



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

Eva K.M. Meyers<sup>1,6</sup> · Nadia Faure<sup>2,10</sup> · David Jiménez-Alvarado<sup>3,6</sup> · Joanna Barker<sup>4,6</sup> · Hector Toledo-Padilla<sup>6</sup> · Fernando Tuya<sup>3</sup> · Charlotte Pike<sup>4,6</sup> · Lucy R. Mead<sup>5,6</sup> · Michael J. Sealey<sup>6</sup> · Maria Belén Caro<sup>6</sup> · David M.P. Jacoby<sup>7</sup> · Felipe Ravina Olivares<sup>6</sup> · Tomas Bañeras<sup>3,6</sup> · Airam Guerra-Marrero<sup>3</sup> · Ana Espino-Ruano<sup>3</sup> · Jose Juan Castro<sup>3</sup> · Caroline Bousquet<sup>8</sup> · Ioannis Giovos<sup>9</sup> · Dennis Rödder<sup>1</sup> · Stéphanie Manel<sup>2,13</sup> · Julie Deter<sup>10,11</sup> · Kevin A. Feldheim<sup>6,12</sup>

Received: 9 August 2024 / Accepted: 15 October 2024  
© The Author(s) 2024

## 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.

**Keywords** Conservation · Genetic diversity · Microsatellites · SNPs · Elasmobranchs · Atlantic

✉ Eva K.M. Meyers  
e.meyers@leibniz-lib.de

<sup>1</sup> LIB, Museum Koenig Bonn, Leibniz Institute for the Analysis of Biodiversity Change Bonn, Bonn, Germany

<sup>2</sup> CEFE, Univ Montpellier, CNRS, EPHE-PSL University, IRD, Montpellier, France

<sup>3</sup> IU-ECOQUA, Universidad de Las Palmas de Gran Canaria, Las Palmas, Spain

<sup>4</sup> Conservation and Policy, Zoological Society of London, London, UK

<sup>5</sup> Institute of Zoology, Zoological Society of London, London, UK

<sup>6</sup> Angel Shark Project: Canary Islands, Islas Canarias, Spain

<sup>7</sup> Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

<sup>8</sup> Protected Areas of the Sea, Islands and Coasts Service, Environment Agency of Corsica, Bonifacio, France

<sup>9</sup> iSea, Environmental Organisation for the Preservation of the Aquatic Ecosystems, Thessaloniki, Greece

<sup>10</sup> MARBEC, Univ Montpellier, CNRS, IFREMER, IRD, Montpellier, France

<sup>11</sup> Andromède Océanologie, Mauguio, France

<sup>12</sup> Pritzker Laboratory for Molecular Systematics and Evolution, Field Museum of Natural History, Chicago, IL 60605, USA

<sup>13</sup> Institut Universitaire de France, Paris, France

## 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

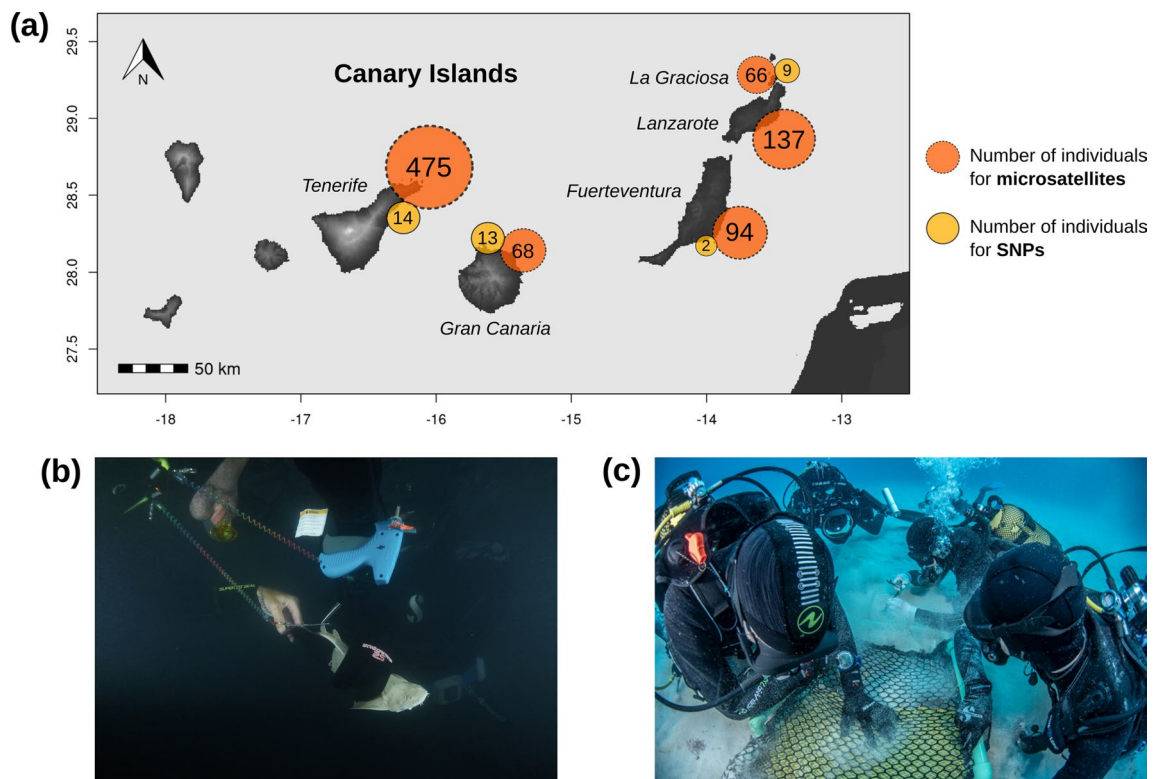
The Canary Islands consist of eight main islands (from east to west: La Graciosa, Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro (Fig. 1a)). They are situated approximately 100 km west of the African coastline, while the westernmost island is almost 400 km away (Fernández-Palacios 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.  $1 \times 1$  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>; Kearsse 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  $\mu$ l volumes with 1x PCR buffer (10mM Tris-HCl, 50mM KCl, pH 8.3), 0.12 mM of each dNTP, 1.5 mM MgCl<sub>2</sub>, 10x BSA, 0.04  $\mu$ M forward primer tagged with an M13 sequence on the 5' end (Schuelke 2000), 0.16  $\mu$ M 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 °C for four minutes was followed by 30 cycles of 94 °C for 15 s, Ta for 15 s (Table 1), and 72 °C for 45 s, followed by 8 cycles of 94 °C for 15 s, 53 °C for 15 s, and 72 °C for 45 s. A final elongation step of 72 °C 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  $\mu$ l each) and 1.0  $\mu$ l of an internal ladder (ALEXA-725, Maddox and Feldheim 2014) were combined with 8.5  $\mu$ l 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 <sub>m</sub> (°C)	Size range (bp)	A	H <sub>o</sub>	H <sub>e</sub>	GenBank accession #
Ssq29	F: CTTGGCTTTCCTGGTCCACA R: GGCTCGAAGGGCCTAATAGC	(TG) <sub>14</sub>	55	304–326	8	0.381	0.416	OQ606924
Ssq145	F: GAGCTCCAATGGGCCAAT R: TCTTGGTTCATTCTCTTTACCC	(AG) <sub>12</sub>	55	248–252	3	0.418	0.441	OQ606925
Ssq168	F: AATTATGGCTGACCCGGTGC R: TGAGTCATCCCAGCCTGTTG	(TG) <sub>23</sub>	55	235–269	13	0.305	0.298	OQ606926
Ssq363	F: TTACAAGCTTCCCGTCCAG R: CCAGAATATCCACACAGAGCCC	(TACA) <sub>12</sub>	58	162–206	7	0.367	0.402	OQ606927
Ssq540	F: CCCTGTGAATCCTCTACGCA R: AAACCCGCATATCCCCCTCC	(AG) <sub>26</sub>	58	368–422	17	0.687	0.678	OQ606928
Ssq604	F: CAATCTGCACGCTGATTGCA R: ACCTGTGGTAGCGAATCCA	(AG) <sub>17</sub>	58	208–218	4	0.397	0.391	OQ606929
Ssq605	F: AGCAGTTGGTCTGATTAGCCT R: AITCAGTGGTCCGAGTGTGT	(TC) <sub>24</sub>	58	196–210	8	0.501	0.494	OQ606930
Ssq606	F: GCCATGGAGGTCITGGGATT R: GTGCTTCAGTTAAAGACAGGGC	(TC) <sub>24</sub>	55	274–302	6	0.603	0.586	OQ606931
Ssq610	F: TCCCATGGTTAACAACCTCCGG R: CAAGAAGCCTCCTCTGTGCC	(TC) <sub>23</sub>	58	243–255	7	0.125	0.165*	OQ606932
Ssq612	F: TCCAGCTTTTGTGGTCTGCT R: TGCAGCTGACGGCATACTTA	(TC) <sub>22</sub>	55	344–372	10	0.481	0.540	OQ606933
Ssq616	F: ATGCTGTGTACACAGGGAGTG R: TTTTGGCCCATCGTGTCTGT	(AG) <sub>17</sub>	TD to 58	217–235	8	0.150	0.146	OQ606934
Ssq618	F: ACCGAGAAAGTGTATGGCACCC R: AAGTTGGTGGCTGCAAGT	(TC) <sub>21</sub>	TD to 58	284–296	6	0.499	0.492	OQ606935
Ssq623	F: GGTCAAGGAAACTGCTCCAGTA R: TGTCAAGGAACAGGAAGTGT	(AC) <sub>20</sub>	58	232–244	7	0.360	0.352	OQ606936
Ssq625	F: GCATGTCCCCCTCCCAATCA R: GCGAATCCAGAATAAGGGGCC	(TG) <sub>21</sub>	58	230–271	17	0.711	0.650	OQ606937
Ssq626	F: ACACACTTTGCAGAGAGGTGT R: TGCAGTATCTCTTTTGTAAACGC	(TG) <sub>23</sub>	TD to 58	359–392	14	0.664	0.542	OQ606938
Ssq627	F: TGGATAATAATGTTTTGCCAGAGG R: TGATTTGGTGGTGGTATGTTTCA	(AG) <sub>23</sub>	58	150–192	21	0.793	0.623	OQ606939
Ssq628	F: TGCCCTTTTCTCTGTGTCTGT R: TTAACCTTGGCCCTAICCCC	(TC) <sub>24</sub>	TD to 58	216–232	6	0.087	0.175*	OQ606940
Ssq629	F: TGCTGGGCCAAATACTCCTG R: TGCAGTTGTGACACCTGTCT	(TG) <sub>19</sub>	58	180–242	24	0.858	0.868	OQ606941
Ssq630	F: TTGCATCGGTGGACAGAGAC R: AACTCAGCTTCTGCCCATCT	(AG) <sub>22</sub>	TD to 58	183–197	6	0.461	0.456	OQ606942
Ssq631	F: GATGTCATCTCGCTCTCCGG R: TGGCTGACAAATAGTGGAGCA	(TC) <sub>20</sub>	55	224–252	10	0.395	0.384	OQ606943



Table 1 (continued)

Locus	Primer sequence 5'-3'	Repeat motif	$T_m$ (°C)	Size range (bp)	A	$H_o$	$H_e$	GenBank accession #
Ssq632	F: CACACCCCTGTGGTAGCAAGT R: GGA AAGGATGAACCTGGCATGG	(TC) <sub>21</sub>	TD to 58	269–304	16	0.765	0.792	OQ606944
Ssq633	F: GCTGGGCACAACCTCCATTG R: TGAGGAGGACACCCCTACA	(AC) <sub>20</sub>	55	286–294	3	0.434	0.428	OQ606945
Ssq634	F: TCTGCCATTTGACTTCCCCA R: GCAAGAGTCTTTCATGAATGCCA	(AC) <sub>21</sub>	58	242–256	5	0.526	0.524	OQ606946

$T_m$  = 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 TGTA AACGACGGCCAGT, 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 STRUCTURESELECTION (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). STRUCTURESELECTION 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.fst" from the R package dartR.base version 0.49 (Gruber et al. 2018; Mijangos et al. 2022).

## Results

### Microsatellites

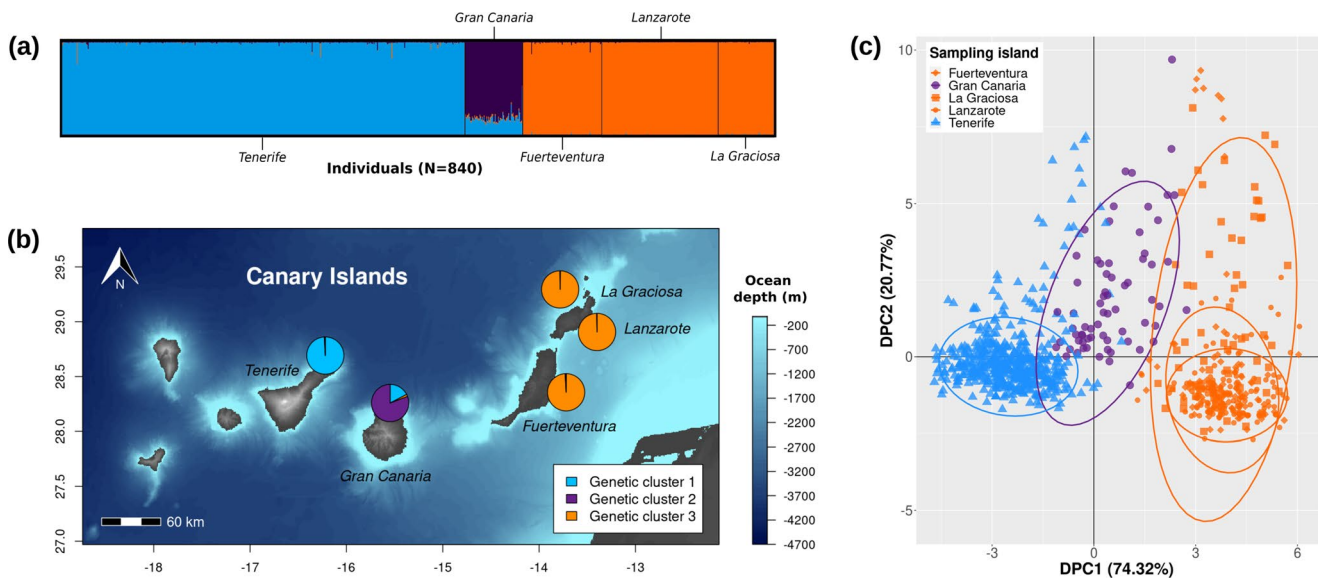
Microsatellite loci had from 3 to 24 alleles, while observed and expected heterozygosity ranged from 0.087 to 0.858 and from 0.146 to 0.868 respectively (Table 1). When the entire dataset was analyzed, several loci were out of HWE (data not shown), likely due to the fact that there are several related 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  $\ln P(K)$  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

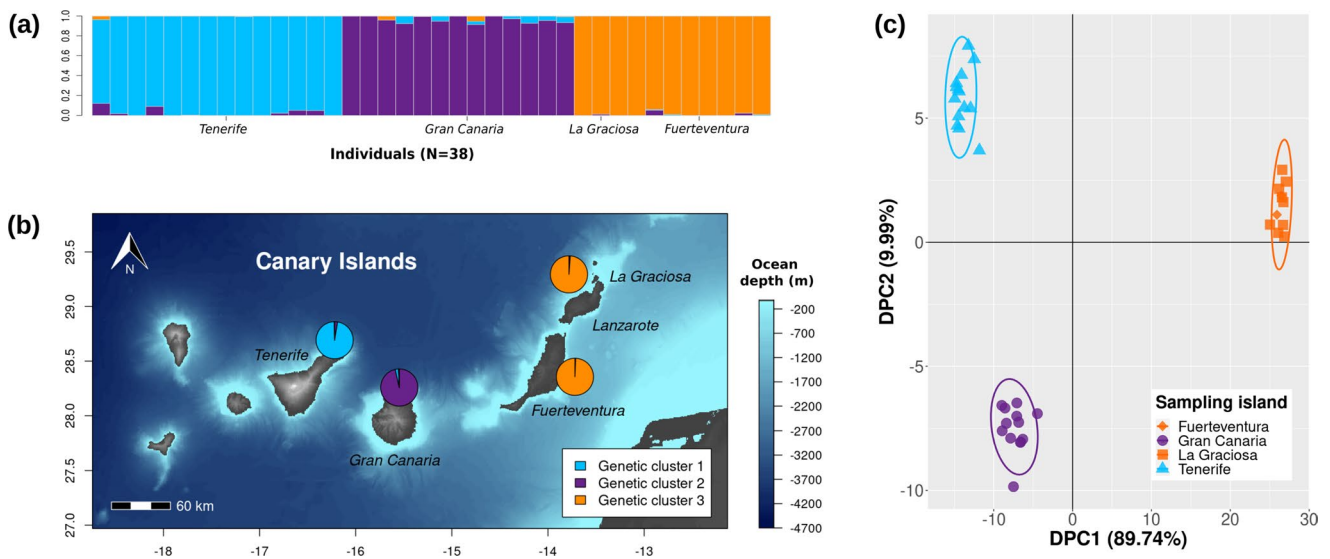
		FV	GC	LG	LZ
$F''_{ST}$	GC	0.269*			
$G''_{ST}$		0.269*			
Jost's D		0.160*			
$F''_{ST}$	LG	0.004	0.257*		
$G''_{ST}$		0.005	0.259*		
Jost's D		0.002	0.152*		
$F''_{ST}$	LZ	0.009*	0.297*	0.013*	
$G''_{ST}$		0.009*	0.299*	0.013*	
Jost's D		0.005*	0.182*	0.007*	
$F''_{ST}$	TN	0.371*	0.184*	0.364*	0.387*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

\*  $p \leq 0.001$



**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)** Dis-

criminant 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 (*Sphyrna lewini*) (Daly-Engel et al. 2012; Duncan et al. 2006), Blue Sharks (*Prionace glauca*) (Verissimo 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

**Table 3** Pairwise genetic differentiation calculated between islands for angelsharks genotyped at 5,633 SNP loci. For each pair of islands, estimates of  $F_{ST}$  (Weir and Cockerham 1984),  $G_{ST}$  (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*

\*  $p \leq 0.001$

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

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.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10592-024-01655-1>.

**Acknowledgements** We thank the local government of the Canary Islands and the Ministry of Environment and Ecological Transition of Spain for the necessary fieldwork permits and their trust in the Angel

Shark Project. We are grateful to all the fantastic volunteers and collaborating dive centres in the Canary Islands that have supported us during fieldwork and collecting samples. Thanks to Ursula Bott for the technical support. We specially thank and dedicate this study to Toni Sanchez for helping set up this project and developing the restraining device used to capture and sample angelsharks underwater. May you rest in peace.

**Author contributions** EKM wrote the main manuscript text with extensive support from KAF and NF, and all authors contributed to critical revisions of the paper editing. EKM, KAF, DJA, JB, FT, JJC, DR, CB, IG designed the study, conceived the central idea of the paper and made substantial contributions to the interpretation of data and critical revision of the content. EKM, DJA, JB, MJS, HT, MBC, CP, TB, DMPJ, AGM, AME, FRO and LRM conducted fieldwork and collected samples from the Canary Islands. KAF and NF conducted laboratory work and statistical analyses with support from SM and JD. All authors reviewed the manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL. For this study was provided by Agence de l'eau Rhône-Méditerranée-Corse, CanBio, Deutsche Elasmobranchier Gesellschaft, Disney Conservation Fund, Oceanario de Lisboa, Office Français de la Biodiversité, Save our Seas Foundation and Shark Conservation Fund.

**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

- Acosta Yepes J, Uchupi E, Muñoz A, Herranz P, Palomo C, Ballesteros M, Group ZEE (2003) Geophysics of the Canary Islands. *Mar Geophys Res* 24:1–40. <https://doi.org/10.1007/s11001-004-1513-3>
- Allendorf FW (2022) Small populations and genetic drift. In: Allendorf FW, Funk WC, Aitken SN, Byrne M, Luikart G (eds) *Conservation and the genomics of populations*. Oxford University Press, Oxford, pp 113–132
- Barker AM, Nosal AP, Lewallen EA, Burton RS (2015) Genetic structure of leopard shark (*Triakis semifasciata*) populations along the Pacific coast of North America. *J Exp Mar Biol Ecol* 472:151–157. <https://doi.org/10.1016/j.jembe.2015.06.020>
- Barker J, Bartoli A, Clark M, Dulvy NK, Gordon C, Hood A, Alvarado DJ, Lawson J, Meyers E (2016) Angelshark Action Plan for the Canary Islands, 32pp edn. Zoological Society of London
- Barker J, Davies J, Goralczyk M, Patel S, O'Connor J, Evans J, Sharp R, Gollock M, Wood FR, Rosindell J, Bartlett C, Garner BJ, Jones D, Quigley D, Wray B (2022) The distribution, ecology and predicted habitat use of the critically endangered angelshark (*Squatina squatina*) in coastal waters of Wales and the central Irish Sea. *J Fish Biol* 101(3):640–658. <https://doi.org/10.1111/jfb.15133>
- Benavides MT, Horn RL, Feldheim KA, Shivji MS, Clarke SC, Wintner S, Natanson L, Braccini M, Boomer JJ, Gulak SJB, Chapman DD (2011) Global phylogeography of the dusky shark *Carcharhinus obscurus*: implications for fisheries management and monitoring the shark fin trade. *Endanger Species Res* 14:13–22. <https://doi.org/10.3354/esr00337>
- Bernard AM, Finnegan KA, Pavinski Bitar P, Stanhope MJ, Shivji MS (2021) Genomic assessment of global population structure in a highly migratory and habitat versatile apex predator, the tiger shark (*Galeocerdo cuvier*). *J Hered* 112(6):497–507. <https://doi.org/10.1093/jhered/esab046>
- Boussarie G, Momigliano P, Robbins WD, Bonnin L, Cornu JF, Fauvelot C, Kiszka JJ, Manel S, Mouillot D, Vigliola L (2022) Identifying barriers to gene flow and hierarchical conservation units from seascape genomics: a modelling framework applied to a marine predator. *Ecography* 7, e06158
- Brito A, Pascual PJ, Falcón JM, Sancho A, González G (2002) Peces De Las Islas Canarias: Catálogo comentado e ilustrado. Lémur, La Laguna (Tenerife)
- Castillo-Páez A, Sosa-Nishizaki O, Sandoval-Castillo J, Galván-Magaña F, Blanco-Parra M, Rocha-Olivares A (2014) Strong Population structure and shallow mitochondrial phylogeny in the Banded Guitartfish, *Zapteryx exasperata* (Jordan Y Gilbert, 1880), from the Northern Mexican Pacific. *J Hered* 105:91–100. <https://doi.org/10.1093/jhered/est067>
- Chapman DD, Simpfendorfer CA, Wiley TR, Poulakis GR, Curtis C, Tringali M, Carlson JK, Feldheim KA (2011) Genetic diversity despite population collapse in a critically endangered marine fish: the smalltooth sawfish (*Pristis pectinata*). *J Hered* 102:643–652. <https://doi.org/10.1093/jhered/esr098>
- Chapman DD, Feldheim KA, Papastamatiou YP, Hueter RE (2015) There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. *Ann Rev Mar Sci* 7:547–570. <https://doi.org/10.1146/annurev-marine-010814-015730>
- Corrigan S, Lowther AD, Beheregaray LB, Bruce BD, Cliff G, Duffy CA, Foulis A, Francis MP, Goldsworthy SD, Hyde JR, Jabado RW, Kacev D, Marshall L, Mucientes GR, Naylor GJP, Pepperell JG, Queiroz N, White WT, Wintner SP, Rogers PJ (2018) Population connectivity of the highly migratory shortfin mako (*Isurus oxyrinchus Rafinesque* 1810) and implications for management in the Southern Hemisphere. *Front Ecol Evol* 6:187
- Daly-Engel TS, Seraphin KD, Holland KN, Coffey JP, Nance HA, Toonen RJ, Bowen BW (2012) Global phylogeography with mixed-marker analysis reveals male-mediated dispersal in the Endangered Scalloped Hammerhead Shark (*Sphyrna lewini*). *PLoS ONE* 7:e29986. <https://doi.org/10.1371/journal.pone.0029986>
- Davenport R, Never S, Helmke P, Pérez-Moreno J, Llinás O (2002) Primary productivity in the northern Canary Islands region as inferred from Seawifs imagery. *Deep Sea Res Part II* 49:3481–3496. [https://doi.org/10.1016/S0967-0645\(02\)00095-4](https://doi.org/10.1016/S0967-0645(02)00095-4)
- Delaval A, Frost M, Bendall V, Hetherington SJ, Stirling D, Hoarau G, Jones CS, Noble LR (2022) Population and seascape genomics of a critically endangered benthic elasmobranch, the blue skate *Dipturus batis*. *Evol Appl* 15:78–94. <https://doi.org/10.1111/evo.13327>

- Domingues RR, Hilsdorf AWS, Gadig OBF (2018) The importance of considering genetic diversity in shark and ray conservation policies. *Conserv Genet* 19:501–525. <https://doi.org/10.1007/s10592-017-1038-3>
- Dulvy NK, Fowler S, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LN, Fordham SV, Francis MP, Pollock CM, Simpfendorfer CA, Burgess G, Carpenter KE, Compagno LJ, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT (2014) Extinction risk and conservation of the world's sharks and rays. *eLife* 3, e00590
- Dulvy NK, Pacoureau N, Rigby CL, Pollom RA, Jabado R, Ebert DA et al (2021) Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Curr Biol* 31:4773–4787. <https://doi.org/10.1016/j.cub.2021.08.062>
- Duncan KM, Martin AP, Bowen BW, De Couet HD (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Mol Ecol* 15:2239–2251. <https://doi.org/10.1111/j.1365-294X.2006.02933>
- Ellis JR, Barker J, Phillips SRM, Meyers EKM, Heupel M (2021) Angel sharks (Squatinae): a review of biological knowledge and exploitation. *J Fish Biol* 98:592–621. <https://doi.org/10.1111/jfb.14613>
- Espino F, Tuya F, del Rosario A, Bosch N, Coca J, González-Ramos AJ, del Rosario F, Otero-Ferrer FJ, Moreno AC, Haroun R (2019) Geographical range extension of the Spotfin burrfish, *Chilomycterus reticulatus* L. 1758, in the Canary Islands: response to ocean warming? *Diversity* 11:230. <https://doi.org/10.3390/d111120230>
- Espino-Ruano A, Castro JJ, Guerra-Marrero A, Couce-Montero L, Meyers EKM, Santana-del-Pino A, Jimenez-Alvarado D (2023) Aggregative Behaviour of Spiny Butterfly Rays (*Gymnura altavela* Linnaeus, 1758) in the shallow Coastal zones of Gran Canaria in the Eastern Central Atlantic. *Animals* 13:1455. <https://doi.org/10.3390/ani13091455>
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol Ecol* 14:2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. *Genetics* 164:1567–1587. <https://doi.org/10.1093/genetics/164.4.1567>
- Feldheim KA, Jabado RW, Chapman DD, Cardenaosa D, Maddox JD (2020) Microsatellite primer development in elasmobranchs using next generation sequencing of enriched libraries. *Mol Biol Rep* 47:2669–2675. <https://doi.org/10.1007/s11033-020-05357-y>
- Fernández-Palacios JM, Martín Esquivel JL (2001) Las islas como experimento de laboratorio. In: Fernández-Palacios JM, Martín Esquivel JL (eds) *Naturaleza de las Islas Canarias. Ecología y Conservación*. Publicaciones Turquesa, Santa Cruz de Tenerife, pp 39–44
- Feutry P, Berry O, Kyne PM, Pillans RD, Hillary RM, Grewe PM, Marthick JR, Johnson G, Gunasekera RM, Bax NJ, Bravington M (2017) Inferring contemporary and historical genetic connectivity from juveniles. *Mol Ecol* 26:444–456. <https://doi.org/10.1111/mec.13929>
- Flowers KI, Ajemian MJ, Bassos-Hull K, Feldheim KA, Hueter RE, Papastamatiou YP, Chapman DD (2016) A review of batoid philopatry, with implications for future research and population management. *Mar Ecol Prog Ser (MEPS)* 562:251–261. <https://doi.org/10.3354/meps11963>
- Frankham R (2010) Where are we in conservation genetics and where do we need to go? *Conserv Genet* 11:661–663. <https://doi.org/10.1007/s10592-009-0010-2>
- Frichot E, François O (2015) LEA: an R package for landscape and ecological association studies. *Methods Ecol Evol* 6:925–929. <https://doi.org/10.1111/2041-210X.12382>
- Fumagalli M (2013) Assessing the effect of sequencing depth and sample size in population genetics inferences. *PLoS ONE*, 8(11), e79667
- Gaida IH (1997) Population structure of the Pacific angel shark, *Squatina californica* (Squatinae: Squatinidae), around the California Channel Islands. *Copeia* 1997:738–744. <https://doi.org/10.2307/1447291>
- Gordon CA, Hood AR, Barker J, Bartoli A, Dulvy NK, Jiménez Alvarado D, Lawson JM, Meyers EKM (2017) *The Shark Trust*, UK.
- Gordon CA, Hood AR, Al Mabruk SAA, Barker J, Bartoli A, Ben Abdelhamid S, Bradai MN, Dulvy NK, Fortibuoni T, Giovos I, Jimenez Alvarado D, Meyers EKM, Morey G, Niedermuller S, Pauly A, Serena F, Vacchi M (2019) *Mediterranean Angel Sharks: Regional Action Plan*. The Shark Trust, United Kingdom
- Green ME, Appleyard SA, White WT, Tracey SR, Heupel MR, Oven- den JR (2022) Updated connectivity assessment for the scalloped hammerhead (*Sphyrna lewini*) in Pacific and Indian oceans using a multi-marker genetic approach. *Fisher Res* 251:106305. <https://doi.org/10.1016/j.fishres.2022.106305>
- Gruber B, Unmack PJ, Berry O, Georges A (2018) Dart: an r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Mol Ecol Resour* 18:691–699. <https://doi.org/10.1111/1755-0998.12745>
- Hedrick PW (1999) Perspective: highly variable genetic loci and their interpretation in evolution and conservation. *Evolution* 53:313–318. <https://doi.org/10.2307/2640768>
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59:1633–1638. <https://doi.org/10.1111/j.0014-3820.2005.tb01814.x>
- Hirschfeld M, Dudgeon C, Sheaves M, Barnett A (2021) Barriers in a sea of elasmobranchs: from fishing for populations to testing hypotheses in population genetics. *Glob Ecol Biogeogr* 30:2147–2163. <https://doi.org/10.1111/geb.13379>
- Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Mol Ecol Resour* 9:1322–1332
- Jiménez-Alvarado D, Meyers EKM, Caro MB, Sealey MJ, Barker J (2020) Investigation of juvenile angelshark (*Squatina squatina*) habitat in the Canary Islands with recommended measures for protection and management. *Aquat Conservation: Mar Freshw Ecosyst* 30:2019–2025. <https://doi.org/10.1002/aqc.3337>
- Jombart T (2008) Adegnet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24(11):1403–1405
- Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet* 11. <https://doi.org/10.1186/1471-2156-11-94>
- Jost L (2008) G(ST) and its relatives do not measure differentiation. *Mol Ecol* 17:4015–4026. <https://doi.org/10.1111/j.1365-294x.2008.03887.x>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kilian A, Wenzl P, Huttner E, Carling J, Xia L, Blois H, Caig V, Heller-Uszynska K, Jaccoud D, Hopper C, Aschenbrenner-Kilian M, Evers M, Peng K, Cayla C, Hok P, Uszynski G (2012) Diversity arrays technology: a generic genome profiling technology on open platforms. *Data production and analysis in*



- population genomics. *Methods Protocols* 888:67–89. [https://doi.org/10.1007/978-1-61779-870-2\\_5](https://doi.org/10.1007/978-1-61779-870-2_5)
- Kohler NE, Casey JG, Turner PA (1998) NMFS Cooperative Shark Tagging Program, 1962-93: an atlas of shark tag and recapture data. *Mar Fisher Rev* 60:1–87
- Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Mol Ecol Resour* 15:1179–1191. <https://doi.org/10.1111/1755-0998.12387>
- Kyne PM, Jabado RW, Rigby CL, Dharmadi, Gore MA, Pollock CM, Herman KB, Cheok J, Ebert DA, Simpfendorfer CA, Dulvy NK (2020) The thin edge of the wedge: extremely high extinction risk in wedgefishes and giant guitarfishes. *Aquat Conservation: Mar Freshw Ecosyst* 30:1337–1361. <https://doi.org/10.1101/595462>
- Lascelles B, Notarbartolo Di Sciara G, Agardy T, Cuttelod A, Eckert S, Glowka L, Hoyt E, Llewellyn F, Louzou M, Ridoux V, Tetley MJ (2014) Migratory marine species: their status, threats and conservation management needs. *Aquat Conservation: Mar Freshw Ecosyst* 24:111–127. <https://doi.org/10.1002/aqc.2512>
- Lawson JM, Pollom RA, Gordon CA, Barker J, Meyers EKM, Zidowitz H, Ellis JR, Bartoli Á, Morey G, Fowler SL, Alvarado DJ, Fordham SV, Sharp R, Hood AR, Dulvy NK (2020) Extinction risk and conservation of critically endangered angel sharks in the Eastern Atlantic and Mediterranean Sea. *ICES J Mar Sci* 77:12–29. <https://doi.org/10.1093/icesjms/fsz222>
- Lesturgie P, Lainé H, Suwalski A, Chifflet-Belle P, Delser PM, Clua E, Jaquemet S, Magalon H, Mona S (2022) Life history traits and biogeographic features shaped the complex evolutionary history of an iconic apex predator (*Galeocerdo cuvier*). *Res Square*. <https://doi.org/10.21203/rs.3.rs-1635778/v1>
- Lesturgie P, Braun CD, Clua E, Mourier J, Thorrold SR, Vignaud T, Planes S, Mona S (2023) Like a rolling stone: colonization and migration dynamics of the gray reef shark (*Carcharhinus amblyrhynchos*). *Ecol Evol* 13:e9746. <https://doi.org/10.1002/ece3.9746>
- Li YL, Liu JX (2018) Structure selector: a web based software to select and visualize the optimal number of clusters using multiple methods. *Mol Ecol Resour* 18:176–177. <https://doi.org/10.1111/1755-0998.12719>
- Li C, Olave M, Hou Y, Qin G, Schneider RF, Gao Z, Tu X, Wang X, Qi F, Nater A, Kautt AF, Wan S, Zhang Y, Liu Y, Zhang H, Zhang B, Zhang H, Qu M, Liu S, Chen Z, Zhong J, Zhang H, Meng L, Wang K, Yin J, Huang L, Venkatesh B, Meyer A, Lu X, Lin Q (2021) Genome sequences reveal global dispersal routes and suggest convergent genetic adaptations in seahorse evolution. *Nat Commun* 12:1094. <https://doi.org/10.1038/s41467-021-21379-x>
- López KER, Guadalupe JJ, Gordillo-Romero M, Montero-Oleas A, Pazmiño DA, Guerrero M, de Lourdes Torres M (2022) Exploring the genetic diversity and population structure of *Mobula birostris* in two key aggregation zones in the Eastern Tropical Pacific. *Mar Ecol Prog Ser (MEPS)* 699:75–89. <https://doi.org/10.3354/meps14171>
- Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Mol Ecol* 19:3038–3051. <https://doi.org/10.1111/j.1365-294X.2010.04688.x>
- Maddox JD, Feldheim KA (2014) A cost-effective size standard for fragment analysis that maximizes throughput on five dye set platforms. *Conserv Genet Resour* 6:5–7. <https://doi.org/10.1007/s12686-013-0019-1>
- Manuzzi A, Zane L, Muñoz-Merida A, Griffiths AM, Verissimo A (2019) Population genomics and phylogeography of a benthic coastal shark (*Scyliorhinus canicula*) using 2b-RAD single nucleotide polymorphisms. *Biol J Linn Soc* 126:289–303. <https://doi.org/10.1093/biolinnean/bly185>
- Mead LR, Alvarado DJ, Meyers E, Barker J, Sealey M, Caro MB, Toledo H, Pike C, Gollock M, Piper A, Schofield G, Herraiz E, Jacoby DMP (2023) Spatiotemporal distribution and sexual segregation in the critically endangered angelshark *Squatina squatina* in Spain's largest marine reserve. *Endanger Species Res* 51:233–248. <https://doi.org/10.3354/esr01255>
- Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution* 60:2399–2402
- Meirmans PG, Hedrick PW (2011) Measuring differentiation: G<sub>st</sub> and related statistics. *Mol Ecol Resour* 11:5–18. <https://doi.org/10.1111/j.1755-0998.2010.02927.x>
- Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>
- Meyers EKM, Tuya F, Barker J, Alvarado DJ, Castro-Hernández JJ, Haroun R, Rödder D (2017) Population structure, distribution and habitat use of the critically endangered angelshark, *Squatina squatina*, in the Canary Islands. *Aquat Conservation: Mar Freshw Ecosyst* 27:1133–1144. <https://doi.org/10.1002/aqc.2769>
- Mijangos JL, Gruber B, Berry O, Pacioni C, Georges A (2022) dartR v2: an accessible genetic analysis platform for conservation, ecology and agriculture. *Methods Ecol Evol* 13:2150–2158. <https://doi.org/10.1111/2041-210X.13918>
- Morey G, Barker J, Hood A, Gordon C, Bartoli A, Meyers EKM, Ellis J, Sharp R, Jiménez D, Pollom RA (2019) *Squatina squatina*. The IUCN Red List of Threatened Species 2019: e.T39332A117498371. <https://doi.org/10.2305/IUCN.UK.2019-1.RLTS.T39332A117498371.en>
- Nikolic N, Devloo-Delva F, Bailleul D, Noskova E, Rougeux C, Delord C, Borsa P, Liatard-Haag C, Hassan M, Marie AD, Feutry P, Grewe P, Davies C, Farley J, Fernando D, Biton-Porsmoguer S, Poisson F, Parker D, Leone A, Aulich J, Lansdell M, Marsac F, Arnaud-Haond S (2023) Stepping up to genome scan allows stock differentiation in the worldwide distributed blue shark *Priocnace glauca*. *Mol Ecol* 32:1000–1019. <https://doi.org/10.1111/mec.16822>
- Noviello N, McGonigle C, Jacoby DMP, Meyers EKM, Jiménez-Alvarado D, Barker J (2021) Modelling critically endangered marine species: Bias-corrected citizen science data inform habitat suitability for the angelshark (*SQUATINA SQUATINA*). *Aquat Conservation: Mar Freshw Ecosyst* 31:3451–3465. <https://doi.org/10.1002/aqc.3711>
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research— an update. *Bioinform Application Note* 28:2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Pearce J, Fraser MW, Sequeira AM, Kaur P (2021) State of shark and ray genomics in an era of extinction. *Front Mar Sci* 8:744986. <https://doi.org/10.3389/fmars.2021.744986>
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959. <https://doi.org/10.1093/genetics/155.2.945>
- Puechmaille SJ (2016) The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Mol Ecol Resour* 16:608–627. <https://doi.org/10.1111/1755-0998.12512>
- R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered*



- 86:248–249. <https://doi.org/10.1093/oxfordjournals.jhered.a111573>
- Reed DH (2004) Extinction risk in fragmented habitats. *Anim Conserv* 7:181–191. <https://doi.org/10.1017/S1367>
- Rousset F (2008) Genepop '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour* 8:103–106. <https://doi.org/10.1111/j.1471-8286.2007.01931.x>
- Sansaloni C, Petroli C, Jaccoud D, Carling J, Detering F, Grattapaglia D, Kilian A (2011) Diversity arrays technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus. *BMC Proc* 5(Suppl 7):P54. <https://doi.org/10.1186/1753-6561-5-S7-P54>. (2011)
- Schmidt JV, Schmidt CL, Ozer F, Ernst RE, Feldheim KA, Ashley MV, Levine M (2009) Low genetic differentiation across three Major Ocean populations of the Whale Shark, *Rhincodon typus*. *PLoS ONE* 4:e4988. <https://doi.org/10.1371/journal.pone.0004988>
- Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nat Biotechnol* 18:233–234. <https://doi.org/10.1038/72708>
- Tuya F, Boyra A, Sánchez-Jerez P, Haroun RJ, Barberá C (2004) Relationships between rocky-reef fish assemblages, the sea urchin *Diadema antillarum* and macroalgae throughout the Canarian Archipelago. *Mar Ecol Prog Ser* 278:157–169
- Tuya F, Aguilar R, Espino F, Bosch NE, Meyers EK, Jiménez-Alvarado D, Castro JJ, Otero-Ferrer F, Haroun R (2021) Differences in the occurrence and abundance of batoids across an oceanic archipelago using complementary data sources: implications for conservation. *Ecol Evol* 11:16704–16715. <https://doi.org/10.1002/ece3.8290>
- Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3—new capabilities and interfaces. *Nucleic Acids Res* 40:e115. <https://doi.org/10.1093/nar/gks596>
- Venables SK, Marshall AD, Armstrong AJ, Tomkins JL, Kennington WJ (2021) Genome-wide SNPs detect no evidence of genetic population structure for reef manta rays (*Mobula alfredi*) in southern Mozambique. *Heredity* 126:308–319. <https://doi.org/10.1038/s41437-020-00373-x>
- Veríssimo A, Sampaio Í, McDowell JR, Alexandrino P, Mucientes G, Queiroz N, da Silva C, Jones CS, Noble LR (2017) World without borders-genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). *Ecol Evol* 7:4768–4781. <https://doi.org/10.1002/ece3.2987>
- Walsh CA, Momigliano P, Boussarie G, Robbins WD, Bonnin L, Fauvelot C, Kiszka JJ, Mouillot D, Vigliola L, Manel S (2022) Genomic insights into the historical and contemporary demographics of the grey reef shark. *Heredity* 128:225–235. <https://doi.org/10.1038/s41437-022-00514-4>
- Wang J (2017) The computer program STRUCTURE for assigning individuals to populations: easy to use but easier to misuse. *Ecol Resour* 17:981–990. <https://doi.org/10.1111/1755-0998.12650>
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370. <https://doi.org/10.2307/2408641>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.