Title:
Mapping Risk and Conservation Potential Across the Indo-Pacific with Reefshark Genomescapes

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ABSTRACT:
Overfishing has severely depleted marine populations worldwide, including within protected areas. Illegal and unreported fishing are major contributors to this decline. Large-bodied apex predators such as sharks are among the most affected, with overfishing causing dramatic species declines and ecosystem destabilization due to trophic downgrading. Key barriers to effective marine conservation and management include: Data deficiencies that hinder population benchmarks and impact assessments, limited surveillance, allowing illegal fisheries to disproportionately affect apex predators, and insufficient capacity in vulnerable nations to monitor and protect species within their waters.

Our study addresses these challenges through a novel genomic framework that enables assessment of shark population diversity and health, while also improving fisheries traceability by detecting instances of illegal fishing across the Indian and Pacific Oceans. We present the *Reefshark Genomescape*, the first genome-wide reference database for Indo-Pacific reef sharks, an assessment of genetic diversity, structure, and connectivity of two key species across their Indo-Pacific range and geographic assignment of fished individuals using population-specific genetic signatures.

We show that grey reef shark (*Carcharhinus amblyrhynchos*) populations exhibit high genetic diversity, strong population structure, and elevated F_{st} values, with previously unknown connectivity between the central and western Indian Ocean and clear isolation of populations in the Andaman Sea. In contrast, silvertip sharks (*Carcharhinus albimarginatus*) display high connectivity, but show genomic signals of declining population health, supporting a reassessment of their IUCN status. Using supervised machine learning with Monte Carlo cross-validation, we assigned geographic origins to fished grey reef sharks with 96% accuracy.

These findings provide critical insights into population structure, connectivity, and health of two ecologically important reef shark species, while establishing a robust method for assigning geographic origin. We anticipate this framework will support regional conservation assessments and targeted management. Moreover, by enabling the identification of fishing hotspots and detection of IUU fishing, it lays the groundwork for a broader traceability system in marine ecosystems. Much like the landmark elephant ivory tracing study, our approach has the potential to transform marine conservation globally.

INTRODUCTION:

Marine ecosystems across the globe are experiencing unprecedented biodiversity loss due to overfishing and exploitation, driven by the rising global demand for seafood. Coral reefs, one of the most biodiverse ecosystems on earth, and home to over one-third of all marine fish species (Plaisance et al. 2011) are especially vulnerable to overfishing. This has contributed significantly to reef degradation by direct removal of key predator species (Sherman et al. 2023; Dulvy et al. 2021) and the indirect disruption of ecological dynamics once they are gone (Graham et al. 2017; Sandin et al. 2008). Illegal, unreported, and unregulated (IUU) fishing is a major driver contributing to overfishing in reef ecosystems especially in East and West Africa, North Pacific and Asia, which are frequent hotspots for IUU fisheries (Belhabib and Le Billon 2020).

The threats faced by reefs often vary spatially, necessitating region-specific conservation strategies (D. Pazmiño et al. 2018). However, due to technical, legal, and geopolitical loopholes in vessel monitoring systems and as a result of extensive IUU fishing activity, it is challenging to assess where fishing efforts are focused, which species and stock populations are being depleted and which reef ecosystems need bolstered protections. While many studies have elucidated species targeted by reef fisheries (Fields et al. 2015), there has been a very scarce examination of targeted stock populations (Fields et al. 2020).

Reef sharks comprise a significant portion of the large predator biomass in coral reef ecosystems (Sandin et al. 2008; Friedlander et al. 2014) and are among the primary targets of coral reef fisheries (Graham et al. 2017). As a result, reef shark populations have declined sharply worldwide due to overfishing and exploitation (Sherman et al. 2023). Many reef-associated shark species, display strong site fidelity and limited gene flow across distant reef systems, resulting in genetically distinct populations (D. A. Pazmiño et al. 2017; Momigliano et al. 2017; Maisano Delser et al. 2019). While such population structure increases vulnerability to local extinctions, it also provides an opportunity to establish population-specific baseline indicators of ocean health. These genetic patterns can be used to build reference libraries capable of identifying the geographic origin of individuals, offering a valuable tool for genomic monitoring, conservation and management. Here we use whole genome resequencing to identify population structure across the Indo-Pacific basin in grey reef sharks (*Carcharhinus amblyrhynchos*) and silver tip sharks (*Carcharhinus albimarginatus*) and to develop tools for identification of geographic origin of shark catches within fisheries.

Grey reef sharks are a dominant and ecologically important predator species across a majority of the tropical Indo-Pacific reef ecosystems and serve as valuable indicators of ecosystem integrity (Friedlander et al. 2014). Despite their ecological importance, they are listed as Endangered on the International Union for Conservation of Nature (IUCN) Red list index, having suffered an estimated global biomass decline of 50–79% (Ali et al. 2020). Similarly, the silvertip shark, which exhibits a patchy distribution across the Indo-Pacific, is one of the most frequently observed reef shark species in its range (Bond et al. 2015). It is currently classified as Vulnerable, with a predicted global biomass decline of approximately 30% over the past 54 years (Rigby, C.L. et al. 2023). Both species are frequently captured in IUU fisheries and trafficked through illegal trade (Bennett et al. 2022). While both *C. amblyrhynchos* and *C. albimarginatus* are listed under Appendix II of the Convention on International Trade in Endangered Species (CITES) – enforcement of regulation in international trade remains a persistent challenge (Bond et al. 2025).

Analyses of movement patterns and long-term reef fidelity studies showed that greef sharks (GRS) have a very small activity space with an average radius of 58 km (Jacoby et al. 2020) and high reef residency (Espinoza et al. 2014). Further, a regional genetic connectivity study using reduced representation

nuclear and mitochondrial marker suggests that grey reef shark populations separated by open ocean in the Chagos archipelago and Australia had limited gene flow, while local populations in eastern and western Australia, had higher connectivity (Momigliano et al. 2017). In contrast, movement analyses of silvertip sharks showed that they are more dynamic, wide-ranging, and less resident than grey reef sharks, with an activity space at 175 km, nearly three times larger than that of grey reef sharks (Jacoby et al. 2020). A regional genetic study of silvertip sharks in the southwest Pacific, using reduced representation mitochondrial and nuclear markers, showed high connectivity and no structure within the region, but similar to grey reef sharks showed strong genetic isolation between populations separated by open ocean in the southwest Pacific and Seychelles (Green et al. 2019).

Given their broad but discontinuous distributions and ongoing population declines, both species urgently require fine scale spatial assessments of genetic connectivity, population structure, and demographic health across their distribution range. While regional studies have provided important insights into the genetic connectivity patterns of the species, notably, no study till date has investigated population structure across the full Indo-Pacific range of either species using a genome-wide approach. As a result status of several sub-populations remains unexamined, potentially leaving critical gaps in conservation and our understanding of how ecological, oceanographic, and historical factors shape population connectivity.

Given their frequent capture in fisheries across the Indo-Pacific region (Bennett et al. 2022), developing a genetic reference database spanning their full distribution, and matching fished individuals to this database, could provide valuable insights into spatial fishing pressures across Indo-Pacific reefs. In this context, both species, particularly grey reef sharks, show strong potential as biological indicators for identifying fishing hotspots and detecting instances of illegal, unreported, and unregulated fishing and trade across the Indo-Pacific. Establishing such a database and using it to determine the geographic origin of fished sharks could significantly enhance traceability in fisheries. This approach has the potential to transform global fisheries monitoring, improve stock assessments, and support more effective, adaptive conservation and management of marine ecosystems. Importantly, these efforts would not only enable targeted protection of reef shark populations but also contribute to the broader conservation of coral reef ecosystems, by detecting spatial explicit fishing pressures.

In this study, we present a genomic framework capable of both assessing the demographic health and connectivity of reef shark populations and supporting fisheries traceability and conservation enforcement. We constructed a comprehensive genetic reference database for two ecologically significant reef shark species, *Carcharhinus amblyrhynchos* and *Carcharhinus albimarginatus*, which also serve as indicators of reef ecosystem health. By collecting samples from across their Indo-Pacific distribution, we conducted the first range-wide population genomic analysis of these species, integrating complete mitochondrial genome sequences with hundreds of thousands to millions of genome-wide single nucleotide polymorphisms (SNPs).

Our dataset spans multiple regions, including the western Indian Ocean, Chagos Archipelago, Andaman Sea, eastern Indian Ocean, and the broader Pacific. These regions vary in reef density, geographic isolation, and exposure to fishing pressure, providing an ideal framework for investigating how ecological and anthropogenic factors shape genetic structure and gene flow. Through this approach, we address critical knowledge gaps in reef shark conservation and demonstrate how genomic data can be applied to improve fisheries traceability, CITES reporting and sustainable marine resource management.

Ultimately, this study provides a robust, baseline assessment of two apex reef predators across their

Indo-Pacific range and offers a significant advancement in efforts to overcome the traceability limitations in current fisheries monitoring. The data and analytical tools presented here contribute a much-needed framework for evaluating the conservation status of marine species and identifying region-specific threats from fishing activities, paving the way for more targeted, effective management strategies.

METHODS:

***Biological Materials*:**

Fin clips were taken during catch and release operations while tagging grey reef and silvertip sharks across sampling locations. Fin clips or muscle tissue were collected from samples collected at landing sites, markets or on fishing vessels. Samples were stored immediately after collection in RNA later tubes or in 90% ethanol at room temperature and then stored once ethanol was drained off in a -80 C freezer until DNA isolation. Samples were collected at the following locations indicated in Figure 1. The number of samples per site is listed in Supplementary Table 1.

DNA extraction and library preparation

Each fin clip sample was subsampled and DNA was extracted from a 10 mg piece using the DNeasy Blood & Tissue Kit (Qiagen N.V., Venlo, The Netherlands), including a 3 hour lysis step. The concentration and quality of genomic DNA was checked using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Inc., Waltham, MA, USA) and an Agilent Tape Station 4150 (Agilent Technologies, Inc., Santa Clara, USA). For most of the samples DNA was diluted to be within 10-100ng/ul and sent for Library Preparation and sequencing to Genomics & Bioinformatics Services, College Station, TX, USA.

Libraries were prepared using the PerkinElmer NextFlex DNA Library Prep Kit for Illumina (Perkin Elmer Inc., Connecticut, USA). Library quality and quantity was checked on an Agilent Tape Station 4150 (Agilent Technologies, Inc., Santa Clara, USA). Sequencing was performed on the Illumina NovaSeq 6000 S4 X 2x150 v1.5 platform (Illumina Inc., San Diego, USA) at the sequencing core Genomics & Bioinformatics Services, College Station, TX, USA. Samples from India were processed and sequenced using the same methods and platform at Clevergene Inc., Bangalore, India.

Mitochondrial DNA Analyses

Whole mitochondrial genomes were generated from the trimmed paired whole genome sequence reads of 316 grey reef sharks and 190 silvertip sharks using the software package Mitos and MitoFinder (Bernt et al. 2013). Annotated coding and non-coding regions for each mitogenome were assembled into mitochondrial contigs and aligned to the reference mitogenome (Dunn et al. 2020; Johri et al. 2020) for the respective species to generate a multi sample nucleotide alignment file using Geneious^{R} software. Diversity indices (number of haplotypes NH), within population diversity and measures of pairwise genetic differentiation (Φ_{ST}) were estimated in the software Popart v. 1.7 (Leigh and Bryant 2015). A minimum spanning network of whole mitochondrial genome haplotypes was constructed in the software Popart v. 1.7 (Leigh and Bryant 2015).

Whole Genome Data Processing

Adapters, low quality bases and 5' and 3' start or end sequences were trimmed with Trimmomatic v.0.39 (Bolger et al. 2014). Cleaned reads were then mapped to the respective grey reef or silvertip shark reference genomes (NCBI accession number JBNGOJ000000000 and JBNGOK000000000, Johri et al. manuscript in review) using BWA-MEM v.0.7.17-r1188 (Li and Durbin 2009). Reads were sorted by query

name and duplicates were removed via Picard v. 2.26.6 ("Picard Tools - By Broad Institute," n.d.). Sorted bam files for 316 samples for the grey reef shark and 190 samples for the silvertip shark were combined into two vcf files respectively using BCFtools mpile-up (Li et al. 2009). VCFtools was used for downstream SNP quality control which included filtering the VCF files for : a minimum base call and mapping quality threshold of 30, only reads mapping to a single location of the genome retained, Minor Allele Frequencies (MAF) > 1%, Hardy–Weinberg Equilibrium (HWE), read depth of 3X, biallelic loci only, and a call rate threshold > 75% (i.e. a total of 25% missing data) (Danecek et al. 2011). Finally the VCF file was filtered for linked loci using Plink at linkage disequilibrium (LD) r2 > 0.6 (Chang 2020).

Analysis of genetic structure and diversity

A relatedness analysis was performed using VCFtools to prevent biased intra-population diversity results. In order to test for genetic homogeneity between locations we calculated pairwise fixation indices, F_{ST} for nuclear SNP markers using the R package 'StAMPP' (https:// cran.r-project.org/web/packages/StAMPP) (Pembleton et al. 2013). Each analysis consisted of 100,000 bootstraps generating confidence intervals and P-values for each pairwise comparison of F_{ST}. Significance levels of all pairwise tests were corrected for multiple comparisons with a sequential Bonferroni procedure (BF P ¼ conventional P-value 0.05 divided by the number of tests per marker type; Rice 1989).

The VCF file was converted to BED format and Principal Component Analyses conducted using Plink2 . Using the same BED files, admixture proportions were estimated using the Admixture software package (Alexander et al. 2009) for k = 2-6 for both the grey reef shark and silvertip shark populations. The cross validation (CV) error was estimated for each of the K=2-6 and the Ks with lowest CV error was used for subsequent admixture analyses of both species' population groups. The PCA and Admixture plots were generated using the eigenvec and eigenval files and the .Q and .P files respectively in RStudio.

Gene flow patterns were examined under the assumption of asymmetric bidirectional gene flow, using the Genepop file with a set of 11,345 SNPs across all locations and 316 individuals for the grey reef shark and 190 individuals for the silvertip shark. This analysis was executed using the R-function divMigrate from the diveRsity R-package (Keenan et al. 2013). The divMigrate function computes migration rates between all sites and subsequently normalizes them, generating relative migration rates from zero to one. Furthermore, significant asymmetry in gene flow direction between two sites was assessed employing statistics introduced by Alcala et al. This approach combines information from both genetic differentiation measures, GST and D, to estimate the number of migrants per generation Nm, from which relative migration is derived. To evaluate the presence of significant asymmetric migration, 1,000 bootstraps were conducted.

Nucleotide diversity was estimated by generating a VCF file using BCFtools that contained variant and non-variant sites for each of the grey reef and silvertip shark populations. The VCF file thus generated along with the population data for each sample was used to calculate nucleotide diversity per population using the software package pixy v0.95.02 (Korunes and Samuk 2021).

Demographic History

Demographic histories of the grey reef shark and the silvertip shark were reconstructed using Pairwise Sequential Markovian Coalescence (PSMC) v0.6.5 (Schiffels and Durbin 2014). The Illumina sequence reads from each species were mapped to the respective chromosomal scaffolds for the species using BWA-MEM v0.7.17 (Li and Durbin 2009) and the consensus sequence was called using BCFtools v1.21-79-gcef68bc+ mpileup (Li et al. 2009), BCFtools call, and vcfutils "vcf2fastq." function in the PSMC software package (https:// github.com/lh3/psmc/utils). Minimum depth cutoffs of 5 and maximum

depth cutoffs of 100 were applied to all genomes using vcfutils. Remaining positions with an average mapping quality greater than 30 were used to create the input file for PSMC. The program `fq2psmcfa' in the PSMC software package, was used to transform the consensus sequence into a fasta-like format. In order to perform bootstrapping, we ran 'splitfa' function in PSMC, to split long chromosome sequences to shorter segments. PSMC was run for 25 iterations with parameter values: -N25 -t15 -r5 -p "4 + 25*2 + 4+6". We then performed 100 rounds of bootstrapping to evaluate variance in effective population size (N_E) estimates. Results from the 100 rounds were concatenated into a combined PSMC file, which was then used to generate plots using 'psmc_plot.pl' from the PSMC package. In order to visualize the PSMC plot, we applied a mutation rate of 1.9e−08 (Lesturgie et al. 2022) and a generation time of 7-10 years for the grey reef shark (Bm et al. 1997; Lesturgie et al. 2022). For the silvertip shark we applied the mutation rate 1.9e−08, same as the grey reef shark as has been used previously for multiple reef shark species (Lesturgie et al. 2022). A generation time of 9.5 years was used for the silver tip shark (Kato, S. and Hernandez 1967).

Heterozygosity* and *Runs of Homozygosity

After self-alignment of Illumina short reads from the same individual to the reference genome, heterozygosity estimates were calculated using ANGSD v.940 (Korneliussen et al. 2014). Heterozygosity estimates for the whole genome and on a per site basis were obtained using these methods. We calculated homozygosity for the two genomes by aligning short reads from the same individual to the genome and assessing variant and non-variant sites across the genome. Short reads were mapped to the genome using BWA-MEM v0.7.17 (Li and Durbin 2009) and BCFtools mpile-up (Li et al. 2009; Danecek et al. 2021) was used to generate a VCF file. BCFtools ROH (Narasimhan et al. 2016) was used to identify runs of homozygosity across the genome. Illumina short read data at 30x or higher genome coverage was used to create a vcf file which was then used to determine ROH length and location across the two genomes using BCFtools ROH.

Population Assignment of Fished Sharks

For the population assignment of fished shark specimens with unknown source populations we generated a supervised machine learning model using Monte Carlo Cross Validation in the Rpackage assignPOP (Chen et al. 2018). Monte-Carlo cross-validation was conducted using 75%, 80% and 95% of individuals from each population by the top 10%, 25%, 50% of high F_{ST} and all loci as training sets and each training set combination was resampled for 30 iterations. A total of 360 assignment tests were performed. Support vector machine (model = "svm") classification model was used to build predictive models. Assignment accuracy of the Monte–Carlo cross-validation results was calculated and the accuracy plot generated. After an optimal model with high assignment accuracy of >95% for the training dataset was generated, the model was used to assign populations for fished shark specimens. Each of the svm, naïveBayes, random forest or lda models were tested to the best assignment accuracy of unknown samples.

RESULTS:

Genome-wide SNP Analysis in *Carcharhinus amblyrhynchos*

Principal component analysis (PCA) of 6 million genome-wide SNPs from 323 *C. amblyrhynchos* individuals identified four distinct genetic clusters and one intermediate group. The intermediate cluster consisted of two individuals from northern Indonesia, likely reflecting recent admixture between the Andaman and Chagos populations (Figure 3A). These four clusters were further supported by admixture analysis (Figure 3B). Cross-validation errors were lowest for K = 2, 3, and 4, with K = 4 providing the most

informative resolution of population structure of *C. amblyrhynchos*.

At K = 4, four well-differentiated clusters were resolved: (i) the WIO, (ii) Chagos archipelago, (iii) the Andaman–northern Indonesia group, and (iv) the EIO–Pacific group, which included southern Indonesia, Western Australia, Palau, and Palmyra (Figure 3B). At K = 2, two major genetic clusters were recovered: one comprising the EIO and Pacific populations, and the other including all WIO populations along with the Chagos archipelago (Supplementary Figure 1). At K = 3, the Chagos archipelago population emerged as a distinct genetic cluster (Supplementary Figure 1).

Genome-wide F_{ST} analyses between individual populations and regional clusters, confirmed strong genetic differentiation consistent with PCA and admixture results (Figure 3C, Table 2 & 3). The Chagos population showed the highest differentiation from the Pacific (F_{ST} = 0.284), followed by the EIO cluster (F_{ST} = 0.242), moderate differentiation from the Andaman Sea (F_{ST} = 0.107), and the lowest from the WIO (F_{ST} = 0.007). The smallest differences were between the Eastern IO and Pacific (F_{ST} = 0.008) and between the WIO and Chagos (F_{ST} = 0.007).

Pairwise FST values between individual populations mirrored regional trends (Supplementary Figure 2). Populations within the same regional cluster showed low genetic differentiation, while FST values increased with geographic distances. The WIO populations were most differentiated from those in the Andaman and EIO clusters. Notably, the Chagos population exhibited greater genetic distance from Andaman and EIO populations than from the WIO, despite geographic proximity to the Andamans. Similarly, populations within the Andaman cluster were highly differentiated from those in the EIO and broader Indo-Pacific, underscoring pronounced genetic structuring over relatively short distances.

To assess gene flow among regions, we analyzed 10,000 high F_{ST} SNPs from 293 individuals, using 1,000 bootstrap replicates and assuming asymmetric bidirectional migration. Results showed significantly high gene flow between Chagos and the WIO, low gene flow between the EIO and Pacific, and no detectable gene flow between any other regions (Figure 3D).

Genome-wide SNP Analysis in *Carcharhinus albimarginatus*

PCA of 500,000 SNPs from 195 *C. albimarginatus* individuals revealed three main genetic clusters corresponding to the WIO, Chagos, and the EIO–Indo-Pacific region, with some individuals exhibiting intermediate genotypes (Figure 4A). Admixture analysis refined these patterns (Figure 4B), with both K = 2 and K = 3 showing similarly low cross-validation errors.

At K = 3, Chagos was resolved as a distinct cluster, and individuals from the Andamans, Indonesia, and Scott Reef showed signs of recent admixture between Chagos and EIO lineages (Figure 4B). At K = 2, populations grouped into two broad clusters: one comprising the EIO and Pacific, and the other including the WIO and Chagos (Supplementary Figure 3). At K = 4, additionally admixed individuals were detected, particularly between Chagos and the Andaman/Indonesia cluster (Supplementary Figure 3).

Genome-wide F_{ST} analyses revealed similar regional population structures to those observed in *C. amblyrhynchos*, though with lower overall differentiation (Figure 4C, Table 4 & 5). The Chagos population showed the greatest differentiation from the Pacific (F_{ST} = 0.009), followed by the EIO (F_{ST} = 0.006), and the Andaman region (F_{ST} = 0.007), while it was least differentiated from the WIO (F_{ST} = 0.005). The Andaman population exhibited the highest differentiation from other regions, whereas the EIO and Pacific populations were the least differentiated (F_{ST} = 0.001).

Although pairwise F_{ST} values were generally lower than in *C. amblyrhynchos*, regional trends were consistent in population wise comparisons (Supplementary Figure 4). Populations within geographic clusters showed low differentiation, while F_{ST} values increased with geographic distances. WIO populations were most distinct from those in the Andaman and Indo-Pacific clusters.
As with grey reef sharks, we evaluated gene flow in *C. albimarginatus* using 10,000 SNPs from 195 individuals. Results showed significantly high gene flow between Chagos and the WIO, and lower levels of gene flow between all other regions, except the Andaman Sea, where no significant gene flow was detected (Figure 4D).

Mitogenome Variation and Structure

Mtochondrial genome sequences, ~14,030 bp in length, were analyzed from 205 *Carcharhinus amblyrhynchos* individuals, along with one *Rhinoptera brasiliensis* used as an outgroup, to investigate phylogenetic relationships and haplotype sharing across the Indo-Pacific. A total of 1,040 variable sites (~7%) were identified, including 129 parsimony-informative positions, resulting in 19 distinct mtDNA haplotypes. Haplotypes, 1, 2 and 3 were the most dominant and originated in the Andaman Sea, eastern Indian Ocean (EIO). In the Pacific, haplotypes 4 and 5 predominated in the western Indian Ocean (EIO) and the Chagos archipelago (Figure 2A). Nucleotide diversity across regional populations was $\pi = 0.0031$ (Table 1), with a sample size of 223 and an estimated mutation rate of $\mu \approx 3.74 \times 10^{-6}$.

Phylogenetic reconstructions using both Median- joining networks and Bayesian inference revealed congruent topologies and well-supported clades, indicating spatially structured populations of *C. amblyrhynchos* (Figure 2C). Genetic distances between *C. amblyrhynchos* and the outgroup exceeded 25%. Among-region distances ranged from 0.3% to 8%, while within-region distances ranged from 0.1% to 4% in Chagos, 0.1% to 5% in the WIO, and 0.3% to 5% in the EIO. AMOVA results revealed significant genetic differentiation between regions (pairwise ΦST = 0.5647, $p = 0.001$), though pairwise ΦST values among individual populations were not significant. Tajima's D values indicated no evidence of recent population expansion.

For *Carcharhinus albimarginatus*, mitochondrial genomes (14,091 bp) were analyzed from 93 individuals and one *R. brasiliensis* outgroup. We identified 1,464 variable sites (~10%), including 102 parsimony-informative sites, and 13 haplotypes. Among the five main haplotypes 1 was predominant in the EIO, 2 in the Pacific, 3 in the EIO, Pacific and Chagos, 4 was frequent in the WIO and 5 was the main haplotype in the Chagos and upto a lesser extent in the WIO(Figure 2B). Nucleotide diversity was lower than in *C. amblyrhynchos* ($\pi = 0.0012$, Table 1), with a sample size of 195 and mutation rate $\mu \approx 1.54 \times 10^{-6}$.

Phylogenetic analyses again revealed geographically structured clades with high bootstrap values and significant posterior probability support (Figure 2D). Genetic distances between *C. albimarginatus* and the outgroup exceeded 25%. Among-region distances ranged from 0.2% to 9%, and within-region distances ranged from 0.2% to 6% in Chagos, 0.1% to 5% in the WIO, and 1% to 7% in the EIO. AMOVA indicated low but significant ΦST values between regions and among populations. Neutrality tests (Tajima's D) were non-significant, suggesting a lack of recent demographic expansion.

Nucleotide Diversity

We estimated the average nucleotide diversity (π) within population groups of the grey reef and silvertip shark populations, identified by analyses of F_{ST} and gene flow between the species populations. A total of 203833 neutral variant and non-variant SNP loci were used to calculate π for grey reef shark population groups and these resulted in π values in the range of 0.02 to 0.05 (Table 6, Figure 5A). Each

cluster was significantly different with respect to π and the EIO and Pacific Ocean clusters had the highest π values of ~0.05.

We used 289045 neutral variant and invariant SNP loci to calculate π for silvertip shark population groups. The π values were significantly lower compared to that for the grey reef shark populations, and most were in the range of 0.001 while the Chagos population had a π of 0.006 and the Pacific Ocean cluster had the highest π of 0.01 (Table 6, Figure 5B). Distribution is skewed for all silvertip populations, with long whiskers and higher maximums suggesting a few individuals with elevated diversity.

Demographic History

Analyses of demographic history in the grey reef shark populations using Pairwise Sequential Markovian Coalescence, suggested historical population sizes in the range of 200,000 to 110, 000 for the western Indian Ocean populations (Figure 6A, B), and 110,000 to 75, 000 in the Central Indian Ocean populations (Figure 6C) and the eastern Indian and Pacific Ocean populations (Figure 6D). All grey reef populations experienced a precipitous decline around 3-5 million years ago, with stabilization at a lower population level of ~30,000-50,000 individuals ~500, 000 years ago. All populations except for the eastern Indian Ocean and Pacific Ocean populations continued to decline beyond this point with a final stabilization at 10,000-15,000 individuals ~100,000 years ago (Figure 6A, D). The Eastern Indian Oceana and Pacific Ocean populations stabilized at around 30,000 individuals ~100,000 years ago (Figure 6A, D).

Demographic history analyses in the silvertip shark populations using PSMC, revealed historical population sizes in the range of 130,000 to 90, 000 for the western Indian Ocean populations (Figure 7A, B), 60,000 for the Chagos population (Figure 7C) and 90,000 to 75, 000 in the Andaman Sea, and Eastern Indian Ocean populations (Figure 7D). Silvertip shark populations experienced a precipitous decline around 2-3.5 million years ago, with stabilization at ~15,000 individuals starting at ~750, 000 years ago (Figure 7A).

Tracking Shark Fisheries using Genomics

To evaluate the use of genomics to identify independent samples, a model was trained using supervised machine-learning to discriminate between population groups of the grey reef shark. Monte-Carlo cross-validation was utilized on the trained model to estimate the mean and variance of assignment of samples and accuracy was evaluated by resampling of random individuals from the training dataset. Overall assignment accuracy of the model for the training data was greater than 93% for all population groups when 95% of individuals in each population and all loci from the training data set were utilized(Figure 7A). Assignment accuracy was greater than 93% for the Western IO, greater than 96% for the Chagos, and ~100% for the Easter IO and Pacific Ocean populations (Figure 8A).

The overall assignment accuracy was tested with varying proportions of individuals and loci from the training dataset, to assess the accuracy of the model when using smaller datasets. The model achieved median assignment accuracy of greater than 93% when the proportion of individuals was changed between 75%, 85% or 95% of individuals in each population group and the proportion of training loci was changed between 75%, 90% and 100% of the total loci for each population (Figure 8B). However, when 95% of individuals from each population were used in the training dataset the variance of accuracy skewed towards 100% accuracy, suggesting a higher probability of accurate results when 95% or higher percentage of individuals in the training dataset are used (Figure 8B).

We then tested the impact of using smaller proportions of training data with respect to identifying individuals and loci for assignment accuracy of individual population groups. Proportions of individuals

used were 50%, 70% or 90% and proportion of loci varied between 10%, 25% , 50% and 100%. Overall median assignment accuracy was at 85% or higher even when 50% of individuals and 10% of training loci were used (Figure 8C). Cumulative assignment accuracy as well as assignment accuracy for each individual population group was most optimal when 90% of the individuals in each population were used, and 25% or higher proportion of training loci were used (Figure 8C). In summary, the number of individuals had a greater impact on assignment accuracy than the proportion of loci, when loci above a threshold of 25% were used.

To evaluate the discriminatory power of this model in assigning unknown samples (those not included in the training data and never previously encountered by the model) we tested this using genetic data from 27 samples confiscated from a vessel illegally fishing in the Chagos archipelago. These samples were treated as unassigned during the assignment test. The model correctly assigned 26 out of 27 samples (96%) to Chagos, based on assignment probabilities greater than 50% for the region (Figure 8D). One of the 27 samples had a less than 50% assignment probability for the Chagos and therefore it was not assigned to the Chagos grey reef shark population.

Admixture analyses of the confiscated grey reef shark samples from the Chagos Archipelago grouped them with the local Chagos population, supporting our assignment based on a parallel analysis discussed above (Supplementary Figure 5A).

In contrast, the silvertip shark samples were assigned to the Western Indian Ocean population (Supplementary Figure 5B). However, high gene flow, due to likely due to migration between the Western Indian Ocean and Chagos, makes it difficult to confidently assign these individuals to a specific population.

DISCUSSION:

This study presents the most comprehensive population genomic assessment of two reef shark species, *Carcharhinus amblyrhynchos* and *Carcharhinus albimarginatus*, across their entire Indo-Pacific distribution. Our findings reveal strong and spatially structured population differentiation in grey reef sharks, with comparatively lesser but still significant structuring in silvertip sharks. Genetic structure and gene flow patterns aligned broadly with recognized biogeographic provinces, but also exposed striking exceptions, such as unexpectedly high connectivity between populations in the Chagos Archipelago and the WIO, and near-complete isolation of populations in the Andaman Sea. These patterns suggest that both historical and ecological barriers, including oceanographic currents and reef fragmentation, have shaped the asymmetric gene flow observed in both species. Our results show that grey reef sharks have higher nucleotide diversity than silvertip sharks, with particularly elevated diversity in the Eastern Indian and Pacific Ocean grey reef populations. Demographic reconstructions indicate both species experienced sharp population declines 2–5 million years ago, but grey reef sharks stabilized at higher effective sizes compared to silvertips, with regional differences in recovery and persistence. Importantly the genomescape approach provides an novel framework to enable traceability of fisheries by genetic tracking of source populations of fished sharks and to identify reef ecosystems targeted by fisheries across the Indo-Pacific.

Placing these genomic insights in the context of current conservation challenges reveals their critical importance. Overfishing, incidental and targeted are the largest drivers of decline among sharks and rays (Dulvy et al. 2021). The Indian Ocean is recognized as a biodiversity hotspot, supports prolonged residency of ecologically significant megafauna (Sequeira et al. 2025) and is targeted for global seafood supplies by some of the largest fishing nations including the largest shark landing nations of the world

(Dulvy et al. 2014; 2021). A large proportion of these fisheries are illegal, unreported or unregulated (Belhabib and Le Billon 2020). Despite efforts to curb IUU fishing through vessel monitoring systems, technical, legal, and geopolitical loopholes, undermine their effectiveness, allowing vessels to operate without detection (Paolo et al. 2024). This problem is exacerbated by practices such as transshipment and flag-hopping, which are commonly associated with IUU fishing (Belhabib and Le Billon 2020; 2022). These blind spots make it difficult to pinpoint fishing locations, identify potentially illegal or unreported activity and enforce existing regulations designed to protect marine ecosystems. For example, the inability to detect fishing within no-take marine protected areas (MPAs) has compromised the effectiveness of these conservation zones, contributing to severe declines in shark populations in the Chagos MPA (Graham et al. 2010). As a result, biodiversity baselines are shifting quickly within the Indian ocean region and regional or national management agencies are unable to assess where fishing efforts are focused, which reef ecosystems are declining rapidly, which species and stock populations are being depleted and which regional ecosystems need bolstered protections. Together, these limitations undermine sustainable fisheries governance, threaten marine biodiversity, and harm the livelihoods of lawful fishers (Okafor-Yarwood et al. 2022; Belhabib and Le Billon 2022). The current study provides a robust framework to track the health, connectivity and fishing pressures on reef shark populations and by extension reef ecosystems across the Indo-Pacific ocean basins.

The assessment of the grey reef shark across their entire Indo Pacific range reveals five genetic clusters in the WIO, all parts of the Chagos, Andaman Sea, EIO and the Pacific. An intermediary population in the Persian Gulf with intermixed lineage from the Andaman Sea and WIO was also identified. For grey reef sharks there is almost no gene flow between populations in the Andaman Sea and other populations. On the other hand, there is significant connectivity between shark populations in the WIO and Chagos and the EIO and the Pacific despite separation by large open ocean distance. Silvertip shark populations in the Chagos archipelago had little to no gene flow and connectivity with the Andaman Sea, EIO and the Pacific populations. However, the Eastern IO and Pacific populations had high gene flow and connectivity amongst themselves. Notably, this genetic flow is much higher than that seen in grey reef sharks from the same locations. This is the first study to investigate the genetic connectivity of silvertip sharks in the Chagos archipelago and Western Australia as well as the far east populations in Taiwanese waters. A key finding of this study is that both grey reef and silvertip shark populations show significant gene flow and connectivity between the Chagos Archipelago and the Western Indian Ocean. In contrast, populations in the Andaman Sea are remarkably isolated, with almost no gene flow to neighboring populations in the Eastern Indian Ocean or Pacific. These results highlight the need to treat Andaman Sea grey reef sharks as a distinct management unit, as intense fishing pressure coupled with the absence of genetic mixing leaves them highly vulnerable to rapid local extinction.

Gene flow patterns observed in the current study are reflective of and consistent studies assessing the movement boundaries of the two species through acoustic telemetry studies that show silvertips move across larger distances and hence enable intermixing of species' populations (Jacoby et al. 2020; Williamson et al. 2021). These findings suggest that conservation strategies must be species-specific: cross-jurisdictional measures for silvertips, and more localized, national-level protections for grey reef sharks.

Beyond advancing fundamental biogeographic understanding, this study establishes the 'Reefshark Genomescape', the largest genome-wide reference database for any reef shark species to date and a novel population assignment framework with immediate applications for conservation and fisheries management. By generating population-level genetic baselines, we enable forensic tracing of shark products to their geographic source, offering a science-based tool for enforcing CITES trade restrictions,

identifying fishing hotspots, instances of IUU fishing and guiding the creation of spatially explicit Fisheries Management Zones (FMZs).

The Indian Ocean is one of the least studied areas with respect to connectivity and migration of sharks, as such connectivity studies between shark populations in the Western IO and Chagos are lacking. However, connectivity between the Western Indian Ocean and the Chagos Archipelago have been observed for other taxa such as turtles which use Chagos as the breeding grounds and the Western_IO regions of Seychelles as the foraging areas (Hays et al. 2014). Juvenile turtles are thought to drift to the foraging areas in the Seychelles through the south equatorial ocean current. In addition satellite tagging data from whale sharks tagged along the west coast of India indicate migrations towards to the Gulf of Oman and the Somali coast in the Western IO (Arrowsmith et al. 2021). The migration of whale sharks, a filter feeder, is thought to be driven by frontal zones between cold and warm currents rich in chlorophyl and prey (Arrowsmith et al. 2021). The connectivity between grey reef and silvertip shark populations of the Chagos and Western IO could be similarly impacted by ocean currents and prey availability along the Chagos- Western IO corridors. Nevertheless, further investigations are needed to elucidate the drivers of connectivity through careful analysis of animal age, migration timing, oceanographic conditions, and prey availability and the influence of key behaviors, such as foraging, mating, and periodic migrations, during observed movement patterns. Furthermore, migration corridors for green sea turtles have been identified between the Andaman Islands and the southeastern Indian Ocean. Turtles from certain nesting sites in the Andamans appear to be isolated to the eastern Indian Ocean and/or the Andaman Sea (Wallace et al. 2010). Similarly, the current study found negligible or no connectivity between the Andaman Sea and the broader Indian Ocean, as indicated by the observed genetic differentiation observed in grey reef and silvertip shark populations from the Andamans compared to those in the broader Indian Ocean. The study therefore provides evidence for a previously unknown oceanographic break which prevents gene flow between the Andaman Sea and the rest of the species' the Indo-Pacific distribution range. Although our sample size of populations in the Andaman Sea was lower compared to other study areas, we see similar patterns of genetic differentiation across both species. Future studies will be needed to explore the underlying causes of the observed high genetic differentiation and break in connectivity. Additionally, a larger sample size from the region will help capture the full spectrum of genetic diversity within the species' population and strengthen the findings of this study.

Further, connectivity of the Chagos and the Western IO populations may suggest that these populations could potentially replenish each other if one or more populations in the region was depleted. However, the gene flow between the Chagos and WIO is below the threshold which allows complete intermixing of the stocks and the Chagos stock is still distinct from the Western IO stock populations. Notably, even within the WIO, the population in the Persian Gulf is distinct from the rest of the WIO countries. Also, the demographic patterns for all the WIO populations and Chagos indicate severe declines in the populations in recent times requiring higher protections for these populations through cross-country and regional collaborative regulations. These findings underscore the urgent need to list grey reef and silvertip sharks under the Convention on the Conservation of Migratory Species of Wild Animals (CMS) and to implement binding protections that safeguard both species along their migratory corridors. In addition, satellite telemetry studies should be undertaken in the future to access the movement of the species across these ocean basins to understand behaviors and timing of the migration for both species in order to inform spatial and temporal protection and conservation measures for both species which are listed on CITES Appendix II.

The current study demonstrates through analyses of nucleotide diversity that silvertip populations throughout their Indo-Pacific distribution range had much lower nucleotide diversity values except for

the Pacific Ocean and Chagos populations which had relatively higher π values. Overall, these trends suggest extremely small population sizes and inbreeding within silvertip populations. This is alarming especially given the large movement boundaries of silvertip sharks (Jacoby et al. 2020), allowing them to interbreed across populations. The low genetic diversity suggests a severe decline in populations and lower effective population sizes across all populations resulting in inbreeding and low genetic diversity. Results presented here indicate a need for IUCN reassessment of the silvertip populations and enact urgent measures for the management and protection of the species throughout their distribution range.

Grey reef populations in the central IO and western IO have low diversity while the EIO and Pacific populations have higher diversity. The higher π values reflect a greater nucleotide diversity, thus indicating that these are genetically more diverse and healthier populations potentially resulting from larger effective population sizes, more historical gene flow, and/or the absence or lower frequency of genetic bottlenecks. In contrast, Chagos, the WIO and Andaman Sea populations have relatively lower π values (i.e., lower genetic diversity) indicating that these populations experienced lower effective population sizes, isolation and/or genetic bottlenecks. These observations are supported by our PSMC analyses, which show higher residual populations and a more gradual decline of grey reef sharks in the EIO and Pacific during the last glacial period ~100,000–11,700 years ago (Ludt and Rocha 2015), in contrast to steeper population declines in the western and central Indian Ocean. This pattern likely reflects region-specific environmental stability and habitat continuity through glacial cycles, as sea-level fluctuations and global cooling had uneven effects across ocean basins (Ludt and Rocha 2015). Notably, the Pacific appears to have served as a climatic refugium, for sperm whale populations, whose global genetic origin traces back to a Pacific refugium approximately 100,000 years ago, followed by expansion into the Atlantic (Morin et al. 2018). A similar pattern may have occurred in grey reef sharks, with populations in the Pacific and eastern Indian Ocean maintaining higher numbers throughout the glacial period.

Traceability of Fisheries and Trade

This study presents the most comprehensive genomic reference database to date for reef sharks, coupled with a novel population assignment framework capable of resolving regionally distinct stocks across the Indo-Pacific. Given the trophic impacts of predators in maintaining ecological balance, beyond tracking population structure and genetic health of sharks, the framework offers a means to infer the health of reef ecosystems in the Indo-Pacific. This is the first fine-scale, spatially explicit genomic reference database for any marine species, providing critical insight to guide both immediate and long-term conservation strategies for reef sharks and Indo-Pacific reef ecosystems. A comparable genomic framework developed for African elephants (Wasser et al. 2015; 2018), has revolutionized conservation enforcement, underscoring the potential impact of this approach in the marine realm.

Similar to the elephant database, the IO 'Reefshark Genomescape' presented here, allows for the assignment of fished grey reef sharks to their source populations, at a regional or local level, across the western, central, and eastern Indian Ocean, as well as the Pacific, using population-specific SNPs. Grey reef and silvertip sharks, are both frequently targeted in Indian Ocean fisheries (Bennett et al. 2022), and therefore, the species can serve as effective biological indicators of coral reef fisheries in the region. By enabling assignment of individual grey reef sharks to their source populations, this framework can reveal fishing hotspots, ecosystem targets, and instances of illegal, unreported, and unregulated fishing. These data would provide a powerful basis for localized, data-driven fisheries regulation, including targeted spatial closures and temporal protections.

The framework thus has practical applications for conservation, offering managers a powerful tool to assess which regions are overfished, which may still support current pressures, and which require urgent protection. Crucially, the genomics framework is designed to be adaptive, capable of integrating new data from ongoing monitoring of reef sharks and other key species in the food web, to improve sensitivity and resolution of the framework for increasingly targeted and effective management. By supporting evidence-based, region-specific decision-making, the framework has the potential to contributes to more sustainable and long-lasting conservation and fisheries management outcomes.

Both grey reef and silvertip sharks are listed under CITES Appendix II ("Appendices | CITES," n.d.), which restricts international trade of the species unless non-detriment findings are undertaken. By identifying local and regional population stocks, this framework enables the detection of illegal trade and supports the enforcement of existing fisheries and trade regulations. It strengthens capacity to identify instances of illegal fishing or trafficking, particularly in violation of CITES listings. Moreover, by revealing which regions are being disproportionately targeted, the framework allows for more precise regulation of non-compliant fishing actors and supports timely conservation interventions in vulnerable marine habitats.

CONCLUSION:

Our findings demonstrate that populations of both reef shark species are neither homogeneous nor panmictic, but instead comprise genetically distinct management units that must be recognized in conservation and fisheries policy. Isolated populations, such as those in the Chagos Archipelago and the Andaman Sea, are particularly vulnerable and warrant targeted protection. The current IUCN Red List assessment of *Endangered* for grey reef sharks (Ali et al. 2020) are strongly supported by our analyses of genomic diversity and demographic history, which indicate significant declines in genetic health and effective population size, especially in the Central and Western Indian Ocean. However, the findings of this study suggest that the silvertip shark is at a greater risk across its Indo-Pacific range than currently recognized, warranting a reassessment of its IUCN Red List status to a higher threat level, from its presently listed category as Vulnerable (Rigby, C.L. et al. 2023).

Beyond advancing fundamental biogeographic understanding, this study establishes the 'Reefshark Genomescape', the largest genome-wide reference database for any reef shark species to date and a novel population assignment framework with immediate applications for conservation and fisheries management. By generating population-level genetic baselines, we enable forensic tracing of shark products to their geographic source, offering a science-based tool for enforcing CITES trade restrictions, identifying fishing hotspots, instances of IUU fishing and guiding the creation of spatially explicit Fisheries Management Zones (FMZs). Analogous to the landmark genetic tracing of elephant ivory that reshaped wildlife crime enforcement in Africa, this study lays the foundation for similarly transformative regulatory impact in marine systems. In doing so, we address a long-standing challenge in marine conservation: the untraceability of fisheries. By linking fished individuals to their source populations, we identify ecosystems under pressure, reveal patterns of legal and illegal exploitation, and provide critical insights for the management of apex predators that structure coral reef ecosystems. This work also offers a scalable, incorruptible framework for monitoring biodiversity decline and enforcing sustainable fishing practices across national and regional boundaries.

In an era of unprecedented biodiversity loss and rapidly shifting ecological baselines, the integration of high-resolution genomics with applied conservation tools is not only timely, it is essential. Our study represents a significant advancement in marine conservation genomics, delivering both data and

practical solutions. It offers a model for species-wide monitoring, international cooperation, and ecosystem-level protection, essential for preserving the ecological integrity of coral reefs and the apex predators that maintain them.

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AUTHOR CONTRIBUTIONS:
Conceptualization: SJ., and BB. Sampling: SJ co-ordinated the sampling by co-authors across the study area and conducted sampling in Lakshadweep and in small part in Seychelles. GA made a significant contribution to coordinating and supporting of sampling and shipping of samples from Seychelles, Mozambique Channel and Indonesia. RS, BB collected samples in the Chagos archipelago. All authors contributed to aspects of fieldwork, data collection, and data formatting. Methodology: SJ with input from all authors. Investigation: SJ. Visualization: SJ. Funding acquisition: SJ. and BB. Project administration: SJ. and BB. Writing – original draft: SJ. Writing – review & editing: SJ., BB., and all authors.

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The authors declare no conflicts of interest.

DATA AVAILABILITY:
TBD on Dryad and Genbank

REFERENCES:
Alexander, David H., John Novembre, and Kenneth Lange. 2009. "Fast Model-Based Estimation of Ancestry in Unrelated Individuals." *Genome Research* 19 (9): 1655–64. https://doi.org/10.1101/gr.094052.109.

Ali, Ahmad Bin (IUCN SSC Shark Specialist, Colin (James Cook Simpfendorfer, Indonesian Institute of Sciences) Fahmi (Research Centre for Oceanography, et al. 2020. "Carcharhinus Amblyrhynchos. The IUCN Red List of Threatened Species 2020: E.T39365A173433550. Https://Dx.Doi.Org/10.2305/IUCN.UK.2020-3.RLTS.T39365A173433550.En. Accessed on 18 April 2023." *IUCN Red List of Threatened Species*, July 3. https://www.iucnredlist.org/en.

"Appendices | CITES." n.d. Accessed July 28, 2025. https://cites.org/eng/app/appendices.php.

Arrowsmith, Lucy M., Charan Kumar Paidi, Farukhkha Husenkha Bloch, et al. 2021. "First Insights Into the Horizontal Movements of Whale Sharks (Rhincodon Typus) in the Northern Arabian Sea." *Frontiers in Marine Science* 8 (July). https://doi.org/10.3389/fmars.2021.682730.

Belhabib, Dyhia, and Philippe Le Billon. 2020. "Editorial: Illegal Fishing as a Trans-National Crime." *Frontiers in Marine Science* 7. https://doi.org/10.3389/fmars.2020.00162.

Belhabib, Dyhia, and Philippe Le Billon. 2022. "Adjacency and Vessel Domestication as Enablers of Fish Crimes." *Frontiers in Marine Science* 9: 936174.

Bennett, Rhett, David Van Beuningen, Amie Bräutigam, et al. 2022. *Chondrichthyans of the Western Indian Ocean: Biodiversity, Fisheries and Trade, Management and Conservation*. Wildlife Conservation Society. https://doi.org/10.19121/2022.report.44805.

Bernt, Matthias, Alexander Donath, Frank Jühling, et al. 2013. “MITOS: Improved de Novo Metazoan Mitochondrial Genome Annotation.” *Molecular Phylogenetics and Evolution* 69 (2): 313–19. https://doi.org/10.1016/j.ympev.2012.08.023.

Bm, Wetherbee, Crow Gl, and Lowe Cg. 1997. “Distribution, Reproduction and Diet of the Gray Reef Shark Carcharhinus Amblyrhynchos in Hawaii.” *Marine Ecology Progress Series* 151 (May): 181–89. https://doi.org/10.3354/meps151181.

Bolger, Anthony M., Marc Lohse, and Bjoern Usadel. 2014. “Trimmomatic: A Flexible Trimmer for Illumina Sequence Data.” *Bioinformatics* 30 (15): 2114–20. https://doi.org/10.1093/bioinformatics/btu170.

Bond, Mark E., Hollie Booth, Akshay Tanna, et al. 2025. “Trade Regulations Drive Improved Global Shark and Ray Management.” *Marine Policy* 180 (October): 106733. https://doi.org/10.1016/j.marpol.2025.106733.

Bond, Mark E., Emily Tolentino, Sangeeta Mangubhai, and Lucy A. Howey. 2015. “Vertical and Horizontal Movements of a Silvertip Shark (Carcharhinus Albimarginatus) in the Fijian Archipelago.” *Animal Biotelemetry* 3 (1): 19. https://doi.org/10.1186/s40317-015-0055-6.

Chang, Christopher C. 2020. “Data Management and Summary Statistics with PLINK.” *Methods in Molecular Biology (Clifton, N.J.)* 2090: 49–65. https://doi.org/10.1007/978-1-0716-0199-0_3.

Chen, Kuan-Yu, Elizabeth A. Marschall, Michael G. Sovic, Anthony C. Fries, H. Lisle Gibbs, and Stuart A. Ludsin. 2018. “Assign: An r Package for Population Assignment Using Genetic, Non-Genetic, or Integrated Data in a Machine-Learning Framework.” *Methods in Ecology and Evolution* 9 (2): 439–46. https://doi.org/10.1111/2041-210X.12897.

Cortés, Enric. 2000. “Life History Patterns and Correlations in Sharks.” *Reviews in Fisheries Science* 8 (January): 299–344. https://doi.org/10.1080/10408340308951115.

Danecek, Petr, Adam Auton, Goncalo Abecasis, et al. 2011. “The Variant Call Format and VCFtools.” *Bioinformatics* 27 (15): 2156–58. https://doi.org/10.1093/bioinformatics/btr330.

Danecek, Petr, James K Bonfield, Jennifer Liddle, et al. 2021. “Twelve Years of SAMtools and BCFtools.” *GigaScience* 10 (2): giab008. https://doi.org/10.1093/gigascience/giab008.

Dulvy, Nicholas K., Sarah L. Fowler, John A. Musick, et al. 2014. “Extinction Risk and Conservation of the World’s Sharks and Rays.” *eLife* 3 (January): e00590. https://doi.org/10.7554/eLife.00590.

Dulvy, Nicholas K., Nathan Pacoureau, Cassandra L. Rigby, et al. 2021. “Overfishing Drives over One-Third of All Sharks and Rays toward a Global Extinction Crisis.” *Current Biology: CB* 31 (21): 4773-4787.e8. https://doi.org/10.1016/j.cub.2021.08.062.

Dulvy, Nicholas K., Colin A. Simpfendorfer, Lindsay N. K. Davidson, et al. 2017. “Challenges and Priorities in Shark and Ray Conservation.” *Current Biology* 27 (11): R565–72. https://doi.org/10.1016/j.cub.2017.04.038.

Dunn, Nicholas, Shaili Johri, David Curnick, et al. 2020. “Complete Mitochondrial Genome of the Gray Reef Shark, Carcharhinus Amblyrhynchos (Carcharhiniformes: Carcharhinidae).” *Mitochondrial DNA Part B* 5 (3): 2080–82. https://doi.org/10.1080/23802359.2020.1765208.

Espinoza, Mario, Michelle Heupel, Andrew Tobin, and Colin Simpfendorfer. 2014. “Residency Patterns and Movements of Grey Reef Sharks (Carcharhinus Amblyrhynchos) in Semi‐isolated Coral Reef Habitats.” *Marine Biology*, ahead of print, November 20. https://doi.org/10.1007/s00227-014-2572-x.

Fields, A. T., G. A. Fischer, S. K. H. Shea, H. Zhang, K. A. Feldheim, and D. D. Chapman. 2020. “DNA Zip-Coding: Identifying the Source Populations Supplying the International Trade of a Critically Endangered Coastal Shark.” *Animal Conservation* 23 (6): 670–78. https://doi.org/10.1111/acv.12585.

Fields, Andrew T., Debra L. Abercrombie, Rowena Eng, Kevin Feldheim, and Demian D. Chapman. 2015. "A Novel Mini-DNA Barcoding Assay to Identify Processed Fins from Internationally Protected Shark Species." *PLOS ONE* 10 (2): e0114844. https://doi.org/10.1371/journal.pone.0114844.

Friedlander, Alan M., Jennifer E. Caselle, Enric Ballesteros, Eric K. Brown, Alan Turchik, and Enric Sala. 2014. "The Real Bounty: Marine Biodiversity in the Pitcairn Islands." *PLoS ONE* 9 (6): e100142. https://doi.org/10.1371/journal.pone.0100142.

Graham, Nicholas A. J., Tim R. McClanahan, M. Aaron MacNeil, et al. 2017. "Human Disruption of Coral Reef Trophic Structure." *Current Biology* 27 (2): 231–36. https://doi.org/10.1016/j.cub.2016.10.062.

Graham, Nicholas A. J., Mark D. Spalding, and Charles R. C. Sheppard. 2010. "Reef Shark Declines in Remote Atolls Highlight the Need for Multi-Faceted Conservation Action." *Aquatic Conservation: Marine and Freshwater Ecosystems* 20 (5): 543–48. https://doi.org/10.1002/aqc.1116.

Green, M. E., S. A. Appleyard, W. White, S. Tracey, F. Devloo-Delva, and J. R. Ovenden. 2019. "Novel Multimarker Comparisons Address the Genetic Population Structure of Silvertip Sharks (Carcharhinus Albimarginatus)." *Marine and Freshwater Research* 70 (7): 1007–19. https://doi.org/10.1071/MF18296.

Hays, Graeme C., Jeanne A. Mortimer, Daniel Ierodiaconou, and Nicole Esteban. 2014. "Use of Long-Distance Migration Patterns of an Endangered Species to Inform Conservation Planning for the World's Largest Marine Protected Area." *Conservation Biology* 28 (6): 1636–44. https://doi.org/10.1111/cobi.12325.

Jabado, Rima W., Peter M. Kyne, Riley A. Pollom, et al. 2018. "Troubled Waters: Threats and Extinction Risk of the Sharks, Rays and Chimaeras of the Arabian Sea and Adjacent Waters." *Fish and Fisheries* 19 (6): 1043–62. https://doi.org/10.1111/faf.12311.

Jacoby, David M. P., Francesco Ferretti, Robin Freeman, et al. 2020. "Shark Movement Strategies Influence Poaching Risk and Can Guide Enforcement Decisions in a Large, Remote Marine Protected Area." *Journal of Applied Ecology* n/a (n/a). https://doi.org/10.1111/1365-2664.13654.

Johri, Shaili, Nicholas Dunn, Taylor K. Chapple, et al. 2020. "Mitochondrial Genome of the Silvertip Shark, Carcharhinus Albimarginatus, from the British Indian Ocean Territory." *Mitochondrial DNA Part B* 5 (3): 2085–86. https://doi.org/10.1080/23802359.2020.1765210.

Kato, S., and Carvallo Hernandez A. 1967. "Shark Tagging in the Eastern Pacific Ocean, 1962–1965." *In Gilbert, P.W.; Mathewson, R.F. & Rail, D.P. (Eds.). Sharks, Skates, and Rays. Baltimore: The Johns Hopkins Press*., 93-109.

Keenan, Kevin, Philip McGinnity, Tom F. Cross, Walter W. Crozier, and Paulo A. Prodöhl. 2013. "diveRsity: An R Package for the Estimation and Exploration of Population Genetics Parameters and Their Associated Errors." *Methods in Ecology and Evolution* 4 (8): 782–88. https://doi.org/10.1111/2041-210X.12067.

Korneliussen, Thorfinn Sand, Anders Albrechtsen, and Rasmus Nielsen. 2014. "ANGSD: Analysis of Next Generation Sequencing Data." *BMC Bioinformatics* 15 (1): 356. https://doi.org/10.1186/s12859-014-0356-4.

Korunes, Katharine L., and Kieran Samuk. 2021. "Pixy: Unbiased Estimation of Nucleotide Diversity and Divergence in the Presence of Missing Data." *Molecular Ecology Resources* 21 (4): 1359–68. https://doi.org/10.1111/1755-0998.13326.

Leigh, Jessica W., and David Bryant. 2015. "Popart: Full-Feature Software for Haplotype Network Construction." *Methods in Ecology and Evolution* 6 (9): 1110–16. https://doi.org/10.1111/2041-210X.12410.

Lesturgie, Pierre, Serge Planes, and Stefano Mona. 2022. “Coalescence Times, Life History Traits and Conservation Concerns: An Example from Four Coastal Shark Species from the Indo-Pacific.” *Molecular Ecology Resources* 22 (2): 554–66. https://doi.org/10.1111/1755-0998.13487.

Li, Heng, and Richard Durbin. 2009. “Fast and Accurate Short Read Alignment with Burrows–Wheeler Transform.” *Bioinformatics* 25 (14): 1754–60. https://doi.org/10.1093/bioinformatics/btp324.

Li, Heng, Bob Handsaker, Alec Wysoker, et al. 2009. “The Sequence Alignment/Map Format and SAMtools.” *Bioinformatics (Oxford, England)* 25 (16): 2078–79. https://doi.org/10.1093/bioinformatics/btp352.

Ludt, William B., and Luiz A. Rocha. 2015. “Shifting Seas: The Impacts of Pleistocene Sea-Level Fluctuations on the Evolution of Tropical Marine Taxa.” *Journal of Biogeography* 42 (1): 25–38. https://doi.org/10.1111/jbi.12416.

Maisano Delser, Pierpaolo, Shannon Corrigan, Drew Duckett, et al. 2019. “Demographic Inferences after a Range Expansion Can Be Biased: The Test Case of the Blacktip Reef Shark (Carcharhinus Melanopterus).” *Heredity* 122 (6): 759–69. https://doi.org/10.1038/s41437-018-0164-0.

Momigliano, P., R. Harcourt, W. D. Robbins, et al. 2017. “Genetic Structure and Signatures of Selection in Grey Reef Sharks (Carcharhinus Amblyrhynchos).” *Heredity* 119 (3): 3. https://doi.org/10.1038/hdy.2017.21.

Morin, Phillip A., Andrew D. Foote, Charles Scott Baker, et al. 2018. “Demography or Selection on Linked Cultural Traits or Genes? Investigating the Driver of Low mtDNA Diversity in the Sperm Whale Using Complementary Mitochondrial and Nuclear Genome Analyses.” *Molecular Ecology* 27 (11): 2604–19. https://doi.org/10.1111/mec.14698.

Narasimhan, Vagheesh, Petr Danecek, Aylwyn Scally, Yali Xue, Chris Tyler-Smith, and Richard Durbin. 2016. “BCFtools/RoH: A Hidden Markov Model Approach for Detecting Autozygosity from next-Generation Sequencing Data.” *Bioinformatics (Oxford, England)* 32 (11): 1749–51. https://doi.org/10.1093/bioinformatics/btw044.

Okafor-Yarwood, Ifesinachi, Nelly I. Kadagi, Dyhia Belhabib, and Edward H. Allison. 2022. “Survival of the Richest, Not the Fittest: How Attempts to Improve Governance Impact African Small-Scale Marine Fisheries.” *Marine Policy* 135 (January): 104847. https://doi.org/10.1016/j.marpol.2021.104847.

Paolo, Fernando S., David Kroodsma, Jennifer Raynor, et al. 2024. “Satellite Mapping Reveals Extensive Industrial Activity at Sea.” *Nature* 625 (7993): 85–91. https://doi.org/10.1038/s41586-023-06825-8.

Pazmiño, Diana A., Gregory E. Maes, Colin A. Simpfendorfer, Pelayo Salinas-de-León, and Lynne van Herwerden. 2017. “Genome-Wide SNPs Reveal Low Effective Population Size within Confined Management Units of the Highly Vagile Galapagos Shark (<Emphasis Type="Italic">Carcharhinus Galapagensis</Emphasis>).” *Conservation Genetics* 18 (5): 1151–63. https://doi.org/10.1007/s10592-017-0967-1.

Pazmiño, Diana, Gregory Maes, Madeline Green, et al. 2018. “Strong Trans-Pacific Break and Local Conservation Units in the Galapagos Shark (Carcharhinus Galapagensis) Revealed by Genome-Wide Cytonuclear Markers.” *Heredity* 120 (January). https://doi.org/10.1038/s41437-017-0025-2.

Pembleton, Luke W., Noel O. I. Cogan, and John W. Forster. 2013. “StAMPP: An R Package for Calculation of Genetic Differentiation and Structure of Mixed-Ploidy Level Populations.” *Molecular Ecology Resources* 13 (5): 946–52. https://doi.org/10.1111/1755-0998.12129.

“Picard Tools - By Broad Institute.” n.d. Accessed July 28, 2025. https://broadinstitute.github.io/picard/.

Plaisance, Laetitia, M. Julian Caley, Russell E. Brainard, and Nancy Knowlton. 2011. “The Diversity of Coral Reefs: What Are We Missing?” *PLOS ONE* 6 (10): e25026. https://doi.org/10.1371/journal.pone.0025026.

Pollom, Riley A., Jessica Cheok, Nathan Pacoureau, et al. 2024. “Overfishing and Climate Change Elevate Extinction Risk of Endemic Sharks and Rays in the Southwest Indian Ocean Hotspot.” *PLOS ONE* 19 (9): e0306813. https://doi.org/10.1371/journal.pone.0306813.

Rigby, C.L., Charles, R., Espinoza, M., et al. 2023. “Carcharhinus Albimarginatus: The IUCN Red List of Threatened Species 2024.” June 8. https://doi.org/10.2305/IUCN.UK.2024-1.RLTS.T161526A124499982.en.

Sandin, Stuart A., Jennifer E. Smith, Edward E. DeMartini, et al. 2008. “Baselines and Degradation of Coral Reefs in the Northern Line Islands.” *PLOS ONE* 3 (2): e1548. https://doi.org/10.1371/journal.pone.0001548.

Sant, Glenn, Sarah Fowler, Nicola Okes, and Amie Brautigam. 2021. *Conservation, Fisheries, Trade and Management Status of CITES-Listed Sharks.* https://doi.org/10.19217/skr607.

Schiffels, Stephan, and Richard Durbin. 2014. “Inferring Human Population Size and Separation History from Multiple Genome Sequences.” *Nature Genetics* 46 (8): 8. https://doi.org/10.1038/ng.3015.

Sequeira, Ana M. M., Jorge P. Rodríguez, Sarah A. Marley, et al. 2025. “Global Tracking of Marine Megafauna Space Use Reveals How to Achieve Conservation Targets.” *Science* 388 (6751): 1086–97. https://doi.org/10.1126/science.adl0239.

Sherman, C. Samantha, Colin A. Simpfendorfer, Nathan Pacoureau, et al. 2023. “Half a Century of Rising Extinction Risk of Coral Reef Sharks and Rays.” *Nature Communications* 14 (1): 15. https://doi.org/10.1038/s41467-022-35091-x.

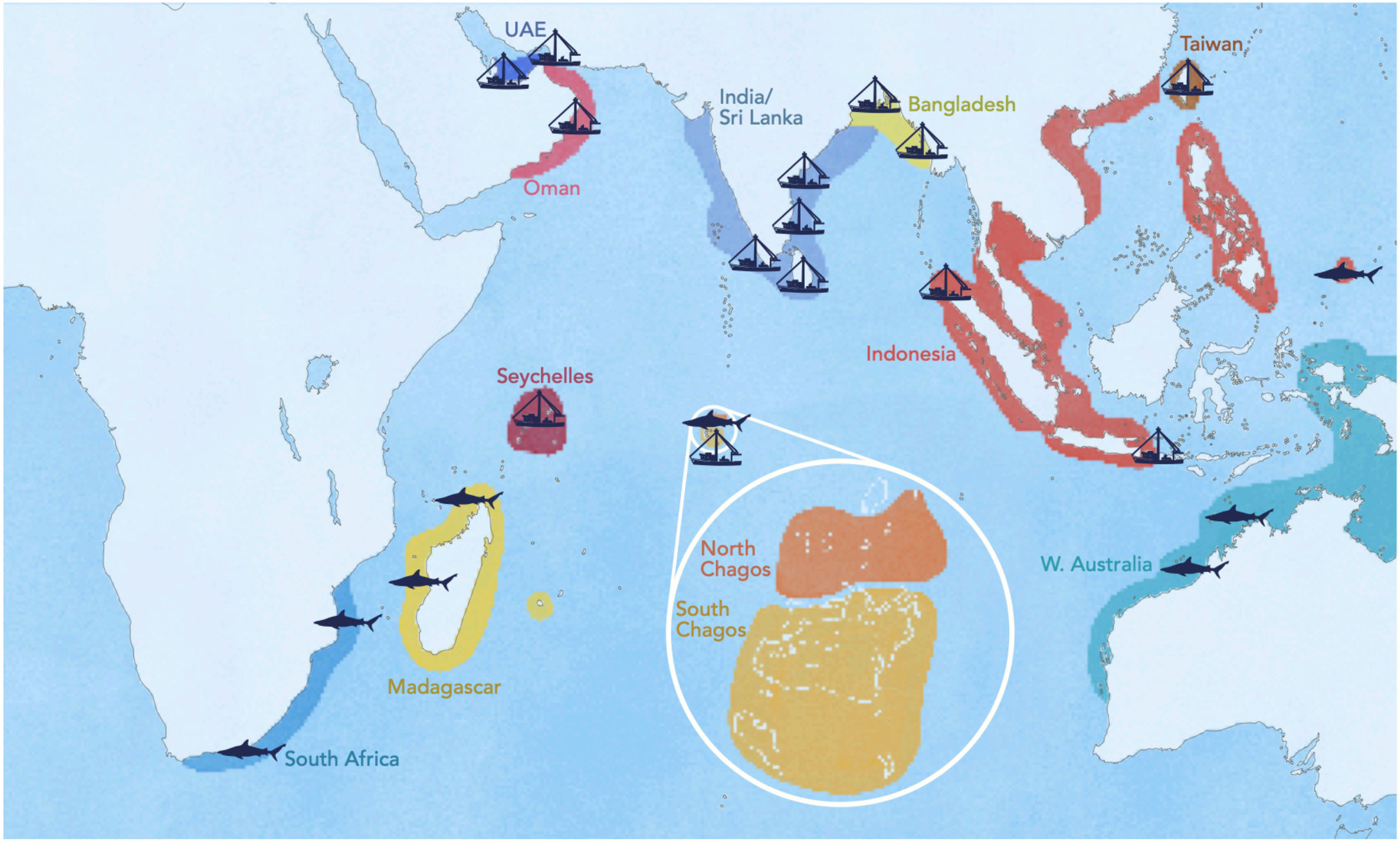
Wallace, Bryan P., Andrew D. DiMatteo, Brendan J. Hurley, et al. 2010. “Regional Management Units for Marine Turtles: A Novel Framework for Prioritizing Conservation and Research across Multiple Scales.” *PLoS ONE* 5 (12): e15465. https://doi.org/10.1371/journal.pone.0015465.

Wasser, S. K., L. Brown, C. Mailand, et al. 2015. “Genetic Assignment of Large Seizures of Elephant Ivory Reveals Africa’s Major Poaching Hotspots.” *Science* 349 (6243): 84–87. https://doi.org/10.1126/science.aaa2457.

Wasser, Samuel K., Amy Torkelson, Misa Winters, et al. 2018. “Combating Transnational Organized Crime by Linking Multiple Large Ivory Seizures to the Same Dealer.” Research Article. *Science Advances* 4 (9): eaat0625. https://doi.org/10.1126/sciadv.aat0625.

Williamson, Michael J., Emma J. Tebbs, Terence P. Dawson, et al. 2021. “Analysing Detection Gaps in Acoustic Telemetry Data to Infer Differential Movement Patterns in Fish.” *Ecology and Evolution* 11 (6): 2717–30. https://doi.org/10.1002/ece3.7226.

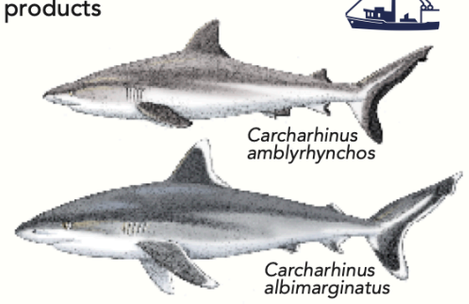
Infographic



1. SAMPLING LOCATIONS

Sampling for local populations

Sampling for illegal fishing products



2. POPULATION STRUCTURE & GENETIC SIGNATURES

	1 5 10 15 20
Reference	**ATGCGCATCCTACGCATGGG**
Madagascar	ATGCGCATCC**G**ACGCATGGG
Seychelles	AT**T**CGCATCC**G**ACGCATGGG
India/Sri Lanka	ATGCGCATCC**A**ACGCATGGG
North Chagos	A**A**GCGCA**G**CCTACGCAT**C**GG
Indonesia	ATGCGCATCCTACGCGTGGG
W. Australia	ATGCGCATC**A**TACGC**G**TGGG

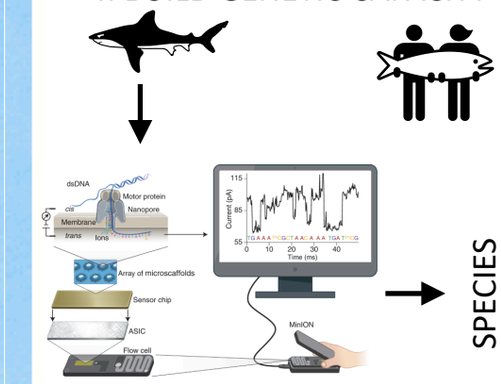
3. COMPARISON WITH GENETIC SIGNATURES OF FISHED SHARKS

	1 5 10 15 20
Madagascar	ATGCGCATCC**G**ACGCATGGG
Illegally fished	ATGCGCATCC**G**ACGCATGGG

ID of targeted populations

ID of nations participating in illegal fishing

4. BUILD GENETIC CAPACITY



Reference Populations

Reference Sample

Fished Sharks

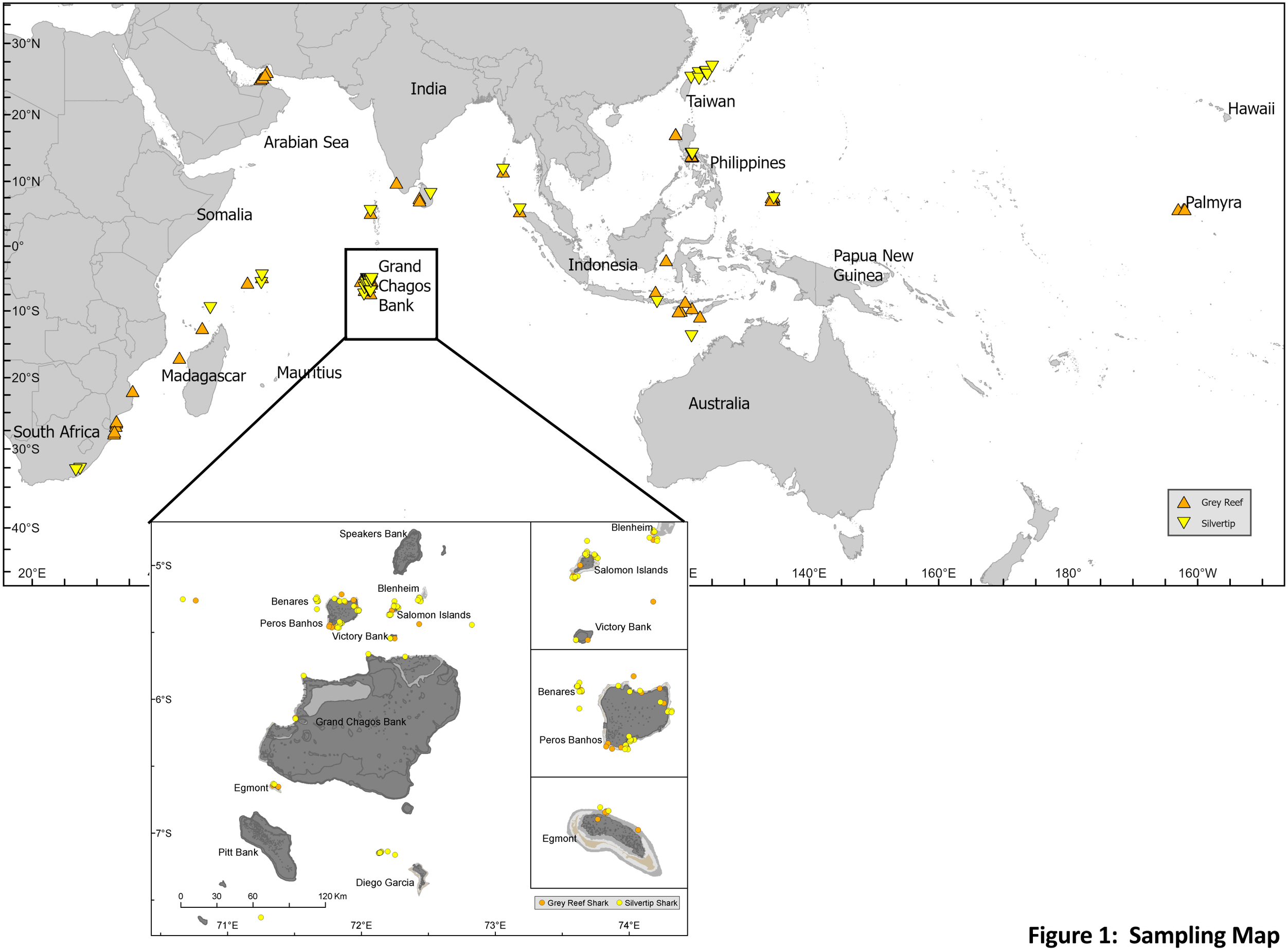


Figure 1: Sampling Map

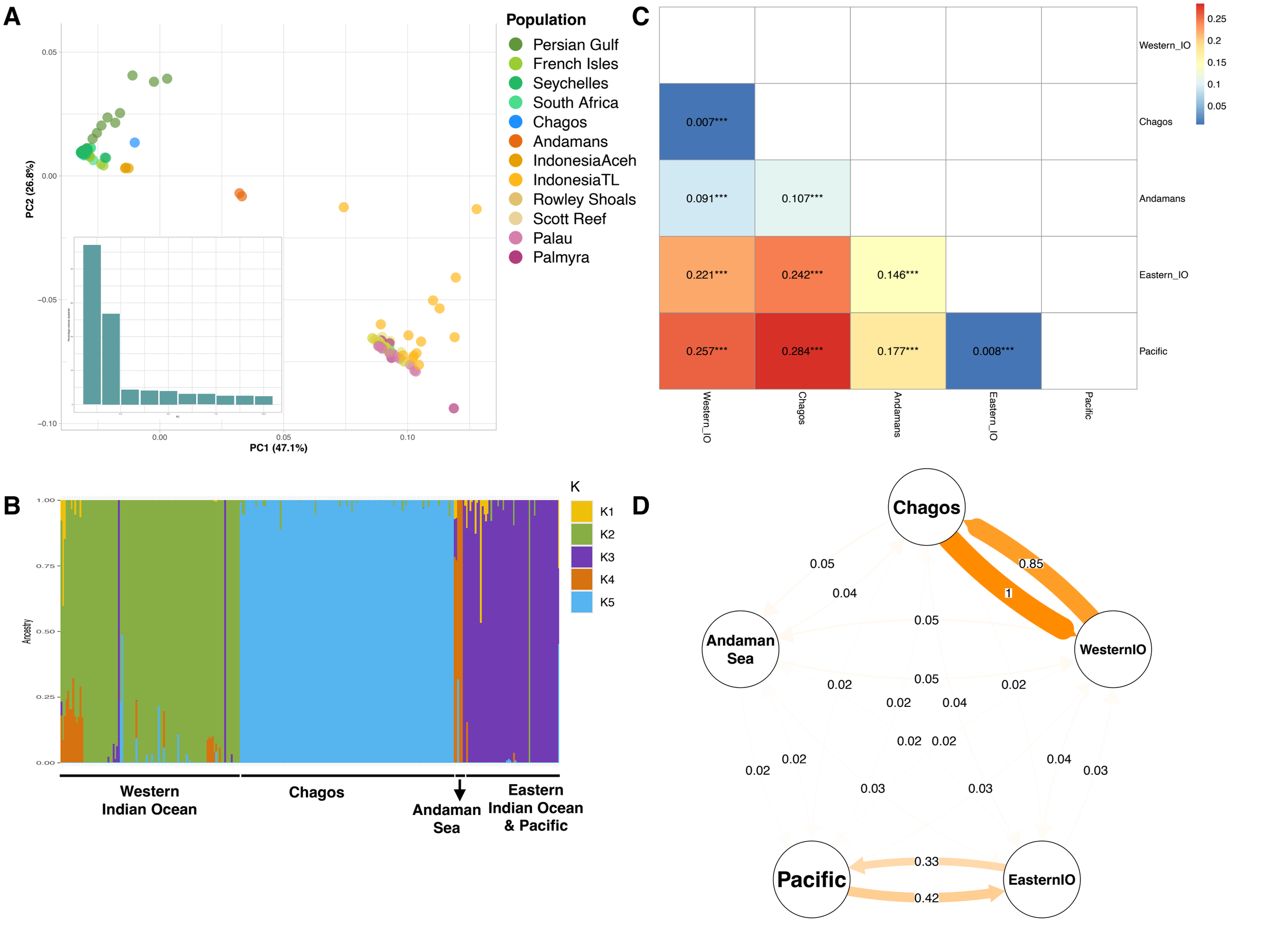
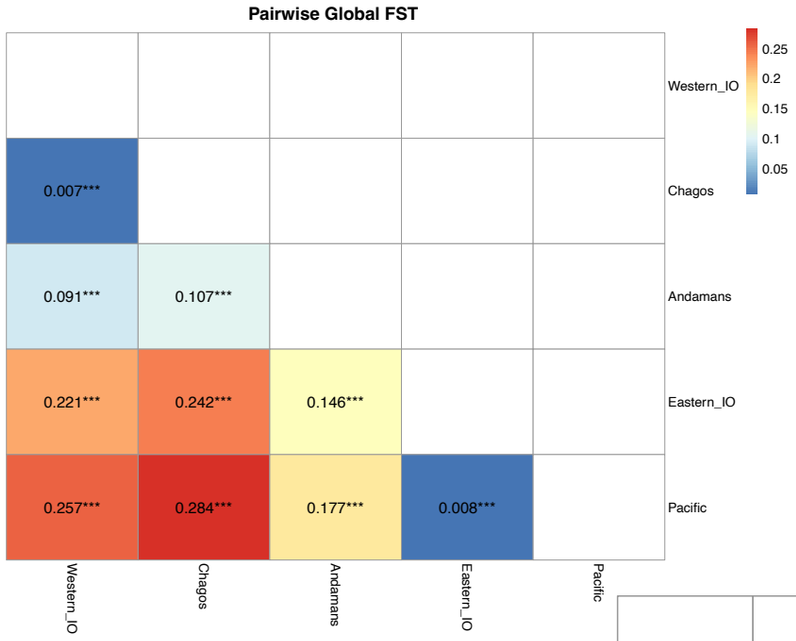


Figure 3: PopulationStructure_Grey Reef Shark: A) PCA, B) Admixture, C) Group Fst, D) Gene Flow E) Mapped Population Clusters



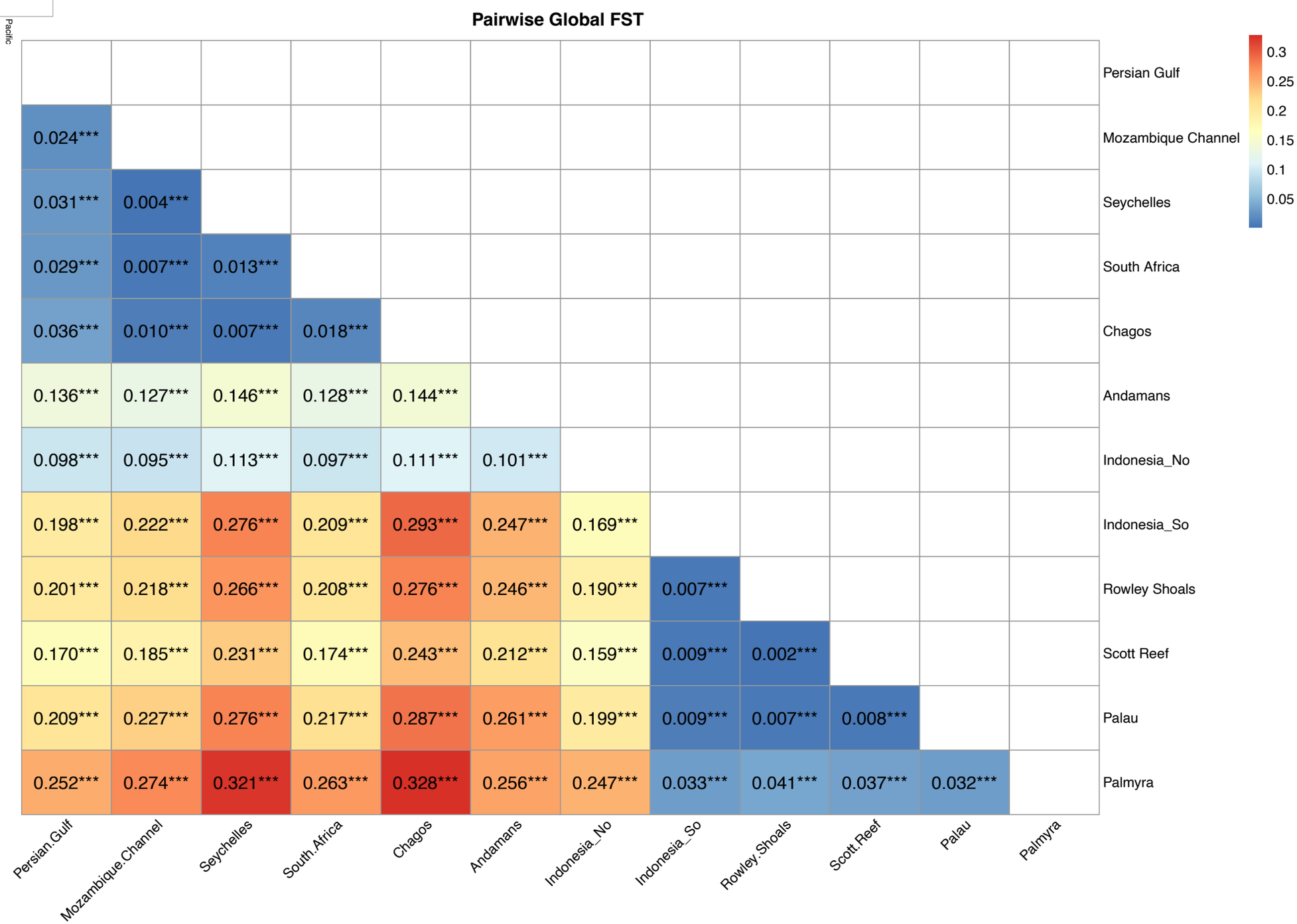
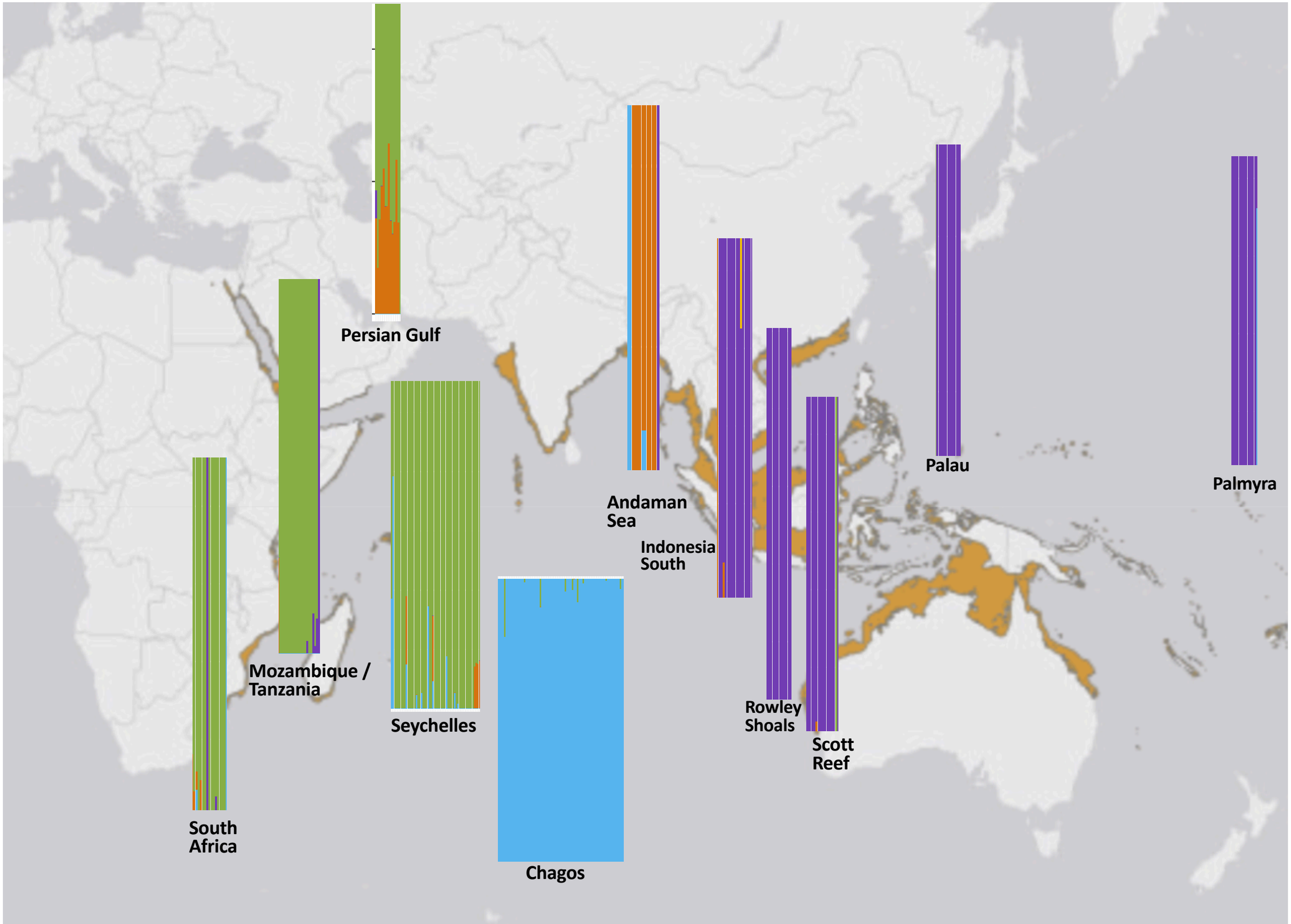
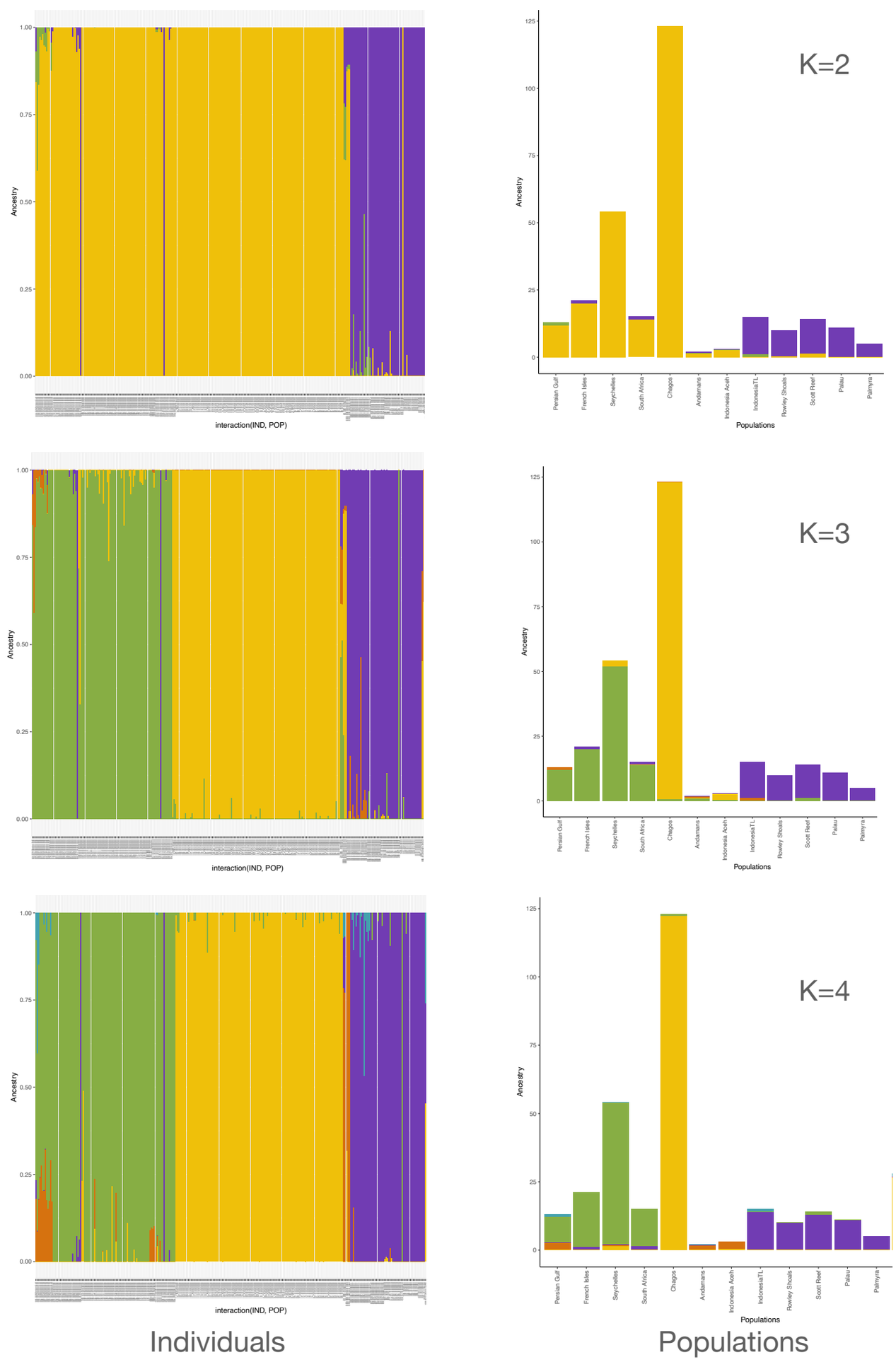


Figure 5: Fst_GRS

Figure 3E: Mapped Population Clusters in Grey Reef Sharks In the Indo-Pacific

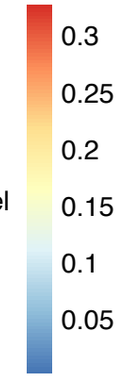




Supplementary Figure 1: Admixture_Grey Reef Shark

Population Structure in Grey Reef Sharks

	Persian.Gulf	Mozambique.Channel	Seychelles	South.Africa	Chagos	Andamans	Indonesia_No	Indonesia_So	Rowley.Shoals	Scott.Reef	Palau	Palmyra
Persian Gulf												
Mozambique Channel	0.024***											
Seychelles	0.031***	0.004***										
South Africa	0.029***	0.007***	0.013***									
Chagos	0.036***	0.010***	0.007***	0.018***								
Andamans	0.136***	0.127***	0.146***	0.128***	0.144***							
Indonesia_No	0.098***	0.095***	0.113***	0.097***	0.111***	0.101***						
Indonesia_So	0.198***	0.222***	0.276***	0.209***	0.293***	0.247***	0.169***					
Rowley Shoals	0.201***	0.218***	0.266***	0.208***	0.276***	0.246***	0.190***	0.007***				
Scott Reef	0.170***	0.185***	0.231***	0.174***	0.243***	0.212***	0.159***	0.009***	0.002***			
Palau	0.209***	0.227***	0.276***	0.217***	0.287***	0.261***	0.199***	0.009***	0.007***	0.008***		
Palmyra	0.252***	0.274***	0.321***	0.263***	0.328***	0.256***	0.247***	0.033***	0.041***	0.037***	0.032***	



Supplementary Figure 2: Populationwise_Fst_Grey Reef Shark

Table 2: *C. amblyrhynchos* group pairwise FST_matrix

GRSgroup_pairwise_FST_matrix					
	Western_IO	**Chagos**	**Andaman Sea**	**Eastern_IO**	**Pacific**
Western_IO	NA	NA	NA	NA	NA
Chagos	0.00728	NA	NA	NA	NA
Andamans	0.09088	0.10657	NA	NA	NA
Eastern_IO	0.22129	0.24241	0.14583	NA	NA
Pacific	0.25748	0.28353	0.17735	0.00819	NA

Table 3: *C. amblyrhynchos* population pairwise FST_matrix

GRSpopulation_pairwise_FST_matrix												
	Chagos	**South Africa**	**Scott Reef**	**Palmyra**	**Palau**	**Persian Gulf**	**Rowley Shoals**	**Mozambique Channel**	**Seychelles**	**Andamans**	**Indonesia_So**	**Indonesia_No**
Chagos	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
South Africa	0.0181	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Scott Reef	0.2428	0.17412	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Palmyra	0.3277	0.26291	0.03692	NA	NA	NA	NA	NA	NA	NA	NA	NA
Palau	0.2872	0.21657	0.00788	0.0322	NA	NA	NA	NA	NA	NA	NA	NA
Persian Gulf	0.0358	0.02873	0.17049	0.25163	0.2095	NA	NA	NA	NA	NA	NA	NA
Rowley Shoals	0.2764	0.20766	0.00224	0.04079	0.0074	0.20089	NA	NA	NA	NA	NA	NA
Mozambique Channel	0.0098	0.0075	0.18482	0.27362	0.2273	0.02426	0.21796	NA	NA	NA	NA	NA
Seychelles	0.0072	0.01293	0.23094	0.32136	0.2763	0.03112	0.26639	0.00406	NA	NA	NA	NA
Andamans	0.144	0.12763	0.21212	0.25638	0.2611	0.13577	0.24558	0.12728	0.14611	NA	NA	NA
Indonesia_So	0.2932	0.20885	0.0087	0.03284	0.0093	0.19834	0.00654	0.22244	0.27608	0.24675	NA	NA
Indonesia_No	0.1111	0.09744	0.15937	0.24716	0.1987	0.09838	0.18963	0.09535	0.11309	0.1008	0.16856	NA

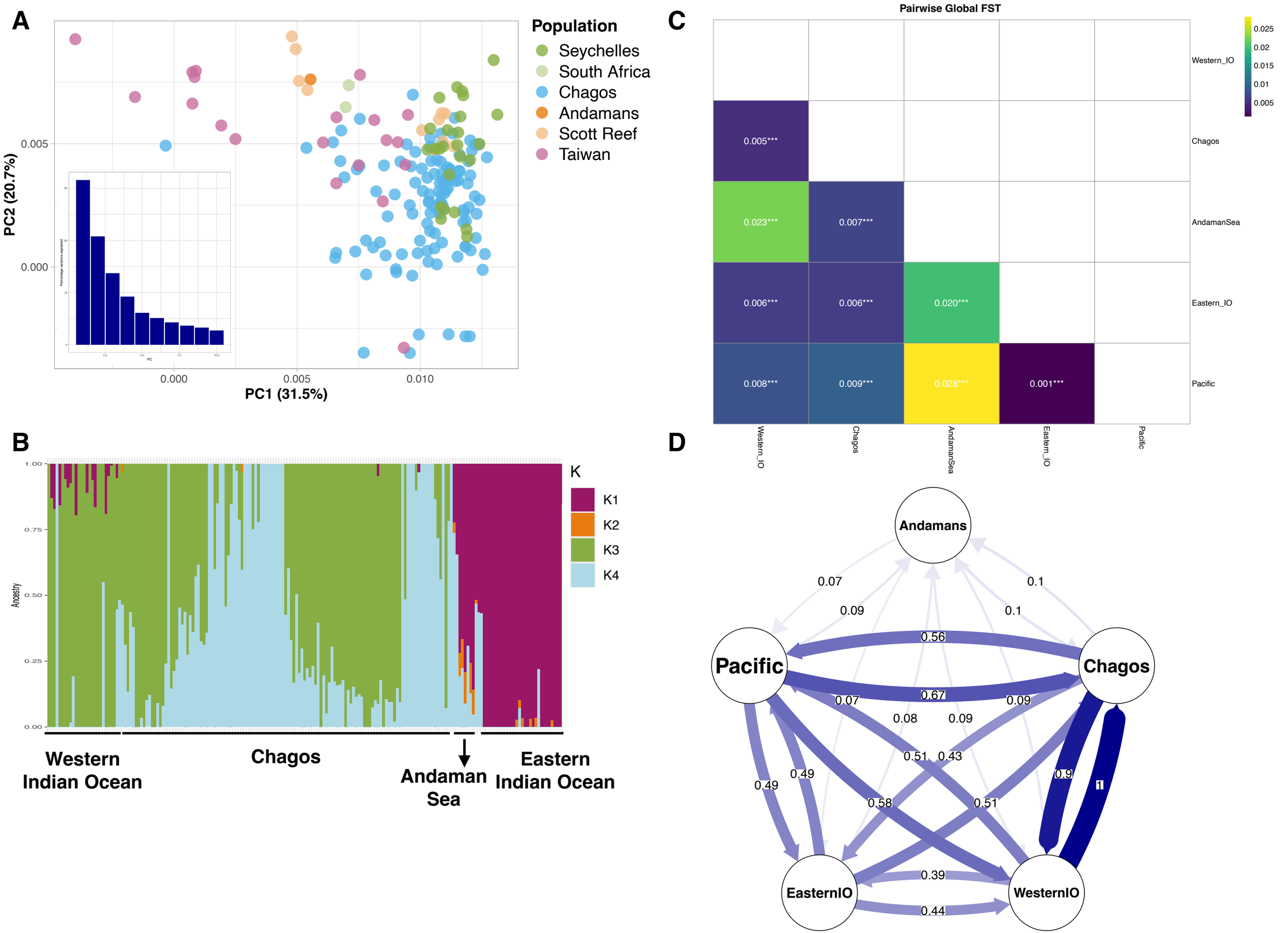
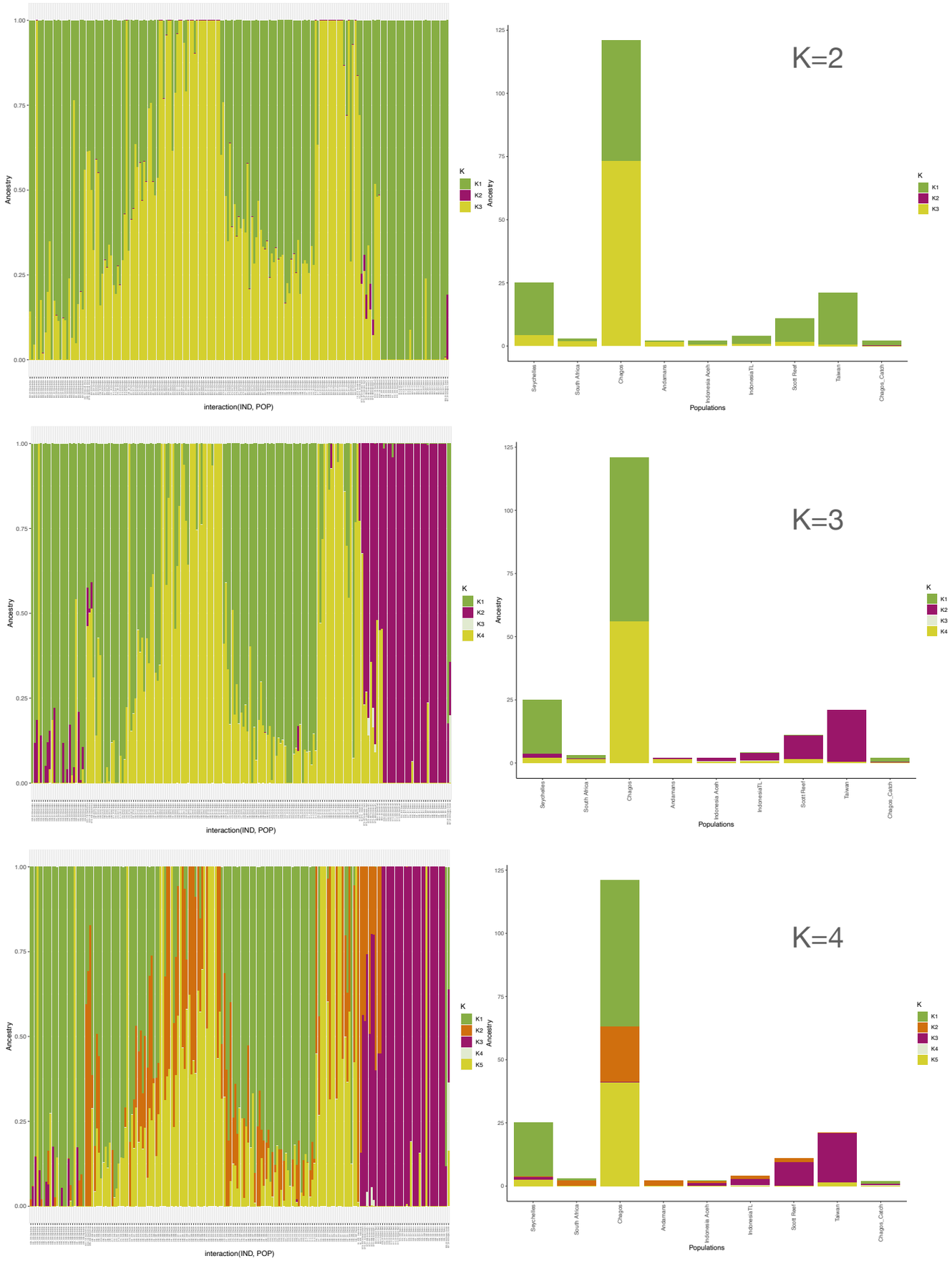
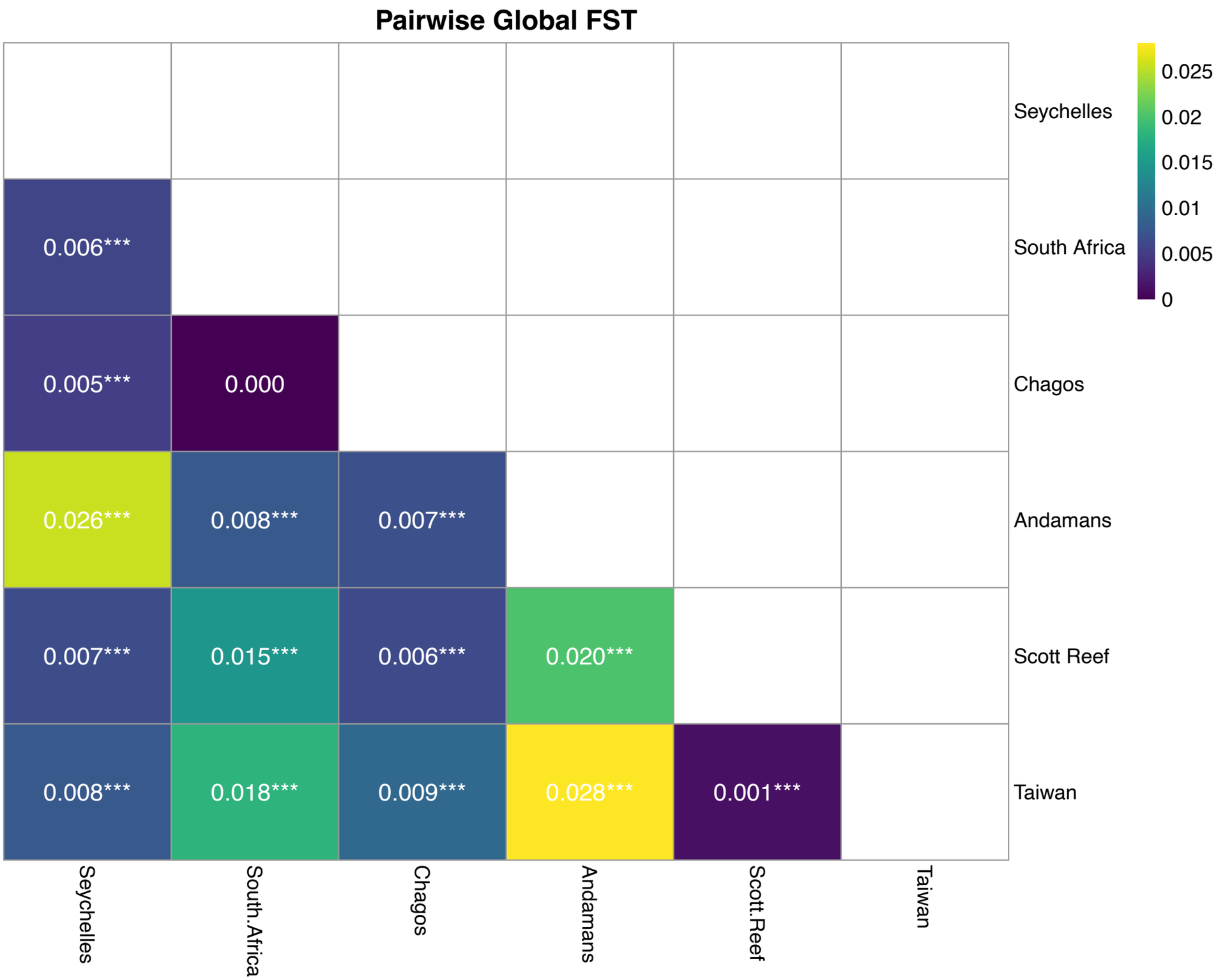


Figure 4: PopulationStructure_ Silvertip Sharks A) PCA, B) Admixture, C) Group Fst, D) Gene Flow



Supplementary Figure 3: Admixture_ Silvertip Sharks



Supplementary Figure 4: Populationwise_Fst_ Silvertip Sharks

Table 4: *C. albimarginatus* group pairwise FST_matrix

STSgroup_pairwise_FST_matrix					
	Chagos	**Western_IO**	**Andaman Sea**	**Eastern_IO**	**Pacific**
Chagos	NA	NA	NA	NA	NA
Western_IO	0.00495	NA	NA	NA	NA
Andaman Sea	0.00685	0.02264	NA	NA	NA
Eastern_IO	0.00632	0.00635	0.02003	NA	NA
Pacific	0.0094	0.00805	0.02808	0.00132	NA

Table 5: *C. albimarginatus* population pairwise FST_matrix

STSpopulation_pairwise_FST_matrix						
	Seychelles	**South Africa**	**Chagos**	**Andamans**	**Scott Reef**	**Taiwan**
Seychelles	NA	NA	NA	NA	NA	NA
South Africa	0.01637	NA	NA	NA	NA	NA
Chagos	0.00521	0.00498	NA	NA	NA	NA
Andamans	0.02582	0.02289	0.0069	NA	NA	NA
Scott Reef	0.00673	0.02189	0.0063	0.02003	NA	NA
Taiwan	0.00779	0.02696	0.0094	0.02808	0.00132	NA

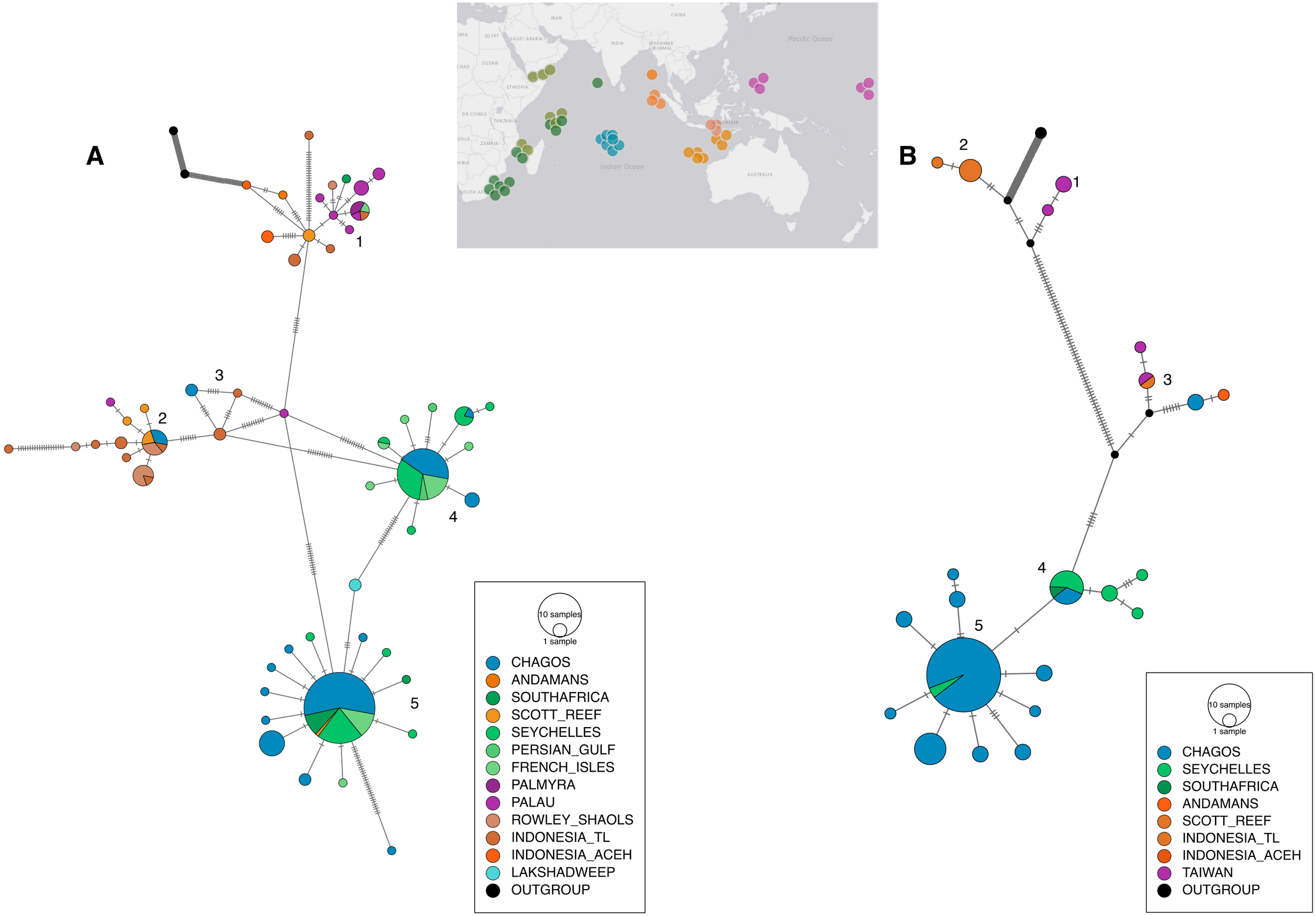


Figure 2: Mitochondrial Haplotype Network- A) Grey Reef and B) Silvertip Sharks

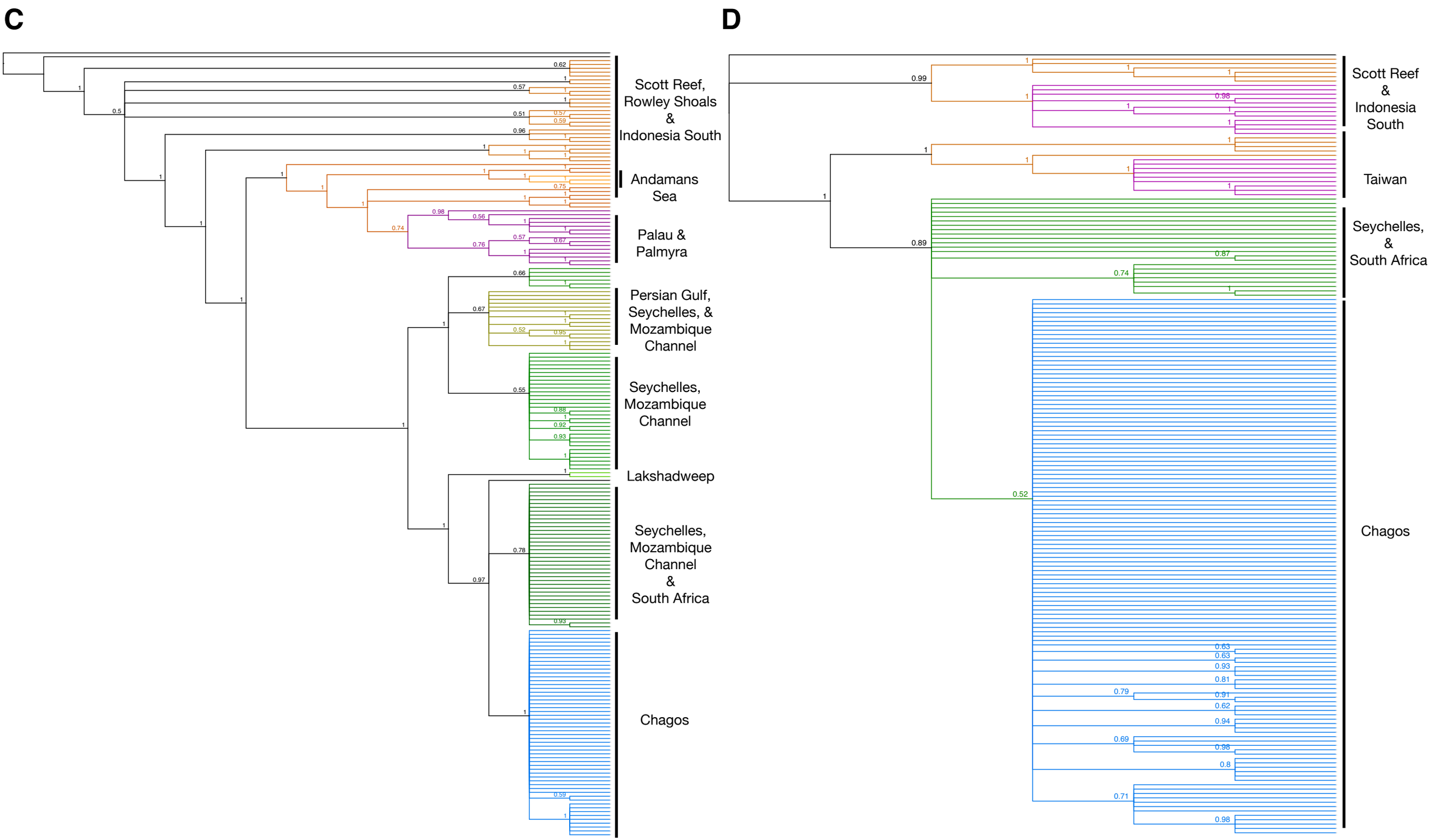


Figure 2: Mitochondrial Genome Phylogeny- C) Grey Reef and D) Silvertip Sharks

Table 1: Mitochondrial Haplotype Network Statistics

Species	Variable Sites	Parsimony informative sites	Number of haplotypes	Nucleotide diversity (π)	Phi_ST	Pvalue	Tajima's D
C. amblyrhynchos	1040	129	19	0.00306594	0.56472	0.001 ***	NS
C. albimarginatus	1464	102	13	0.0012143	NA	<0.001 ***	NS

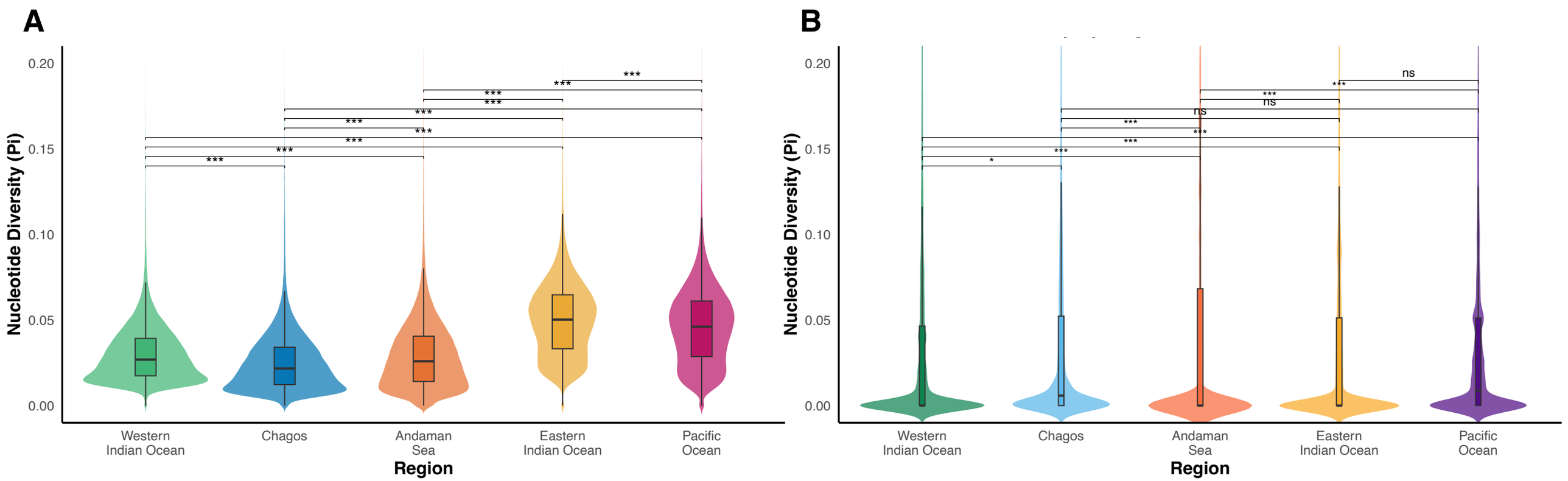


Figure 5: Nucleotide Diversity by Population Group A) Grey Reef Sharks, B) Silvertip Sharks

Table 6: Nucleotide Diversity

GRSavg_pi_per_grp				STSavg_pi_per_grp		
pop	**mean_pi**	**number of sites**		**pop**	**mean_pi**	**number of sites**
Western Indian Ocean	0.03054663	203833		**Western Indian Ocean**	0.001	289045
Chagos	0.02541538	203833		**Chagos**	0.0064	289045
Andaman Sea	0.03000888	203833		**Andaman Sea**	0.001	289045
Eastern Indian Ocean	0.05084639	203833		**Eastern Indian Ocean**	0.001	289045
Pacific Ocean	0.04674426	203833		**Pacific Ocean**	0.01	289045

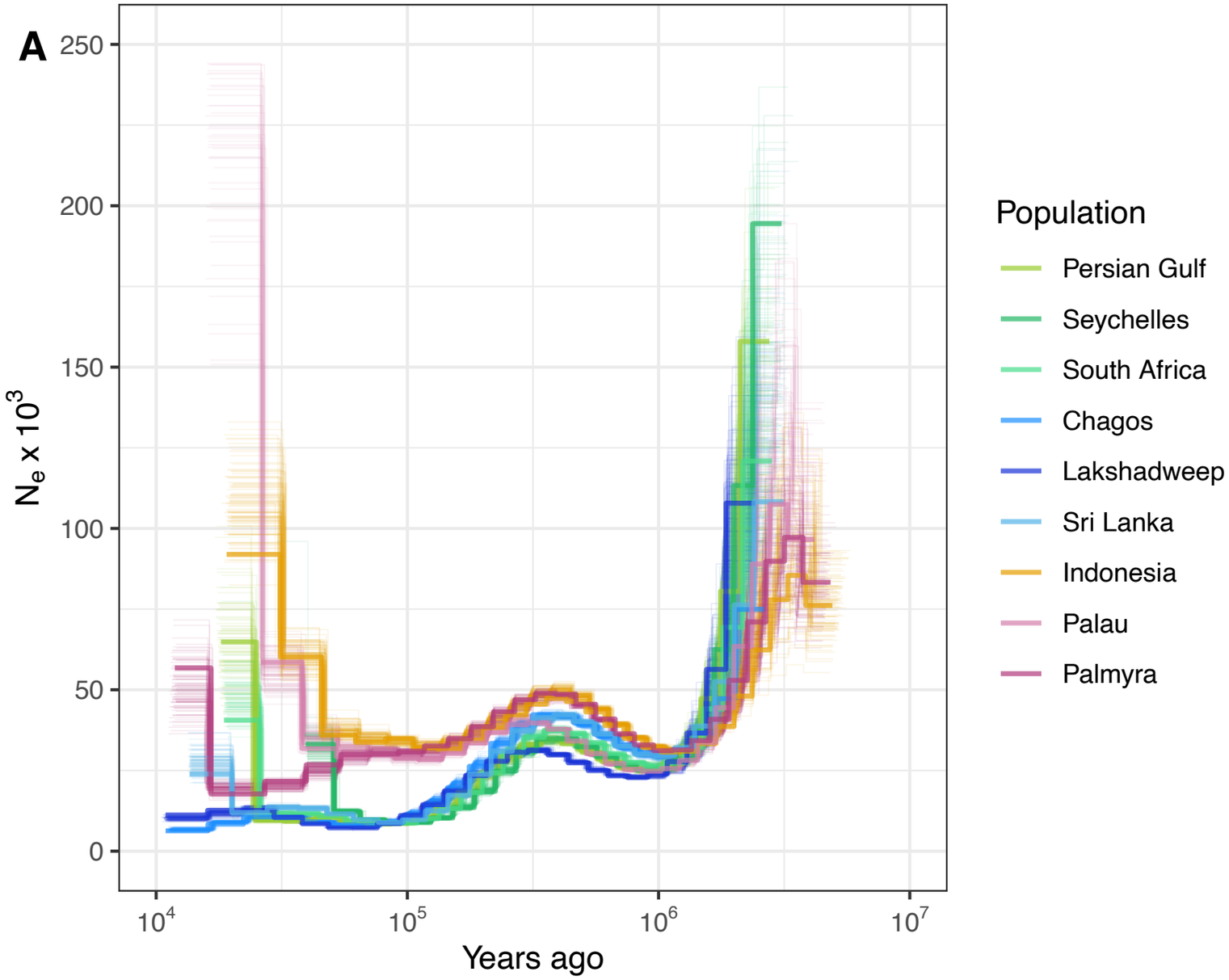
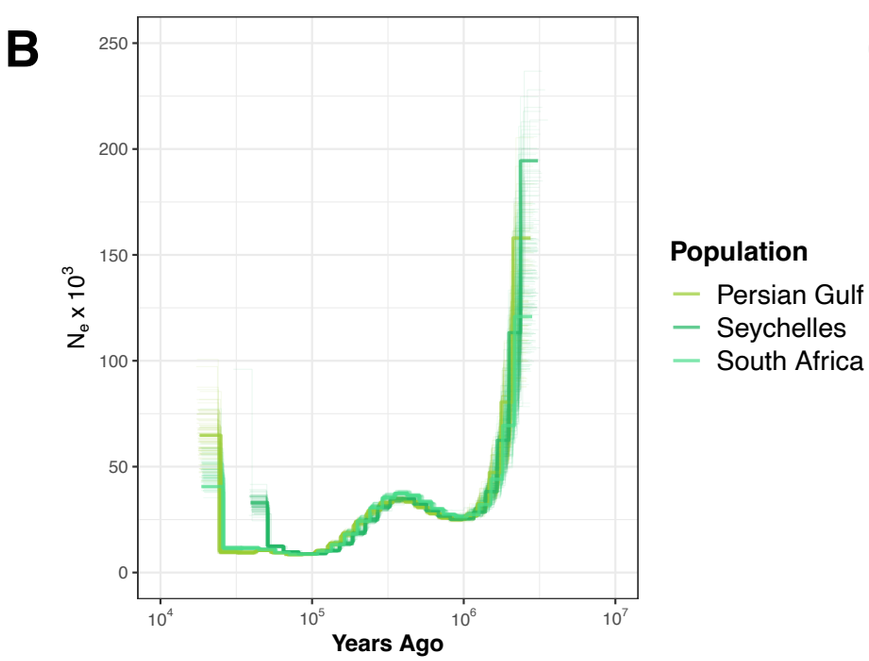
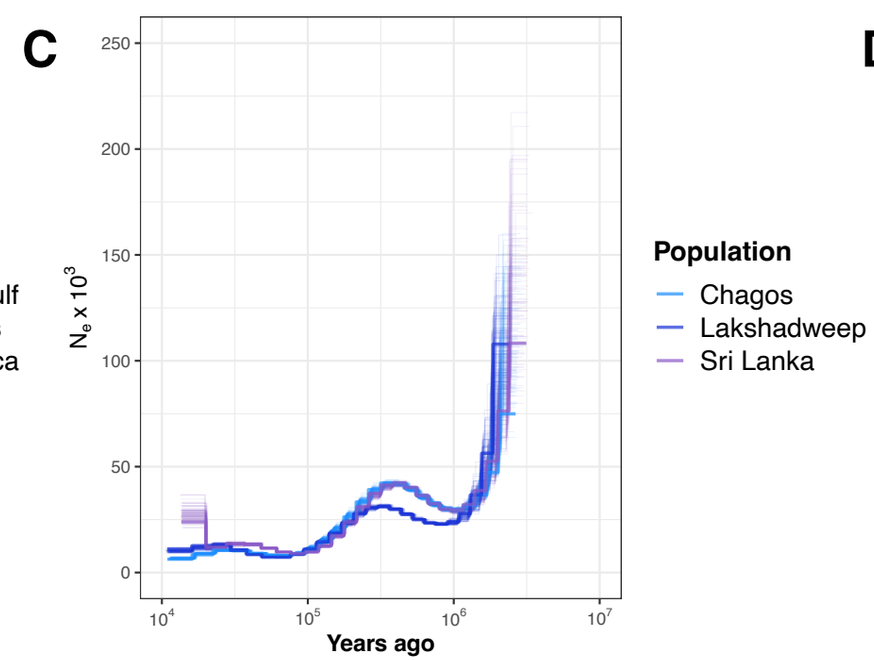
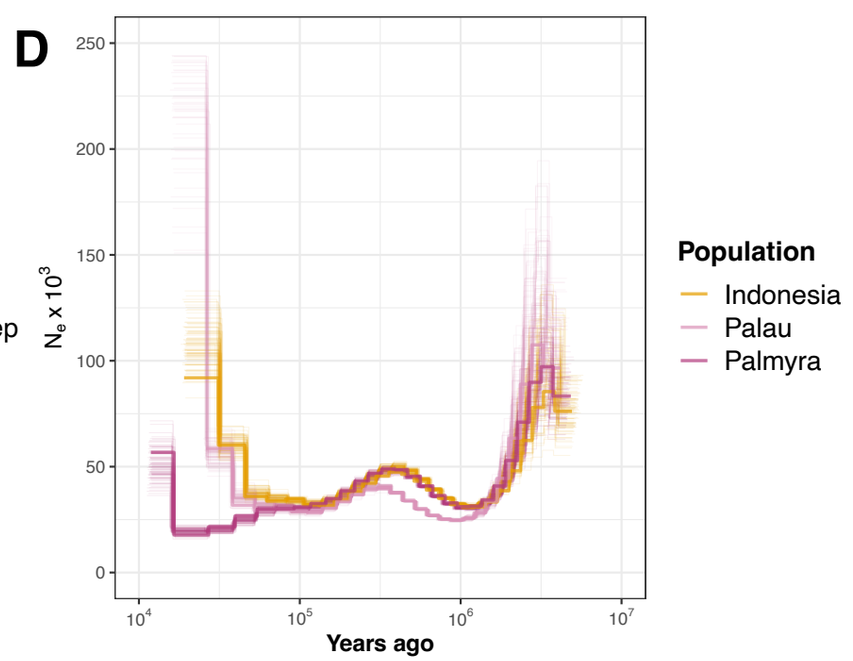


Figure 6: PSMC_ Grey Reef Sharks







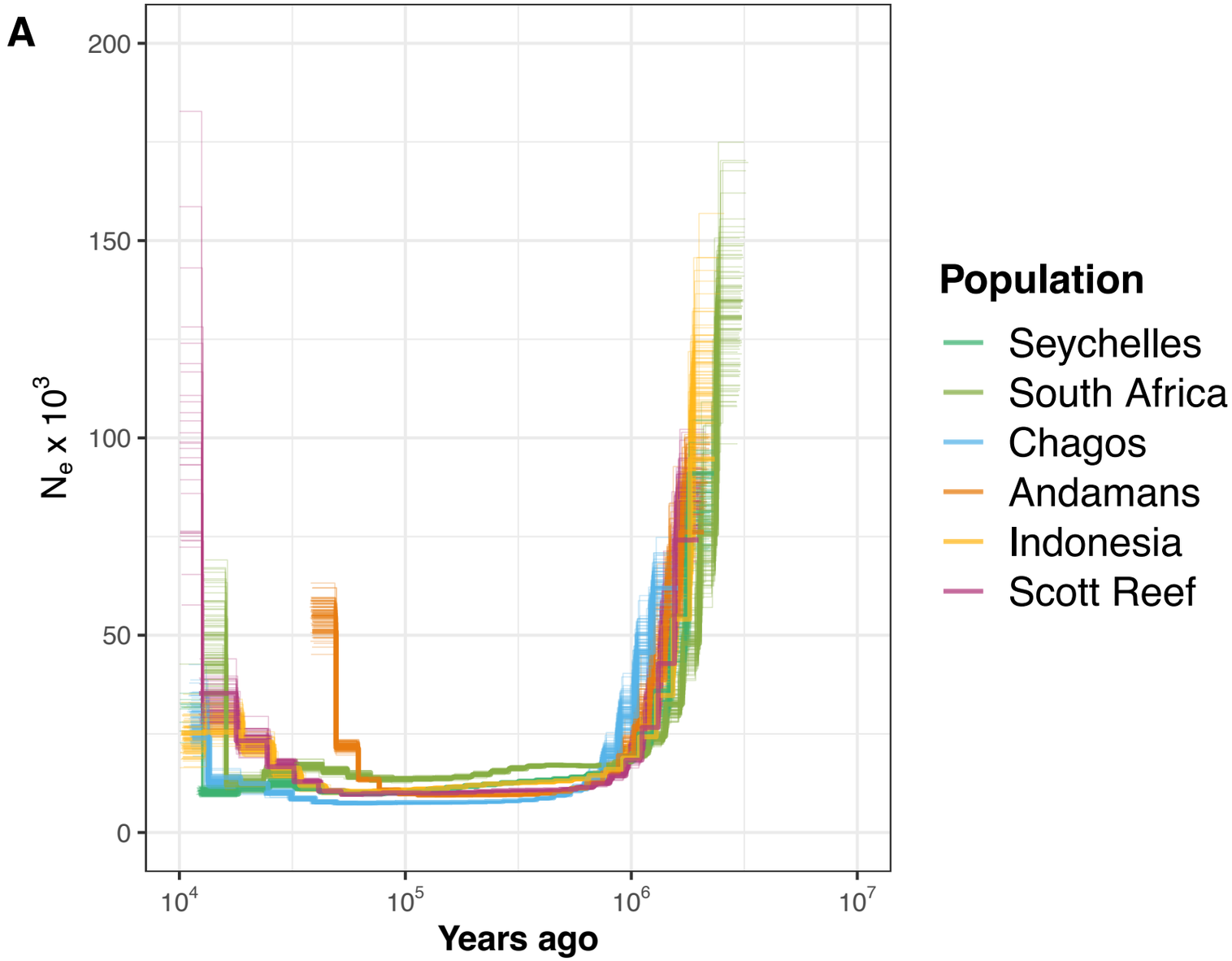
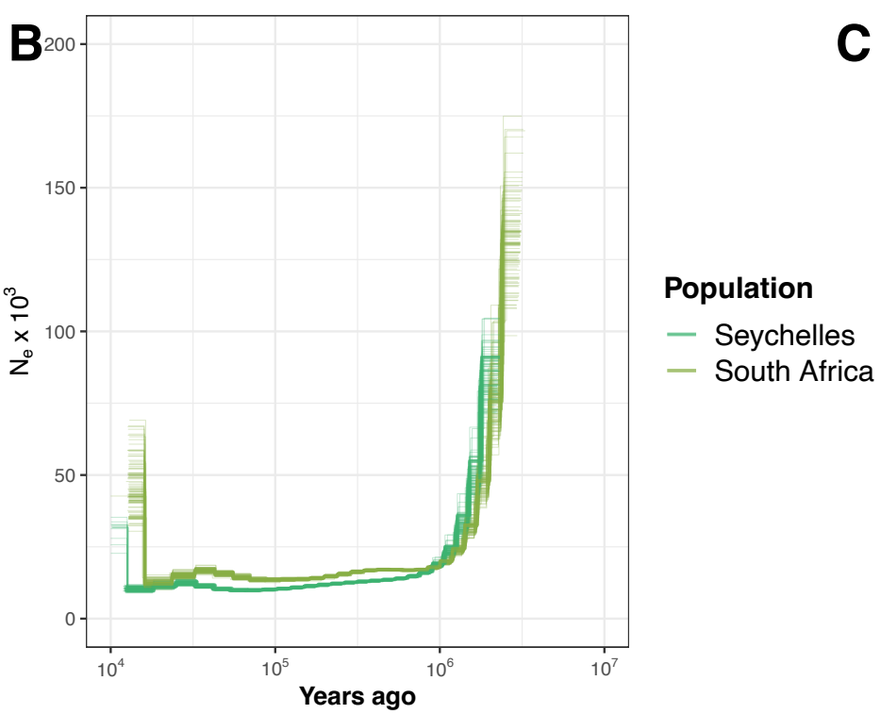
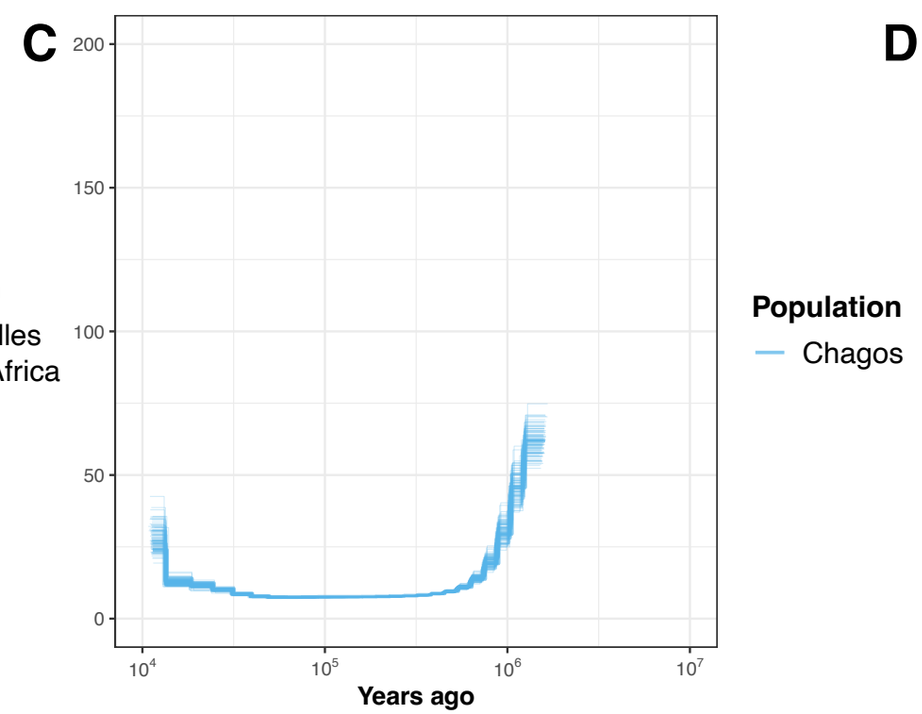
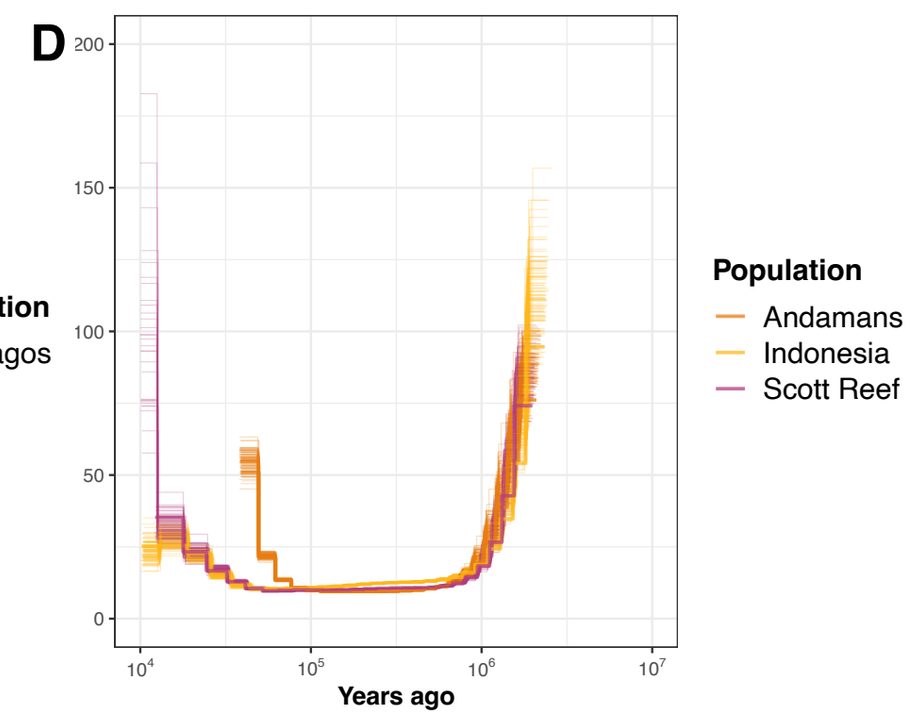
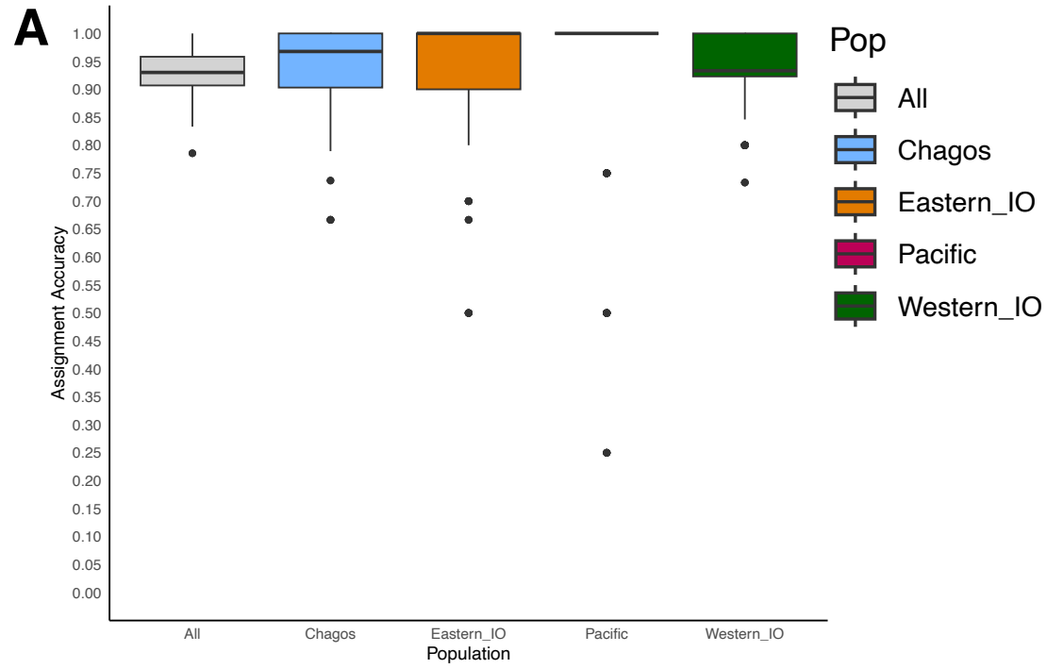


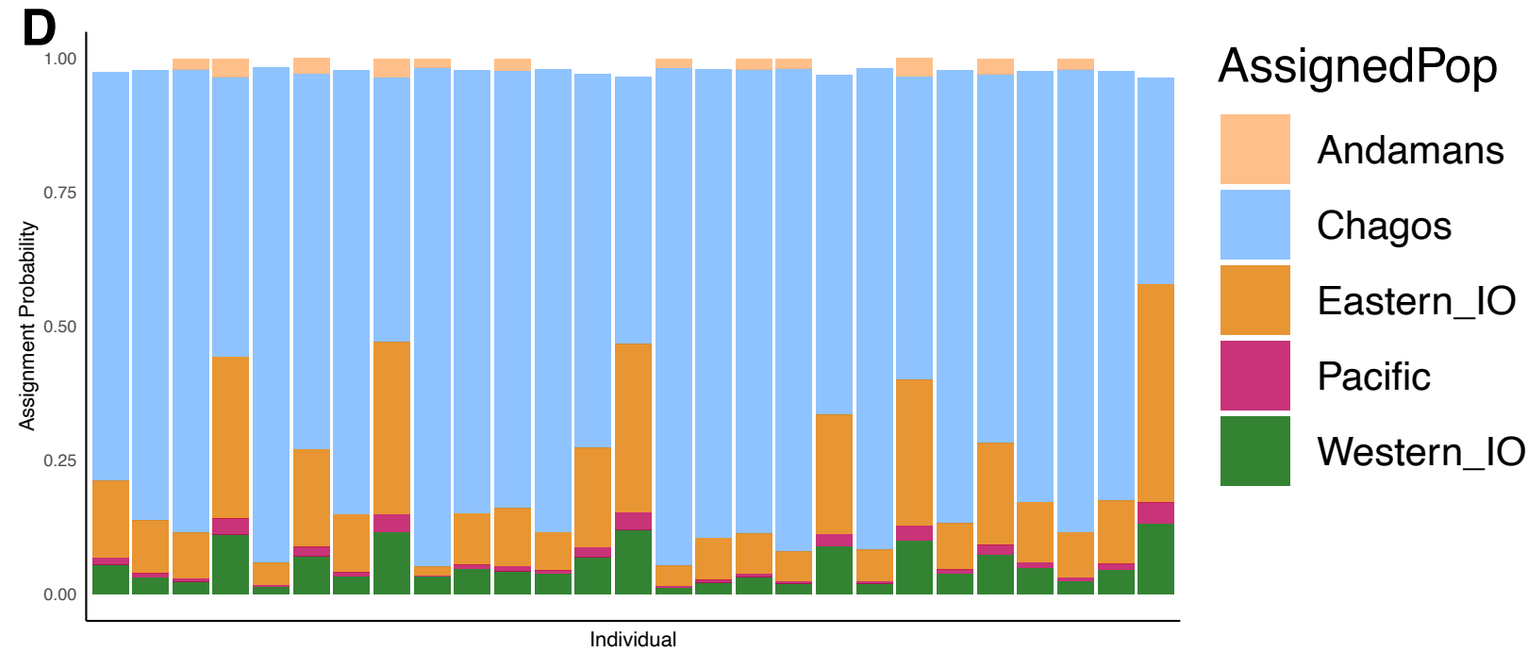
Figure 7: PSMC_Silvertip Sharks

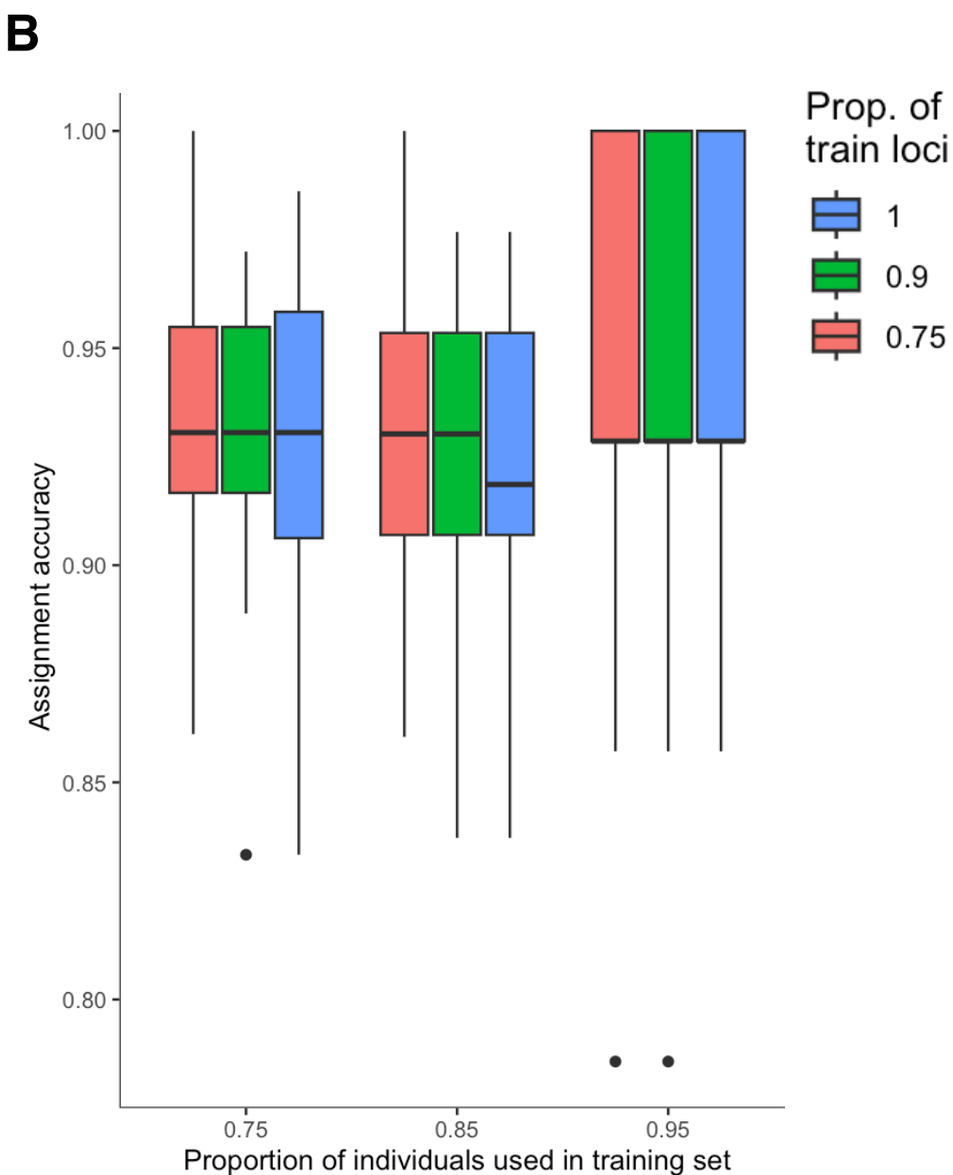












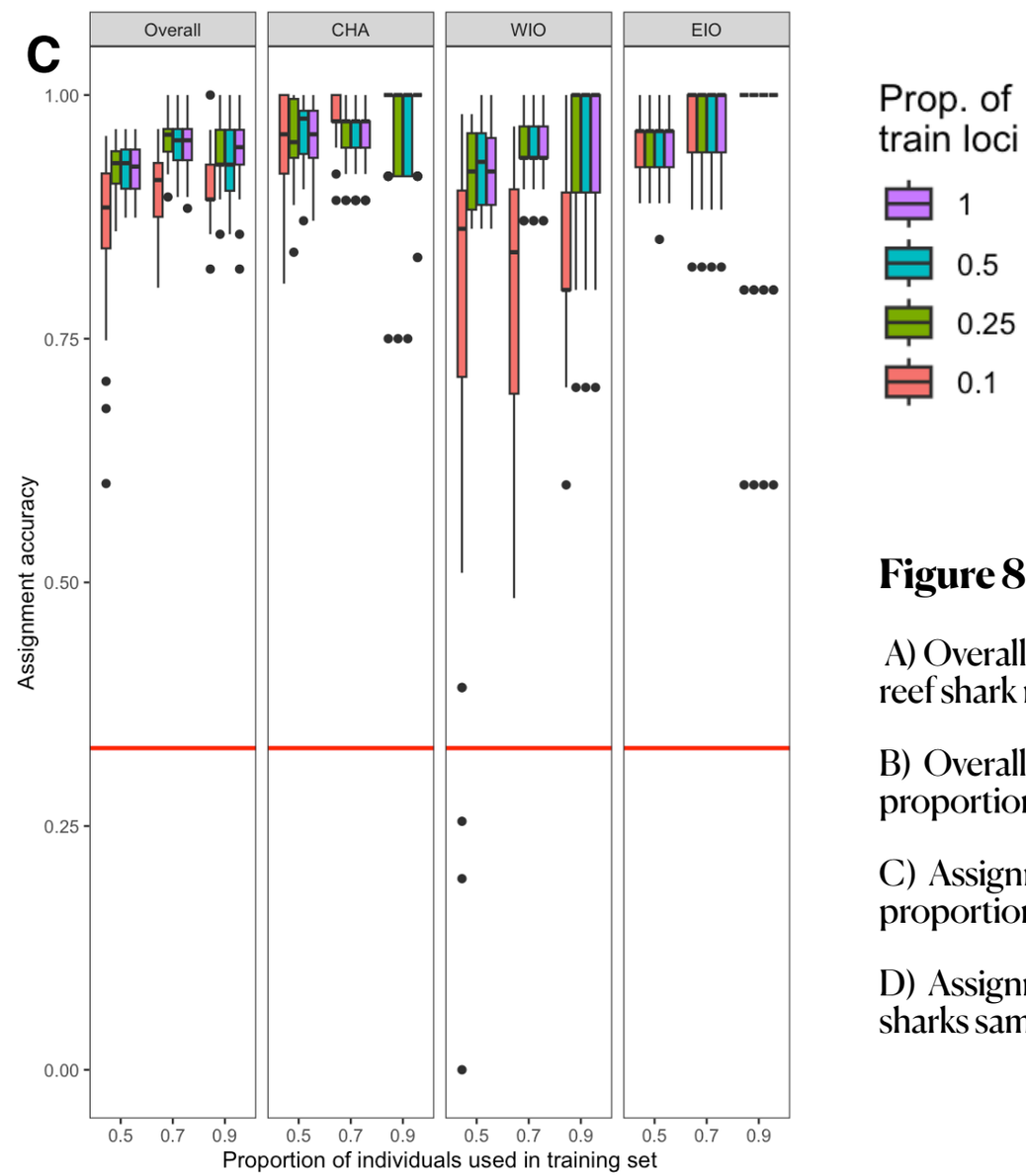


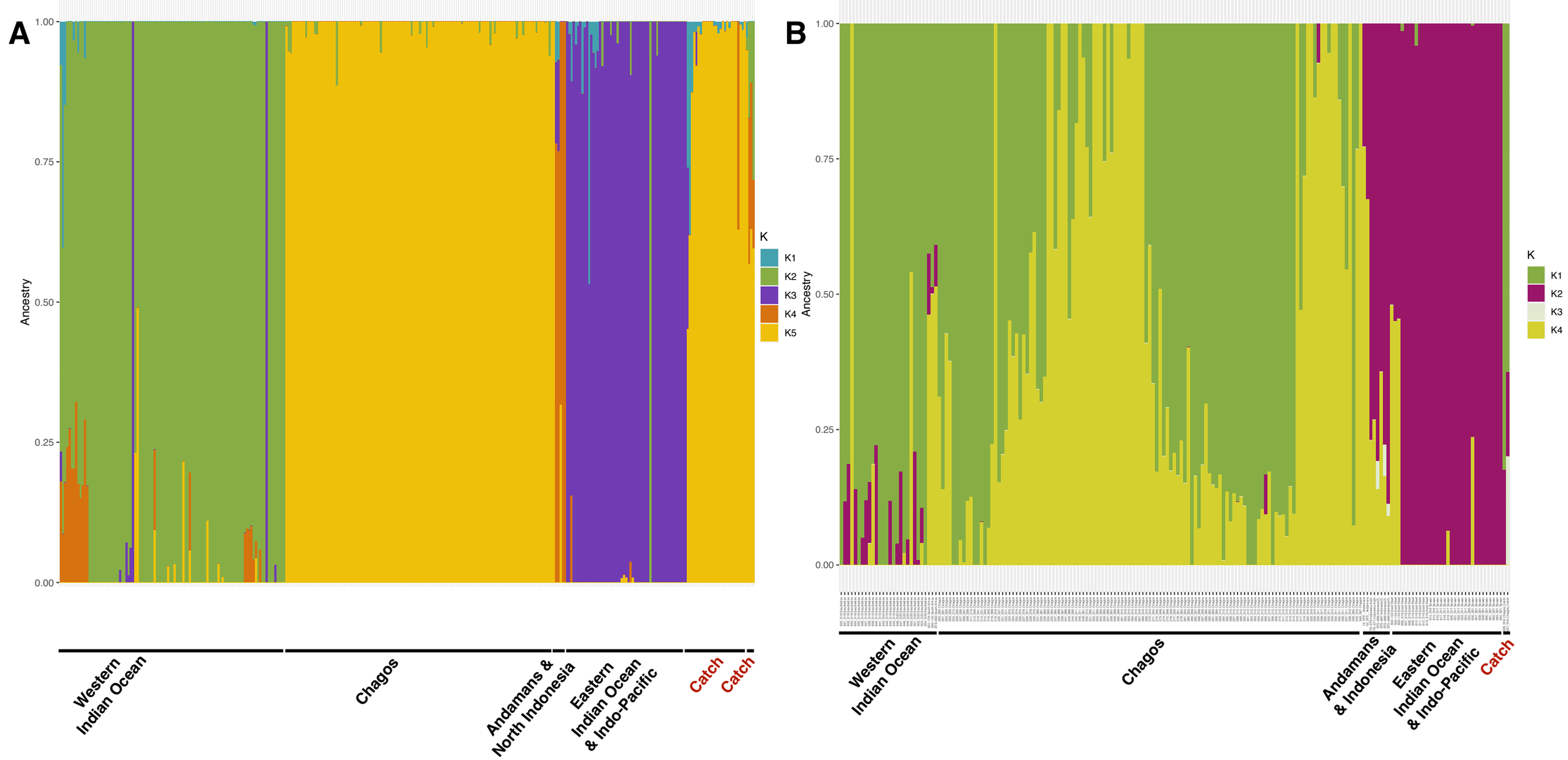
Figure 8 : Tracking fisheries:

A) Overall assignment accuracy of grey reef shark reference populations

B) Overall assignment accuracy by proportion of individuals and training loci

C) Assignment accuracy per group by proportion of individuals and training loci

D) Assignment probability of fished sharks sampled in the Chagos



Supplementary Figure 5 : Tracking fisheries using admixture analyses A) Grey Reef Sharks, B) Silvertip Sharks