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PRIMARY RESEARCH PAPER

Regional philopatry of scalloped hammerhead sharks (*Sphyrna lewini*) to nursery areas in the Mexican Pacific

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Abstract The population genetic structure and female philopatry to nursery grounds of the scalloped hammerhead shark (*Sphyrna lewini*) were studied in different mangrove estuaries along the Mexican Pacific coast containing putative nurseries. These nurseries were grouped into northern (Sinaloa-Nayarit), central (Jalisco), and southern (Oaxaca-Chiapas) regions. Neonates and young of the year were collected near estuaries or river inlets, and their genetic variation was compared based on mitochondrial DNA (mtDNA) genome sequences and 11 nuclear microsatellite loci. The mtDNA analysis showed significant differences between the abovementioned regions, accompanied by genetic homogeneity of microsatellites. Based on the genetic divergence of mtDNA and the lack of differences in nuclear markers, our results are congruent with female philopatry to nursery areas, as observed in other shark species. The parentage analysis applied to the microsatellite data showed moderate levels of relatedness among individuals within nurseries, suggesting philopatry as a cause of the observed results. The pattern of nursery grounds of the scalloped hammerhead shark in the Mexican Pacific seems to be regional, as no differences were observed between

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neighboring estuaries within each studied region. These findings are relevant for delineating conservation plans to preserve key populations and minimize the effects of commercial fisheries.

Keywords Scalloped hammerhead · Site fidelity · Mitochondrial genome · Gene diversity

Introduction

Marine resources are being highly impacted by fisheries, with annual catches worldwide showing a decrease in recent decades (Watson et al., 2013). An increasing number of exploited species have started to exhibit signals of severe reductions in abundance, while other species are showing an increased extinction risk (McClenachan et al., 2012). Sharks are of special interest because of their inability to overcome the effects of commercial catches throughout their ranges, in contrast to teleost fishes (Dulvy et al., 2008; Pacoureau et al., 2021).

At present, sharks are globally threatened due to overexploitation, habitat depletion, and pollution; for example, approximately 30% of chondrichthyans are estimated to be threatened with extinction (Consales & Marsili 2021), with almost 16% of shark species being categorized as 'Threatened' and approximately 20% of shark species categorized as 'Data Deficient' by the International Union for Conservation of Nature (IUCN; Dulvy et al., 2014). Therefore, studies providing information on the area-focused protection of species have become essential for increasing the effectiveness of conservation efforts (Davidson & Dulvy, 2017).

Sharks have a profound impact on ecosystem functioning and stability (Ferretti et al., 2010; Hammerschlag et al., 2019). As top predators, sharks connect habitats and ecosystems by transferring energy through food webs, potentially shaping marine communities over large spatial scales (Ferretti et al., 2010; Heupel et al., 2015) and affecting fundamental aspects of ecosystem functioning (Heupel et al., 2015; Roff et al., 2016; Dulvy et al., 2014). Therefore, a better understanding of their habitat requirements, population status, and distribution is critical for their conservation and management.

The identification of biological units is crucial for the conservation and management of exploited resources. This goal involves genetic studies for the identification of discrete populations across the species range, including populations showing genetic differences resulting from the repeated use of specific habitats to improve the survival of their progeny (philopatry). Philopatry was initially defined by Mayr (1963) as the affinity or propensity of individuals to return to or remain in the area where they were born. In a recent review by Chapman et al. (2015), the authors defined philopatry as the selective return of gravid females to specific birth sites or regions, resulting in the multigenerational use of such sites by populations with their own internal dynamics.

Previous genetic studies have assessed philopatry in sharks using mitochondrial and nuclear DNA genetic markers in samples from different nurseries (Dudgeon et al., 2012; Portnoy & Heist, 2012) and focused on sampling juvenile individuals (Feutry et al., 2016; Sandoval-Laurrabquio et al., 2019). Knowledge of philopatric behavior is fundamental for the conservation of threatened and endangered species (Secor, 2002).

The scalloped hammerhead shark, *Sphyrna lewini* (Griffith & Smith, 1834), is a species distributed globally in tropical and subtropical waters. In the Eastern Pacific, it is distributed from southern California USA to Puerto Cabuyal Ecuador. It is a coastal and semioceanic pelagic shark that is found over continental and insular shelves (Compagno et al., 2005). Globally, the scalloped hammerhead is heavily exploited, which has resulted in major declines in most areas of the species' range due to its low resilience to exploitation as a consequence of its life-history features (Maguire et al., 2006). Because of this, the species has recently been cataloged as Critically Endangered (Rigby et al., 2019). It is one of the most commonly caught hammerhead sharks in Mexico and Central and South America (Anislado-Tolentino & Robinson-Mendoza, 2001; Arauz et al., 2004; Martínez-Ortíz et al., 2015). In addition, neonate and juvenile sharks are caught throughout the Mexican Pacific and, in many cases, account for more than 90% of the total catch (in number) of the species (Torres-Huerta et al., 2008; Furlong-Estrada et al., 2015). The identification of critical habitats, such as nurseries that are used during early stages of life, is crucial for the conservation and management of shark populations. There is an increasing number of reports of the repeated use of estuaries by females of different shark species (Chapman et al., 2015), including the scalloped hammerhead shark (Duncan & Holland, 2006; Marie et al., 2017). Philopatric behavior usually results in genetic divergence between

neighboring estuaries due to the nonrandom segregation of maternal lineages among them. Genetic studies in combination with telemetry data contribute greatly to assessing philopatric behavior and provide essential information for conservation purposes.

Potential nurseries have been identified for this species in the Mexican Pacific based on reports of the abundance of neonates and pregnant females: in the Gulf of California, or the northern region (San Francisquito, Salomón-Aguilar et al., 2009; Bahía Santa Martha, Coiraton et al., 2020); the central region (Jalisco, Corgos & Rosende-Pereiro, 2021); and the Gulf of Tehuantepec, or the southern region (Oaxaca, Alejo-Plata et al., 2007; Bejarano-Álvarez et al., 2011). These three regions are characterized by marked differences in oceanographic conditions and are also considered biogeographic provinces and marine ecoregions (Wilkinson et al., 2009). Genetic studies on the scalloped hammerhead shark in the eastern Pacific have focused mainly on testing population genetic structure as a result of limited dispersal and have revealed evidence of the presence of genetically discrete populations at the regional (Duncan et al., 2006; Nance et al., 2011) or local scale (Castillo-Olguín et al., 2012). Several telemetric studies have shown the extent of fidelity to specific areas during early life stages (Kohler & Turner, 2001; Duncan & Holland, 2006; Bessudo et al., 2011; Diemer et al., 2011; Hoyos-Padilla et al., 2014; Ketchum et al., 2014a); however, the philopatry hypothesis has not been fully tested until now.

Here, we used whole-mtDNA-genome sequences of the scalloped hammerhead shark in combination with nuclear microsatellite markers to assess natal philopatry, as successfully applied in other shark species (Pardini et al., 2001; Portnoy et al., 2010; Karl et al., 2011; Sandoval-Laurrabquio et al., 2019). We used samples of neonates and young of the year (YOY) of the scalloped hammerhead shark taken from putative nursery grounds to recover the genetic signal generated by the segregation of haplotypes in different nurseries along the Mexican Pacific.

Materials and methods

Sample collection and DNA extraction

A total of 335 tissue samples of neonates and YOY individuals of scalloped hammerheads were collected at eight fishing grounds representing potential nurseries within three main regions of the Mexican Pacific from 2013 to 2016 (Fig. 1). Four locations were sampled in the northern region (La Reforma $n=29$; Chametla $n=29$; Teacapan $n=63$, and Boca de Camichín $n=22$), two in the central region (Bahía de Navidad $n=58$ and Rebalsito $n=20$), and two in the southern region (Salina Cruz $n=54$ and Puerto Madero $n=60$). The sex of each specimen was recorded when possible, as was the total length (TL), measured from the tip of the snout to the posterior end of the dorsal caudal lobe in a natural position. When specimens were incomplete, TL was estimated by measuring the interdorsal length (sensu Gallegos-Camacho & Tovar-Ávila, 2011). Individuals were considered (i) neonates when an unhealed umbilical scar could be observed or they showed a TL of less than 65 cm and (ii) YOY when they showed a TL of 65–75 cm (based on Alejo-Plata et al., 2007). Tissue samples were preserved in 70% ethanol, and DNA extraction was performed using the Wizard ® Genomic DNA Purification Kit from PROMEGA.

PCR amplification and sequencing

For mtDNA analysis, DNA was sheared by sonication with Bioruptor®, followed by library preparation using the KAPA BIOSYSTEMS® Hyper Prep Kit (KK8504) protocol with slight modifications. In brief, fragmented DNA was ligated to Illumina universal TruSeq adapters containing eight custom nucleotide indices (Glenn et al., 2019), and fragments in the size range of ~ 250–450 bases were selected. Sized fragments were enriched through PCR, purified, and normalized. Libraries for sequencing were prepared using the Illumina NextSeq v2 300 cycle kit to produce paired-end 150 nucleotide reads at the Georgia Genomics Facility of the University of Georgia in Athens (UGA). The resultant reads were quality filtered, trimmed, assembled, and annotated in Geneious® 8.1.7 using the mtDNA genome of the scalloped hammerhead shark as a reference (GenBank accession number JX827259). Only sequences with more than 97% high-quality reads were aligned using the Muscle application implemented in Geneious to identify variable sites in the mtDNA genome sequences.

Fifteen microsatellite loci developed for scalloped hammerhead sharks by Nance et al. (2009)

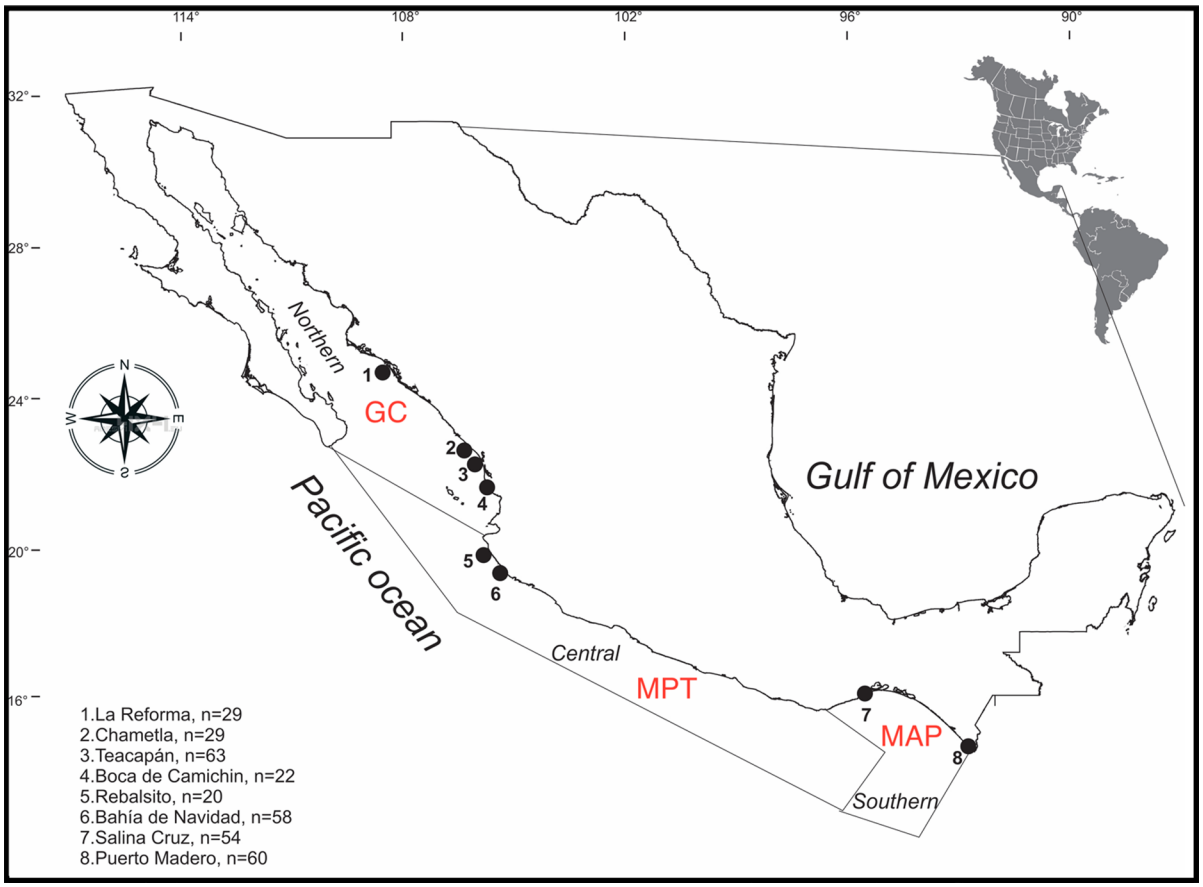

Fig. 1 Sampling locations for scalloped hammerhead collection. Northern region: La Reforma (RE), Chametla (CH), Teacapan (TEC), and Boca de Camichín (NAY). Central region: Bahía de Navidad (BN) and El Rebalsito (REB). Southern region: Salina Cruz (SC) and Puerto Madero (PM). The ecoregions according to Wilkinson et al. (2009) are the Gulf of California (GC), Mexican Pacific Transition (MPT), and Middle American Pacific (MAP)

were amplified and scored. All loci were fluorescently tagged (6-FAM, VIC, NED or PET; APPLIED BIOSYSTEMS®). PCR for fragment amplification was performed in a 5 µl final volume using a Type it-microsatellite kit from QIAGEN containing the following components: 2.5 µl of Type-it Master Mix, 1.5 µl of 0.4 µM primer mix (Forward and Reverse), and ~ 5–20 ng of DNA (~ 1 µl). The PCR profile consisted of 5 min at 95°C, followed by 28 cycles of 30 s at 95°C, 90 s at 60°C, and 30 s at 72°C, with a final extension of 30 min at 60°C. The PCR products for microsatellite loci were sized with the GENESCANTM 500LIZ™ standard. Capillary electrophoresis was performed with an automatic 24 capillary array sequencer 3500 xl (LIFE TECHNOLOGIES TM®). For visualization and scoring, GENEMAPPER v. 3.7 (APPLIED BIOSYSTEMS) software was used.

Data analyses

For the mtDNA genome sequences, nucleotide (π) and haplotypic (H) diversities were estimated, and the number of haplotypes (h) and polymorphic sites (S) per population/region were calculated using DNASP 5.2 (Librado & Rozas, 2009). To evaluate the monophyly of scalloped hammerhead haplotypes, sequences of the 191 mtDNA genomes were used to construct a phylogeny based on a Bayesian approach, using a mtDNA genome sequence of *S. lewini* from the Gulf of Mexico as external group. For phylogenetic reconstruction, the substitution

model was determined using JModelTest2 (Darriba et al., 2012), and the Generalized Time Reversible" (GTR) + gamma (G) + invariable sites (I) model was selected. The phylogenetic analysis was performed in MrBayes v3.2 (Ronquist et al., 2012) via two independent runs with 10 million generations each and sampling every 100 generations, with 25% of the data discarded as burn-in. The convergence of the two runs was assessed on TRACER v1.6 (Rambaut et al., 2018). Finally, a haplotype network of the mtDNA sequences was reconstructed using the program PopART (Leigh & Bryant, 2015) to visualize the relationships among haplotypes relative to their geographic origin.

All microsatellite loci were tested for the presence of null alleles, miss-genotyping and stuttering using Micro-Checker (Van Oosterhout et al., 2004). Deviations from Hardy–Weinberg equilibrium (H-W) and its probability (No. of Markov chain steps: 1,000,000 and No. of dememorization steps: 100,000) and linkage disequilibrium tests (No. of permutations: 1000 and No. of initial conditions for EM: 2) were assessed with ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010). This software was also used to estimate genetic diversity, measured as expected and observed heterozygosities (H_E and $H_{O,}$ respectively), and the number of alleles (*Na*).

A parentage analysis within and between localities was performed using COLONY software (v. 2.0.5.8) (Jones & Wang, 2010) to assess the effect of the relatedness of individuals due to the 'Allendorf-Phelps' effect (Allendorf & Phelps, 1981), which is associated with a potential excess of sibs because of the sampling of immature individuals of scalloped hammerhead sharks in nurseries. The parentage relationships among individuals of both full-siblings (FS) and half-siblings (HS) were determined based on the maximum likelihood value. Runs were set up under female and male polygamy with inbreeding and a medium run length using full likelihood and no prior sibship. Comparisons of individuals showing a probability above 0.95 for FS and HS were considered true siblings, and one of the related individuals in these comparisons was removed for population differentiation analyses according to both mtDNA and microsatellite data. To preserve the maximum number of individuals for subsequent analyses, individuals showing sibling relationships that were sampled in different years were retained. When individuals were involved in multiple paired comparisons, they were unequivocally removed.

For mtDNA genome sequences, ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010) was used to obtain the molecular analogs of the unbiased Wright's F-statistics (Φ-statistics). For microsatellite data, ARLEQUIN was used to calculate F_{ST} and R_{ST} statistics. The statistical significance of the estimates was assessed with 50,000 permutations. For R_{ST} estimation, the smallest allele was considered one repetition. The significance level for multiple testing was adjusted using the Benjamini & Yekutieli (2001) correction (B-Y), as proposed by Narum (2006), by dividing the critical value of α by the sum of the number of tests according to the following formula:

$$\alpha / \sum_{i=1}^{k} \left(\frac{1}{i} \right)$$

AMOVA was also used to test panmixia among samples grouped into the following regions: northern (La Reforma, Teacapan, Chametla, and Boca de Camichín), central (Rebalsito and Bahía de Navidad), and southern (Salina Cruz and Puerto Madero), with differentiation assessed both among (F_{CT}) and within (F_{SC}) regions. These regions are based on the marine ecoregions reported by Wilkinson et al. (2009). The northern region corresponds to the Gulf of California (GC) ecoregion, whereas the central and southern regions correspond to the Mexican Pacific Transition (MPT) and the Middle American Pacific (MAP), respectively (Fig. 1). Ecoregions have been determined based on differences in the oceanography and the extent of the continental shelf and according to the existence/abundance of coastal lagoons, which represent suitable shark nursery habitats.

A discriminant analysis of principal components (DAPC) was performed to identify and describe clusters of genetically related individuals without prior knowledge (*k*-means) based on the lowest Bayesian information criterion (BIC) score using the *dapc* function implemented in the R package *adegenet* v. 2.1.1 (Jombart & Ahmed, 2011). To calculate the number of informative PCs and accurately estimate membership probability, the associated *alpha* score was also computed. The number of clusters defined by the alpha score was used to plot the DAPC and membership probabilities using the *compoplot* function from the same R package. The *find.clusters*

function using K = 5 was also employed to identify the number of genetic clusters based on the lowest Bayesian information criterion score (BIC).

The effective population size (N_e) was estimated using NeEstimator (Do et al., 2014) under the linkage disequilibrium method for microsatellite data. The settings considered a critical allele frequency value of $P_{CRIT} = 0.05$. The upper and lower bounds of the 95% confidence intervals (CI) were obtained for estimates of N_e using the jackknife procedure to reduce the bias associated with the estimation of CI when using linkage disequilibrium (Waples & Do, 2010). Gene flow estimates were obtained for the microsatellite dataset using BayesAss version 3.0.4 (Wilson & Rannala, 2003). Five independent runs were performed using 2 million iterations with burn-in every 5 million chains and sampling every 1000 steps. The adjustment of mixing parameters was set as follows: $a = 0.3$, $m = 0.3$, and $f = 0.5$ (for allele frequencies, migration rates, and inbreeding coefficients, respectively).

Results

Parentage analysis

The analysis performed with COLONY resulted in a low number of fully related individuals; 1,116 analyzed pairs included 7 (0.62%) full-siblings. However, a moderate number of half-siblings (224; 20%) were also observed (Supplementary Table S1). FS were found in Bahía de Navidad (4), Teacapan (2), and Salina Cruz (1), whereas HS were found mainly in locations in the southern region, in Salina Cruz (69) and Puerto Madero (51), and in Bahía de Navidad (44) in the central region and Teacapan in the northern region (33), whereas all other locations showed low numbers of HS pairs (0–12). Considering the criteria for the elimination of related individuals, 39 individuals were removed from the analysis of mtDNA and 61 from the analysis of microsatellite data.

Gene diversity

The analysis of the complete mtDNA genome resulted in a total of 16,729 base pairs (bp) and a dataset of 191 YOY individuals after excluding FS and HS individuals. The sequences of the mtDNA genome contained 106 polymorphic sites (S), including 88 transitions and 15 transversions, which resulted in 72 haplotypes (*nh*) among sampled individuals (Table 1). The mean haplotype diversity was high ($h = 0.929 \pm 0.012$), whereas nucleotide diversity was low ($\pi = 0.00021 \pm 0.00012$). Genetic diversity was similar across locations, although the lowest estimates were observed for Rebalsito (S = 13; $nh = 7$; $h = 0.863 \pm 0.079$; $\pi = 0.00017 \pm 0.00011$), while Salina Cruz exhibited the highest genetic diversity (S = 45; $nh = 18$; $h = 0.969 \pm 0.018$; $\pi = 0.00050 \pm 0.00027$). Moreover, the highest gene diversity was observed in the southern region (S = 56; $nh = 44$; $h = 0.964 \pm 0.015$; $\pi = 0.00037 \pm 0.00019$), followed by the northern (S = 48; $nh = 39$; $h = 0.924 \pm 0.017$; $\pi = 0.00016 \pm 0.0009$) and central regions (S = 26; $nh = 22$; $h = 0.888 \pm 0.035$;

Table 1 Summary statistics for the mtDNA genome of *Sphyrna lewini* in the Mexican Pacific

Location	*N*	*S*	nh	*h*	π	*k*
Northern	98	48	39	0.924 ± 0.017	0.00016 ± 0.0009	23.5
1. La Reforma	26	18	13	0.926 ± 0.028	0.00014 ± 0.00009	9.7
2. Chametla	20	20	13	0.910 ± 0.053	0.00018 ± 0.00011	15.0
3. Teacapan	36	16	19	0.925 ± 0. 027	0.00015 ± 0.00009	15.6
4. Boca de Camichín	16	15	11	0.941 ± 0.046	0.00017 ± 0.00011	14.3
Central	45	26	22	0.888 ± 0.035	0.00015 ± 0.00009	16.4
5. Rebalsito	12	13	7	0.863 ± 0.079	0.00017 ± 0.00011	6.2
6. Bahía de Navidad	33	21	17	0.910 ± 0.037	0.00015 ± 0.00090	13.4
Southern	48	60	31	0.964 ± 0.015	0.00037 ± 0.00019	36.8
7. Salina Cruz	21	45	18	0.969 ± 0.018	0.00050 ± 0.00027	24.6
8. Puerto Madero	27	49	19	0.960 ± 0.023	0.00027 ± 0.00015	27.2
Overall	191	106	72	0.929 ± 0.012	0.00021 ± 0.00012	41.6

N sample size, *S* polymorphic sites, *nh* number of haplotypes, *h* haplotypic diversity, π nucleotide diversity, *k* mean number of differences

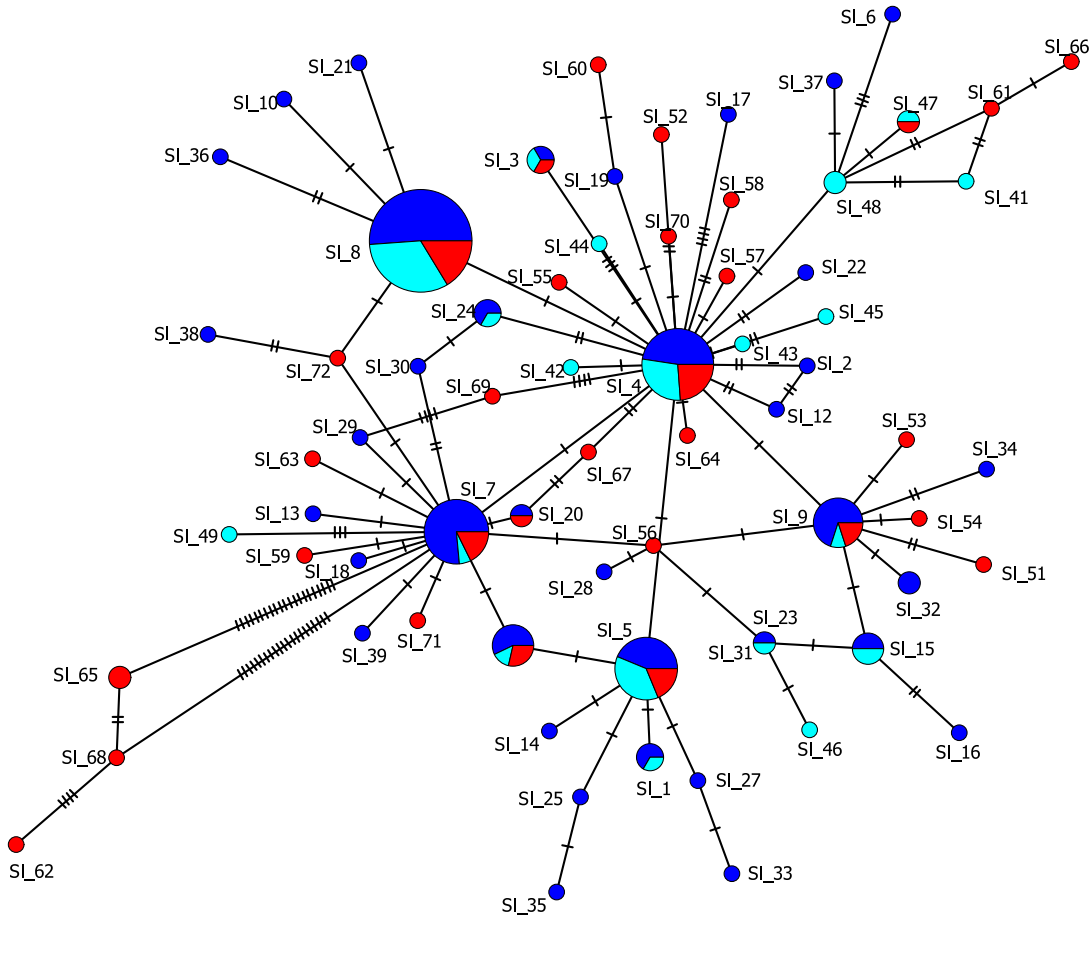

Fig. 2 Minimum spanning network showing the relationships between haplotypes in terms of the numbers of differences and their proportions in each region. Each analyzed region is shown in a different color (box on the right), and the numbers inside the circles represent the frequency of haplotypes

$\pi = 0.00015 \pm 0.00009$; Table 1). Gene diversity estimates obtained by using the full dataset showed minor changes relative to estimates obtained when related individuals were excluded.

Phylogenetic analysis confirmed the monophyly of scalloped hammerhead haplotypes, with the exception of three divergent haplotypes (*Sl-62*, *Sl-65,* and *Sl-68*) that were separated from the rest of the haplotypes by 22–27 nucleotide substitutions (Supplementary Fig. S1; Fig. 2). The minimum spanning network showed no specific arrangement of haplotypes related to the geographical location of the samples; instead, the haplotypes were uniformly distributed among regions, showing strong evidence of random segregation of haplotypes (Fig. 2). However, there were five abundant haplotypes separated by one or two mutations: haplotype *Sl-8* was the most abundant (21.99%), followed by haplotypes *Sl-5* (8.37%), *Sl-4* (7.85%), *Sl-7* (7.85%), and *Sl-9* (5.23%). Although these five haplotypes contained almost equal frequencies of individuals from the three regions, haplotype *Sl-7* showed a low frequency of individuals from the central region while showing a predominance of haplotypes from the northern region. The rest of the haplotypes were mostly unique < 3 (60), came predominantly from the northern (45.0%) and southern (36.7%) regions, and were randomly distributed within the network,

except for haplotype *Sl-7*, which presented a low frequency of individuals from the central region.

Microchecker analysis of the nuclear microsatellite data indicated a lack of errors in the scoring of alleles caused by stuttering or large allele drop out. Nevertheless, four loci (*Sl-18*, *Sl-25*, *Sl-71,* and *Sl-77*) presented null alleles at distinct localities due to an excess of homozygotes. As a result, these loci showed H-W deviations even after applying the BY correction ($P<0.01$). Similarly, eight out of 120 paired locus comparisons showed linkage disequilibrium involving loci *Sl-18*, *Sl-25*, *Sl-71*, and *Sl-77* (Supplementary Table S2). Hence, these loci were eliminated from the analysis. The remaining 11 microsatellite loci exhibited an average of 8.08 alleles (range: 6.182–9.909 for La Reforma and Teacapan, respectively). The overall observed heterozygosity was 0.704 ± 0.13 (range: 0.666–0.741), whereas the expected heterozygosity was 0.701 ± 0.012 (range 0.679–0.715; Table 2). As in the case of mtDNA, the gene diversity was similar when related individuals were excluded, but a small increase in observed heterozygosity was registered.

Population genetic divergence

Thirty-six individuals showing recurrent full-sibling (FS) and half-sibling (HS) relationships were excluded from the mtDNA dataset ($n=191$). Pairwise sample comparisons of Φ_{ST} between locations showed significant differences in three comparisons involving the Salina Cruz location from the southern region (Table 3); two comparisons involving locations from the northern region (La Reforma and Teacapan); and one comparison involving a location from the central region (Bahía de Navidad; Table 3). However, among these comparisons, only Salina Cruz *vs.* Teacapan and Salina Cruz *vs.* Bahía de Navidad remained significant after correcting for multiple testing ($k=28$ tests; $\Sigma 1/k=3.891$; initial $\alpha=0.05/3.891=0.0128$). Hierarchical AMOVA resulted in significant differences ($\Phi_{ST}=0.013$; $P=0.024$; Table 4) when the locations were grouped into the northern (La Reforma, Teacapan, Chametla, and Boca de Camichín), central (Rebalsito and Bahía de Navidad), and southern (Salina Cruz and Puerto Madero) regions.

Pairwise-sample $F_{ST}$and R_{ST} estimates resulted in 8 significant comparisons out of 28 total comparisons for microsatellite data (Table 3) after excluding FS and HS individuals ($n=243$). Only two comparisons of F_{ST} (Chametla vs. Teacapan and Teacapan vs. Bahía de Navidad) and three comparisons of R_{ST} (La Reforma vs. Rebalsito and Salina Cruz vs. Teacapan and Bahia de Banderas) remained significant after correcting for multiple testing. The AMOVA performed to compare the three regions resulted in non-significant differences for both F_{ST} ($F_{CT}=0.00005$; $P=0.446$) and R_{ST} ($F_{CT}=0.012$; $P=0.122$; Table 4).

The DAPC of the microsatellite dataset identified $K=5$ as the best number of genetic clusters, with no a priori information, based on the BIC score. Nevertheless, although the clusters showed separated groups in the DAPC based on the *alpha* score (Fig. 3A), the membership probability graphs failed to assign individuals to well-defined groups (Fig. 3B). The number of individuals from each original group (locations of

Table 2 Summary statistics for the eleven Microsatellite loci used in this study averaged across locations and regions

Location	*N*	Na	Ho	He
Northern	103	11.455 ± 1.391	0.716 ± 0.037	0.721 ± 0.034
1. La Reforma	14	6.182 ± 0.553	0.682 ± 0.048	0.685 0.037
2. Chametla	21	7.182 ± 0.630	0.737 ± 0.035	0.707 ± 0.035
3. Teacapan	50	9.636 ± 0.975	0.715 ± 0.045	0.718 ± 0.035
4. Boca de Camichin	18	7.091 ± 0.610	0.719 ± 0.049	0.697 ± 0.035
Central	60	9.545 ± 1.098	0.714 ± 0.029	0.714 ± 0.031
5. Rebalsito	15	6.636 ± 0.778	0.707 ± 0.036	0.683 ± 0.039
6. Bahía de Navidad	45	8.273 ± 0.776	0.715 ± 0.028	0.712 ± 0.029
Southern	78	10.455 ± 1.224	0.700 ± 0.044	0.718 ± 0.040
7. Salina Cruz	36	7.909 ± 0.958	0.684 ± 0.040	0.703 ± 0.041
8. Puerto Madero	42	9.000 ± 0.709	0.715 ± 0.050	0.723 ± 0.039
Overall	241	10.485 ± 0.709	0.710 ± 0.021	0.717 ± 0.020

N number of samples per region, *Na* number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity and ± standard deviation

Table 3 Pairwise-sample Φ_{ST} estimates for mtDNA sequences (below diagonal)

	RE	CH	TEC	BC	REB	BN	SC	PM
RE	–	0.015* − 0.001	0.002 0.001	0.006 0.039*	0.016* 0.062	0.012* − 0.002	0.006 0.091*	0.009 0.044**
CH	− 0.002	–	0.013** − 0.011	0.001 0.002	0.011 0.008	0.010* − 0.006	0.006 0.034	0.009* 0.003
TEC	0.013	0.015	–	− 0.002 0.008	0.0005 0.013	0.009** − 0.003	− 0.0002 0.053**	0.002 0.007
BC	0.004	− 0.011	0.011	–	− 0.001 − 0.023	− 0.001 0.013	− 0.004 0.049*	− 0.005 0.008
REB	− 0.014	− 0.010	0.010	− 0.009	–	0.011* 0.026*	0.001 0.033	0.010 − 0.001
BN	− 0.012	− 0.006	0.010	0.012	− 0.028	–	0.003 0.061**	0.005 0.018*
SC	0.062*	0.046	0.081**	0.035	0.038	0.078**	–	− 0.002 0.014
PM	− 0.002	0.0008	0.004	0.005	− 0.00004	0.008	0.013	–

Values above diagonal are F_{ST} (upper value) and R_{ST} (lower value) for the 11 microsatellite loci excluding related individuals (FH and HS)

*$P<0.05$ and **$P<0.0128$ after adjusting for multiple testing ($\alpha/\sum_{i=1}^{k}\left(\frac{1}{i}\right)$; for 28 comparisons = 3.927; initial $\alpha = 0.05/3.927 = 0.0128$). 1. La Reforma (RE), 2. Chametla (CH), 3. Teacapán (TEC), 4. Boca de Camichin (BC), 5. Bahía de Navidad (BN), 6. Rebalsito (REB), 7. Salina Cruz (SC), and 8. Puerto Madero (PM)

Table 4 Hierarchical AMOVA analysis for mtDNA and 11 microsatellite loci excluding related individuals (FS and HS) with locations pooled into regions; Northern (La Reforma, Teacapán, Chametla and Boca de Camichín), Central (Bahía de Navidad and Rebalsito), and Southern (Salina Cruz and Puerto Madero)

	Genetic marker	Variance (%)	*F*-statistics	*P*	R_{ST}	*P*
Among groups (Northern–Central–Southern)	mtDNA	2.42	0.024	**0.024**		
	Micros	0.00	0.00005	0.446	0.012	0.122
Among locations within groups	mtDNA	0.28	0.003	0.256		
	Micros	0.45	0.004	**0.0176**	0.011	**0.049**
Within locations	mtDNA	97.3	0.027	**0.012**		
	Micros	99.5	0.004	**0.006**	0.022	**0.0005**

origin) assigned to inferred clusters also showed no correspondence of individuals to any specific cluster (Supplementary Fig. S2).

Effective population size and gene flow

Effective population size estimates showed the lowest value for La Reforma in the northern region ($N_e = 34.1$), followed by Rebalsito from the central region ($N_e = 45.6$), whereas these estimates were moderate for the rest of the northern locations (Chametla = 131.1; Boca de Camichín = 276.0; Teacapan = 348.3; Supplementary Table S3). In contrast, the southern locations and Bahía de Navidad from the central region presented negative values, indicative of large estimates of N_e; in this case, the value of the lower boundary of the 95% confidence interval (Salina Cruz = 440.5; Puerto Madero = 223.1; Bahía de Navidad = 188.7) can be used as a reliable estimation, as noted by Waples & Do (2010).

Three out of five gene flow estimation runs showed consistent convergence for locations pooled into regions (Northern–Central–Southern; Supplementary Table S4). The mean estimates of the three

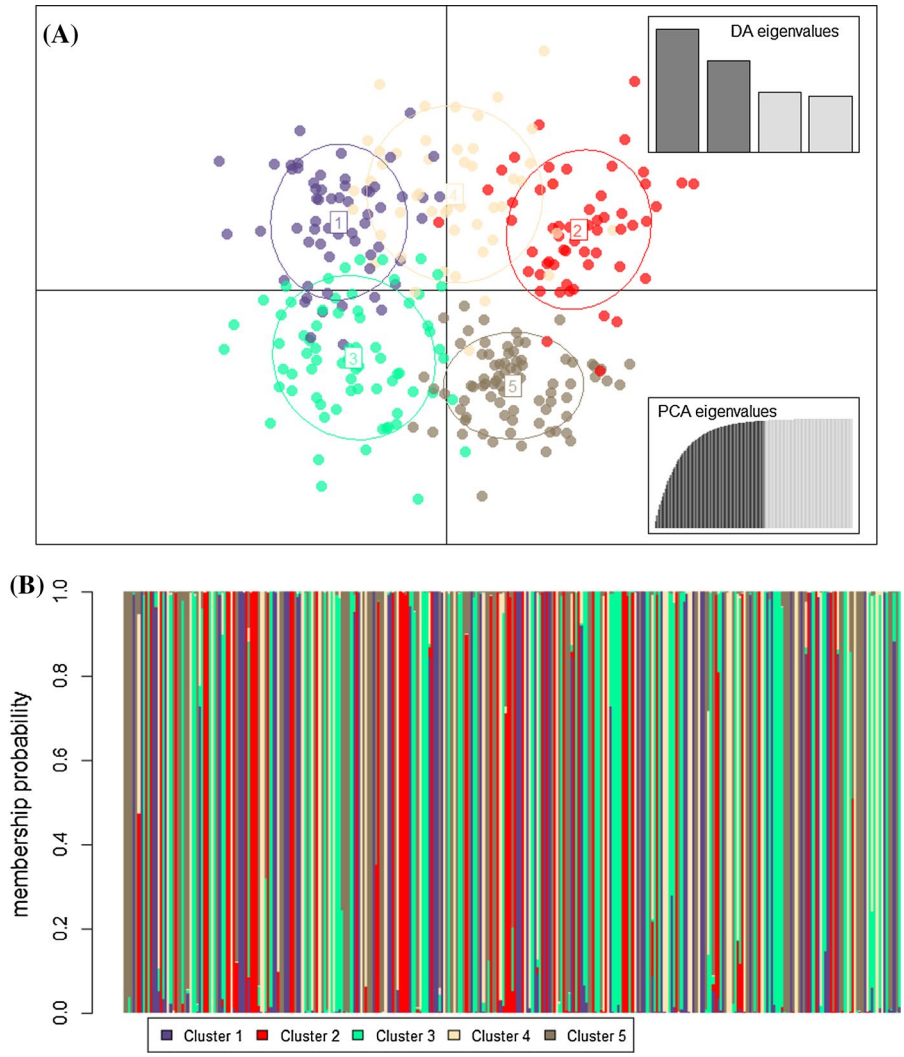

Fig. 3 **A** Discriminant spatial analysis of principal components (DAPC) for individuals of the scalloped hammerhead shark, *S. lewini*, from the Mexican Pacific using 11 microsatellite loci. **B** Posterior assignment probabilities to $K=5$ clusters in the scalloped hammerhead shark, *S. lewini*

trials between locations in different regions were high ($m_{\text{northern}}=0.983\pm0.014$; $m_{\text{central}}=0.671\pm0.004$; $m_{southern}=0.672\pm0.006$). Gene flow estimates showed a pattern of movements predominantly from southern and central regions toward the northern region ($m_{\text{central-northern}}=0.321\pm0.016$ and $m_{\text{southern-northern}}=0.325\pm0.006$, respectively) but low estimates in the opposite direction ($m_{\text{northern-central}}=0.003\pm0.003$ and $m_{\text{northern-southern}}=0.014\pm0.014$). Finally, gene flow estimates between the central and southern regions were remarkably low ($m_{\text{central-southern}}=0.008\pm0.015$; $m_{\text{southern-central}}=0.003\pm0.003$).

Discussion

Our study provides new insights into the patterns of genetic differentiation in the scalloped hammerhead shark, particularly in YOY individuals, along the Mexican Pacific coast. Genetic diversity using the mtDNA genome was higher for haplotype diversity than previous studies where only the mtDNA-control region was utilized. Thus, we demonstrate that using the whole mtDNA genome has the potential to increase the resolution in detecting significant population structure between nurseries, including estuaries

separated by relatively short distances. Previous studies in YOY individuals based on the mtDNA-control region failed to identify differences due to philopatry along the eastern Pacific (Duncan et al., 2006), although evidence of female philopatry has been obtained at a global scale based on mtDNA sequences (Daly-Engel et al., 2012). Discrepancies in genetic divergence between nuclear and mtDNA molecular markers are resulted of independent evolutionary histories of the two genomes, influenced by ecological and evolutionary factors related to the life history of elasmobranchs, such as sex-biased dispersal. Therefore, the matrilineal inheritance in addition to the lack of recombination of mtDNA supports the genetic structure pattern observed in the mtDNA genome, which may be the result of regional female philopatry.

Gene diversity

The mean number of nucleotide differences between whole mtDNA sequences was similar but slightly higher than that reported in the control region for scalloped hammerhead in other studies. In contrast to the gene diversity estimates obtained for mtDNA, microsatellites showed high gene diversity based on the average number of alleles and heterozygosity, similar to the reported in samples from the Tropical Eastern Pacific (Nance et al., 2011; Daly-Engel et al., 2012). Multiple paternity, which has been reported in the scalloped hammerhead, is thought to increase genetic diversity in nuclear DNA (Chapman et al., 2004; Daly-Engel et al., 2006). Multiple paternity has been considered an evolutionary strategy for overcoming the low level of gene diversity associated with long gestation, slow growth, and a small number of offspring (Daly-Engel et al., 2010; Domingues et al., 2018).

When sampling YOY individuals in nurseries, the chance to collect related individuals increases, and consequently, estimates of gene diversity can be influenced by the biased sampling of a portion of the genetic pool contained in a population. In the parentage analysis tests, the percentages of full-siblings (0.62%) and half-siblings (20%) were lower than the value of 59.2% reported by Nance et al. (2011). Although an important number of related individuals were found, the similarities between the levels of diversity that we estimated based on nuclear data and those from previous studies in the same area (Nance et al., 2011; Daly-Engel et al., 2012) suggest that the relatedness among individuals has little or no influence on estimates of gene diversity.

Genetic divergence and philopatry

The results based on nuclear and mtDNA data showed dissimilarities in the patterns of genetic divergence among the sampled localities in the Mexican Pacific. The mtDNA data showed differences among the three major regions analyzed, while nuclear DNA data failed to reveal these differences. The existence of genetic structure in mtDNA and its absence from nuclear DNA is usually explained by the philopatric behavior of females and gene flow mediated by males and has been widely documented in a variety of shark species (Hueter et al., 2005; Keeney et al., 2005; Tillett et al., 2012). In the present study, this pattern was confirmed in the scalloped hammerhead shark in the Mexican Pacific, suggesting that differences in mtDNA data originate from female philopatry. As philopatry originates from the repeated use of nurseries (usually estuaries, river mouths, or small bays) by females, the nonrandom segregation of haplotypes across generations results in genetic differences in mtDNA lineages between nurseries.

The strategy of including neonates and YOY sampled in nurseries increased our ability to detect differences, as young individuals maintain the genetic signature of their area of origin, avoiding the bias resulting from sampling migrating adult individuals and neonates, as YOY typically remain closer to their natal site (Feutry et al., 2016; Chapman et al., 2015). In contrast, the use of neonates and YOY may also result in the overestimation of the frequency of haplotypes resulting from sampling related individuals and drive the detection of artificial differences between nurseries. However, since the extent of the differences detected between the groups representing the studied regions when full-sibling and half-sibling individuals were removed was similar to those obtained when using the full dataset, the results seem to confirm the existence of female philopatry. Similarly, the use of the complete mtDNA genome improved the resolution of the detection of slight differences resulting from philopatry. Previous results based on the mtDNA-control region and a greater number of samples ($n = 301$; data not shown) did not allow these differences to be detected. In addition,

since differences were resolved among estuaries separated by 1000–2000 km, whereas no differences were found between nearby estuaries (400–500 km), our data provide evidence that females exhibit philopatric behavior, at least at the regional scale, which is consistent with previous reports of regional philopatry in the scalloped hammerhead shark in the western Atlantic and the Gulf of Mexico (Daly-Engel et al., 2012). Chapman et al. (2015) emphasized that regional philopatry consists of the return of females not necessarily to the exact place of their birth but to the region within which it occurred.

Genetic divergence and site fidelity

Site fidelity has been defined by Chapman et al. (2015) as the return of individuals to a previously inhabited location after leaving it for a certain period of time, for the purpose of specific activities such as feeding or shelter (feeding site fidelity) and may include return to an individual's birthplace only for feeding or refuge (natal site fidelity) but not for the purpose of breeding. This behavior differs from philopatry in that philopatric individuals return to their birthplace for reproductive purposes (parturition or breeding) across generations. The scalloped hammerhead shark is a species in which both juveniles and adults show a notable ability to undergo long displacements. However, in the eastern Pacific, telemetric studies have reported site fidelity and seasonal residence in specific areas near the coast, with intermittent movements offshore to islands or seamounts and returns to the coast (Hearn et al., 2010; Bessudo et al., 2011; Ketchum et al., 2014a; Zanella et al., 2019). Aldana-Moreno et al. (2020) found high residency of adult female scalloped hammerheads around San Benedicto Island in the Revillagigedo Archipelago and seamounts in the Gulf of California (Klimley & Nelson, 1984), which constitute important refuges and cleaning stations (Bessudo et al., 2011; Ketchum et al., 2014b). Islands and seamounts along the eastern Pacific coast show a high abundance of pelagic fish species (Klimley et al., 1988; Worm et al., 2003; Harding et al., 2011), promoting fidelity to aggregation areas and consequently yielding genetic differences between distant populations.

Sex-biased dispersal caused by both philopatry and site fidelity results in patterns of gene divergence at different levels. Whereas slight differences in mtDNA can be observed among populations due to philopatry between relatively nearby locations, deeper differences may be detected among populations inhabiting spatially separated aggregation sites, accompanied by differences in nuclear DNA due to site fidelity.

Because differences resulting from philopatry originate from the nonrandom segregation of haplotypes, the accumulation of variants will be slower and may result in slight divergence, which is usually evident in mtDNA. In contrast, the effects of genetic drift under the isolation of populations over time will result in highly divergent mtDNA lineages, in addition to differences in nuclear DNA. Differences for both molecular markers observed in this study among locations between groups (F_{SC}; Table 4) represents high temporal heterogeneity of allelic/haplotype frequencies possibly due to genetic interchange of individuals of populations from distinct sites. Highly divergent haplotypes have been reported between spatially separated populations of scalloped hammerheads (in the western Atlantic Chapman et al., 2009). Although these differences were attributed to philopatry, the extent of the reported differences was similar to that reported for populations representing subspecies of the bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758), a congeneric species distributed in the western Atlantic and eastern Pacific (Fields et al., 2016).

Significant genetic differences in scalloped hammerhead sharks based on nuclear microsatellite data and mtDNA have been reported in the eastern Pacific (Nance et al., 2011) and between the Gulf of Mexico and the Atlantic coast of the US (Daly-Engel et al., 2012).

Effective population size and gene flow

Estimates of gene flow for locations pooled into regions (northern–central–southern) showed a predominance of movements from the southern and central regions to the northern region and little exchange in the opposite direction. This result is compatible with the microchemical data (Coiraton, 2019), which showed that the proportion of trace elements

in individuals from La Reforma, the northernmost location, was highly compatible with fidelity to their site of origin. In relation to Puerto Madero, the southernmost location, individuals from this site seem to exhibit wider movements, reaching locations in the central region (Coiraton, 2019). Therefore, whereas females from La Reforma exhibited high residence in the area, females from the southern region showed a higher proclivity to move northward (Coiraton, 2019). Microchemical data also showed a high probability of females at La Reforma belonging exclusively to this location, which is compatible also with low N_e estimates.

Microchemical data also support the migration patterns of adult females from offshore to coastal areas while showing as well that some proportion of adult individuals remain in coastal waters for their entire lives (Coiraton et al., 2020). This is in accord with tagging data supporting a diel pattern of movements of juveniles undergoing offshore migrations into the pelagic environment at night to feed on pelagic fish and squid (Hoyos-Padilla et al., 2014). However, it has also been observed that the movements of this species could be related to behavioral plasticity in its movements, rather than its life-history characteristics related to the reduction of vulnerability to stressors among regions (Coiraton et al., 2020).

The high levels of genetic diversity observed in populations from the southern region in this study are consistent with the large estimates of N_e and could be a result of the interaction between gene drift and gene flow explaining the increased levels of gene diversity. Gene flow estimates indicating that individuals predominantly move from southern locations toward the northern region are largely in accord with their high levels of gene diversity and high N_e estimates observed, as gene contributions from females can influence both mtDNA and nuclear DNA. However, it should be noted that individuals from a far southern region of Central America may have also contributed to increasing the levels of gene diversity at locations in the southern region, as highly divergent haplotypes associated with these locations were detected. These haplotypes belonged to the southernmost locations, Salina Cruz and Puerto Madero, which were the locations with the highest gene diversity and gene flow estimates. The presence of highly divergent haplotypes in the southern region may suggest that some haplotypes may be introduced by migrants coming from populations farther south, originated in Central and/or South America. If this is the case, this evidence would indicate that the spatial separation of populations represents a factor promoting genetic divergence.

Migrating individuals play a paramount role in redistributing the genetic variation among populations. The identification of regions containing higher levels of gene diversity should be a conservation priority since these regions may constitute reservoirs contributing to the recovery of gene diversity caused by overexploitation. Special attention should be paid to populations from the southern area in the Mexican Pacific, as they may interact with other populations from the northern region and Central America. Additionally, individuals from the central region showed lower estimates of gene diversity and N_e and may constitute a vulnerable population that should be protected to preserve its evolutionary history.

The delineation of conservation plans requires differentiation between site fidelity and philopatry, since these processes will need the adoption of different criteria for determining priority areas of conservation. Better scenarios for assessing philopatry result from the combination of data from different approximations, such as tagging data, genetics, and, recently, microchemical studies. The YOY individuals used in this study were also analyzed using vertebral microchemistry (Coiraton et al., 2020). The evidence of regional philopatry in the scalloped hammerhead obtained in this study is in accord with microchemical data on the use of estuaries and coastal areas by neonates during early life stages, estimated based on the proportions of trace elements in vertebrae (Sr and Ba), which have demonstrated high residence in estuaries (Coiraton & Amezcua-Martínez, 2020).

Despite the ban on shark fishing in the months in which birth takes place in the scalloped hammerhead and other elasmobranch species (May to July), there is a need to delineate an integrated conservation strategy for the scalloped hammerhead by considering restrictions on fishing in nurseries of the species. Additionally, a comprehensive conservation strategy should be developed for the eastern Pacific to maintain the viability of populations and protect the gene pool of scalloped hammerhead shark populations.

Conclusions

Our results in the present study provide new insights into the genetic differentiation patterns of the scalloped hammerhead shark along the Mexican Pacific coast. While we identified differences in mtDNA between estuaries representing potential nurseries, no differences in nuclear DNA were observed, indicating female philopatry. However, highly significant differences have also been detected in spatially separated populations of the scalloped hammerhead shark based on both mtDNA and nuclear DNA, which can be attributed to site fidelity, as reported from tagging studies. This evidence may suggest that the genetic divergence of the scalloped hammerhead has a philopatric component, but it is also the result of the fidelity of individuals to aggregation sites for feeding, reproduction, and/or refuge. The delineation of conservation plans requires differentiation between fidelity to aggregation sites and philopatry, since these phenomena will result in different criteria for determining priority habitats for the protection and management of this critically endangered species in the Mexican Pacific.

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Data availability The authors will make the data available upon request.

Declarations

Conflict of interest The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

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