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Global Genetic Stock Structure of the Copper (*Carcharhinus brachyurus*) and Dusky Sharks (*Carcharhinus obscurus*): Interspecific Comparisons and Implications for Management

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Abstract of the Thesis

Global Genetic Stock Structure of the Copper (*Carcharhinus brachyurus*) and Dusky Sharks (*Carcharhinus obscurus*): Interspecific Comparisons and Implications for Management

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Genetic stock structure information is needed to help delineate management units and monitor trade in sharks, many of which are heavily exploited and declining. Among these, the copper (*Carcharhinus brachyurus*) and dusky sharks (*Carcharhinus obscurus*) are considered some of the most vulnerable to overexploitation, given their K-selected life history strategy. The International Union for the Conservation of Nature (IUCN) categorizes the copper shark as "Near-Threatened" globally and the dusky shark as "Vulnerable" globally, with the Northwest Atlantic-Gulf of Mexico population qualifying for "Endangered." Global mitochondrial stock structure is assessed for both *C. brachyurus* and *C. obscurus* by analyzing part of the mitochondrial control region (mtCR). Samples from 120 copper sharks and 255 dusky sharks were obtained from 8 geographically dispersed locations. For *C. brachyurus*, I found 20 mtCR haplotypes and detected significant genetic structure between three sampling areas that were separated by oceanic expanses: "Australia/New Zealand (ANZ)," "Africa (AFR)" and "Peru (PER)" (Φ_{ST} = 0.95, $p < 0.000001$). For *C. obscurus*, I found 25 mtCR haplotypes and detected significant genetic structure between three sampling areas: "U.S. Atlantic (USATL)", "South Africa (SAF)" and "Australia (AUS)" ($\Phi_{ST} = 0.55$, $p < 0.000001$). Copper sharks showed a major phylogeographic discontinuity between Africa and the two Pacific poulations, which indicates an absence of female-mediated gene flow for millions of years. Dusky shark analysis suggested some recent female-mediated gene flow between SAF and AUS, but not between either of these locations and USATL. Preliminary evidence supports structure between USATL and Southwest Atlantic (Brazil) *C. obscurus*, suggesting that replenishment of the collapsed USATL population via immigration of females from elsewhere is unlikely. Mixed Stock Analysis (MSA) simulations showed that reconstruction of the relative contributions of sampling areas to shark fins in trade is possible using mtCR sequences. Once global genetic stock structure of these species is fully resolved, region-specific reconstruction of landings from genetic surveys of Asian fin markets will be possible.

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Introduction

Sharks are a group of exploited marine species that are in dire need of effective management. There are over 400 species, most of which are extremely vulnerable to overexploitation given life history characteristics such as slow growth, late maturity, long gestation periods and small litter sizes (Compagno et al. 2005). Despite this, the majority of shark fisheries around the globe are unmonitored and unmanaged, leading to severe population declines as demand for shark products, especially dried fins, has escalated (Bonfil 1994, Rose 1996). Shark fins are among the most valuable seafood products in the world because they are used to make the Asian luxury dish shark fin soup, which can retail for over $100 USD per bowl. Recent estimates suggest that from 22 to 73 million sharks are killed annually to supply the fin trade (Clarke et al. 2006b).

Much of the global trade in shark fins flows through Hong Kong, where fins come from all over the world for traders to auction (Vannuccini 1999). It is because of this that there is an interest in being able to monitor the trade here instead of the many areas where sharks are harvested (Clarke 2004a). However, the route from fishing grounds to the fin – market can be convoluted, rendering import records unreliable for individual fins (Clarke 2004b). A more robust assessment of fin provenance involves using region-specific genetic signatures to trace fins in trade back to their area of origin. This method, which is in its infancy for sharks (Chapman et al. 2009), hinges upon the availability of robust genetic population structure information. Once the global population structure of key shark species in the trade is resolved, it will be possible to obtain samples of their fins in trade, reconstruct the contribution of different stocks to the trade, and then proceed with regional stock assessment and management (Chapman et al. 2009).

In the late 1980's, scientists began to use molecular markers to investigate population structure of sharks. MacDonald (1988) conducted the first of these studies using allozymes to investigate population structure of the gummy shark (*Mustelus antarcticus*) around Australia. While the use of allozymes continued throughout the next decade, the norm quickly changed to using both allozymes and Restriction Fragment Length Polymorphism (RFLP) of mtDNA until allozymes were completely phased out due to low levels of polymorphism (Lavery and Shaklee 1989, Heist et al. 1995, Heist et al. 1996b, a, Gaida 1997, Gardner and Ward 1998, Heist 1999). With the availability of sequencing technology improving throughout the 1990's, shark population genetic studies began to employ sequencing of mtDNA as opposed to RFLP (Keeney et al. 2003, Keeney and Heist 2006, Stow et al. 2006, Castro et al. 2007, Mendonca et al. 2009, Jorgensen et al. 2009). Heist and Gold (1999) conducted the first published study of microsatellite loci in an elasmobranch. Following this, microsatellites have been used, many times in conjunction with mtDNA sequencing, as a way to differentiate male and female-mediated gene flow (Pardini et al. 2001, Keeney et al. 2005, Schultz et al. 2008, Ahonen et al. 2009, Dudgeon et al. 2009, Ovenden et al. 2009).

While the methods and scope of shark population genetic studies can vary tremendously, a few common patterns have emerged. Several large pelagic sharks displayed a pattern of low genetic structure worldwide, indicating the possibility of one cosmopolitan population (Hoelzel et al. 2006, Castro et al. 2007). Other species showed a similar pattern, but at a much more regional scale (Heist et al. 1995, Heist et al. 1996b,

Mendonca et al. 2009). Another common pattern was isolation by distance, both along continuous continental coastlines (Gaida 1997, Gardner and Ward 1998, Keeney et al. 2003, Lewallen et al. 2007, Dudgeon et al. 2009) and between ocean basins (Ahonen et al. 2009, Chabot and Allen 2009). Finally, some species showed structure in their mtDNA, but high gene flow in nuclear markers, indicating female philopatry and male-biased dispersal (Pardini et al. 2001, Schrey and Heist 2003, Schultz et al. 2008), which has been validated in lemon sharks at Bimini, Bahamas (Feldheim et al. 2002, 2004).

The dusky shark (*Carcharhinus obscurus*) and the copper shark (*Carcharhinus brachyurus*) are two species of the family Carcharhinidae that exhibit similar life-history characteristics. Both are large-bodied sharks {383cm PCL for *C. obscurus* (Dudley et al. 2005) and 294cm TL for *C. brachyurus* (Compagno et al. 2005)}and have a broad distribution, utilizing both coastal and pelagic habitat (Compagno et al. 2005). Tagging studies have shown both species to undertake long-range movements (1,320 km for *C. brachyurus* and 3,800 km for *C. obscurus*) (Cliff and Dudley 1992, Kohler et al. 1998). They both exhibit a late age at maturity, small litter size, and long reproductive cycle (Compagno et al. 2005). The dusky shark is assessed by the International Union for the Conservation of Nature (IUCN) as "Vulnerable A2bd" worldwide, with populations in the Northwest Atlantic and Gulf of Mexico being considered "Endangered" (Musick et al. 2007). The copper shark is assessed as "Near Threatened" globally by the IUCN, with populations in Asia classified as "Vulnerable" (Duffy and Gordon 2003). Both of these species are fished around the world to supply the fin trade, with the dusky shark being traded under the name "Haihu" and comprising up to 1.7% of fins auctioned annually (Clarke et al. 2006a, Clarke et al. 2006b).

The management of both the copper and dusky shark would benefit from an understanding of their global mitochondrial population structure. In my thesis I have used a partial sequence of the mitochondrial control region to (1) examine mitochondrial stock structure and (2) use these mitochondrial stocks to begin to define management units. I also performed simulations aimed at assessing the ability to trace fins of these species from unknown geographic origin back to the stocks and management units I defined.

Chapter I: Phylogeography of the copper shark (*Carcharhinus brachyurus*) in the Southern Hemisphere.

Abstract

The copper or bronze whaler shark (*Carcharhinus brachyurus*) is a large, coastally-oriented requiem shark (F. Carcharhinidae) that exhibits a life history strategy that makes it extremely vulnerable to overexploitation. The International Union for the Conservation of Nature (IUCN) categorizes this species as "Near-Threatened" globally, but assumes each coastal population is distinct and assesses them independently. I test the hypothesis that there are distinct coastal populations of copper sharks by analyzing part of the mitochondrial control region (mtCR) in 120 individuals from eight sampling areas. I found 20 mtCR haplotypes and detected significant genetic structure between three sampling areas that were separated by oceanic expanses: "Australia/New Zealand (ANZ)," "Africa (AFR)" and "Peru (PER)" (Φ_{ST} = 0.95, $p < 0.000001$). I discovered a major phylogeographic discontinuity in this species between Africa and the two Pacific populations, which indicates an absence of female-mediated gene flow for millions of years. Comparisons of populations separated by narrow seas (Australia and New Zealand) and along continental coastlines (Namibia and South Africa) indicated high female gene flow. Mixed Stock Analysis (MSA) simulations showed that reconstruction of the relative contributions of ANZ, AFR, and PER to copper shark fins in trade is possible using mtCR sequences. Once the global genetic stock structure of this species is better resolved, region-specific reconstruction of landings from genetic surveys of Asian fin markets will be possible.

Introduction

The copper shark or bronze whaler (*Carcharhinus brachyurus* Günther, 1870) is a large apex predator (max length = 3.25 m) belonging to the family Carcharhinidae (requiem sharks), which includes the majority of heavily exploited large sharks worldwide (Clarke et al. 2006a). Shark populations are constrained by life history characteristics such as long lifespan, production of relatively few offspring and late age at sexual maturity, which limits their ability to replenish populations impacted by fisheries (Compagno et al. 2005). Among them the copper shark is especially vulnerable because it reaches sexual maturity at a very late age (13 years for males and 20 years for females) and produces litters of 13-24 pups on what is probably a biennial cycle (Compagno et al. 2005).

Copper sharks occur in most warm temperate waters across the globe and use both coastal and offshore areas (Compagno et al. 2005). Most abundant in the Southern Hemisphere, the species has been given several common names in different regions (Garrick 1982). Major population centers occur in the western South Atlantic from southern Brazil to northern Argentina, the eastern Atlantic in northwest Africa and the southwest coast of South Africa, the Mediterranean, the Indian Ocean off southeast South Africa on the western border and western Australia on the eastern border, the western Pacific off Australia and New Zealand and Japan, and the eastern Pacific from southern California to Baja California and off Perú (Garrick 1982). In contrast to other members of

the genus, copper sharks tend to give birth and have nursery areas at the highest latitudes of their range (Lucifora et al. 2005).

Copper sharks are fished throughout their range. In east Asia, intensive fisheries and nursery ground destruction and degradation may be causing a regional collapse of the species (Duffy and Gordon 2003). Commercial catches in Australia appear to be stable, while New Zealand saw an almost four-fold increase in catches followed by a decrease in the last decade (Cavanagh et al. 2003). South African copper sharks are taken in commercial fisheries and protective beach meshing programs (Dudley and Simpfendorfer 2005). In its eastern Pacific ranges the copper shark is considered rare and data on this species is deficient. In many regions population declines are likely to go unnoticed because copper sharks are grouped with other carcharhinid species (Duffy and Gordon 2003).

In a global assessment of copper sharks for the International Union for the Conservation of Nature (IUCN), Duffy and Gordon (2003) list this species as globally "Near Threatened", "Vulnerable" in East Asia, "Data Deficient" in the Eastern Pacific and "Least Concern" in Australia, New Zealand and Southern Africa. They suggest that each coastal population is demographically independent, but indicate that this needs to be confirmed with genetic data. A recent genetic study of the zebra shark (*Stegostoma fasiciatum*) revealed that current IUCN regional classifications of this species did not reflect the underlying population structure well, highlighting the need for more population genetic studies of sharks to inform these and more quantitative assessments for sharks (Dudgeon et al. 2009).

The hypothesis that most coastal populations of copper sharks are distinct (Duffy & Gordon 2003) seems plausible given that some other coastally-oriented shark species exhibit highly structured populations across the globe. Wide expanse of pelagic habitat appears to be the most serious impediment to female dispersal in many of these species, as evidenced by sharp discontinuities in mitochondrial haplotype frequencies observed on different continental shelves (Duncan et al. 2006, Keeney and Heist 2006, Stow et al. 2006, Schultz et al. 2008, Chabot and Allen 2009, Chapman et al. 2009). In some cases there is also evidence of population structure along continuous continental coastlines, which may be related to limited dispersal or natal homing by females (Keeney and Heist 2006, Chapman et al. 2009). One of the most potent and lasting barriers to female-mediated gene flow in coastal sharks appears to be the Indian Ocean from southern Africa to western Australia. In several large sharks (e.g. *Carcharodon carcharias*, Pardini et al. 2001) this barrier is reflected in highly divergent, monophyletic lineages that are almost perfectly segregated on either side. In other cases there are marked haplotype frequency differences between southern Africa and Australia (Chapter 2). I hypothesize that (1) oceanic expanses are a barrier to female-mediated gene flow and (2) copper sharks exhibit a phylogeographic break across the Indian Ocean. I therefore used mitochondrial DNA sequence data to describe the phylogeography of copper sharks in the Southern Hemisphere, with a focus on beginning to define mitochondrial stocks for assessment and management purposes.

Materials and Methods

Sample acquisition

A total of 117 copper sharks were sampled from five areas in the Southern Hemisphere: Namibia (NB), South Africa (SA), Australia (AU), New Zealand (NZ) and Perú (Fig. 1). Samples from one individual each were also collected in Spain, Brazil and the Pacific coast of Mexico. Specimens were obtained by a combination of recreational and commercial fishery sampling, research cruises, and beach meshing captures. Tissue was preserved in 95% reagent grade ethanol and stored at room temperature. Tissue types included fin and muscle. Total genomic DNA was extracted from 25 mg of tissue using the DNeasy® Blood and Tissue kit (QIAGEN, Valencia, CA) following the manufacturer's protocol with some adjustments of final elution volumes based on tissue type. Samples from fin generally contained a higher concentration of DNA and were eluted into a final volume of 300 µl, whereas muscle extractions were eluted in 150 µl. Genomic DNA was checked for quality and approximate quantity on a 0.8% agarose gel run at 60V for ~ 45 minutes.

Mitochondrial control region amplification, sequencing and analysis

Polymerase chain reaction (PCR) was used to amplify the mitochondrial control region (hereafter abbreviated "mtCR") from all samples. Reactions were carried out in 50 µL volumes containing 1 µL of genomic DNA, 1X PCR buffer (QIAGEN Inc.), 40 µM dNTPs, 12.5 pmol of each of the primers Pro-L (5'-AGGGRAAGGAGGGTCAAACT-3') and 12S (5'- AAGGCTAGGACCAAACCT – 3'), and 1 unit of HotStar Taq™ DNA Polymerase (QIAGEN Inc.). PCR was performed in a LabnetMultigene TC9600-G thermocycler for 35 cycles of 1 min at 95°C, 1 min at 65°C and 2 min at 72°C, followed by a final extension step of 72°C for 10 min. PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase according to the manufacturer's protocol (ExoSAP-IT®, USB Corporation, Cleveland, Ohio). Dye termination sequencing was performed using the Pro-L forward primer and BigDye®. Cycle sequencing reactions were performed in a Bio-RAD Dyad thermocycler for 25 cycles of 96°C for 10s, 50°C for 5s, and 60°C for 4 min. Sequencing reactions were precipitated with ethanol and 125mM EDTA and run on an ABI 3730 DNA Analyzer.

Sequences were validated by eye in the program Chromas 2.33 (http://www.technelysium.com.au) and aligned and trimmed in the program GeneDoc (http://www.nrbsc.org/gfx/genedoc/). All distinct haplotypes were verified by sequencing them in both the forward and reverse direction. A maximum parsimony haplotype network was drawn in TCS 1.21 at the 95% confidence interval to show the evolutionary relationships between haplotypes (Clement et al. 2000). Genetic diversity indices for each sampling region, as well as overall diversity indices, were calculated in DnaSP 4.0 (Rozas et al. 2003). Genetic differentiation (Φ_{ST}; Kimura 2 distances) between the sampling sites and tests of significance were calculated in Arlequin 3.1 (Schneider et al. 2000), using AMOVA.

Migration, Divergence and Mixed Stock Analysis (MSA)

Divergence time and migration rates were estimated using the program MDIV (Nielsen and Wakeley 2001), carried out using the resources of the Computational Biology Service Unit from Cornell University. The Bayesian Markov chain Monte Carlo method was performed on comparisons between Australia/New Zealand population (based on the Φ_{ST} results below) versus Africa, Australia/ New Zealand vs. Peru, as well as between Africa and Peru, using the finite series model. For all comparisons, one long chain was run for a length of 5,000,000 steps, with a burn-in of 500,000 steps. Several repetitions were performed with different random number seeds to assure proper chain length. Posterior probability distributions were used to obtain estimates (mode) of M ($M = 2N_em$, Where m = migration rate) and T ($T = t/2N_e$, where t = divergence time). Effective population size (N_e) was calculated using the mutation rate published for the scalloped hammerhead (*Sphyrna lewini*), a member of the sister family to Carcharhinidae (Duncan et al 2006). For the comparison between Australia/New Zealand and Africa, an initial run showed M was not significantly different from 0 and T was likely greater than 10, therefore Mmax was set to 0 and Tmax was set to 15 for subsequent runs in order to better assess T alone. For the comparison between Africa and Perú, Mmax was set to 0 and Tmax to 10 for the reasons stated above. Both Mmax and Tmax were left at default for the comparison between Australia/New Zealand and Perú.

In order to assess the potential efficacy of using mtCR sequences to trace fins of unknown origin to mitochondrial stocks with a known geographic origin, I ran simulations in a commonly used MSA program (Statistics Program for Analyzing Mixtures [SPAM 3.7b; http://www.cf.adfg.state.ak.us/geneinfo/research/genetics/genetics.php]). The SPAM program randomly resampled the baseline mitochondrial haplotype frequency of each mitochondrial stock delineated in this study to construct mixtures (n = 100 animals) with a specified contribution from each stock. The program then used maximum likelihood (1000 iterations) to reassign each individual in the mixture back to its most probable mitochondrial stock of origin and thus reconstruct the contribution of each stock to the mixture. The level of concordance between the mean estimated contribution of each mitochondrial stock to the mixture and the known, user-specified contribution assesses the potential accuracy of future MSA. I ran multiple simulations using a range of user-specified contributions from each mitochondrial stock defined in my phylogeographic study.

Results

A total of 120 partial mtCR sequences were obtained and analyzed in this study. The 645bp segment of the mtCR obtained was composed of 20.7% cytosine, 37.9% thymine, 30.7% adenine, and 10.8% guanine. There were 27 polymorphic sites, comprising 16 transitions, 11 transversions and 2 indels and characterizing 20 haplotypes. Two highly divergent mtCR lineages were present in the global sample, comprising haplotypes separated from each other by a minimum of 15 substitutions (Table 1, Figure 2). TCS 1.21 was unable to connect the two lineages at the 95% confidence level (Figure 2). The two lineages were perfectly segregated by geography: the first was found in Africa, Brazil and Spain, while the second was restricted to Australia, New Zealand, Perú and the Mexican Pacific (Table 2, 3). Of the 20 haplotypes discovered in 120 individuals,

2 occurred in Australia and New Zealand, 14 occured in South Africa, Namibia, Brazil and Spain, 4 occured in Perú and 1 occured in Mexico (Table 2). Of these, only a single haplotype was shared between Australia/New Zealand and Perú. Overall diversity (*h*) of the global sample was 0.76 $\pm$ 0.06 and nucleotide diversity (π) was 0.016 $\pm$ 0.0007 (Table 3). Because of small sample size, samples from Brazil (n = 1), Mexico (n = 1), and Spain (n = 1) were not included in further analyses of population structure, except to note that the sequence from Mexico displayed a unique haplotype.

Southern Hemisphere copper sharks are structured into at least three distinct mitochondrial stocks (overall $\Phi_{ST} = 0.95$, $p < 0.000001$; Table 4, 5): 'Australia/New Zealand' (ANZ, comprised of Australia and New Zealand animals, pairwise Φ_{ST} non-significant), 'Africa' (AFR, comprised of South Africa and Namibia, pairwise Φ_{ST} non-significant), and 'Perú' (PER). Haplotype and nucleotide diversities were highest in AFR ($h = 0.77$ and $\pi = 0.0017$) and PER and lowest in ANZ ($h = 0.20$ and $\pi = 0.0003$) (Table 3). The analysis of divergence time between ANZ and AFR produced an estimate of *t* of 3.48×10^6 years and the comparison between AFR and PER produced an estimate of 2.36×10^6. Both these had effectively zero migrants per generation. The analysis of the migration rate between ANZ and PER yielded an estimate of *m* of 1.47×10^{-6} (M = 0.12 migrants per generation) and the estimated divergence time (*t*) for this comparison was 1.61×10^5 years.

Mixed Stock Analysis (MSA) simulations were executed with a range of user-specified mitochondrial stock contributions to a hypothetical mixture of 100 *Carcharhinus brachyurus* products in trade (Table 6). Simulation results indicate that there is sufficient differentiation in mtCR haplotype frequencies between the AFR, ANZ, and PER populations to allow for reasonably accurate reconstruction of each of their contributions to mixtures of products in trade (Table 6). Mean simulation-recovered contributions were very close to the user-specified contributions regardless of which stocks were being resolved, with a fairly narrow deviation around the mean.

Discussion

Copper sharks exhibit high mitochondrial control region genetic diversity in the Southern Hemisphere. Overall nucleotide diversity ($\pi = 0.016 \pm 0.0007$) is one of the highest observed in any large shark species (Duncan et al. 2006, Keeney and Heist 2006, Castro et al. 2007, Ahonen et al. 2009, Chabot and Allen 2009). This is due to the presence of two highly divergent, monophyletic mtCR lineages that exhibited strong association with sampling regions: one lineage was only found in the Atlantic and southwestern Indian Ocean (South Africa, Namibia, Brazil and Spain), while the other was only found in the eastern Indian and Pacific oceans (Australia, New Zealand, Perú and the Pacific coast of Mexico). A similar phylogeographic break across the Indian Ocean was described in white sharks, *Carcharhodon carcharias* (Pardini et al. 2001), but there was evidence of movement of Indian/Atlantic derived white sharks from South Africa to Australia and male-mediated gene flow (Pardini et al. 2001, Bonfil et al. 2005). I observed extremely high Φ_{ST} values and did not find any evidence of haplotype sharing between South Africa and the Pacific populations, which indicates the Indian Ocean is a strong and long-lasting barrier to female-mediated gene flow in copper sharks. The estimated divergence time between the two clades was nearly 3.5 million years. Copper

shark teeth are present in the fossil record during the Miocene (23-5 mya) at a site in the Northwest Atlantic (http://www.elasmo.com/frameMe.html?file=genera/cenozoic/sharks/carcharhinus.html&menu=bin/menu_genera-alt.html). The divergence time calculated roughly coincides with the timing of the rise of the Isthmus of Panama, suggesting that the separation of the Pacific and the Atlantic played a role in separating these two lineages. Another cosmopolitan shark species, the scalloped hammerhead (*Shpyrna lewini*), is also thought to have undergone population division during this separation (Duncan et al. 2006).

I also found evidence of population structure within South Pacific copper sharks. The most common mtCR haplotypes in Perú were not found in New Zealand or Australia, both of which were dominated by one haplotype (haplotype 1), as reflected by the significant pairwise Φ_{ST} values between Perú and the other two sampling areas. My analyses indicated a small number of migrants per generation (0.12), with a divergence time of around 160,000 years. Although I need more samples from Perú to verify this finding, it makes sense that the large ocean expanse of the South Pacific would inhibit female-mediated gene flow in a coastally-oriented shark.

Copper sharks are known to move long distances along continental coastlines and are occasionally recorded in pelagic habitats (Cliff and Dudley 1992, Compagno et al. 2005). Walter and Ebert (1991) hypothesized that copper sharks in Namibia and South Africa may be distinct from one another given differing seasonal reproductive patterns and a seemingly disjoint distribution. My data do not support this hypothesis because I found substantial sharing of mtCR haplotypes and non-significant pairwise Φ_{ST} values between sharks sampled in two these regions. I cannot fully refute this hypothesis, however, and more variable genetic markers or a sampling strategy that targets newborn sharks that are segregated into their nursery areas may be needed to resolve this issue.

I also failed to detect mitochondrial stock structure between copper sharks in Australia and New Zealand. Tagging studies have shown movements by copper sharks of up to 1,320 km in South Africa, which is near the minimum distance I calculated in Google Earth between Australia and New Zealand of 1,500 km (Cliff and Dudley 1992, Google 2010). Since this species is capable of offshore movement, it is possible that the Tasman Sea is not a strong or lasting barrier to female-mediated gene flow in copper sharks. However, the very low level of mtCR diversity in these two populations limits the power of this locus for resolving weak but significant population structure (i.e., due to recent contact or short time since divergence).

My analyses support the hypothesis that copper sharks inhabiting distinct continental shelves separated by large pelagic expanses often comprise distinct populations, at least in terms of the movement and reproductive mixing of females. This provides rationale for assessing, monitoring and managing distinct populations separated this way. I failed to find evidence of structure along continental landmasses or across relatively narrow oceanic expanses, but caution that more samples and genetic markers are needed to resolve finer scale population structure in copper sharks. Nevertheless, the mtCR data I present here indicates that copper shark body parts in trade, such as dried fins, can be sorted to broad regions or different continental shelves using mtCR sequences. First, copper sharks and their body parts from Atlantic-Indian Ocean and

Pacific lineages are easily sortable into their natal basin-of-origin essentially by eye using multiple diagnostic SNPs in this portion of their genome. Secondly, simulations run to assess MSA efficiency proved that differences in haplotype frequencies occurring among my sampling regions were sufficient to be able to assign fins of unknown origin back to these regions. These characteristics would be particularly useful for monitoring the global trade in dried copper shark fins, because fins landed all over the world are exported to a reasonably centralized Asian marketplace that could be monitored and sampled more cost-effectively than all of the countries participating in the fisheries (Chapman et al. 2009). Future work could build on this study by adding new samples and sampling locations, together with adding the perspective of biparentally inherited loci (microsatellites). My study indicates that once their global population structure is fully resolved it will be possible to estimate relative contributions of breeding areas in different ocean basins and continental shelves to global copper shark fin landings.

Chapter II: Global phylogeography of the dusky shark, *Carcharhinus obscurus*: implications for fisheries management and trade-monitoring.

Abstract

Genetic stock structure information is needed to help delineate management units and monitor trade in sharks, many of which are heavily exploited and declining. The dusky shark, *Carcharhinus obscurus*, is a large-bodied species that is sought after for its fins and is considered one of the most susceptible vertebrates to overexploitation. The International Union for the Conservation of Nature (IUCN) categorizes this species as globally "Vulnerable" and "Endangered" in the Northwest Atlantic-Gulf of Mexico. I make the first assessment of global mitochondrial stock structure of *C. obscurus* by analyzing part of the mitochondrial control region (mtCR) in 255 individuals sampled from 8 geographically dispersed locations. I found 25 mtCR haplotypes and detected significant genetic structure between three sampling areas: "U.S. Atlantic (USATL)", "South Africa (SAF)" and "Australia (AUS)" ($\Phi_{ST} = 0.55$, $p < 0.000001$). My analyses suggest some recent female-mediated gene flow between SAF and AUS, but not between either of these locations and USATL. I also found preliminary evidence of population structure between USATL and Southwest Atlantic (Brazil). These analyses suggest that replenishment of the collapsed USATL population via immigration of females from elsewhere is unlikely. Mixed stock analysis (MSA) simulations showed that reconstruction of the relative contributions of USATL, SAF, and AUS mitochondrial stocks to dusky shark fins in trade is possible using mtCR sequences. Once the global stock structure of this species is fully resolved it will be possible to reconstruct region-specific landings of dusky sharks from genetic surveys of Asian shark fin markets.

Introduction

Many shark populations worldwide are declining due to intense fishing pressure, largely fueled by high prices paid for shark fins that are used in the Asian delicacy shark fin soup (Bonfil 1994, Rose 1996, Baum et al. 2003). Despite the importance of delineating management units for these biologically vulnerable apex predators, there are relatively few robust studies of the population genetic structure of sharks. Existing studies reveal high population structure in some coastally-oriented sharks based on mitochondrial DNA sequence data (Duncan et al. 2006, Keeney and Heist 2006, Stow et al. 2006, Schultz et al. 2008, Chabot and Allen 2009, Chapman et al. 2009). Notwithstanding the importance of also obtaining perspective from bi-parentally inherited loci, delineating these "mitochondrial stocks" is a critical part of establishing management units because they represent discrete pools of breeding females that are associated with a specific geographic area for reproduction. The DNA polymorphisms associated with each mitochondrial stock are also a potentially informative signature of natal-area-of-origin for sharks and shark parts in trade (Chapman et al. 2009). Distinct mitochondrial stocks are often found on either side of wide expanses of pelagic habitat in coastal sharks, which indicates that the open ocean can be a substantial and lasting impediment to female-mediated gene flow (Duncan et al. 2006, Keeney and Heist 2006, Stow et al. 2006, Schultz et al. 2008, Chabot and Allen 2009, Chapman et al. 2009). In

some cases structure has also been detected within seemingly contiguous coastal populations, which may be explained by natal homing by females, isolation by geographic distance or environmental barriers to dispersal (Duncan et al. 2006, Keeney and Heist 2006, Stow et al. 2006, Schultz et al. 2008, Chabot and Allen 2009, Chapman et al. 2009).

Sharks have life history characteristics that make them particularly susceptible to overexploitation (Compagno et al. 2005). The dusky shark (*Carcharhinus obscurus*) is among the most vulnerable due to its extremely late age at maturity (17-23 years), small litter size (3-14 offspring per litter), and 3-year reproductive cycle including 24 month gestation period (Last and Stevens 1994, Natanson et al. 1995, Simpfendorfer et al. 2002, Romine et al. 2009). Dusky sharks have a cosmopolitan, but patchy distribution in warm temperate and subtropical regions, with major population centers along the east and Gulf coasts of North America, southern Africa, and Australia (Musick et al. 2007). Their fins are sorted by Chinese dealers under the trade category "Haihu" and it has been estimated that 150,000-750,000 individuals are harvested annually to supply the fin trade (Clarke et al. 2006b). The contribution of different populations and breeding areas to these landings remains unknown, making it difficult to determine if this level of global harvest is sustainable. Indeed, the absence of stock-specific landings data is widely regarded as a serious obstacle for quantitative assessment and management of sharks.

Dusky sharks have experienced serious population declines in some regions, arguing against the sustainability of recent harvest levels. Along the U.S. east coast and Gulf of Mexico, for example, a recent stock assessment has shown declines of over 80% with respect to virgin biomass (Cortés et al. 2006). Similarly, adult bycatch in Australia has led to reduced recruitment, with Catch Per Unit Effort (CPUE) declining over 75% (McAuley et al. 2005). Overall, dusky sharks are assessed as "Vulnerable A2bd" globally by the International Union for the Conservation of Nature (IUCN), while the U.S. East coast and Gulf of Mexico population is assumed by IUCN assessors to be a single distinct unit and is classified as "Endangered"(Musick et al. 2007). Dusky sharks have been prohibited from the landings of U.S. fisheries since 2000, although they are still caught incidentally and their fins are sometimes traded illegally (Romine et al. 2009). They are also currently listed as a "Species of Concern" for potential listing under the U.S. Endangered Species Act.

For a variety of reasons, dusky sharks might be expected to exhibit high gene flow over wide geographic areas, more similar to pelagic fish than the coastal sharks studied thus far (Bremer et al. 2005, Garber et al. 2005, McDowell et al. 2007). Tagging studies show that the dusky shark undertakes long coastal migrations related to seasonal temperature changes, with individual movements of > 3000 km recorded along continental coastlines (Davies and Joubert 1967, Kohler 1996, Hussey et al. 2009). They are also known to frequent pelagic habitat, even from a very early age (Beerkircher et al. 2002). It is therefore possible that large coastal distances and wide expanses of pelagic habitat are not as serious an impediment to movement between regions in dusky sharks as in some other coastal shark species.

The objectives of this study are two-fold. I first describe the global population structure of dusky sharks using mitochondrial DNA sequence data, defining discrete

"mitochondrial stocks" as a contribution towards establishing robust management units for this vulnerable species. My particular focus is to assess female-mediated connectivity between "Endangered" U.S. east coast and Gulf of Mexico populations and other regions. My second objective is to assess the potential for using mitochondrial DNA sequences to trace fins and other products of dusky sharks from unknown geographic origin obtained in trade back to their mitochondrial stock of origin.

Materials and Methods

Tissue samples were collected from a total of 255 dusky sharks from 8 globally-distributed sampling areas (Fig. 3): United States East Coast (USEC), United States Gulf of Mexico (USGOM), Brazil (BRA), South Africa (SAF), west Australia (WAUS), east Australia (EAUS), Taiwan (TAI) and Perú (PER). Samples were obtained by a combination of fishery sampling (both recreational and commercial), research cruises, and beach meshing captures. Ten of these samples were taken from dried fins obtained from 2 different shark fin dealers in Hong Kong (Clarke et al. 2006a,b), who stated the fins came from Brazilian suppliers (S. Clarke, pers com). I analyzed these samples assuming the animals were caught in Brazil, but stress that findings related to them are tentative until such time as additional dusky sharks directly sampled in Brazil become available. All tissues were preserved in 95% reagent grade ethanol and stored at room temperature. Tissue types included fins (wet and dried), muscle and soft tissue excised from vertebrae that had been archived for age and growth studies. Total genomic DNA was extracted from 25mg of tissue using the DNeasy® Blood and Tissue kit (QIAGEN, Valencia, CA) following the manufacturer's protocol with some adjustments of final elution volumes based on tissue type. Samples from fins generally contained a higher DNA concentration than the other tissues and were eluted into a final volume of 300 µl, while muscle and vertebrae-soft tissue extractions were eluted in 150 µl. Genomic DNA was checked for quality and approximate quantity on a 0.8% agarose gel run at 60V for ~ 45 minutes.

Dusky sharks are sometimes confused with closely related carcharhinid sharks so I genetically verified that all of the samples used in this study were the correct species, even if they were identified as such by experienced biologists. To achieve this, I tested all samples with the species-diagnostic Polymerase Chain Reaction (PCR) assay of Pank et al. (2001) and sequenced the entire internal transcribed spacer (ITS2) locus in ~ 40% of specimens. PCR was used to amplify the mitochondrial control region (hereafter abbreviated to "mtCR") from all samples. Reactions were carried out in 50 µL volumes containing 1 µL of genomic DNA, 1X PCR buffer (QIAGEN Inc.), 40 µM dNTPs, 12.5 pmol of each of the primers Pro-L (5'-AGGGRAAGGAGGGTCAAACT- 3') and 12S (5'- AAGGCTAGGACCAAACCT – 3'), and 1 unit of HotStar Taq™ DNA Polymerase (QIAGEN Inc.). PCR was performed in a Labnet Multigene TC9600-G thermocycler for 35 cycles of 1 min at 95°C, 1 min at 65°C and 2 min at 72°C, followed by a final extension step of 72°C for 10 min. PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase according to the manufacturer's protocol (ExoSAP-IT®, USB Corporation, Cleveland, Ohio). Dye termination sequencing was performed using the Pro-L forward primer and BigDye®. Cycle sequencing reactions were performed in a Bio-RAD Dyad thermocycler for 25 cycles of 96°C for 10s, 50°C for 5s, and 60°C for 4

min. Sequencing reactions were precipitated with ethanol and 125mM EDTA and run on an ABI 3730 DNA Analyzer.

Sequences were validated by eye in the program Chromas 2.33 (http://www.technelysium.com.au) and aligned and trimmed to 558bp in the program GeneDoc (http://www.nrbsc.org/gfx/genedoc/). All distinct haplotypes were verified by sequencing them in both the forward and reverse direction. A maximum parsimony haplotype network was drawn in TCS 1.21 at the 95% confidence interval to show the evolutionary relationships between haplotypes (Clement et al. 2000). Genetic diversity indices for each sampling region, as well as overall diversity indices, were calculated in DnaSP 4.0 (Rozas et al. 2003). The coancestry coefficient, θ_S, was estimated in Arlequin 3.1 (Schneider et al. 2000), based on the sample size, number of segregating sites and θ for non-recombining DNA. Genetic differentiation (Φ_{ST}; Kimura 2 distances) between the sampling sites and tests of their significance were calculated in Arlequin 3.1, using Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992). To test for population structure between dusky sharks on the east and west coast of Australia I combined my sequences (western N = 57, eastern N = 16) with sequences from 20 individuals (western N=13, eastern N=7) that were obtained from a published study (Ovenden et al. 2009; GenBank accession number of base sequence is FJ161688). I trimmed my Australian sequences so that they aligned completely with the 375bp partial mtCR sequence from Ovenden et al. (2009) and tested for genetic differentiation between them using AMOVA.

I assessed the potential efficacy of using mtCR data to reconstruct the relative contribution of different mitochondrial stocks to mixtures of dusky sharks and their parts (e.g., fins) in trade. To achieve this I ran simulations in a commonly used Mixed Stock Analysis (MSA) program (Statistics Program for Analyzing Mixtures [SPAM 3.7b; http://www.cf.adfg.state.ak.us/geneinfo/research/genetics/genetics.php]). The SPAM program randomly resampled the baseline mitochondrial haplotype frequency of each mitochondrial stock delineated in this study to construct mixtures of 100 “fins” with a specified contribution from each stock. The program then used maximum likelihood (1000 iterations) to reassign each “fin” in the mixture back to its most probable mitochondrial stock of origin and thus reconstructed the contribution of each stock to the “fin” mixture. The level of concordance between the mean estimated contributions of each mitochondrial stock to the mixture and the known, user-specified contributions assessed the accuracy of future MSA. We ran multiple simulations using a range of user-specified contributions from each mitochondrial stock we defined in our global phylogeographic study.

Results

A total of 255 partial dusky shark mtCR sequences were obtained and analyzed in this study. The 558 bp segment of the mtCR obtained was composed of 20.6% cytosine, 37.6% thymine, 30.7% adenine, and 11.2% guanine. There were 22 polymorphic sites among the 255 individuals, which were composed of 16 transition mutations, five transversions and 2 indels. Of the 25 haplotypes in the sample set, 10 were found only in the U.S. East Coast, U.S. Gulf of Mexico and Brazil samples, all of which were endemic to this region (Table 7, Fig. 3). Assuming that the Hong Kong dealers provided accurate

provenance information about their "Brazil" fins, it appears that there are major haplotype frequency differences between the Southwest Atlantic and the U.S. (Fig.3, Fig.4.). The putative Brazil sample (N=12) consisted of the second most common U.S. haplotype (N=3, haplotype 16 from Fig.4.) and a more common haplotype (N=9, haplotype 25 from Fig.4.) that was not seen anywhere else in the world but is intermediate between Western Atlantic and Indo-Pacific haplotypes. Eight haplotypes were found in South Africa (2 endemic to this location in our dataset) and 13 were found in Australia (east and west combined, 7 endemic, Fig 3, 4; Table 7). Six haplotypes were shared between Australia and South Africa. I also obtained partial mtCR sequences from dusky sharks sampled in Taiwan (N = 4) and Perú (N=1) that comprised three haplotypes, all of which were also found in Australia and/or South Africa. Overall haplotype diversity (h) of the global sample was 0.84 ± 0.03 and nucleotide diversity (π) was 0.005 ± 0.0005 (Table 8). Because of the small sample sizes, mtCR sequences obtained from Perú and Taiwan were not included in further analyses of population structure. I also elected not to include Brazil (N=12) in the subsequent quantitative analyses of population structure given that most of the samples were obtained from Asian fin dealers rather than having been taken directly from wild-captured animals.

AMOVA shows that dusky sharks comprise at least three distinct mitochondrial stocks in the areas we sampled (overall $\Phi_{ST} = 0.55$, $p < 0.000001$; Table 9). These are 'U.S. Atlantic ("USATL")' (comprised of USEC and USGOM, pairwise Φ_{ST} non-significant; Table 10), 'South Africa ("SAF")', and 'Australia ("AUS")' (comprised of EAUS and WAUS, pairwise Φ_{ST} non-significant). Haplotype and nucleotide diversities were higher in AUS and SAF than USATL (Table 8). A second AMOVA comparing the east and west coasts of Australia using a smaller mtCR fragment and haplotype frequency data from Ovenden et al. (2009) also failed to reject the null hypothesis of panmixia within Australia ($\Phi_{ST} = 0.015$, $p > 0.17$).

Mixed Stock Analysis (MSA) simulation results for a hypothetical mixture of 100 "fins" in trade indicate that there is sufficient differentiation in mtCR haplotype frequencies between the USATL, SAF and AUS mitochondrial stocks to allow for reasonably accurate reconstruction of each of their contributions (Table 11). Mean simulation-recovered contributions were very close to the user-specified contributions regardless of which stocks were being resolved, with a fairly narrow deviation around the mean. MSA was generally less accurate in resolving the relative contributions of mitochondrial stocks to mixtures when their contributions were small (~ 5%), at least in the sense that the standard deviations of simulation-estimated contributions were quite large relative to the mean in these cases (Table 11). However, as the contributions of the stocks increased the standard deviation tightened around the mean, suggesting that meaningful reconstructions will be possible for large fin mixtures.

Discussion

Dusky sharks exhibit values of numbers of haplotypes (25) and haplotype diversity ($h = 0.84 \pm 0.03$) within the range of those calculated for other large, globally-distributed sharks (Duncan et al. 2006, Keeney and Heist 2006, Castro et al. 2007, Ahonen et al. 2009, Chabot and Allen 2009). In contrast, overall nucleotide diversity is among the lowest recorded for any large, globally-distributed shark ($\pi = 0.005 \pm 0.0005$,

(Duncan et al. 2006, Keeney and Heist 2006, Castro et al. 2007, Ahonen et al. 2009, Chabot and Allen 2009). The dusky shark is a derived carcharhinid (Naylor 1992, Musick et al. 2004) and its recent divergence and global radiation may explain why nucleotide diversity is still relatively low. I also found that the Indo-Pacific populations in our dataset had higher genetic diversity than the USATL, which is concordant with patterns seen in other sharks (Duncan et al. 2006, Keeney and Heist 2006, Castro et al. 2007, Ahonen et al. 2009, Chabot and Allen 2009).

I did not find evidence of differentiation between the USEC and USGOM dusky shark populations, which is consistent with the findings for other species of shark studied in this region (Heist et al. 1996b, Chapman et al. 2009). The absence of structure found here is supported by conventional tagging data from the National Marine Fisheries Service (NMFS), in which movements by individual dusky sharks of up to 3,800 km (from the USEC to the Yucatán peninsula) are documented and contemporary exchange between the USEC and USGOM appears to be quite common (Kohler 1996). The combined genetic and tagging data support the current U.S. policy of assessing and managing a single stock of dusky sharks within this region. However, I cannot completely rule out that migratory female dusky sharks home back to their natal region of origin to breed, which could generate genetically distinct stocks that could only be recognized by sampling neonates in their natal area or females undergoing parturition (e.g., see sampling design of Keeney et al. 2005). This may prove very difficult to resolve because young-of-the year dusky sharks are mobile and are found in coastal and even pelagic habitats as opposed to being concentrated in discrete estuarine nursery areas (Beerkircher et al. 2002).

I also failed to detect genetic differentiation between Australia's east (Pacific Ocean) and west (Indian Ocean) coasts, suggesting female-mediated gene flow occurs around this coastline. Ovenden et al. (2009) suggested that their discovery of two unique mtCR haplotypes in Indonesia could indicate population structure between Indonesia and Australia. Although I was unable to test this hypothesis due to a lack of new sequences from Indonesia, my expanded Australian dataset yielded several individuals with the 'Indonesian' haplotypes. Moreover, the dusky sharks sequenced from Taiwan had haplotypes also found in Australia. Although fine-scale population structure may exist in this region, it does not appear to involve endemicity of mtCR haplotypes.

My analyses provide quantitative evidence of at least three distinct mitochondrial stocks of dusky sharks worldwide ("United States Atlantic"-USATL, "South Africa"-SAF, "Australia"-AUS), each of which represents a discrete pool of breeding females that should be assessed and managed independently. I found high Φ_{ST} values and an absence of haplotype sharing between the USATL and both Indo-Pacific mitochondrial stocks in my survey. This demonstrates that there is a robust barrier to female-mediated gene flow between these regions, which has also been observed in several other species of marine fish (Bremer et al. 1996, Heist et al. 1996a, Bremer et al. 1998, Duncan et al. 2006, Keeney and Heist 2006). We speculate that the large oceanic expanse separating these regions inhibits female-mediated gene flow in dusky sharks. It is also possible that equatorial regions inhibit female-mediated gene flow, given that dusky shark populations are centered in warm temperate as opposed to tropical latitudes (Musick et al. 2004). I

tentatively also report qualitative evidence of population structure between dusky sharks in the USATL and Brazil. Interestingly, the most common putative "Brazil" haplotype in my sample (H25, Fig. 4.) is intermediate between the USATL and Indo-Pacific haplotype clusters in my parsimony network, suggesting that the Atlantic coast of South America may have provided the historical connection between the western Atlantic and Indo-Pacific populations. Overall, my study indicates that the recovery of collapsed USATL dusky shark populations is likely to rely on reproduction by surviving local females as opposed to replenishment from immigrant females from the neighboring Southwest Atlantic or from the Indo-Pacific. More direct sampling in Brazil is necessary to validate (or refute) these tentative findings.

Dusky sharks are further significantly differentiated into at least two mitochondrial stocks within the Indo-Pacific: SAF and AUS. The Indian Ocean has been a potent barrier to female-mediated gene flow across evolutionary time in several sharks (Pardini et al. 2001, Ahonen et al. 2009, Dudgeon et al. 2009), as evidenced by completely disjunct haplotype composition and/or deeply divergent, monophyletic mitochondrial lineages occurring among these regions. While two species are strongly neritic, white sharks are capable of movement across the Indian Ocean (Bonfil et al. 2005) and current thought is that the existence of significant mitochondrial stock structure in this region is driven by homing of gravid females to their natal coastline of origin for parturition (Pardini et al. 2001). While female-mediated gene flow is restricted between AUS and SAF in dusky sharks, I observed 6 shared mtCR haplotypes and moderate Φ_{ST} values. This may reflect a lesser propensity for natal homing in female dusky sharks, a greater capacity for movement over pelagic habitat than the two neritic sharks, or may be a result of incomplete lineage sorting given the relatively recent radiation of this species (Naylor et al. 1992, Musick et al 2004).

It has become of increasing importance recently to trace the geographic and natal area of origin for wildlife parts in trade to determine whether regional harvests are sustainable. Genetic surveys of reasonably centralized Asian fin markets may be the quickest and most efficient way to obtain this type of data for many sharks that are exploited on a global scale (Chapman et al. 2009), including dusky sharks. My MSA simulations show that the haplotype frequency differences between the USATL, SAF and AUS dusky shark mitochondrial stocks should facilitate robust reconstruction of the contribution of each of them to products in trade. Further sampling, both in terms of locations and individuals, will be necessary before this type of MSA can be applied at its full potential for this species. In addition, surveying of nuclear genetic markers (e.g., microsatellites) is necessary to determine patterns of male-mediated gene flow and further refine my characterization of dusky shark stock structure. Nevertheless, my study indicates that genetic data could enable robust estimation of the contributions of distinct dusky shark breeding areas or stocks to global fin landings and thus help guide future assessment and management efforts.

Conclusion

The global phylogeography or mitochondrial stock structures for both the dusky (*Carcharhinus obscurus*) and copper (*Carcharhinus brachyurus*) sharks indicate female-mediated gene flow occurs along continental coastlines much more than across large oceanic basins. This pattern is seen in other shark species (Ahonen et al. 2009, Chabot and Allen 2009, Chapman et al. 2009). Both species exhibit high population structure across the globe and at least 3 mitochondrial stocks or management units have been identified for each. For the dusky shark, these management units are the Northwest Atlantic (including the U.S. East Coast and Gulf of Mexico), South Africa, and Australia (both east and west coasts). For the copper shark, the management units are Southern Africa (including Namibia and South Africa), Australia/New Zealand, and Perú. Based on these mitochondrial stocks, assessments should be conducted at a regional scale to verify that the level of exploitation is sustainable within each potential management unit. For the dusky shark, this also means that the Northwest Atlantic, where this species has been evaluated as "Endangered" by the International Union for the Conservation of Nature (IUCN), is unlikely to be replenished due to migration of females from adjacent populations.

The copper shark actually shows much more structure than the dusky shark. This may be due to the fact that dusky sharks are a more recently diverged species and thus would exhibit much less genetic diversity overall (Wong et al. 2009). The large nucleotide diversity of copper sharks is driven by the presence of two highly divergent matrilineal clades that are separated by the Indian Ocean. The divergence time calculated between these two lineages roughly coincides with the rise of the Isthmus of Panama, 3.5 million years ago. Fossil remains of copper sharks have been found in California, North Carolina, and Italy from the Miocene to the Pleistocene (Long 1993, Heim and Bourdon 1998, Marsili 2008). It is possible that this species had a historical range in the Atlantic and East Pacific that was separated by the closure, with subsequent colonization of Australia and New Zealand from the East Pacific, which would explain the more recent divergence time between Australia/New Zealand and Peru and the extremely low haplotype diversity in the former. Because copper sharks are a cool temperate species, the Indian Ocean would intuitively be a long-lasting barrier to female mediated gene flow as it contains large expanses of warm, open water. The Indian Ocean proves to be a barrier in two other temperate shark species as well, the white shark (*Carcharadon carcharias*) and the grey nurse shark (*Carcharias taurus*) (Pardini et al. 2001, Stow et al. 2006).

Another useful application of mtCR sequencing, a logical extension of the delineation of management units, is to the identification of broad area of origin of parts in trade. This is extremely important as it applies to the shark fin trade, in which many individual stocks are not being managed appropriately (Bonfil 1994). The concentration of traded fins in a centralized market (Hong Kong) presents an opportunity to cost-effectively sample the global trade and generate region-specific landings data using genetic techniques. My studies both clearly illustrate that tracing fins from coastal carcharhinid sharks in trade back to at least a "continental-shelf-of-origin" can be achieved relatively easily using a short segment of the mitochondrial control region. Given that Chapman et al. (2009) showed the same for a coastal and semi-pelagic

hammerhead shark, we think that the growing literature on shark population genetics will prove extremely useful for this application.

Much more work is needed in the area of shark population genetics. A large proportion of sharks and other species remain to be studied regarding their population structure and many existing studies could be expanded upon with more markers (i.e. nuclear markers to build upon mitochondrial studies) and more samples and sampling locations. Studies that use genetics to examine fins or other body parts in the actual markets are few and await improved fin market access. The listing of additional shark species by the Convention on International Trade in Endangered Species (CITES) or new legislation allowing access to fins markets in Asia are needed to make full use of the ever-improving database of shark population genetics to obtain much-needed region-specific shark landings data.

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Appendix: Figures and Tables

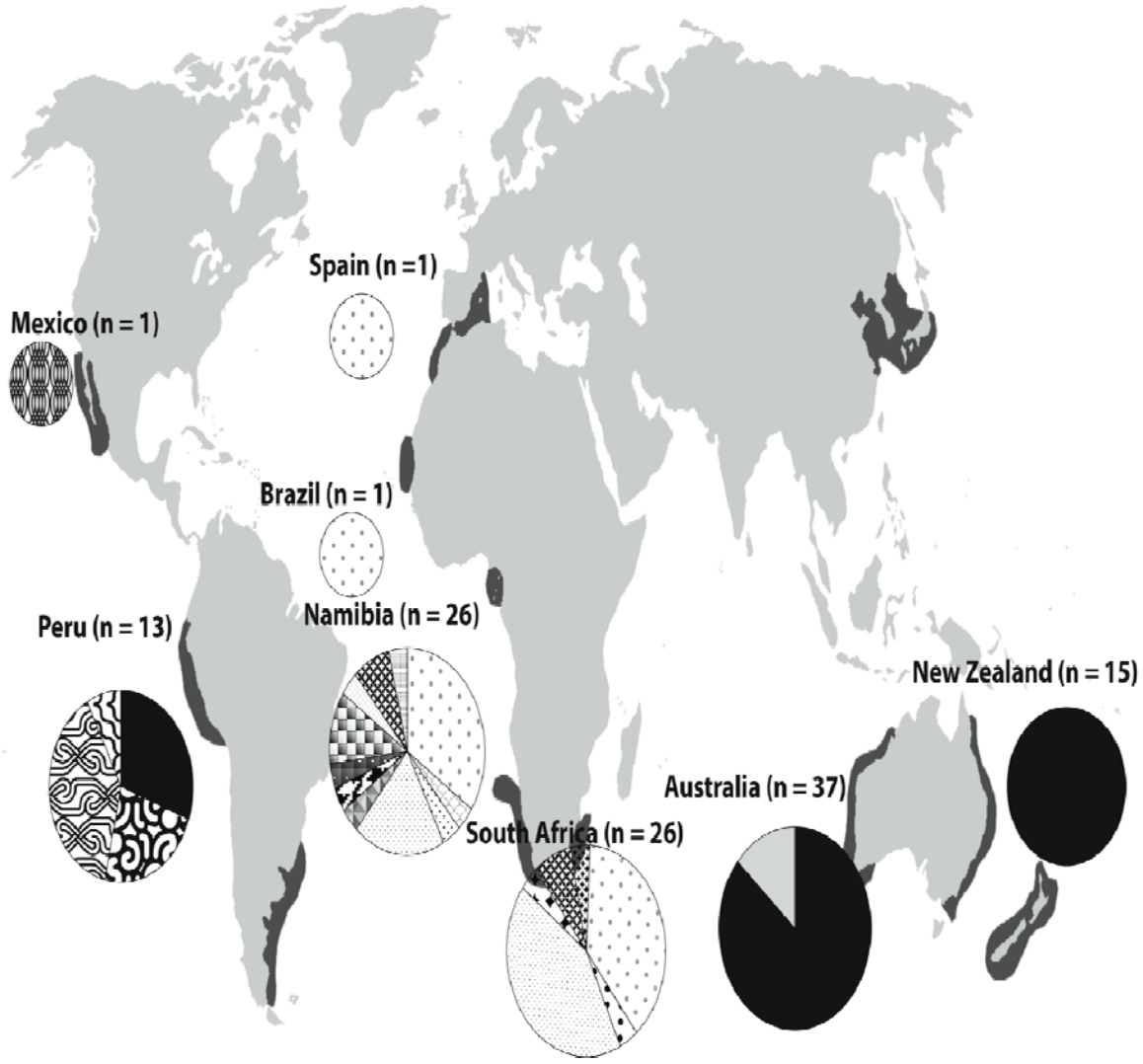

Fig. 1. Mitochondrial control region (mtCR) haplotype frequencies of samples collected from the range of *Carcharhinus brachyurus* (shadowed coastline) in Peru (PER), Namibia (NAM), South Africa (SAF), Australia (AUS) and New Zealand (NZD). Haplotypes are each denoted by separate patterns.

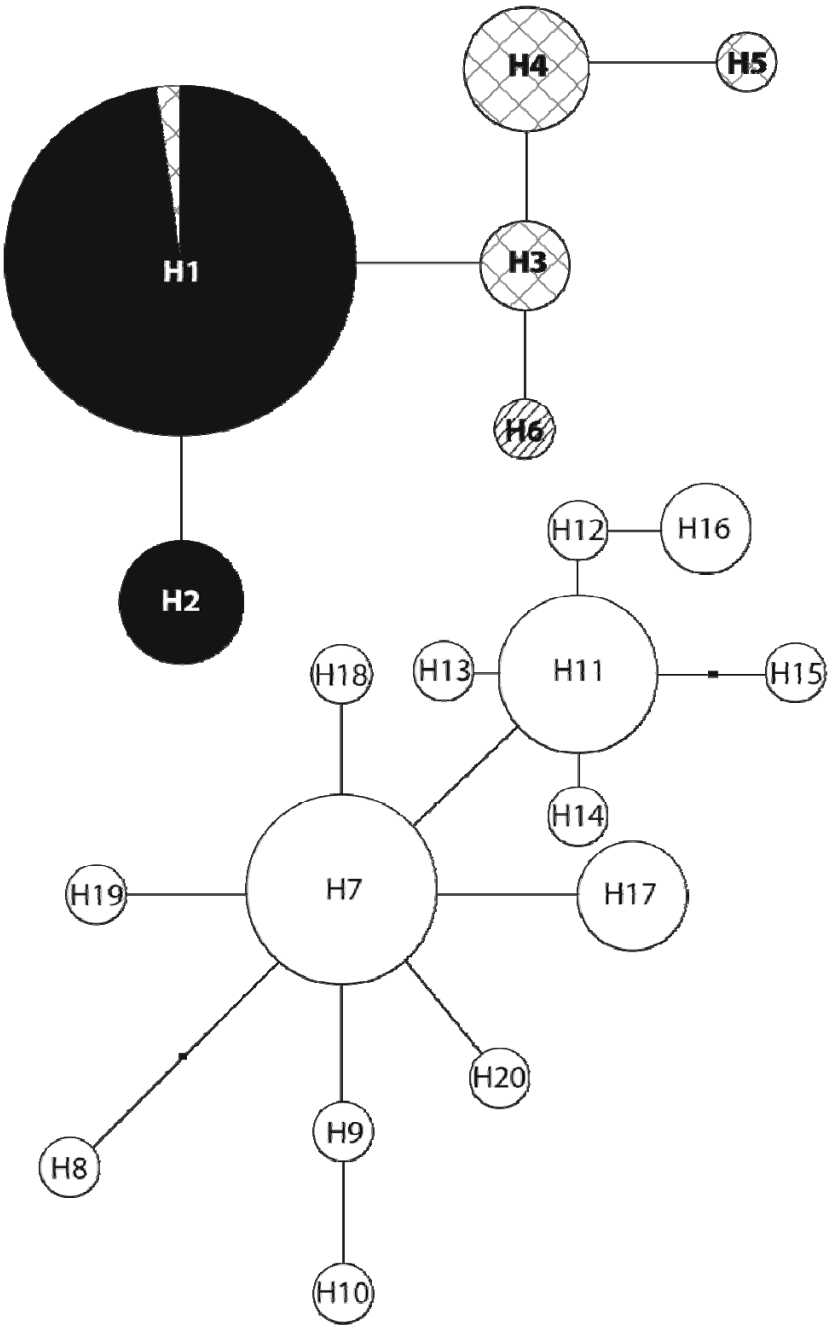

Fig. 2. Mitochondrial control region (mtCR) haplotype network of *Carcharhinus brachyurus,* with haplotypes numbered in circles. Small squares represent inferred mutational steps. Solid circles represent haplotypes displayed in Australia/New Zealand, checkered circles or patterns are Peruvian haplotypes and Mexico's haplotype is a striped circle; empty circles are haplotypes from South Africa and Namibia.

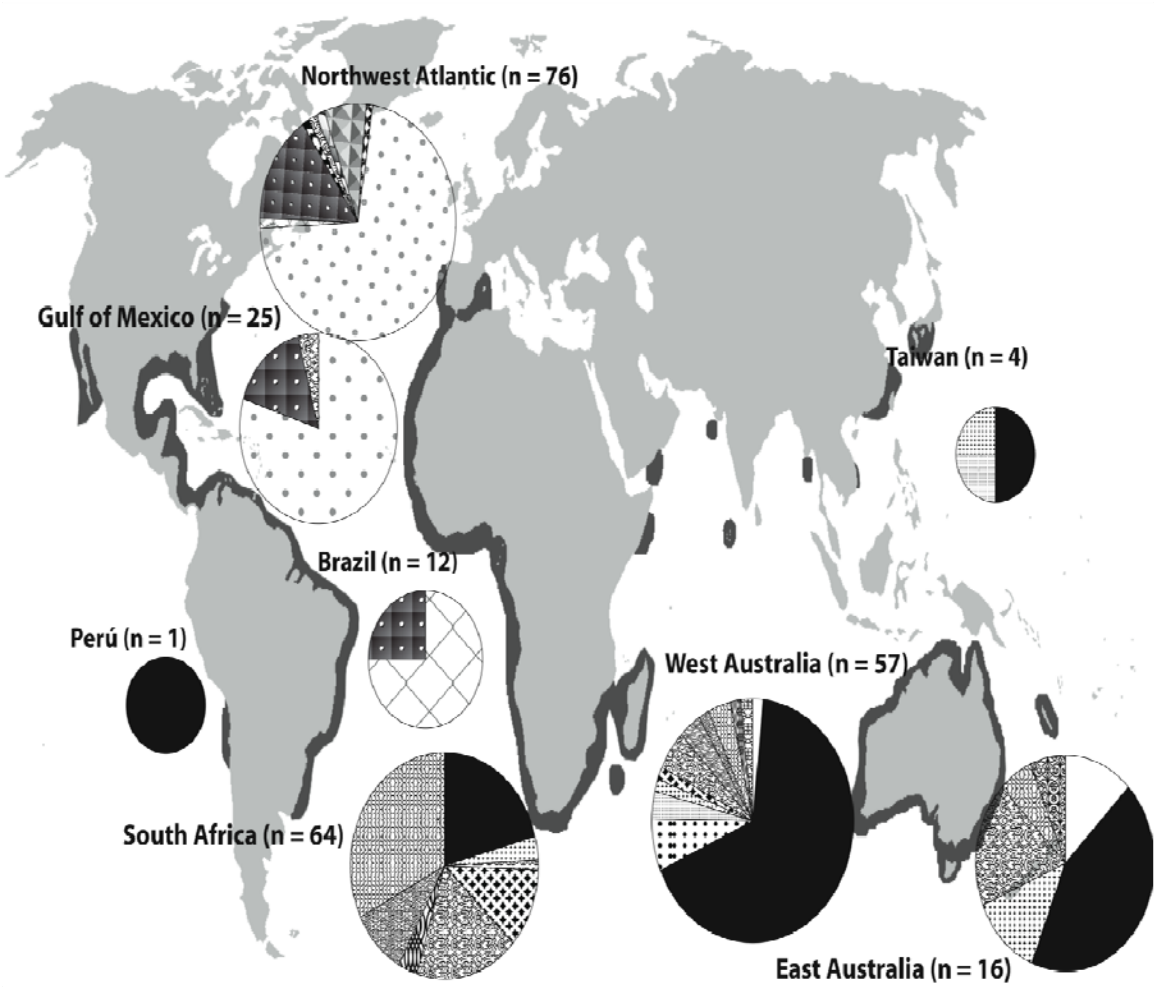

Fig. 3. Mitochondrial control region (mtCR) haplotype frequencies from samples collected across the global distribution of *Carcharhinus obscurus* (shadowed areas): United States East Coast (USEC), United States Gulf of Mexico (USGOM), Brazil (BRZ), South Africa (SAF), west Australia (WAUS), east Australia (EAUS), Taiwan (TAI) and Perú (PER). Note that 10 of 12 samples putatively from "Brazil" were obtained from Hong Kong-based fin dealers who both indicated the fins had been purchased directly from Brazil.

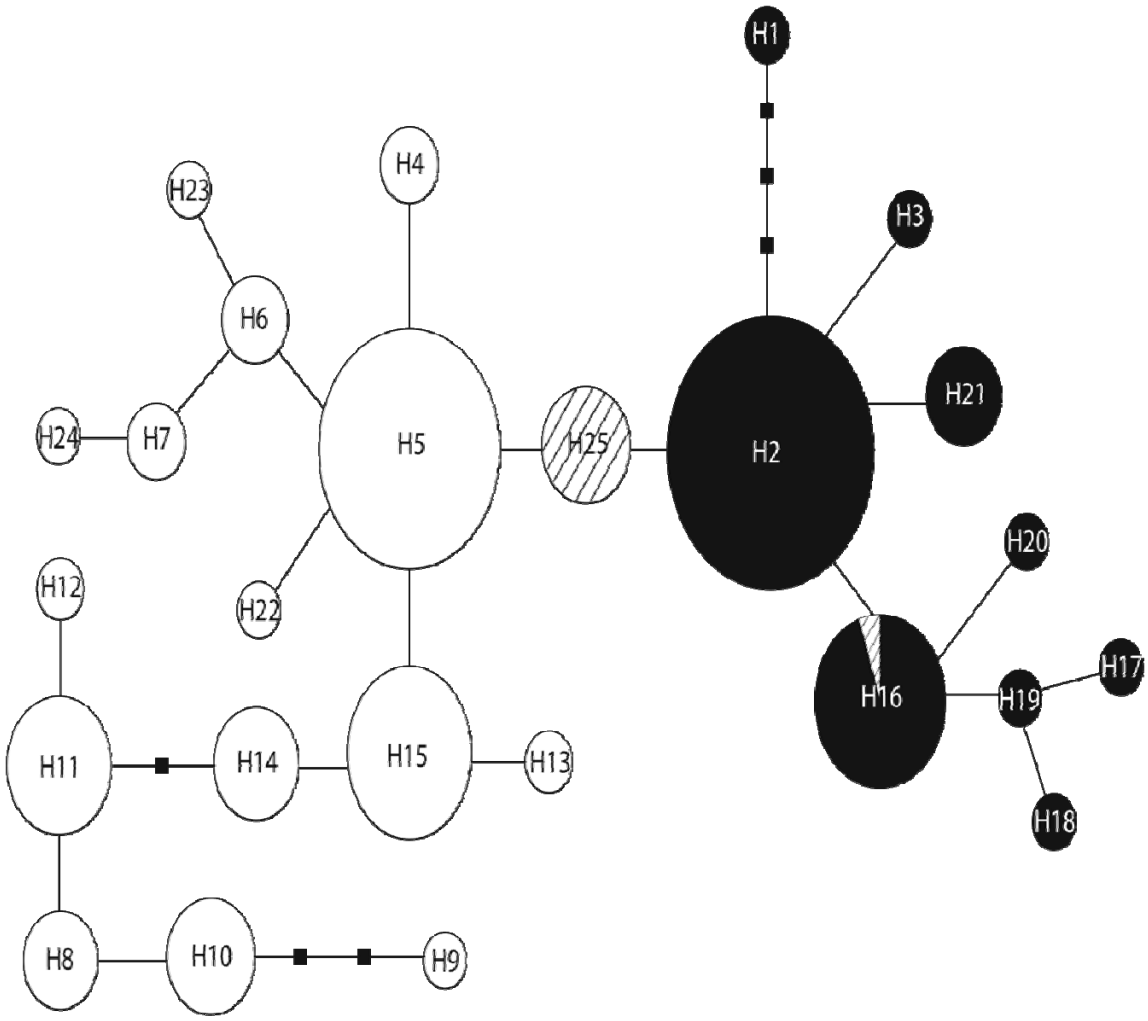

Fig. 4. Mitochondrial control region (mtCR) haplotype network (95% confidence), with individual haplotypes denoted by the number in each circle. The size of the circle is proportional to the frequency of the haplotype in the global sample. Solid-filled circles represent haplotypes found in the United States. Open circles represent haplotypes observed in the Indo-Pacific. Striped circles or fractions of circles represent haplotypes directly collected or putatively originating from Brazil. Small squares represent inferred mutational steps between haplotypes.

Table 1. *Carcharhinus brachyurus* mtCR haplotypes with numbered polymorphic sites.

	Nucleotide Position																													
Haplotype #		3 0	5 2	7 9	1 1 2	1 1 5	1 3 8	1 5 7	1 9 2	1 9 5	2 5 7	2 9 3	2 9 8	3 4 4	3 7 2	3 7 8	3 9 5	4 0 7	4 2 4	4 5 6	5 1 7	5 2 8	5 3 5	5 3 6	5 9 9	6 1 7	6 1 8
1	C	C	G	C	T	T	T	T	-	A	A	C	C	C	-	T	A	T	C	G	C	C	A	T	A	T	C
2	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	C	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	.	T	A	A	A	C	C	C	.	.	.	T	T	T	.	.	C	C	G	A	T	A	.	A	G	C	T
8	.	T	A	A	A	C	C	C	C	T	.	T	T	T	.	.	C	C	G	A	T	A	.	A	G	C	T
9	.	T	A	A	A	C	C	C	.	.	.	T	T	T	.	.	C	C	G	A	T	A	T	A	G	C	T
10	.	T	A	A	A	C	C	C	.	.	.	T	T	T	G	.	C	C	G	A	T	A	T	A	G	C	T
11	.	T	A	A	A	C	C	C	.	.	.	T	.	T	.	.	C	C	G	A	T	A	.	A	G	C	T
12	.	T	A	A	A	C	C	C	.	.	.	T	.	T	G	.	C	C	G	A	T	A	.	A	G	C	T
13	.	T	A	A	A	C	C	C	.	.	.	T	.	T	.	.	C	C	G	A	T	A	.	A	T	C	T
14	.	T	A	A	A	C	C	C	.	.	.	T	.	T	.	.	C	C	G	A	T	A	.	.	G	C	T
15	.	T	A	A	A	C	C	C	C	.	.	T	.	T	.	.	T	C	G	A	T	A	.	A	G	C	T
16	.	T	A	A	A	C	C	C	.	.	.	T	.	T	G	A	C	C	G	A	T	A	.	A	G	C	T
17	.	T	A	A	A	C	C	C	.	.	.	T	T	T	.	.	C	C	G	A	T	A	.	A	T	C	T
18	.	T	A	A	A	C	C	C	.	.	.	T	T	T	.	.	T	C	G	A	T	A	.	A	G	C	T
19	.	T	A	A	A	C	C	C	.	.	.	T	T	T	.	A	C	C	G	A	T	A	.	A	G	C	T
20	.	T	A	A	A	C	C	C	.	.	.	T	T	T	.	.	C	C	G	A	T	A	.	.	G	C	T

Table 2. Global copper shark haplotype frequency distribution with designated haplotype number (Hap. #) along the top row and sampling region in the left-most column. Cells contain the number of times the designated haplotype was observed in a sampling region. Sampling region codes are as follows: AUS = Australia, BRA = Brazil, MEX = Mexico, NAM = Namibia, NZD = New Zealand, PER = Perú, SAF = South Africa, SPA = Spain.

Hap. #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AUS	32	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BRA	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
MEX	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NAM	0	0	0	0	0	0	9	0	1	1	5	1	1	0	1	3	2	0	1	1
NZD	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PER	4	0	3	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SAF	0	0	0	0	0	0	10	1	0	0	11	0	0	1	0	0	2	1	0	0
SPA	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Summary of sample size (N), number of haplotypes, haplotype diversity (h), nucleotide diversity (π), and coancestry coefficient (θ_S) for *Carcharhinus brachyurus* in all sampling regions of this study.

Location	N	Haplotype no.	*h*	π	θ_S
Australia	37	2	0.24024	0.00037	0.23955
Brazil	1	1	N/A	N/A	N/A
Mexico	1	1	N/A	N/A	N/A
Namibia	26	11	0.84615	0.00203	1.57234
New Zealand	15	1	N/A	N/A	N/A
Peru	13	4	0.75641	0.0018	0.96674
South Africa	26	6	0.68923	0.00138	1.04823
Spain	1	1	N/A	N/A	N/A
All Samples	120	20	0.76452	0.01573	4.67844

Table 4. Global Analysis of Molecular Variance of *Carcharhinus brachyurus*

Φ-Statistics Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	4	563.064	6.21491 Va	94.72
Between populations	112	38.763	0.34610 Vb	5.28
Total	116	601.827	6.56101	
Fixation index (Φ_{ST})	0.94725			

$P < 0.000001$

Table 5. Population differentiation among *Carcharhinus brachyurus* samples collected in five regions: Australia (AUS, n=37), Namibia (NAM, n=26), New Zealand (NZD, n=15), Peru (PER, n=13), and South Africa (SAF, n=26). Numbers above diagonal show average pairwise nucleotide divergence beween populations (Kimura-2 distance). Numbers below the diagonal show pairwise Φ_{ST} between populations, with values significantly different from 0 in **bold** ($p<0.000001$).

	AUS	NAM	NZD	PER	SAF
AUS	-	20.47682	0.13593	1.37658	20.24052
NAM	**0.97**	-	20.33587	19.03644	1.09543
NZD	0.06	**0.96**	-	1.24039	20.09962
PER	**0.60**	**0.93**	**0.55**	-	18.80036
SAF	**0.97**	-0.002	**0.97**	**0.95**	-

Table 6. Mixed stock analysis simulation results for *Carcharhinus brachyurus* comparing the concordance between known, user-specified mitochondrial stock contributions and mean reconstructed mitochondrial stock contributions based on stock-specific haplotype frequencies observed in this study. AFR: African stock, AUS: Australian stock, PER: Peruvian stock

User-specified contributions %	Reconstructed contributions (mean ± SD, %)
33 AFR, 33 AUS, 33 PER	33.4 ± 4.8 AFR, 33.4 ± 6.2 AUS, 33.3 ± 6.1 PER
90 AFR, 5 AUS, 5 PER	90.0 ± 3 AFR, 5.01 ± 2.6 AUS, 5.02 ± 2.6 PER
5 AFR, 90 AUS, 5 PER	4.98 ± 2.3 AFR, 90.1 ± 3.5 AUS, 4.89 ± 2.5 PER
5 AFR, 5 AUS, 90 PER	5.06 ± 2.2 AFR, 5.00 ± 4.8 AUS, 89.9 ± 5.1 PER

Table 7. Global dusky shark haplotype frequency distribution with designated haplotype number (Hap. #) along the top row and sampling region in the left-most column. Cells contain the number of times the designated haplotype was observed in a sampling region. Sampling region codes are as follows: WAUS = west Australia, EAUS = east Australia, BRA = Brazil, USGOM = Gulf of Mexico, USEC = northwest Atlantic, PER = Perú, SAF = South Africa, TAI = Taiwan.

Hap. #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
WAUS	0	0	0	1	38	4	2	1	0	1	3	2	0	1	2	0	0	0	0	0	0	1	0	1	0
EAUS	0	0	0	2	7	0	0	2	0	0	3	0	0	0	1	0	0	0	0	0	0	0	1	0	0
BRA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	9
USGOM	0	02	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	0	0	0	0	0	0
USEC	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	1	6	0	0	0	0
PER	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SAF	0	0	0	0	41	0	0	2	1	8	1	0	2	6	02	0	0	0	0	0	0	0	0	0	0
TAI	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 8. Summary of sample size (N), number of haplotypes, haplotype diversity (h), nucleotide diversity (π) and coancestry coefficient (θ_S) in all sampling regions for *Carcharhinus obscurus*. Sampling region codes follow Table 1. Samples from Brazil, Perú and Taiwan are not included in this because of small sample size or uncertain provenance (see text).

Location	N	Haplotypes	*h*	π	θ_S
WAUS	57	12	0.55201	0.00266	2.17
EAUS	16	6	0.78333	0.00466	2.41
USGOM	25	3	0.34667	0.00089	0.79
USEC	76	8	0.48807	0.00121	1.64
SAF	64	8	0.81101	0.0037	1.49

Table 9. Global Analysis of Molecular Variance for *Carcharhinus obscurus*

Φ-Statistics Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	3	148.555	0.85420 Va	55.49
Between populations	234	160.352	0.68527 Vb	44.51
Total	237	308.907	1.53947	
Fixation index (Φ_{ST}) 0.55487				

$P < 0.000001$

Table 10. Population differentiation among *Carcharhinus obscurus* samples collected in five sampling regions. For this analysis, EAUS and WAUS were collapsed into one sample (Australia=AUS) after a parallel AMOVA using these and published sequences failed to reject panmixia between them. Numbers below the diagonal show pairwise Φ_{ST} values between sampling locations. The asterisk (*) on the above diagonal indicates pairwise Φ_{ST} values that were significantly different from 0 ($p<0.000001$). NS=non-significant.

	USEC	GOM	SAF	AUS
USEC	-	NS ($p > 0.54$).	*	*
GOM	-0.009	-	*	*
SAF	0.68	0.63	-	*
AUS	0.62	0.58	0.18	-

Table 11. Mixed Stock Analysis (MSA) simulation for *Carcharhinus obscurus*. The table shows the level of concordance between the known mitochondrial stock (mt stock) contributions (i.e. "User-specified", left column) and mean mt stock contributions reconstructed in SPAM ("Reconstructed contributions", right column). The reconstructions are based on the haplotype frequencies observed in this study for the three mitochondrial stocks I defined from AMOVA analysis: United States Atlantic (USATL), South Africa (SAF), Australia (AUS).

User-specified contributions %	Reconstructed contributions (mean ± SD, %)
33 USATL, 33 SAF, 33AUS	33.3 ± 4.6 USATL, 33.1 ± 6.9 SAF, 33.6 ± 6.6 AUS
90 USATL, 5 SAF, 5 AUS	90.1 ± 3 USATL, 5.03 ± 2.9 SAF, 4.87 ± 2.9 AUS
5 USATL, 90 SAF, 5 AUS	5.11 ± 2.2 USATL, 89.8 ± 4.8 SAF, 5.06 ± 4.5 AUS
5 USATL, 5 SAF, 90 AUS	5.15 ± 2.2 USATL, 5.23 ± 4.5 SAF, 89.6 ± 4.9 AUS

Table 12. *Carcharhinus obscurus* mtCR haplotypes with numbered polymorphic sites.

	Nucleotide Position																				
Haplotype #	30	79	111	132	144	152	169	170	224	225	246	282	371	402	417	418	420	421	422	527
1	C	T	T	A	A	A	T	T	T	A	T	C	-	C	G	C	A	C	-	G
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	T	G	.
3	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A	T	.	T	G	.
4	.	A	.	.	C	.	.	.	C	.	.	.	.	.	A	T	.	T	G	.
5	.	A	.	.	C	.	.	.	.	.	.	.	.	.	A	T	.	T	G	.
6	.	A	.	.	C	.	.	C	.	.	.	.	.	.	A	T	.	T	G	.
7	.	A	.	.	C	G	.	C	.	G	.	.	.	.	A	T	.	T	G	.
8	T	A	.	.	C	.	.	.	.	G	.	T	.	.	A	T	.	T	G	.
9	T	A	.	.	C	.	.	.	.	G	.	T	.	.	.	T	G	T	.	.
10	T	A	.	.	C	.	.	.	.	G	.	T	.	.	A	T	.	T	G	.
11	.	A	.	G	C	.	.	.	.	G	.	T	.	.	A	T	.	T	G	.
12	.	A	.	.	C	.	.	.	.	G	.	T	.	.	A	T	.	T	G	.
13	.	A	.	.	C	.	.	.	.	G	.	T	.	.	A	T	.	T	G	.
14	.	A	.	.	C	.	.	.	.	G	.	.	.	.	A	T	.	T	G	.
15	.	A	.	.	C	.	.	.	.	G	.	.	.	.	A	T	.	T	G	.
16	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	T	G	.
17	.	C	.	.	.	.	.	.	C	.	.	.	.	.	A	T	.	T	G	T
18	.	C	.	.	.	.	C	.	.	.	.	.	.	.	A	T	.	T	G	T
19	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	T	G	T
20	.	C	.	.	.	.	.	.	.	.	.	.	.	A	A	T	.	T	G	.
21	.	T	.	.	.	.	.	.	.	.	.	.	.	A	A	T	.	T	G	.
22	.	A	.	.	C	.	.	.	.	.	.	.	G	.	A	T	.	T	G	.
23	.	A	.	.	C	.	.	C	.	.	C	.	.	.	A	T	.	T	G	.
24	.	A	A	.	C	G	.	C	.	.	.	.	.	.	A	T	.	T	G	.
25	.	A	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	T	G	.