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Distinct management units for the Critically Endangered angelshark (*Squatina squatina*) revealed in the Canary Islands

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Abstract

The angelshark, *Squatina squatina*, is listed as Critically Endangered on the IUCN Red List of Threatened Species, and remaining populations are highly fragmented throughout its historical distribution. The Canary Islands archipelago in the North East Atlantic has been identified as a uniquely large stronghold for the species. In the present study, we compared the population genetic structure of *S. squatina* across different islands of the Canary Island archipelago using both microsatellite and single nucleotide polymorphism (SNP) markers. Both markers revealed significant differentiation of angelsharks between islands in the archipelago, with three main genetic units at: (1) Tenerife, (2) Gran Canaria and (3) the island group consisting of La Graciosa, Lanzarote and Fuerteventura. Our results imply a connectivity barrier between some adjacent islands, most likely driven by abyssal depths, and varying geological history and formation of each island and oceanographic patterns (i.e. seasonal coastal upwellings off the African coast). Therefore, we suggest that in the Canary Islands, *S. squatina* populations should be managed locally, with conservation and research priorities designed and implemented specifically for each of the three genetic units.

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Introduction

Over the last decades, molecular techniques have been used to examine gene flow between populations, including for threatened species, as a basis for their conservation (Frankham 2010; Feutry et al. 2017; Li et al. 2021). Microsatellites, which are short tandem repetitive elements in the genome, have been used to examine the population genetics of several elasmobranch species, such as Whale Shark (*Rhincodon typus*) (Schmidt et al. 2009), Giant Manta Ray (*Manta birostris*) (Rojas Lopez et al. 2022), Scalloped Hammerhead (*Sphyrna lewini*) (Green et al. 2022) and Smalltooth Sawfish (*Pristis pectinata*) (Chapman et al. 2011).

At the same time, while there has been substantial progress in the use of molecular techniques, the application of genetics for the management of threatened species and fragmented populations has progressed simultaneously over the last decades (Frankham 2010; Domingues et al. 2018), pushing the field from genetic to conservation genomics (Allendorf 2022). Knowledge on the degree of species connectivity is crucial for forecasting extinction risks and guiding conservation efforts (Lowe and Allendorf 2010). Species with isolated populations and habitat fragmentation can be expected to face a high risk of local extirpation (Reed 2004). In these situations, management at the local and regional levels, such as considering distinct Management Units (MUs), can be effective for halting population declines (Castillo-Paez et al. 2014). As such, to maintain connectivity and prevent genetic diversity loss in these species, it is essential to examine the genetic structure.

More recently, the cost of next generation sequencing techniques has made it affordable to employ single nucleotide polymorphisms (SNPs) in non-model species, including Chondrichthyans (Manuzzi et al. 2019; Venables et al. 2021; Walsh et al. 2022; Delaval et al. 2022; Lesturgie et al. 2023). Despite this, less than 1% of Chondrichthyan genomes have been sequenced (Pearce et al. 2021). In particular, species listed as Critically Endangered on the IUCN Red List of Threatened Species have been identified to be a priority for genomic population assessments, in order to increase management effectiveness (Pearce et al. 2021).

The angelshark, *Squatina squatina*, belongs to one of the world's most threatened families (Family Squatinidae) of sharks and rays (Dulvy et al. 2014, 2021; Kyne et al. 2020), and is classified as Critically Endangered (CR) on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Morey et al. 2019). Population declines and geographic fragmentations are a result of various threats, including overfishing and habitat loss (Lawson et al. 2020; Dulvy et al. 2021). The life history characteristics of angelsharks, such as their slow reproductive cycle and slow growth rates, as well as their association to

coastal habitats, are the main reasons hindering population recovery from human-induced threats (Barker et al. 2016; Ellis et al. 2021). As such, a series of targeted conservation efforts aiming to restore and safeguard depleted populations throughout the natural distribution range have been put in place on a local, national and regional level (Barker et al. 2016; Gordon et al. 2017, 2019). Yet, important questions remain about the population structure and ecology of *S. squatina* worldwide, which need to be answered to establish population baselines, monitor population trajectories over time, prioritize research efforts and develop effective management practices.

While population declines or extirpations have been observed throughout its range in the Northeast Atlantic and the Mediterranean Sea, the Canary Islands have been identified as a uniquely large stronghold where *S. squatina* is still encountered on a regular basis throughout the entire year (Barker et al. 2016; Meyers et al. 2017; Gordon et al. 2017; Jimenez Alvarado et al. 2020). Despite increased research efforts to improve understanding of the ecology and biology of *S. squatina*, aspects of the genetic diversity, dispersal patterns and reproductive behaviour of this shark remain poorly understood.

In the Canary Islands, *S. squatina* is distributed along the entire coastline; however, the inter-island distribution patterns and density significantly decreases from the easternmost towards the westernmost islands (Meyers et al. 2017). This east-to-west gradient in the composition and abundance of marine species across the archipelago is also common for other coastal fishes (Tuya et al. 2004), including batoids (Tuya et al. 2021; Espino-Ruano et al. 2023), and could be attributed to the large-scale oceanographic variability linked with the proximity of the Canary Islands to the African coast and associated seasonal upwellings (Davenport et al. 2002). Moreover, varying extensions of shelf platforms (due to geological histories) and the resulting depth barriers within the archipelago may constrain movement and gene flow between islands for fauna that have limited pelagic dispersal (Brito et al. 2002; Espino et al. 2019; Tuya et al. 2021).

Despite the recent use of citizen science initiatives, mark–recapture and electronic tagging studies for investigating population connectivity and distribution patterns of *S. squatina* in the Canary Islands (Meyers et al. 2017; Noviello et al. 2021; Barker et al. 2022; Mead et al. 2023), these approaches have not provided a full picture of movement and gene flow patterns. Yet gathering this information is essential to effectively manage angelshark populations at this important hotspot of biodiversity. To date, there is no published study on the population genetic structure of *S. squatina*.

Here, to fill this gap, we examine the genetic structure of angelshark populations in the Canary Islands using both

microsatellite and SNP markers. Our results shed light on the fine-scale (i.e. interisland) connectivity in this species, and we discuss the conservation implications for angelsharks at both local and regional scales.

Materials and methods

Study sites

The Canary Islands consist of eight main islands (from east to west: La Graciosa, Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro (Fig. 1a)). They are situated approximately 100 km west of the African coastline, while the westernmost island is almost 400 km away (Fernández-Palacios and Martín-Esquivel 2001). Waters surrounding the eastern islands are influenced by seasonal coastal upwellings from the West African coast, compared to the warmer (average of 2 °C) waters surrounding the western islands (Davenport et al. 2002). All islands have volcanic origins and distinct geological histories which have influenced their geomorphology (Fernández-Palacios and Martín-Esquivel 2001). Fuerteventura, Lanzarote and La Graciosa share the same insular shelf, while the rest of the islands have very narrow shelves which are separated

from each other by deeper waters up to 3000 m (Acosta et al. 2003).

Animal capture and sampling

Underwater mark-recapture surveys targeting *S. squatina* were completed between June 2015 and December 2022. During these surveys, genetic samples ($N = 840$) were collected from five islands (Fig. 1a): La Graciosa (LG; $N = 66$), Lanzarote (LZ; $N = 137$), Fuerteventura (FV; $N = 94$), Gran Canaria (GC; $N = 68$) and Tenerife (TN; $N = 475$). These islands were identified as having a larger presence of angelsharks, compared to the western islands (El Hierro, La Palma and La Gomera), where the species is less common (Meyers et al. 2017). The sampling sites also included La Graciosa Marine Reserve (LGMR), Spain's largest Marine Reserve, located around La Graciosa and Lanzarote and identified as an important area for angelsharks (Meyers et al. 2017; Mead et al. 2023). Samples of LGMR were included as part of La Graciosa island.

Individuals were captured during snorkeling and SCUBA diving surveys conducted diurnally (for adults and sub-adults) and nocturnally (for juveniles). Upon capture, individuals were measured, tagged and when possible, a skin fragment (fin clip of ca. 1×1 cm) was taken from the back
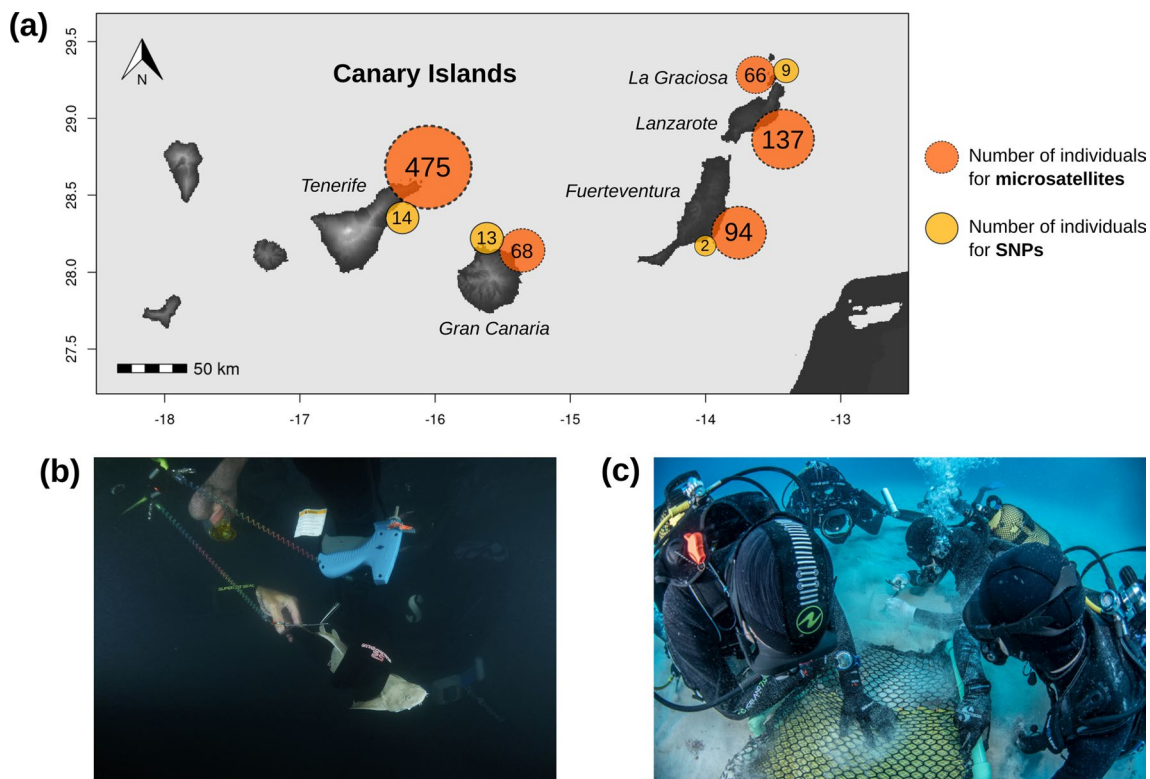

Fig. 1 (**a**) Sampling sites in the Canary Islands and number of genetic samples collected on angelshark (*S. squatina*) individuals for microsatellite (orange) and SNP (yellow) analyses. (**b**) Taking a fin clip from a juvenile angelshark. Photo: Michael J. Sealey (**c**) Taking a fin clip during the underwater tagging procedure of an adult angelshark. Photo: Nuno Vasco Rodrigues

of the second dorsal fin and directly preserved in 20% DMSO until processing (Fig. 1b, c). The genetic sampling process took under 30 s and all animals were released alive.

All surveys were conducted with permits from the Ministry of Environment and Ecological Transition of Spain and the Canary Island Government. Animal handling and sampling methods were assessed and approved by animal ethical committees at the Zoological Society of London and Universidad de Las Palmas de Gran Canaria.

Microsatellite genotyping

We developed species-specific microsatellite markers using an enrichment protocol followed by sequencing on an Illumina platform, as outlined in Feldheim et al. (2020). Fasta files following Illumina sequencing were imported into Geneious v.10.0.3 (http://www.geneious.com; Kearse et al. 2012) and sequences with sufficient flanking sequences were used to develop primers. Primers were developed in Geneious, which uses a modified version of Primer3 (Untergasser et al. 2012). We developed primers for 39 loci (Genbank Accession numbers OQ606924-OQ606962), 23 of which were scoreable and exhibited variation (Table 1). Polymerase Chain Reactions (PCR) were performed in 10 µl volumes with 1x PCR buffer (10mM Tris-HCl, 50mM KCl, pH 8.3), 0.12 mM of each dNTP, 1.5 mM MgCl2, 10x BSA, 0.04 µM forward primer tagged with an M13 sequence on the 5' end (Schuelke 2000), 0.16 µM of both the species-specific reverse primer and a fluorescently labeled universal M13 primer, and 1 U Taq polymerase. Thermal cycling proceeded as follows: an initial denaturation step of 94 ºC for four minutes was followed by 30 cycles of 94 ºC for 15 s, Ta for 15 s (Table 1), and 72 ºC for 45 s, followed by 8 cycles of 94 ºC for 15 s, 53 ºC for 15 s, and 72 ºC for 45 s. A final elongation step of 72 ºC for 10 min concluded each PCR. For loci Ssq616, Ssq618, Ssq626, Ssq628, Ssq630, and Ssq632 a touchdown PCR was performed with the cycling parameters as above with the exception that the first round of thermal cycling was 16 cycles followed by a second round of 20 cycles at the target annealing temperature (Table 1). PCR products (0.6 µl each) and 1.0 µl of an internal ladder (ALEXA-725, Maddox and Feldheim 2014) were combined with 8.5 µl HiDi Formamide and run on an ABI 3730 DNA Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts). Individuals were genotyped using Geneious. Characterization of microsatellite loci, including allele count, observed and expected heterozygosity were performed using GenAlEx v6.5 (Peakall and Smouse 2006, 2012), while tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed using Genepop on the web v4.0 (Raymond and Rousset 1995; Rousset 2008) using the default parameters.

SNP genotyping and filtering

To confirm microsatellite results and examine the presence of more subtle genetic structure, we employed SNP analysis on a subset of $N=38$ samples collected in 2022 from four islands in the Canary Islands: La Graciosa ($N=9$), Fuerteventura ($N=2$), Gran Canaria ($N=13$) and Tenerife ($N=14$) (Fig. 1). Due to limited funds, it was not possible to genotype more individuals using this method.

Tissue samples were transferred into pure ethanol and preserved in a -20 °C freezer at the Centre d'Écologie Fonctionnelle et Évolutive (CEFE, Montpellier, France) until DNA extraction. Samples were then sent to Diversity Arrays Technology (DArT Pty. Ltd.), in Canberra, Australia, for DNA extraction and genotyping, using the standard DArTseq™ protocol (Sansaloni et al. 2011), which combines DArT's genome complexity reduction method (double digestion with restriction enzymes) with Next Generation Sequencing techniques to detect SNPs (Kilian et al. 2012). SNP calling and bioinformatic filtering was performed by DArT using a proprietary pipeline, which results in an initial dataset of 13,299 SNP loci across 38 individuals, with a mean read depth of 8.2x and 3.68% missing data.

We further filtered these data to improve data quality while optimizing the number of loci available for analysis. The data were filtered with the dartR package version 2.7.2 in R (Gruber et al. 2018; Mijangos et al. 2022) to remove SNPs and/or individuals that will not be included in the analysis due to genotyping error or large amounts of missing data. Only loci with a call rate value > 0.90, a Minor Allele Frequency (MAF) > 5% and a read depth between 5 and 100x were retained. All individuals had a call rate > 0.87. These filtering steps resulted in a dataset of 5,633 SNPs across 38 individuals, with 0.51% missing data, used for the population genetics analyses.

Population genetic analyses

Microsatellite analyses

The program Structure v.2.3.2 (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009) was used to examine the number of genetically distinct populations (K) in our sample. To examine how different settings in Structure influenced K, we ran a total of 16 analyses. Structure has been shown to perform poorly when there is uneven sampling between populations (Puechmaille 2016). As such, we randomly chose 137 individuals from the largest sampled island (TN) to match the sample size of the next largest sampled island (LZ). These 137 TN individuals, plus all individuals from the other islands were designated "LOWTN," and the final sample size for these runs was 502 (TN=137, LZ=137,

Table 1 Characteristics of 23 microsatellite loci isolated from *Squatina squatina*

Locus	Primer sequence 5'–3'	Repeat motif	T_a (°C)	Size range (bp)	A	H_o	H_e	GenBank accession #
Ssq29	F: CTTGGCTTTCCTGGTCCACA R: GGCTCGAAGGGCCTAATAGC	$(TG)_{14}$	55	304–326	8	0.381	0.416	OQ606924
Ssq145	F: GAGCTCCAATGGGCCCAAT R: TCTTGGTTCATTCTCTCTTTACCC	$(AG)_{12}$	55	248–252	3	0.418	0.441	OQ606925
Ssq168	F: AATTATGGCCTGACCGGTGC R: TGAGTCATCCCAGCCTGTTG	$(TG)_{23}$	55	235–269	13	0.305	0.298	OQ606926
Ssq363	F: TTACAAGCTCTGCCGTCCAG R: CCAGAATATCCCACAGAGCCC	$(TACA)_{12}$	58	162–206	7	0.367	0.402	OQ606927
Ssq540	F: CCCTGTGAATCCTCTACGCA R: AAACCCGCATATTCCCCTCC	$(AG)_{26}$	58	368–422	17	0.687	0.678	OQ606928
Ssq604	F: CAATCTGCACGCTGATTGCA R: ACCTGTGGTAGCGAATTCCA	$(AG)_{17}$	58	208–218	4	0.397	0.391	OQ606929
Ssq605	F: AGCAGTTGGTCTGATTAGCCT R: ATTCAGTGGTCGAGTGCTGT	$(TC)_{24}$	58	196–210	8	0.501	0.494	OQ606930
Ssq606	F: GCCATGGAGGTCTTGGGATT R: GTGCTTCAGTTAAAGACAGGGC	$(TC)_{24}$	55	274–302	6	0.603	0.586	OQ606931
Ssq610	F: TCCCATGGTTAACAACTCCGG R: CAAGAAGCCTCCTCCTGTCC	$(TC)_{23}$	58	243–255	7	0.125	0.165*	OQ606932
Ssq612	F: TCCAGCTTTTGTGGTCTGCT R: TGCAGCTGACGGCATACTTA	$(TC)_{22}$	55	344–372	10	0.481	0.540	OQ606933
Ssq616	F: ATGCTGTGTCACAGGGAGTG R: TTTTGGCCCATCGTGTCTGT	$(AG)_{17}$	TD to 58	217–235	8	0.150	0.146	OQ606934
Ssq618	F: ACCGAGAAGTGTAATGGCACC R: AAGGTTGGTGAGCTGCAAGT	$(TC)_{21}$	TD to 58	284–296	6	0.499	0.492	OQ606935
Ssq623	F: GGTCAGGAAAACTGCTCCAGTA R: TGTCAGGAACAGGAAGTGCT	$(AC)_{20}$	58	232–244	7	0.360	0.352	OQ606936
Ssq625	F: GCATGTTCCCCTCCCAATCA R: GGGAATCCAGAATAAGGGGCC	$(TG)_{21}$	58	230–271	17	0.711	0.650	OQ606937
Ssq626	F: ACACACTTTGCAGAGAGGTGT R: TGCAGTATCTCTTTTGTTAAACGC	$(TG)_{23}$	TD to 58	359–392	14	0.664	0.542	OQ606938
Ssq627	F: TGGATAAATTGTTTTGCCAGAGG R: TGATTGGTGGTATGTTTCA	$(AG)_{23}$	58	150–192	21	0.793	0.623	OQ606939
Ssq628	F: TGCCCTTTTCTCTGTGTCGT R: TTAACTTGCGGCCTATCCCC	$(TC)_{24}$	TD to 58	216–232	6	0.087	0.175*	OQ606940
Ssq629	F: TGCTGGGCCAAATACTCCTG R: TGCAGTTGTGACACCTGTCT	$(TG)_{19}$	58	180–242	24	0.858	0.868	OQ606941
Ssq630	F: TTGCATCGGTGGACAGAGAC R: AACTCAGCTTCTGCCCATCT	$(AG)_{22}$	TD to 58	183–197	6	0.461	0.456	OQ606942
Ssq631	F: GATGTCATCTCGCTCTCCGG R: TGGCTGACAATAGTGGAGCA	$(TC)_{20}$	55	224–252	10	0.395	0.384	OQ606943

Locus	Primer sequence 5'– 3'	Repeat motif	T_a (°C)	Size range (bp)	A	H_o	H_e	GenBank accession #
Ssq632	F: CACACCCTGTGGTAGCAAGT R: GGAAAGGATGAACTGGCATGG	$(TC)_{21}$	TD to 58	269–304	16	0.765	0.792	OQ606944
Ssq633	F: GCTGGGCACAACTTCCATTG R: TGAGGAGGACACACCCTACA	$(AC)_{20}$	55	286–294	3	0.434	0.428	OQ606945
Ssq634	F: TCTGCCATTTGACTTCCCCA R: GCAAGAGTCTTTCATGAATGCCA	$(AC)_{21}$	58	242–256	5	0.526	0.524	OQ606946

Table 1 (continued)

T_a = annealing temperature, A = no. of alleles, H_e = Expected heterozygosity, H_o = Observed heterozygosity, *Deviation from HWE after Bonferroni correction. Forward primers were 5'-tailed with TGTAAAACGACGGCCAGT, while reverse primers were 5' tailed with GTGTCTT

FV = 94, GC = 68, LG = 66). In addition to the "LOWTN" analyses, we also ran Structure v.2.3.2 on the entire dataset using all 475 individuals from the TN island (i.e. "FULL," N = 840). We ran two Structure analyses (one analysis on each of the "LOWTN" and "FULL" datasets), using the following parameters: admixture with independent allele frequencies between populations with length of burnin period and number of MCMC reps after burnin both sets to 1,000,000. We repeated these two analyses with the exception that the allele frequencies parameter was set to "correlated." All four of these runs were repeated using sampling locations as priors (Hubisz et al. 2009). Wang (2017) found that uneven sampling of populations can be overcome by using an alternative ancestry prior and suggested changing the value of alpha from 1 to 1/K. Thus, we repeated all of the eight runs outlined above with alpha set to 0.20, for a total of 16 separate Structure analyses. Each analysis tested K = 1–6 with ten iterations for each K-value.

To assess the most likely number of population clusters from these analyses, we employed STRUCTURESELECTOR (Li and Liu 2018). This online program uses several estimators to determine the best value of K, including MEDMEDK (median of medians), MEDMEAK (median of means), MAXMEDK (maximum of medians), and MAXMEAK (maximum of means; Puechmaille 2016), Ln Pr(X|K) (Pritchard et al. 2000), and DK (Evanno et al. 2005). The relatively new estimators of Puechmaille are based on each individual's mean membership coefficient to its predefined group (i.e., sampling locale). Mean membership coefficient thresholds were set to 0.5, 0.6, 0.7, and 0.8 as suggested by Puechmaille (2016). As this threshold increases, so does the differentiation required between two subpopulations before they are considered to be different clusters (Puechmaille 2016). STRUCTURESELECTOR also integrates CLUMPAK (Kopelman et al. 2015) to generate graphical representations and allow visualisation of the results.

We also performed a Discriminant Analysis of Principal Components (DAPC), to identify and describe clusters of genetically related individuals (Jombart et al. 2010), using the R package adegenet version 2.1.8 (Jombart 2008). The DAPC relies on transformation of genomic data using Principal Component Analysis (PCA), as a first step, which generates uncorrelated variables, followed by a Discriminant Analysis (DA) on the uncorrelated PCA variables, which produces synthetic discriminant functions (axes) that maximize between-group variation while minimizing within-group variation (Jombart et al. 2010). We determined the optimal number of clusters by performing a DAPC with no prior information with the function "find.clusters". This function uses a sequential k-means clustering algorithm and model selection, based on the Bayesian Information

Criterion (BIC), to infer genetic clusters (Jombart et al. 2010). The resulting clusters were plotted with the first and the second discriminant components.

Genetic differences between islands were examined using three measures of genetic differentiation: Jost's D (Jost 2008), G''st (Meirmans and Hedrick 2011), and F'st (Meirmans 2006). These measures were all calculated using Genodive v. 2.0b27 (Meirmans and Van Tienderen 2004), and significance (i.e. whether or not these populations are statistically differentiated) was determined by 10,000 permutations.

SNPs analyses

All analyses using SNP data were performed in R version 4.3.0 (R Core Team 2023). The pattern of population structure was investigated by carrying out in parallel: (i) a Bayesian-based structure analysis (Frichot and François 2015), (ii) a DAPC (Jombart et al. 2010), and (iii) calculations of pairwise estimates of F_{ST} (Weir and Cockerham 1984), G_{ST} (Hedrick 2005) and Jost's D (Jost 2008).

First, we used the R package LEA version 3.10.2 (Frichot and François 2015) to run the Bayesian structure analysis to determine the most likely number of population clusters (K) within our dataset and obtain admixture coefficient bar-plots. The R function "snmf", which is based on sparse non-negative matrix factorization (sNMF) algorithms, was applied to estimate the cross-entropy criterion, which guides the choice of the number of populations (K) and the results from the best run. After running this function with varying K from 1 to 8, we selected the most likely K, for which the cross-entropy criterion value was the smallest. Then, the "snmf" function was run again with the chosen K to estimate

admixture coefficients (i.e. genetic ancestry proportions) for each individual (Frichot and François 2015).

This unsupervised Bayesian clustering approach was complemented with a DAPC to identify clusters of genetically related individuals (Jombart et al. 2010), using the R package adegenet version 2.1.8 (Jombart 2008), as previously described for our microsatellite analysis.

Genetic differentiation between islands (or between clusters identified by the DAPC) was revealed by calculating pairwise estimates of F_{ST} (Weir and Cockerham 1984) with 10,000 bootstraps using the function "gl.fst.pop" from the R package dartR version 2.9.7 (Gruber et al. 2018; Mijangos et al. 2022). Pairwise estimates of G_{ST} (Hedrick 2005) and Jost's D (Jost 2008) were calculated with 10,000 bootstraps using the function "gl.report.fstat" from the R package dartR.base version 0.49 (Gruber et al. 2018; Mijangos et al. 2022).

Results

Microsatellites

Microsatellite loci had from 3 to 24 alleles, while observed and expected heterozygosity ranged from 0.087 to 0.858 and from 0.146 to 0.868 respectively (Table 1). When the entire dataset was analyzed, several loci were out of HWE (data not shown), likely due to the fact that there are several related individuals in the Tenerife population. When this population was removed from HWE analysis, two loci (Ssq610 and Ssq628) remained out of HWE after Bonferroni correction (Table 1). Likewise, when the Tenerife population was removed from LD tests, one pair of loci remained linked (Ssq627 and Ssq604). Loci Ssq610, Ssq628 and Ssq604 were removed from subsequent analyses, leaving 20 loci.

Significant population differentiation was found between islands, using all three metrics, with the exception of comparisons between La Graciosa and Fuerteventura (Table 2). In all our analyses, the Evanno delta K estimator estimated a value of K = 2 (see supplementary information), with La Graciosa, Lanzarote and Fuerteventura forming one group, while Tenerife and Gran Canaria forming a second and third group respectively (Fig. 2). The MEDMEDK, MEDMEAK, MAXMEDK, and MAXMEAK values typically returned a K = 3 (see supplementary information), with La Graciosa, Lanzarote and Fuerteventura forming one group, and Gran Canaria and Tenerife forming their own unique clusters (Fig. 2). Mean LnP(K) returned a range of K from 3 to 6 (see supplementary information). The DAPC results showed three genetic clusters, matching the west-east geographical locations of the islands (Fig. 3c).

Table 2 Pairwise genetic differentiation between populations of angelsharks in four islands: Fuerteventura (FV), Gran Canaria (GC), La Graciosa (LG) and Lanzarote (LZ), using 20 microsatellites. The top, middle, and bottom values between population pairs are F'st, G''St, and Jost's D, respectively. Significance was determined by 10,000 permutations

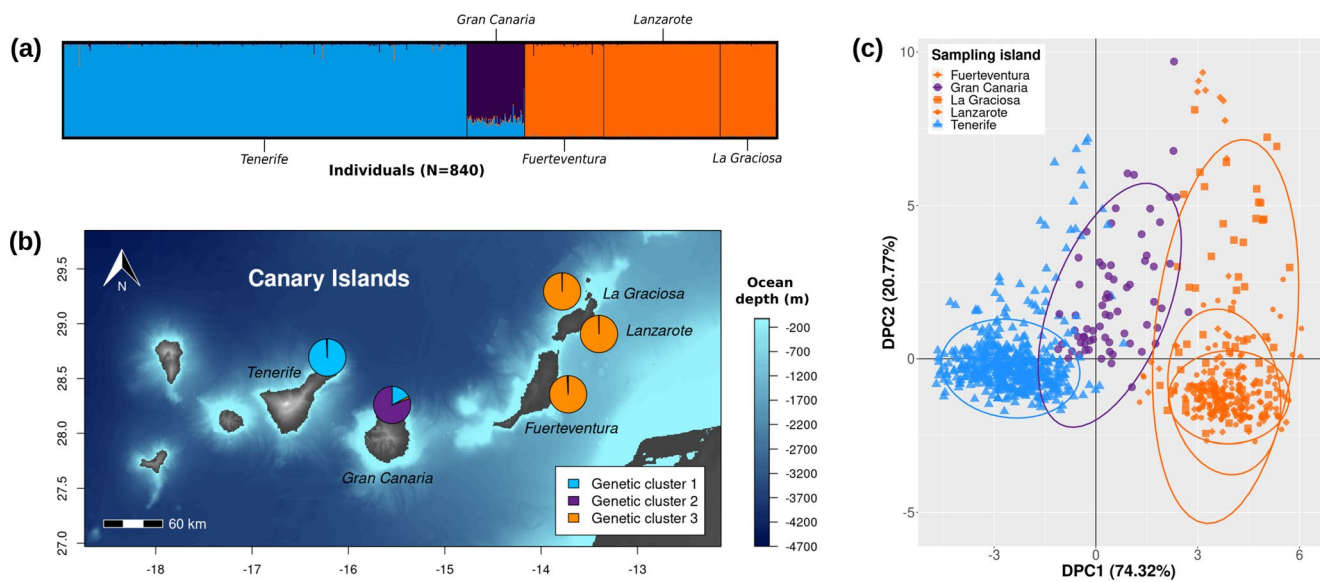|		FV	GC	LG	LZ
F_{ST}	GC	0.269*			
G_{ST}		0.269*			
Jost's D		0.160*			
F_{ST}	LG	0.004	0.257*		
G_{ST}		0.005	0.259*		
Jost's D		0.002	0.152*		
F_{ST}	LZ	0.009*	0.297*	0.013*	
G_{ST}		0.009*	0.299*	0.013*	
Jost's D		0.005*	0.182*	0.007*	
F_{ST}	TN	0.371*	0.184*	0.364*	0.387*
G_{ST}		0.371*	0.185*	0.364*	0.386*
Jost's D		0.235*	0.104*	0.229*	0.249*

* p ≤ 0.001

Fig. 2 Structure results for 20 microsatellites across 840 angelshark individuals from the Canary Islands. (**a**) Structure bar plot, using K = 3 clusters, showing admixture proportions for 840 individuals (vertical bars). (**b**) Map of admixture estimates, using K = 3 clusters, averaged over individuals of each island and displayed in pie charts. (**c**) Discriminant Analysis of Principal Components (DAPC) plot of 840 individuals (each point indicates one individual), with five PCs and two discriminant functions retained to describe the relationships between clusters. The size of the ellipses encompasses 95% of individuals, assuming a t distribution
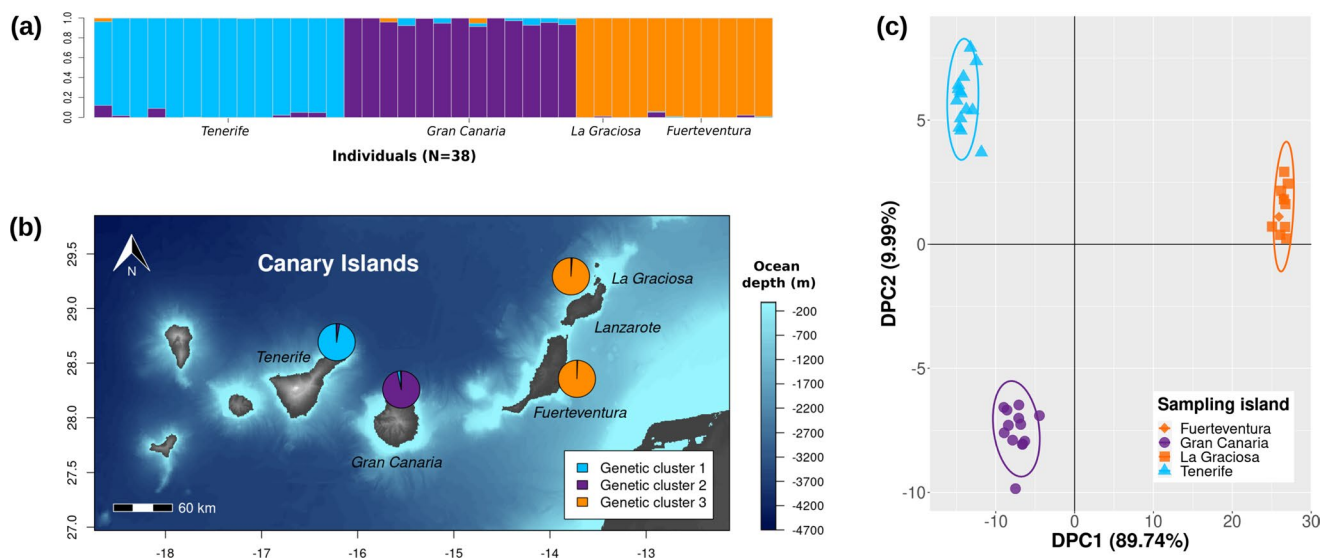
Fig. 3 Structure results for 5,633 SNPs across 38 angelshark individuals from the Canary Islands. (**a**) Structure bar plot, using K = 3 clusters, showing admixture proportions for 38 angelshark individuals (vertical bars) genotyped at 5,633 SNP loci. (**b**) Map of admixture estimates, using K = 3 clusters, averaged over individuals of each island and displayed in pie charts. (**c**) Discriminant Analysis of Principal Components (DAPC) plot of 38 individuals (each point indicates one individual), with three PCs and two discriminant functions retained to describe the relationships between clusters. The size of the ellipses encompasses 95% of individuals, assuming a t distribution

SNPs

The structure analysis (sNMF algorithm) identified a number of genetic clusters corresponding to the different islands (Fig. 3), with La Graciosa and Fuerteventura grouped together. Given that cross-entropy values were the lowest for K = 2 and K = 3 (see Fig. 1 in Supplementary Information),

we run the sNMF algorithm for these two values separately. When choosing two clusters (K = 2), the analysis identified Tenerife and Gran Canaria as one population and La Graciosa and Fuerteventura as another population. When using three clusters (K = 3) for the analysis, the structure plot distinguished Tenerife as a third unique genetic group. These results are consistent with the results from the DAPC, which

also clustered individuals into three very distinct groups (Fig. 3), with non-overlapping 95% ellipses. Two discrimination functions were detected, which explains 89.74% and 9.99% of the variation between clusters (see Fig. 2 in Supplementary Information).

Pairwise F_{ST} comparisons showed significant genetic differentiation between the islands (Table 3): Tenerife– Gran Canaria ($F_{ST} = 0.055$, $p < 0.001$), Gran Canaria– La Graciosa ($F_{ST} = 0.180$, p-value < 0.001) and Tenerife– La Graciosa ($F_{ST} = 0.226$, p-value < 0.001), except between La Graciosa and Fuerteventura. Pairwise estimates of G_{ST} and Jost's D were consistent with the F_{ST} results.

Discussion

Through combining microsatellite and genome-wide SNP markers and various statistical analyses, genetic differentiation in angelshark populations between the Canary Islands was identified. More specifically, we detected three main units: La Graciosa-Lanzarote-Fuerteventura, Gran Canaria and Tenerife. Results were confirmed by statistical significant pairwise genetic differentiation among islands.

Many shark species tend to be highly migratory (Kohler et al. 1998; Lascelles et al. 2014), and genetic differentiation for those species is often lacking or only found at the level of ocean basins, including Tiger Sharks (*Galeocerdo cuvier*) (Bernard et al. 2021; Lesturgie et al. 2022), Scalloped Hammerhead Sharks (*Sphryna lewini*) (Daly-Engel et al. 2012; Duncan et al. 2006), Blue Sharks (*Prionace glauca*) (Veríssimo et al. 2017; Nikolic et al. 2023), Shortfin Mako Sharks (*Isurus oxyrinchus*) (Corrigan et al. 2018), Dusky Sharks (*Carcharhinus obscurus*) (Benavides et al. 2011), and Whale Sharks (*Rhincodon typus*) (Schmidt et al. 2009). In the Canary Islands, acoustic tracking data showed that angelsharks are present year-round in La Graciosa Marine Reserve, with higher levels of localized activity and mobility

in male sharks (Mead et al. 2023). Here, angelsharks were also shown to reside in deeper areas of more than 100 m (Mead et al. 2023).

Our results revealed a significant genetic differentiation between La Graciosa, Lanzarote and Fuerteventura (Table 2). These values were relatively small, however, and statistical significance does not necessarily equate to biological significance (Hedrick 1999). Indeed, comparisons of genetic differentiation between the La Graciosa-Lanzarote-Fuerteventura unit, the Tenerife unit and Gran Canaria unit were at least an order of magnitude greater than those comparisons between La Graciosa, Lanzarote and Fuerteventura (Table 2). These results are similar to those found in Pacific angelsharks (*S. californica*) in the California Channel Gaida (1997) used allozymes to show that angelsharks from the northern islands of Santa Rosa and Santa Cruz were genetically similar, but distinct from those from the southern site of San Clemente. The depth between Santa Cruz and Santa Rosa is only 30 m in many areas, while the depth between these northern sites and San Clemente exceeds 500 m, and the extreme depth serves as a barrier to movement between sites (Gaida 1997). In the Canary Islands, abyssal depths (> 2,000 m) between adjacent islands, excluding La Graciosa, Lanzarote and Fuerteventura that share the same shelf, may constrain the dispersal and movement of angelsharks and other benthic elasmobranchs between islands (Meyers et al. 2017; Tuya et al. 2021).

These results are consistent with previous studies showing that ocean depth acts as a barrier to gene flow for some species of sharks, in particular benthic species (Barker et al. 2015; Boussarie et al. 2022). A study examining how marine barriers shape genetic connectivity among 102 elasmobranch species (Hirschfeld et al. 2021) identified that the most common barriers were related to ocean bathymetry. Depth can create genetic structure at extremely small spatial scales in shallow-water demersal populations that depend on active dispersal for their reproduction (Hirschfeld et al. 2021). Reproductive philopatry (i.e. breeding-site fidelity), which is a common behaviour in elasmobranchs (Chapman et al. 2015; Flowers et al. 2016), can also shape genetic structure (Hirschfeld et al. 2021). This behaviour has not been documented in angelsharks, despite their tendency to show a certain degree of site-fidelity (Ellis et al. 2021; Angel Shark Project unpublished data), which could also explain the lack of genetic connectivity between angelsharks in the Canary Islands.

Our Structure analyses gave conflicting results when determining the most likely K. Structure performs poorly when sampling is uneven between sites (Puechmaille 2016; Wang 2017). To overcome uneven sampling in this study, we created a "LOWTN" dataset, and we set alpha to 1/K (i.e. 0.2). Neither one of these strategies greatly affected

Table 3 Pairwise genetic differentiation calculated between islands for angelsharks genotyped at 5,633 SNP loci. For each pair of islands, estimates of FST (Weir and Cockerham 1984), GST (Hedrick 2005) and Jost's D (Jost 2008) are reported. Significance was determined by 10,000 bootstraps. FV = Fuerteventura, GC = Gran Canaria, LG = La Graciosa, TN = Tenerife

		FV	GC	LG
F_{ST}	GC	0.206*		
G_{ST}		0.197*		
Jost's D		0.113*		
F_{ST}	LG	0.013	0.180*	
G_{ST}		0.010	0.187*	
Jost's D		0.006	0.105*	
F_{ST}	TN	0.274*	0.055*	0.226*
G_{ST}		0.258*	0.049*	0.232*
Jost's D		0.148*	0.024*	0.131*

* $p \leq 0.001$

our results, as delta K always yielded two groups and the Puechmaille estimators generally yielded a K = 3. The mean LnP (K) estimator was affected by different run parameters, however. This statistic is known to overestimate K (Wang 2017), and we found this as well in our analyses (Table 1 in Supplementary Information), as this estimator yielded a K = 6 in five of the 16 analyses. LnP(K) yielded a K = 3 just four times, and only once with the "FULL" dataset. Although the correlated allele frequencies model can overestimate K, this did not seem to be the case. Indeed, in three of the four cases where LnP(K) found a K = 3, the correlated model was used (Table 1 in Supplementary Information). It has been shown that the Puechmaille estimators appear to be more accurate than delta K, particularly when sampling is uneven between sites (Janes et al. 2017). Furthermore, Wang (2017) has shown that, in addition to LnP(K) overestimating structure, delta K tends to underestimate the number of populations. This, coupled with our genetic differentiation results, lead us to the conclusion that La Graciosa-Lanzarote-Fuerteventura are one panmictic population, whereas Tenerife and Gran Canaria are genetically distinct.

It should be noted that sample size was much smaller for SNP-based analyses (particularly for Fuerteventura) causing a possible bias in the results. Although accuracy of the results increases with greater sample size (Fumagalli 2013), our SNP data corroborated our results with the larger microsatellite analyses. Thus, we feel confident in our conclusion of three genetically distinct populations.

Conservation implications and recommendations

The angelshark is an iconic species in the Canary Islands, with an important social and economic value, particularly for the tourism industry. Our findings have direct conservation implications for angelsharks in the Archipelago and may also be worth considering for management of the remaining populations across the biogeographic range. The geographic isolation of angelshark populations likely indicated that angelsharks face a greater risk from local depletion.

In the Canary Islands, strong genetic differentiation of angelsharks between the islands suggests that Tenerife, Gran Canaria and La Graciosa - Lanzarote - Fuerteventura should be treated as three separate management units (MUs). This is particularly relevant for the implementation of the recently developed Angelshark Recovery Plan for the Canary Islands, by the Ministry for the Ecological Transition and the Demographic Challenge of Spain. Archipelago-wide measures outlined in this Recovery Plan, include the regulation of professional and recreational fishing activities, protecting important angelshark habitats and limiting disturbance by water users. However, the application and enforcement of these measures in each of the three MUs, can have a direct impact on the resilience of the entire population.

In addition to the application and enforcement of the Recovery Plan measures, our specific management and research recommendations include assessing the status of angelsharks in each management unit and applying a long-term monitoring programme of identified critical sites in each management unit, including the seasonal distribution patterns, habitat and threats. Furthermore, to better understand population dynamics within each management unit, we recommend investigating angelshark breeding behaviour (i.e. reproductive cycle, parentage, philopatry). Finally, angelshark fishing mortality should be evaluated by developing close partnerships with fishers. Overall, in the Canary Islands we recommend the enforcement of measures to minimize potential threats to angelsharks and their habitats, designed also at smaller scales, specific to each management unit.

Failing to monitor and protect critical sites within each management unit can put the entire Canary Island population at risk. If, for example, key areas are negatively impacted by urban development, contamination events, climate change or fishing, the population within one management unit can decline very quickly with limited potential to recover, as there is limited connectivity.

Moreover, it is likely that given the abyssal barriers between adjacent islands, the populations in La Gomera, La Palma and El Hierro are all similarly isolated from each other and may add three more management units that require further investigation. On a broader geographical scale, our results suggest that the Canary Island population may be isolated from the rest of the fragmented populations in the Northeast Atlantic and Mediterranean Sea. We recommend a comparative analysis of the populations from the Canary Islands with populations from the Northeast Atlantic, West Africa and Mediterranean Sea to shed light on the connectivity between these fragmented populations. Depending on the research question, the availability of samples over time and resources, these analyses can be run both with microsatellites and SNPs, as both methodologies have shown similar results in this study. Considering the conservation status of this species, the sample size should be carefully considered depending on the research questions and ideally, the same samples should be used to answer further research questions, i.e. on the reproductive behaviour of angelsharks.

Declarations

Competing interests The authors declare no competing interests.

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