampling locality. As the number of individuals sampled varied between localities from 16–47, the standardized allelic richness (A_r) and the standardized private allelic richness (A_{pr}) were estimated in HP-RARE (Kalinowski 2005), using a rarefaction method with a minimum number of genes equal to 22.

2.3.3. Genetic differentiation and structure

Genetic differentiation among sampled localities was estimated using the F_{ST} (Weir & Cockerham 1984) and D_{ST} indices (Gerlach et al. 2010). Pairwise F_{ST} estimates and their significance were calculated with an exact G-test in GENEPOP, using 10 000 dememorization numbers, 100 batches, and 5000 iterations per batch. Pairwise D_{ST} estimates and their significance using 1000 bootstrap replicates were obtained using the 'DEMEtics' package (Gerlach et al. 2010) in R v.3.6.1 (R Core Team 2019). For all pairwise comparisons, the false discovery rate (FDR) correction for multiple tests of Benjamini & Hochberg (1995) was applied in R to avoid Type I errors.

The genetic structure was investigated using a Bayesian clustering method implemented in STRUCTURE v.2.3.4 (Pritchard et al. 2000, Falush et al. 2003), which was used to explore the distribution of individuals with known (LOCPRIOR algorithm) and unknown (NO PRIOR) sample localities. The most likely number of groups (K) was searched by testing $K = 1$ to $K = 8$, with a burning period of 150 000 iterations followed by 100 000 recorded iterations and 10 iter-

ations for each *K* value. STRUCTURE Harvester Web v.0.6.94 (Earl & vonHoldt 2012) was used to identify the number of clusters that best captured the structure of the sample, using the plot of the log probability of the data (LnP[D]) as a function of *K*, following Evanno's method (Evanno et al. 2005). Furthermore, given the uneven subpopulations sampled, which can bias STRUCTURE results and lead to an overestimation of the number of clusters, StructureSelector, a web-based software (Puechmaille 2016), was also used to estimate *K*.

2.3.4. Family structure and parentage analysis

Kinship analyses were performed in COLONY v.2.6.5 (Jones & Wang 2010) to infer sibships and parentage relationships among individuals. Normally, only individuals with a shell width greater than 15 cm are considered adults, but given the environmental characteristics of Freus (within the POBR locality), individuals which measured from 13–15 cm in this locality were also considered adults given the low growth rate found in this area (García-March et al. 2020a). In total, 676 individuals were considered potential parents, while all individuals were considered potential offspring (n = 771) with overlapping generation models. *P. nobilis* presents particular hermaphroditism, successive and asynchronous in maturation, in which a succession of alternate spawning and fast gametogenesis occurs without interruptions during the spawning period (de Gaulejac et al. 1995, Deudero et al. 2017). Therefore, since the sex of the individuals could not be determined, parent–offspring (PO) relationships were evaluated using all parental genotypes available in the analysis, as both candidate fathers and mothers. Offspring–offspring relationships (full- and half-sibs) were assessed for the entire data set. COLONY v.2.6.5 was run 3 times using different random seeds and with the following parameters: both sexes are polygamous, and the organism is dioecious, with inbreeding, full-likelihood method with high precision and without sibship prior. The genotyping error rate was set to 1% per locus. As a conservative approach, for the 3 cluster outputs obtained in COLONY v.2.6.5, only the clusters or subclusters that were consistent across the 3 runs were retained as families. In parallel, the program ML-RELATE (Kalinowski et al. 2006) was also run to validate sibling assignments (Schunter et al. 2014, Couvray & Coupé 2018). The program calculates the maximum likelihood relationship between individual pairs and determines which of the PO, full-sibling (FS), half-sibling (HS), and unrelated (U) categories produce the highest likelihood. The U pairs of individuals detected by ML-RELATE were removed from the data set after confirming their non-existent or weak relationship (low sibship probability) computed in each COLONY run. Thus, in order to be conservative in the analyses, only the relationships that were consistent through the 3 COLONY runs and in ML-RELATE were accepted.

3. RESULTS

3.1. Genetic diversity, genetic differentiation, and structure

The total number of alleles (*A*) ranged from 7–34 locus^{-1}, with a mean number of alleles (N_A) per loci of 17.52 (Table S1). The mean H_e over all loci across locations was high (H_e = 0.707), and the mean H_e over all loci within each location ranged between 0.691 (EDD) and 0.724 (PORT) (Table 1). When considering standardized sampling effort, the mean A_r and A_{pr} at all loci were quite similar between localities, ranging from 7.01 (DIM) to 7.50 (EDD) and 0.21 (DIM) to 0.32 (ECI), respectively. All sampled localities except DIM, EDD, and OLLA presented positive and significant F_{IS} values, indicating an excess of homozygotes. Larger F_{IS} values were found in locations with the smallest sample sizes (Table 1).

All pairwise comparisons between localities (F_{ST} and D_{ST}) were low, varying from −0.00060 to 0.0083 for F_{ST} estimates and from −0.0254 to 0.0422 for D_{ST} estimates. Three pairwise F_{ST} and 2 D_{ST} comparisons were significant prior to FDR correction. After FDR correction, no significant genetic differentiation was observed (Table 2). Furthermore, Bayesian clustering (STRUCTURE) did not reveal any significant genetic clustering so the individuals were grouped into a single population, as no differentiation was observed according to sampling localities (Fig. S1).

3.2. Parentage analysis and sibship

By combining the results from COLONY and ML-RELATE and using a conservative approach, we did not identify PO relationships. In contrast, we identified 347 sibship individuals: 8 FS individuals (2.31%), 6 FS and HS individuals (1.73%), and 333 HS individuals (95.96%). For FS individuals, 57.14% were sampled in STM, 21.43% in PORT, 14.29% in OLLA, and 7.14% in RAT (Table S2). A higher percentage of FS

Table 1. Genetic diversity estimates for the 9 sample locations of *Pinna nobilis*. n: number of individuals sampled; *A*: total number of alleles per locality; N_A: mean number of alleles; A_p: number of private alleles; A_r: standardized allelic richness; A_{pr}: standardized private allelic richness H_o: observed heterozygosity; H_e: expected heterozygosity (gene diversity); F_{IS}: inbreeding coefficient. *p < 0.05

Locality	CODE	n	*A*	N_A	A_p	A_r	A_{pr}	H_o	H_e	F_{IS}
Dimoni	DIM	38	199	10.47	1	7.01	0.21	0.702	0.706	0.02
Es Colador des Estells	ECE	17	164	8.63	1	7.21	0.27	0.685	0.696	0.046*
Es Colador des Imperial	ECI	16	164	8.63	0	7.34	0.32	0.651	0.706	0.111*
Estells de Dins	EDD	19	168	8.84	3	7.50	0.26	0.704	0.691	0.01
Olla	OLLA	58	211	11.11	3	7.20	0.24	0.705	0.714	0.022
Na Pobra	POBR	17	153	8.05	1	7.11	0.25	0.684	0.703	0.059*
Port	PORT	93	236	12.42	4	7.19	0.27	0.713	0.724	0.021*
Rates	RAT	36	192	10.11	5	7.07	0.23	0.688	0.702	0.035*
Santa María	STM	477	295	15.53	39	7.18	0.26	0.692	0.719	0.039*
Mean	–	86	198	10.42	6.3	7.20	0.26	0.691	0.707	0.017

Table 2. Pairwise estimates of F_{ST} (below diagonal) and D_{ST} (above diagonal) among the 9 sampled localities of *Pinna nobilis* around Cabrera Island, Balearic Islands. Names of the sampled localities are coded as in Table 1. None of the values are significant

	POBR	DIM	ECE	ECI	EDD	OLLA	PORT	RAT	STM
POBR	–	0.0031	0.0020	0.0205	0.0422	0.0300	0.0010	0.0209	0.0107
DIM	0.0014	–	−0.0213	−0.0078	0.0135	−0.0056	−0.0102	0.0067	−0.0077
ECE	−0.0016	−0.0036	–	−0.0160	0.0021	−0.0065	−0.0044	−0.0254	−0.0137
ECI	0.0061	0.0001	−0.0022	–	−0.0152	−0.0050	−0.0161	−0.0026	−0.0091
EDD	0.0083	0.0036	−0.0006	−0.0045	–	0.0209	0.0028	0.0158	0.0127
OLLA	0.0058	−0.0013	−0.0007	−0.0018	0.0047	–	0.0004	0.0155	0.0024
PORT	0.0006	−0.0019	−0.0010	−0.0033	−0.0005	0.0001	–	0.0092	−0.0018
RAT	0.0016	0.0008	−0.0060	−0.0001	0.0009	0.0035	0.0010	–	0.0014
STM	0.0012	−0.0013	−0.0025	−0.0010	0.0015	0.0001	−0.0006	−0.0004	–

individuals (42.86%) than HS individuals (39.05%) were found to have recruited in the same locality. For FS pairs, the distance between individuals ranged from a few meters within the same locality to 6.70 km between STM and RAT, while for HS pairs, the distances between individuals were longer and varied from a few meters to 11.72 km (RAT–POBR). Furthermore, most FS pairs exhibited a similar maximum shell width (±2.7 cm), except for FS individuals from Family 40, which showed a difference of 4.6 cm between them (Table S2), while larger differences in shell sizes were found among HS pairs varying from 0–21.7 cm.

3.3. Family structure

In total, 126 family clusters were found, involving 347 individuals (45.01% of the individuals sampled). The minimum number of individuals per family cluster was 2, and the maximum was 8. The majority of family clusters consisted of 2 (66.67%) or 3 individuals (12.70%), while larger families (5–8 individuals) were less common (Fig. 2A). All individuals belonging to a given family cluster were related by a HS or FS relationship

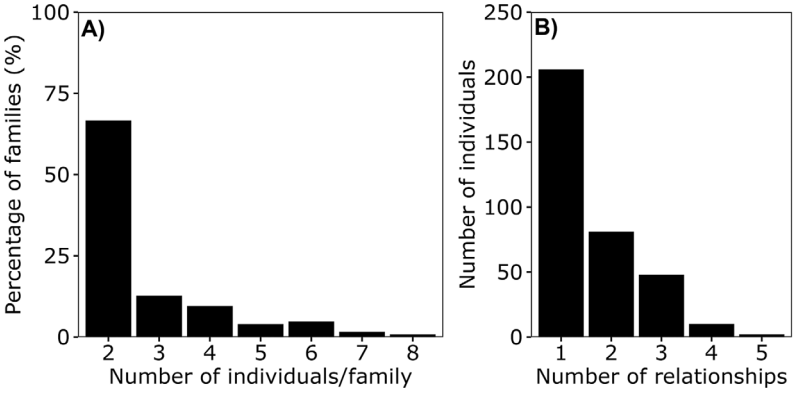

Fig. 2. *Pinna nobilis* family structure and individual relationships: (A) percentage of individuals per family and (B) number of relationships per individual

with at least one individual from the same cluster, forming families of up to 8 individuals. Most individuals belonging to the same family cluster were recruited at different localities and during different recruitment events, as observed by the size of each individual. Specifically, 32.54% of the families were composed of individuals from the same locality, while 51.59, 13.49, and 2.38% of the families had individuals belonging to 2, 3, and 4 different localities, respectively (Fig. 3). Based on the shell size differences observed between individuals from the same family cluster, specimens belonging to different recruitment events recruited in CNP before MMEs (Fig. 3).

3.4. Dispersal patterns

Overall, local dispersal patterns were inferred based on the relationships between pairs of individuals at the scale of the CNP. In total, 281 sibship relationships (FS and HS) were found, involving the 347 related individuals. The minimum number of relationships per individual was 1, and the maximum was 5 (average: 1.62 ± 0.05 relationships ind.$^{-1}$; Fig. 2B). In accordance, in CNP, larvae were heterogeneously dispersed among localities rather than in one particular locality, as observed by the higher number of sibship relationships that occurred between individuals from different localities (62.28%) than within the same locality (37.72%) (Fig. 4B).

Fine-scale dispersal patterns were inferred on the basis of the relationships between pairs of individuals and on the number of related individuals within and among localities. STM was the predominant locality in most sibship relationships (85.41%), followed by PORT (28.47%) and OLLA (12.81%). Of the 175 sibship relationships among localities (62.28%), STM was involved in 50.53% of these relationships; that value was 11.75% among other localities. Most of the sibship relationships from STM occurred between STM–PORT (19.93%), STM–OLLA (9.25%), and STM–RAT (5.34%) (Fig. 4B). Out of the 106 sibship relationships present within localities (37.72%), 34.88% occurred in STM, and the remaining 2.84% occurred in PORT, RAT, and OLLA (Fig. 4B). In parallel, the number of related individuals within and among localities did not follow the same pattern as sibship relationships. In general, high percentages of related individuals were observed in all localities, ranging from 63.16% for EDD to 36.84% for DIM (Table 3). However, all localities except STM presented a higher percentage of individuals that were related to individuals from other localities than individuals related within the same locality (Table 3, Fig. 4A).

4. DISCUSSION

The present study is the first to suggest a possible location for the reintroduction of the Critically Endangered species *Pinna nobilis.* This study provides empirical estimates of family structure and local and fine dispersal patterns based on sibship and genetic parentage analyses from a natural population of *P. nobilis.* Our findings indicate a high number of related individuals despite the low percentage of individuals sampled, which agrees with the lack of genetic structure at the CNP scale, and reveals high levels of relatedness among all localities sampled within the CNP. However, the absence of PO relationships makes it difficult to conclude if the population studied was maintained by self-recruitment or by external donor populations, although the results point to the possibility of both processes occurring. Future genetic studies compiling a larger data set might help elucidate the origin of the larvae.

4.1. Population and family structure

No genetic differentiation or structure was detected in any of the analyses. In addition, high (N_A = 10.42, A_p = 6.3) and homogeneous genetic diversity values were found throughout the entire National Park and were similar to and higher than those found in other localities (Wesselmann et al. 2018) and regions (Peyran et al. 2021). The absence of structure and the homogeneity in genetic diversity values support the idea of a single panmictic homogeneous population within CNP, highlighting a continuous and wide connection among localities and regions rather than the species being isolated and fragmented among localities. These results agree with other studies that found low inter-population differentiation in the western Mediterranean Sea (Wesselmann et al. 2018) and a large homogeneous and highly connected panmictic population across the entire Gulf of Lion (Peyran et al. 2021).

In the present study, a high percentage of related individuals were found despite the conservative analyses applied, similar to analyses applied in other studies (Schunter et al. 2014, Couvray & Coupé 2018). Almost half of the individuals sampled were primarily related through 1 or 2 HS relationships. A

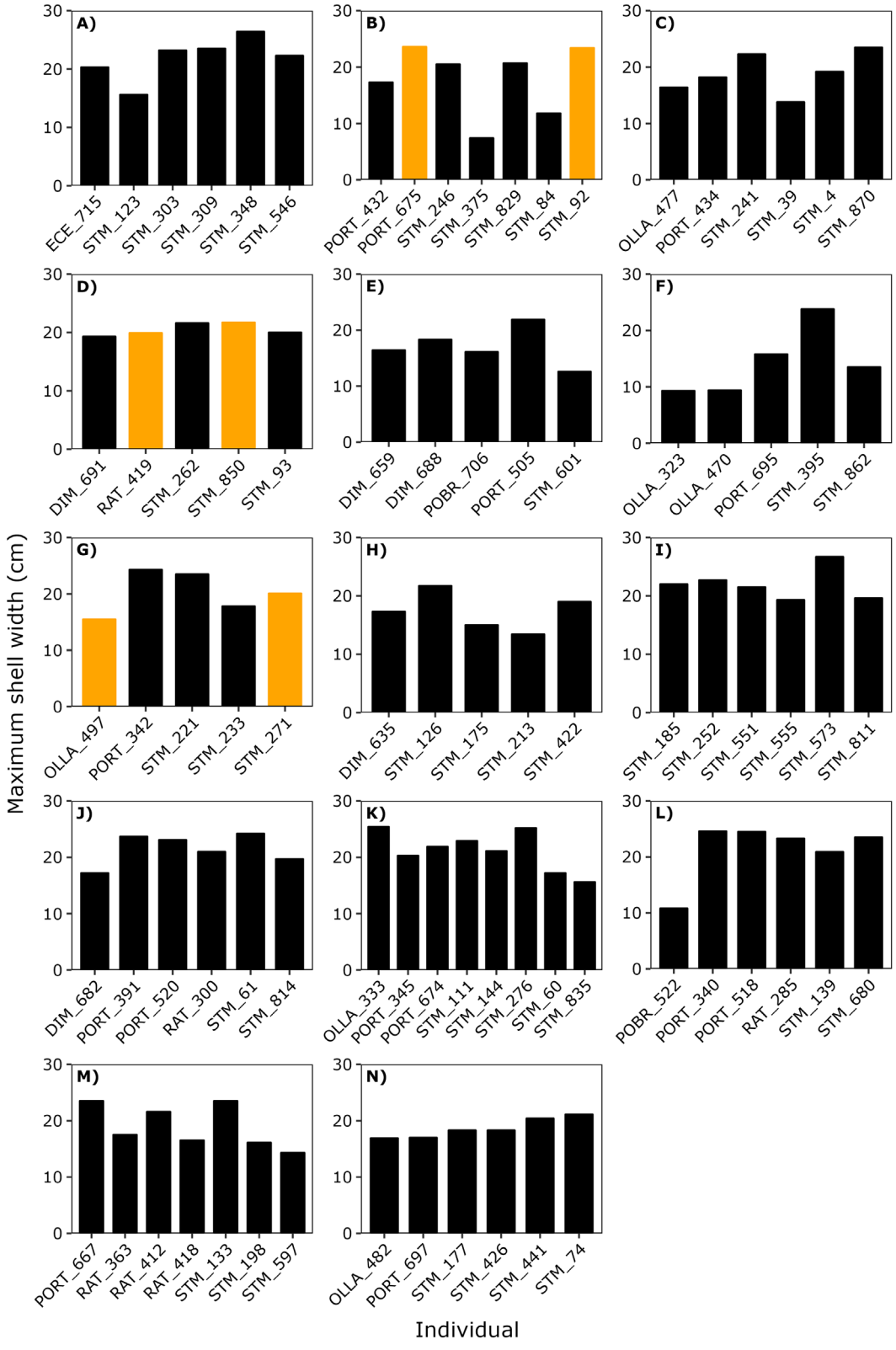

Fig. 3. Family members and size structure of the 14 largest *Pinna nobilis* families. Orange bars: full-sib individuals. (A) Family 2; (B) Family 7; (C) Family 10; (D) Family 17; (E) Family 18; (F) Family 31; (G) Family 40; (H) Family 56; (I) Family 68; (J) Family 77; (K) Family 84; (L) Family 87; (M) Family 88; (N) Family 93

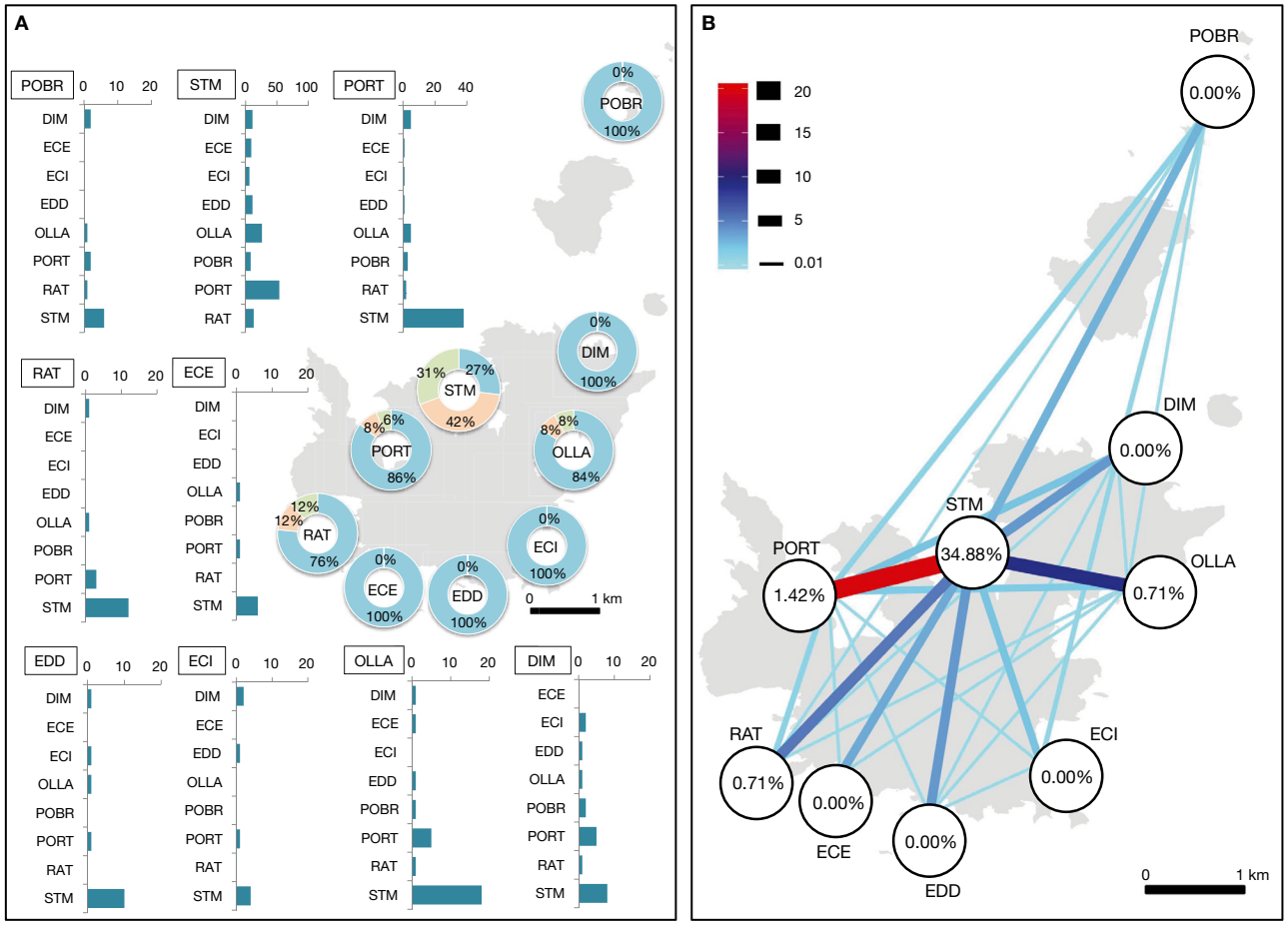

Fig. 4. Family relationships of *Pinna nobilis*. (A) Circles represent sampling locations (names coded as in Table 1); orange: percentage of individuals related to individuals from within the same locality; blue: percentage of individuals related to individuals from other localities; green: percentage of individuals related to individuals from the same and other localities. Each bar plot represents one sampling area and specifies the number of individuals from that locality related to all other localities. (B) Relationships between 2 localities; the number within each circle represents the percentage of relationships occurring within that location

similar percentage of related individuals and a predominance of HS relationships were reported in the Bay of Peyrefite, where a higher percentage of individuals were sampled (Peyran et al. 2022). In the present study, the sampled percentage was lower. Therefore, a higher number of related individuals would be expected with an increase in the number of sampled individuals, which confirms the high percentage of related individuals found in CNP. Most of the HS and FS individuals were involved in family clusters of 2 and 3 individuals. The predominance of families with 2 members is not surprising considering the reproductive biology and post-settlement ecology of the species. *P. nobilis* is an asynchronic and successive hermaphroditism bivalve (de Gaulejac et al. 1995) whose spawning is usually triggered by temperature (Deudero et al. 2017). In this type of reproduction, males and females release their gametes into the water column, generating several mating options according to the reproductively active adult stock in the population at that particular moment. Comparatively, 26 females were reproductively induced in tank installations and released an average of 1.9 × 10^6 oocytes l^{-1} (Trigos et al. 2018). Consequently, in natural populations with a larger number of adults involved in reproduction, the odds that the same males and females continuously match must be low. Even if there is a single match, high mortality rates are expected during the first days of life in its pelagic lifespan (Trigos et al. 2018). Still, post-settlement conditions are quite difficult for the species, producing high mortality rates during the first years of a juvenile's life (Kersting & García-March 2017). In addition, in CNP, high densities of *P. nobilis* were present before the MMEs (Vázquez-Luis et al. 2014, Deudero et al. 2015), which could have made it difficult to

Table 3. Ecological and sib–sib relationships in *Pinna nobilis*. Locality names coded as in Table 1; n: number of sampled individuals; No. ind. rel.: number of related individuals; % rel.: percentage of individuals related. Among: number and percentage of individuals related among other localities; Within: number and percentage of individuals related within the same locality. Highest and lowest values are in **bold**

Locality	Mean depth (m)	Mean density (ind. 100 m^{-2})[a]		Total			Type					
		10 m	20 m	n	No. ind. rel.	% rel.	Among		Within		Among and within	
							n	%	n	%	n	%
DIM	13.06	5.2 ± 1.87	3.5 ± 0.97	38	14	**36.84**	14	**100**	0	**0.00**	0	**0.00**
ECE	18.19	5.23 ± 2.27		17	8	47.06	8	**100**	0	**0.00**	0	**0.00**
ECI	14.50	0.27 ± 0.27	6.83 ± 2.2	16	6	37.50	6	**100**	0	**0.00**	0	**0.00**
EDD	20.41	0.26 ± 0.18	4.33 ± 1.89	19	12	**63.16**	12	**100**	0	**0.00**	0	**0.00**
OLLA	10.58	6.13 ± 1.09		58	25	43.10	21	84.0	2	8.00	2	8.0
POBR	8.21	10.93 ± 3.61		17	7	41.18	7	**100**	0	**0.00**	0	**0.00**
PORT	12.46	11.56 ± 1.59	3.33 ± 0.76	93	50	53.76	43	86.0	4	8.00	3	6.0
RAT	22.55	2.44 ± 0.72	13 ± 2.27	36	17	47.22	13	76.5	2	11.76	2	11.8
STM	10.49	13.07 ± 2.98	16.93 ± 2.34	477	208	43.61	56	26.92	88	**42.31**	64	**30.77**
Total	**14.49**			**771**	**347**		**180**		**96**		**71**	

[a]Data obtained from Deudero et al. (2015)

sample the required percentage of the population to find additional family members. In contrast, although most families were composed of 2 members, the present study revealed the existence of families with up to 8 HS individuals and individuals with up to 5 relationships. Furthermore, these findings suggest that the source population included a large number of reproductively active adults and that it is possible that repetitive mating may occur between parents even through different generations.

4.2. Dispersal patterns

Overall, local dispersal results inferred a possible dispersal pattern for *P. nobilis* within CNP before the MMEs. In particular, a high number of sibship relationships occurred among individuals from different localities, as demonstrated by the existence of families composed of individuals from 2–4 different localities. Additionally, different cohorts of individuals within families were also found, as inferred by the different shell sizes observed within family clusters. Both results revealed a wide, heterogeneous, and repetitive dispersal of larvae around CNP, where the same parents were involved year after year, suggesting that the same source populations participated in the replenishment of CNP. Some cohesive dispersion of larvae is expected, as observed by the number of sibship relationships revealed within the same locality with similar shell size. Similar larval aggregative behavior, where larvae originating from the same spawning event can remain together throughout the planktonic period and settle at the same site, occurs in other species (Selkoe et al. 2006, Couvray & Coupé 2018) and in the *P. nobilis* population from the Bay of Peyrefite (Peyran et al. 2022). Furthermore, it is likely that at least one source population continuously imported larvae to CNP, not homogenously succeeding in all localities, as observed by the differences in densities and the number of related individuals per locality. Such differences might be expected, since larvae could have been heterogeneously dispersed among localities according to the coastal currents that affect each area, or larvae might have had more settlement and/or survival success depending on the locality's environmental conditions. Specifically, STM, PORT, and OLLA bays were the localities that were regularly involved in most relationships. These 3 localities are semi-enclosed bays, which may act as receptor areas that facilitate larvae retention and which may also be optimal localities for survival and growth, as demonstrated by the high densities (Vázquez-Luis et al. 2014) and older individuals observed in these areas (García-March et al. 2020a). Another explanation for these results might be coastal currents that facilitate the connection between these localities and the larval pool. However, further studies on the local and coastal hydrodynamics at the scale of CNP are required.

In parallel, fine-scale results inferred the possible dispersal patterns among and within localities in CNP. In this case, STM showed a differentiated pattern of sibship relationships compared to the other localities. It was the predominant locality in most sibship relationships and the only locality with a higher

number of related individuals residing within the same bay than individuals related with individuals from outside. Therefore, before the MMEs, STM had a large number of individuals with stronger genetic linkages to individuals within the bay than those outside, suggesting some pattern of larval retention. Furthermore, the remaining related individuals from STM were highly related to other localities, as observed by the high percentage of relationships among localities, suggesting some level of larval exportation. However, these results could be considered biased based on the higher sampling effort carried out in STM compared to other areas. Nevertheless, when looking at the highest density observed before the MMEs within CNP and the abundance of the preferred habitat for the species (Vázquez-Luis et al. 2014, Deudero et al. 2015), the percentage sampled as a percentage of the total number of individuals in STM is probably lower than in other localities. Therefore, this dispersal pattern is likely specific to STM and might have been present in CNP before the MMEs.

At this stage, we cannot infer the donor population since no PO dyad was found to confirm self-recruitment. However, the lack of a PO dyad is probably a consequence of the relatively small sample size obtained compared to the expected overall population size given the optimal habitat availability (Vázquez-Luis et al. 2014). In any case, results on dispersal patterns support the idea that 2 dispersal processes probably existed within the limits of CNP, where external and internal donor populations could have been involved in the replenishment of larvae in CNP; thus, our hypothesis could be accepted. In both cases, the donor population needed to have a large effective population size due to the high number of different families and low number of FS and PO dyads found in this study, as a result of the multiple sampling options. In one possible scenario, it is conceivable that larvae originated from an external source population whose larval pool could have traveled in an aggregated form for hundreds of meters until it reached the CNP, where the larvae then successfully settled with respect to coastal currents and environmental characteristics of the locality. The pelagic larval duration of *P. nobilis* (de Gaulejac & Vicente 1990, Butler et al. 1993, Trigos et al. 2018, Wesselmann et al. 2018) and its large genetic connectivity (Wesselmann et al. 2018, Peyran et al. 2021) support such a pattern. Among possible donor populations, large populations with reproductively active adults existed before the MMEs (Prado et al. 2014, Basso et al. 2015, Deudero et al. 2015). Based on Lagrangian simulations, the Ebro Delta is believed to be a source population for Ibiza (Wesselmann et al. 2018), which could have acted as a key population for larval replenishment of Mallorca and Cabrera (González-Wangüemert et al. 2019). Populations situated in the eastern coastal areas of Mallorca could have also contributed (González-Wangüemert et al. 2019). However, more studies are required to determine the degree of contribution of external sources to the population. In any case, larvae may have successfully settled around CNP, predominantly in STM, PORT, and OLLA bays, which probably acted as receptor localities. In another possible scenario, several CNP locations may have acted as donor localities considering the large adult reproductive active aggregation of *P. nobilis* individuals present within CNP before the MMEs (Vázquez-Luis et al. 2014, Deudero et al. 2015, 2017, García-March et al. 2020a). Those larvae could have traveled from a few meters within the same location to a few kilometres to other localities as observed by the distance between siblings individuals. In addition, we would expect that a continued level of self-recruitment existed at the CNP scale, and possibly even at the locality scale, particularly if we consider that some related individuals match through PO relationships that cannot be segregated because both individuals are adults. Among the possible donor localities, the environmental and dispersal characteristics of STM support such a hypothesis, where the larvae could have been retained within their limits as well as exported to adjacent areas such as PORT, RAT, and OLLA. This hypothesis is also supported, considering that more than half of the FS individuals sampled were sampled within STM. Regarding the main hypothesis of our study, we can agree that both scenarios together with the legal protection of the CNP might have benefited the growth and settlement of one of the highest *P. nobilis* populations that ever existed before the MMEs. This hypothesis would agree with the significant increase in *P. nobilis* abundance observed in CNP in the last decades since it was protected (Vázquez-Luis et al. 2020).

4.3. Implications for conservation

The present study demonstrated the importance of CNP for the conservation of the species and confirms results from other studies that have demonstrated the effectiveness of CNP in maintaining a healthy, stable, and mature population (Vázquez-Luis et al. 2014, Deudero et al. 2015, García-March et al. 2020a). This study shows that the *P. nobilis* population of CNP is

highly related between localities and that a high number of related individuals remain within specific areas. Results also indicate that larval replenishment probably depends on one or more donor populations with a large number of mature individuals capable of providing a continuous and differentiated input of immigrants within the CNP. However, the origin of the larvae remains unresolved, and the option to track the donor population through larval recruitment is no longer possible given the absence of recruitment post-MME (Kersting et al. 2020). In any case, it is clear that CNP hosted one of the healthiest, mature, stable, and densest populations (Vázquez-Luis et al. 2014, Basso et al. 2015, Deudero et al. 2015, García-March et al. 2020a), which makes it a good choice for the protection of reintroduced individuals.

Concerning the long-term recovery of the species within the CNP and whether reintroduction programs are developed within its limits, its success will clearly depend on the potential capacity of self-recruitment. Based on the 2013 IUCN/SSC criteria for reintroduction, future reintroduction programs should focus on a few specific CNP localities. Among the possible locations, STM, PORT, and OLLA bays could be selected as priority regions, as they meet all the IUCN criteria for reintroductions (IUCN/SSC 2013). These sites also meet all of the species' biotic and abiotic requirements and contain the appropriate habitat for all life stages of the species: *Posidonia oceanica* seabeds with low hydrodynamic activity (García-March et al. 2007, Vázquez-Luis et al. 2014). These localities had previously hosted *P. nobilis* individuals, and as observed in this study, they have adequate connectivity to a suitable habitat. In addition, they might have acted as receptor and donor locations, retaining a large number of individuals, hosting high levels of genetic diversity, and creating a dispersal network among them. Therefore, larvae of reintroduced individuals might be easily retained and dispersed around the CNP, and with respect to the long-term recovery of the species, this might help to maintain high densities and levels of genetic diversity essential for populations to evolve and to be resilient against other disturbances.

The importance of CNP for the long-term recovery of the species at a larger scale will also depend on the species' ability to reproduce, disperse, and settle successfully in other areas. If reintroduction programs are implemented in STM, PORT, and OLLA bays, it is likely that some larvae may disperse widely and could facilitate the natural repopulation of the species in other areas of the Balearic Islands, such as in coastal areas of Mallorca (González-Wangüemert et al. 2019) and Menorca or even the Columbretes Islands (Kersting et al. 2020). However, further analysis of dispersal patterns and connectivity may help to elucidate the possible donor populations and the importance of CNP as a donor locality on a larger scale. Thus, other parental and sibship analyses are required at larger scales.

In the context of the current pandemic, most *P. nobilis* populations are now extinct (Katsanevakis et al. 2021), and natural repopulation is impossible due to the insufficient number of individuals and the long distances between resistant individuals, as demonstrated by the disruption of recruitment after the MMEs (García-March et al. 2020b, Kersting et al. 2020). The findings reported in this study help to increase our collective knowledge of a well-documented population of *P. nobilis*. This study provides new and essential information on the genetic diversity, family structure, and dispersal patterns at local and fine scales, which may prove to be instrumental in the recovery of the species. However, further studies that incorporate reliable data and information on population densities and distribution, reproduction, age, and growth (García-March et al. 2020a), as well as population genetic structure and connectivity and population dynamics in CNP are needed to ensure the success of any future reintroduction program in the CNP and to potentially ensure the persistence of the species as a whole.

Acknowledgements. Financial support for this work was provided by the 'Estado de conservación del bivalvo amenazado *Pinna nobilis* en el Parque Nacional del Archipiélago de Cabrera' (024/2010) research project, 'Organismo Autónomo de Parques Nacionales, Ministerio de Medio Ambiente y Medio Rural y Marino'; EsMarEs (Ministerio para la Transición Ecológica, Spanish Government), the LIFE IP INTEMARES project, coordinated by the Biodiversity Foundation of the Ministry for the Ecological Transition and the Demographic Challenge, and the European Union's LIFE programme (LIFE15 IPE ES 012). Thanks are due to the staff of CNP for granting permission to work in the park and for use of their facilities. M.V.-L. was supported by a postdoctoral contract Juan de la Cierva-Incorporación (IJCI-2016-29329) of the Ministerio de Ciencia, Innovación y Universidades. We also thank Jeanine Almany and Montse Compa for the English revision of the manuscript.

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Editorial responsibility: David Richardson, Norwich, UK
Reviewed by: 2 anonymous referees

Submitted: November 10, 2021
Accepted: August 24, 2022
Proofs received from author(s): October 5, 2022