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Phylogeography of the copper shark (*Carcharhinus brachyurus*) in the southern hemisphere: implications for the conservation of a coastal apex predator

Martin T. Benavides[A], *Kevin A. Feldheim*[B], *Clinton A. Duffy*[C], *Sabine Wintner*[D], *J. Matias Braccini*[E], *Jessica Boomer*[F], *Charlie Huveneers*[G,H], *Paul Rogers*[G,H], *Jeffrey C. Mangel*[I,J], *Joanna Alfaro-Shigueto*[I,J], *Daniel P. Cartamil*[K] *and Demian D. Chapman*[A]

[A]Institute for Ocean Conservation Science and School of Marine and Atmospheric Science, Stony Brook University, Stony Brook, New York 11794, USA.
[B]Field Museum, Pritzker Laboratory for Molecular Systematics and Evolution, 1400 South Lake Shore Drive, Chicago, IL 60605, USA.
[C]Aquatic and Threats Unit, Department of Conservation, Auckland, New Zealand.
[D]KwaZulu-Natal Sharks Board, Private Bag 2, Umhlanga Rocks 4320, and Biomedical Resource Unit, University of KwaZulu–Natal, Durban 4000, South Africa.
[E]Fisheries Centre, The University of British Columbia, Vancouver, BC V6T1Z4, Canada. Present address: Queensland Department of Employment, Economic Development and Innovation, Southern Fisheries Centre, Deception Bay, Qld 4508, Australia.
[F]Department of Biological Sciences, Macquarie University, NSW 2109, Australia.
[G]Threatened, Endangered, and Protected Species Subprogram, SARDI – Aquatic Sciences, Adelaide, SA 5024, Australia.
[H]School of Biological Sciences, Flinders University, Adelaide, SA 5043, Australia.
[I]Pro Delphinus, Octavio Bernal 572-5, Lima 11, Perú.
[J]Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall, TR10 9EZ, United Kingdom.
[K]Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive (Mail code: 0204), La Jolla, CA 92093-0204, USA.
[L]Corresponding author. Email: demian.chapman@stonybrook.edu

Abstract. The copper or bronze whaler shark (*Carcharhinus brachyurus*) is a large, coastal top predator that is vulnerable to overexploitation. We test the null hypothesis that copper sharks are panmictic throughout the southern hemisphere. We analysed part of the mitochondrial control region (mtCR) in 120 individuals from eight sampling areas, defining 20 mtCR haplotypes ($h = 0.76 \pm 0.06$, $\pi = 0.016 \pm 0.0007$). Significant genetic structure was detected among the following three major coastal regions separated by oceanic habitat: Australia–New Zealand, South Africa–Namibia and Perú (AMOVA $\Phi_{ST} = 0.95$, $P < 0.000001$). A major phylogeographic discontinuity exists across the Indian Ocean, indicating an absence of at least female-mediated gene flow for ~3 million years. We propose that this species originated in the Atlantic, experienced vicariant isolation of Pacific and Atlantic lineages by the rise of the Isthmus of Panama and, subsequently, dispersed across the Pacific to colonise Australasia. Oceanic expanses appear to be traversed over evolutionary but not ecological timescales, which means that regional copper-shark populations should be assessed and managed independently.

Additional keywords: bronze whaler, control region, fin trade, mitochondrial DNA, population genetics.

Introduction

Although there are more than 400 living species of sharks, the phylogeography of very few of them has been examined in detail (Duncan *et al.* 2006; Keeney and Heist 2006; Stow *et al.* 2006; Schultz *et al.* 2008; Ahonen *et al.* 2009; Chabot and Allen 2009; Chapman *et al.* 2009; Portnoy *et al.* 2010). Sharks are unlike many other marine fish in that they do not have a planktonic larval stage, which means that dispersal is entirely mediated by the movement and reproductive mixing of individuals among regions, as opposed to larval drift in ocean currents. Thus, the

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phylogeography of sharks is shaped by a combination of their contemporary movement patterns, the distribution of critical habitats (e.g. nursery areas) and the nature of the species radiation from its centre-of-origin over evolutionary timescales. Delineating the phylogeography of sharks is extremely useful for defining management units (Ahonen *et al.* 2009; Chabot and Allen 2009). Such studies could also enable genetic mixed-stock analysis (MSA) of internationally traded shark products (e.g. dried fins) to estimate stock-specific landings (Chapman *et al.* 2009).

The copper shark or bronze whaler (*Carcharhinus brachyurus* Günther, 1870) is a large apex predator (max length = 3.25 m TL) belonging to the family Carcharhinidae (requiem or whaler sharks), which includes the majority of heavily exploited large sharks worldwide (Clarke *et al.* 2006; Last and Stevens 2009). Whaler sharks 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 affected by fisheries (Smith *et al.* 1998). 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 small litters (up to 24 offspring) on what is probably a biennial cycle (Garrick 1982; Walter and Ebert 1991; Cliff and Dudley 1992).

Copper sharks have an anti-tropical, coastal distribution (Garrick 1982; Compagno *et al.* 2005). Major population centres occur in the western South Atlantic from southern Brazil to northern Argentina (Lucifora *et al.* 2005), the eastern Atlantic in north-western Africa and the south-western coast of South Africa (Walter and Ebert 1991), the Mediterranean, the Indian Ocean off south-eastern South Africa (Cliff and Dudley 1992) and western Australia (Last and Stevens 2009), 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). Copper sharks are fished throughout their range by recreational, commercial and artisanal fisheries and are primarily caught for their meat and their fins, the latter of which are marketed in Asia to make the delicacy shark fin soup (Cavanagh *et al.* 2003; Duffy and Gordon 2003).

In a global assessment of copper sharks for the IUCN, Duffy and Gordon (2003) listed 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 suggested that each coastal population is demographically independent, but indicated 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 IUCN regional classifications and more quantitative assessments (Dudgeon *et al.* 2009).

Despite a widespread distribution, the hypothesis that most coastal populations of copper sharks are distinct (Duffy and Gordon 2003) seems plausible, given that some other large coastally oriented shark species exhibit highly structured populations across the globe (Duncan *et al.* 2006; Schultz *et al.* 2008; Portnoy *et al.* 2010). Most of these studies found structure by using mitochondrial genetic loci, which, by virtue of a maternally inherited mode of inheritance, registers a signal of genetic differentiation when female-mediated gene flow is low even if male-mediated gene flow is high. We therefore hypothesised that oceanic expanses form a barrier to gene flow in copper sharks and predicted discontinuities in mitochondrial haplotype frequencies among collections separated by this type of barrier.

We also begin to test hypotheses about the historic radiation of this species. Some sharks are believed to have originated in the Indo-Pacific and colonised the western Atlantic and eastern Pacific more recently. This pattern is typified by high genetic diversity and ancestral haplotypes occurring in the Indo-Pacific, with closely related but derived haplotypes found in the western Atlantic and eastern Pacific (e.g. scalloped hammerheads, *Sphyrna lewini*, Duncan *et al.* 2006; blacktips, *Carcharhinus limbatus*, Keeney and Heist 2006). Other sharks experienced vicariant isolation of eastern Pacific and western Atlantic populations by the rise of the Isthmus of Panama ~3 million years ago. This pattern is typified by a deep phylogenetic break separating Atlantic and Pacific matrilines (e.g. lemon sharks, *Negaprion brevirostris*, Schultz *et al.* 2008). Given that fossil teeth from copper sharks have been found in Miocene deposits in the Atlantic and eastern Pacific (Long 1993; Heim and Bourdon 1998; Marsili 2008), we hypothesised that this species originated in this region, experienced vicariant isolation and subsequently migrated into the western Pacific.

Materials and methods

Sample acquisition

In total, 117 copper sharks were sampled from the following five sampling areas in the southern hemisphere: Namibia, South Africa, Australia, New Zealand and Perú (Fig. 1). Samples from one individual each were also collected in Spain, Brazil and the Pacific coast of Mexico, which we include to provide preliminary insights into how these regions may be related to the others. Specimens were obtained by a combination of recreational (Namibia, New Zealand) and commercial fishery sampling (Australia, Perú, Mexico, Brazil) and beach-meshing captures (South Africa). Specimens were neither spatially nor temporally clustered in any sampling region (i.e. they were collected in multiple sampling events, spread over at least 1 year). Given the difficulty in procuring samples for this species, specimens consisted of a mix of juvenile and adult individuals in all sampling regions.

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 with the DNeasy® Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol, with some adjustments of final elution volumes based on tissue type. Samples from fins generally contained a higher concentration of DNA than those from muscle and were eluted into a final volume of 300 μL, whereas muscle extractions were eluted into 150 μL. Genomic DNA was checked for quality and approximate quantity on a 0.8% agarose gel run at 60 V for ~45 min.

Mitochondrial control region amplification, sequencing and analysis

Polymerase chain reaction (PCR) was used to amplify the mitochondrial control region (mtCR) from all samples. Reactions

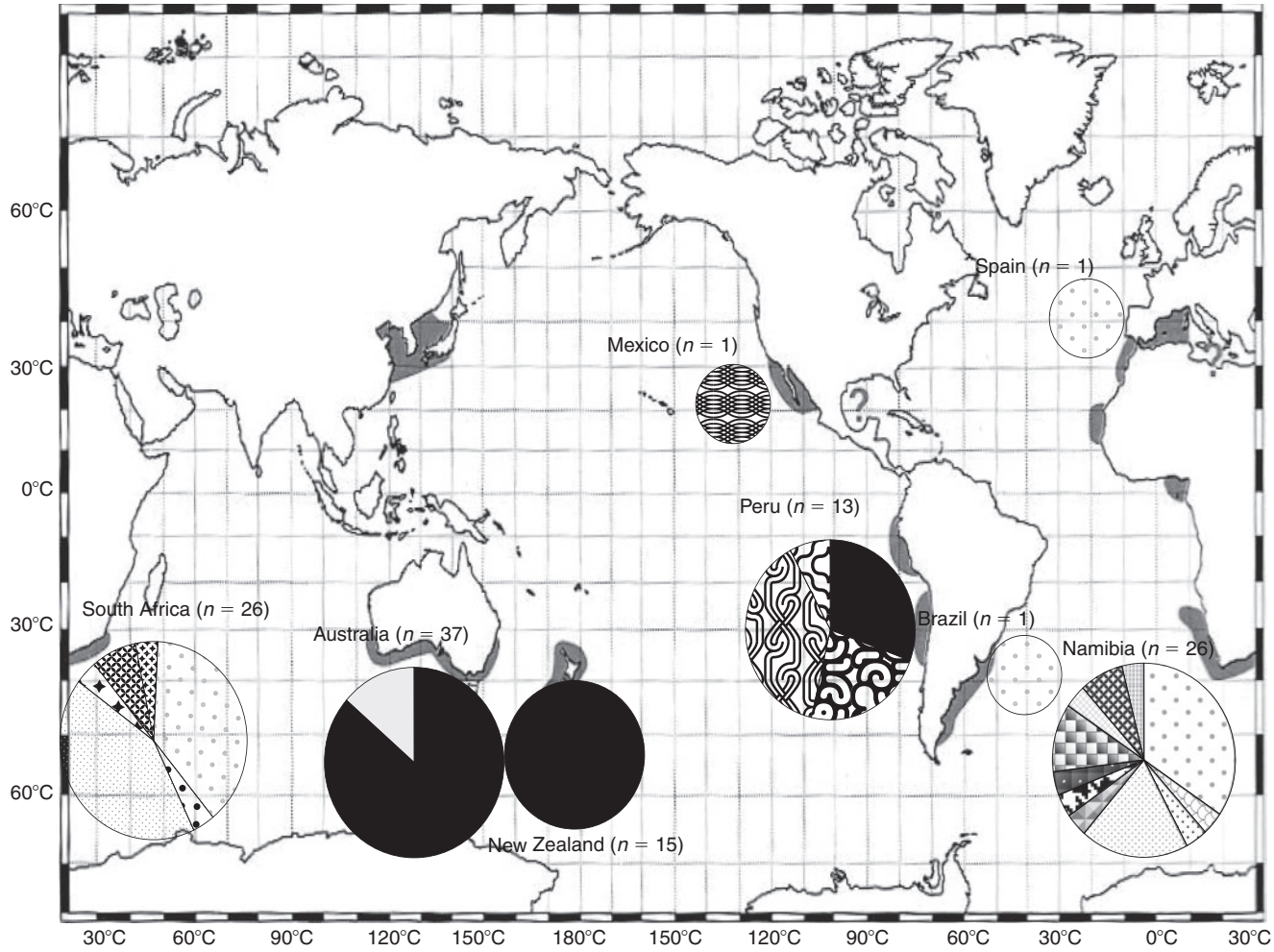

Fig. 1. Mitochondrial control region (mtCR) haplotype frequencies of samples collected from the range of copper sharks (shadowed coastline) in Peru (PER), Namibia (NAM), South Africa (SAF), Australia (AUS) and New Zealand (NZD). Haplotypes are each denoted by a different pattern.

were carried out in 50-μL volumes containing 1 μL of genomic DNA, 1× PCR buffer (Qiagen Inc., Valencia, CA, USA), 40 μM dNTPs, 12.5 pmol of each of the primers Pro-L (5′-AGGGRAAGGAGGGTCAAACT-3′, Keeney *et al.* 2003) and 12S (5′- AAGGCTAGGACCAAACCT-3′, Keeney *et al.* 2003), and 1 unit of HotStar Taq™ DNA Polymerase (Qiagen Inc.). PCR was performed in a LabnetMultigene TC9600-G thermocycler (Woodbridge, CA) 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 10 min at 72°C. PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase according to the manufacturer's protocol (USB Corporation, Cleveland, Ohio, USA). Dye termination sequencing was performed using the Pro-L forward primer. Cycle sequencing reactions were performed in a Bio-RAD Dyad thermocycler (Hercules, CA) for 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reactions were precipitated with ethanol and 125 mM EDTA and run on an ABI 3730 DNA Analyzer (Carlsbad, California, USA).

Sequences were validated by eye in the program Chromas 2.33 (http://www.technelysium.com.au, accessed January 2010) and aligned and trimmed in the program GeneDoc (http://www.nrbsc.org/gfx/genedoc/, accessed January 2010). 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 90% confidence interval to show the evolutionary relationships among haplotypes (Clement *et al.* 2000). Genetic-diversity indices for each collection, as well as overall diversity indices, were calculated in DnaSP 4.0 (Rozas *et al.* 2003).

Our null hypothesis was that copper sharks are panmictic throughout the southern hemisphere. We used analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) run in Arlequin 3.1 (Schneider *et al.* 2000) to partition genetic variance among populations and within populations and then calculate the fixation index Φ_{ST} to assess population subdivision. We analysed one group consisting of the following five putative populations: Namibia, South Africa, Australia, New Zealand and Perú. We used the Tamura Nei substitution model to calculate the genetic distance between sequences because it had the highest log-likelihood score of all of the available models in Arlequin 3.1, according to jModelltest (Posada 2008). We tested for pairwise genetic differentiation between

all populations by calculating pairwise Φ_{ST} between them. The significance of pairwise differences was assessed with 10 000 permutations of the sequence data implemented in Arlequin.

Migration and divergence

Divergence time and migration rates were estimated with the program MDIV (Nielsen and Wakeley 2001), using the resources of the Computational Biology Service Unit from Cornell University. The Bayesian Markov chain Monte Carlo method using the finite series model was performed on comparisons between an aggregated Australia–New Zealand ('Australasia') population (based on non-significant pairwise Φ_{ST} results, described below) and an aggregated South Africa–Namibia population ('southern Africa'). Both aggregated populations were also compared with Perú in the same way. For all comparisons, one long-chain was run for 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 ($M = 2N_e m$, where m = migration rate) and T ($T = t/2N_e$, where t = divergence time). To calculate effective population size (N_e), the mutation rate from a published study on the scalloped hammerhead shark (*Sphyrna lewini*), a member of the sister family to requiem sharks, was used (Duncan *et al.* 2006). For the comparison between Australasia and southern Africa, an initial run showed that M was not significantly different from 0 and T was likely to be >10, therefore M_{max} was set to 0 and T_{max} was set to 15 for subsequent runs so as to better assess T alone. For the comparison between southern Africa and Perú, M_{max} was set to 0 and T_{max} to 10 for the reasons stated above. Both M_{max} and T_{max} were left at default states for the comparison between Australasia and Perú.

Results

Population structure and genetic diversity of copper sharks

In total, 120 partial mtCR sequences (5′ end, 642–643 bp) were obtained and analysed from an amplicon of ~1100 bp that contained the entire mtCR. The partial sequence was composed of 20.71% cytosine, 37.89% thymine, 30.65% adenine and 10.75% guanine. There were 27 polymorphic sites, 16 transitions, 11 transversions and two indels characterising 20 haplotypes (GenBank Accession numbers HQ711308–HQ711327).

Two highly divergent mtCR clades were present in the global sample, comprising haplotypes separated from each other by a minimum of 15 mutational steps (Table 1, Fig. 2). The two clades were perfectly segregated by geography: the first was found in South Africa, Namibia, Brazil and Spain, whereas the second was restricted to Australia, New Zealand, Perú and the Mexican Pacific (Tables 2, 3). Of the 20 haplotypes, two occurred in Australia (one endemic to this collection), one occurred in New Zealand (not endemic), 14 occurred in South Africa, Namibia, Brazil and Spain (all endemic to these collections), four occurred in Perú (3 endemic) and one endemic haplotype occurred in Mexico (Table 2, Fig. 1). Overall haplotype diversity (h) of the global sample was 0.76 ± 0.06 and nucleotide diversity (π) was 0.016 ± 0.0007 (Table 3). Haplotype and nucleotide diversity were higher in Atlantic, western Indian and Peruvian collections than in either Australia or New Zealand (Table 3). Because of a low sample size, samples from Brazil ($n = 1$), Mexico ($n = 1$) and Spain ($n = 1$)

Table 1. Copper-shark mitochondrial control region (mtCR) haplotypes with numbered polymorphic sites
A period indicates that the base in that position is the same as the base in Haplotype 1

Haplotype no.	Nucleotide position																												
		3			1	1	1	1	1	1	2	2	2	3	3	3	3	4	4	4	5	5	5	5	5	6	6
			5	7	1	1	3	5	9	9	5	9	9	4	7	7	9	0	2	5	1	2	3	3	9	1	1
	1	0	2	9	2	5	8	7	2	5	7	3	8	4	2	8	5	7	4	6	7	8	5	6	9	7	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

were not included in the quantitative analyses of the population structure.

We reject the null hypothesis that southern hemisphere copper sharks are panmictic (AMOVA, global $\Phi_{ST} = 0.95$, $P < 0.000001$; Table 4). Pairwise comparisons revealed that there are at least three distinct groups across this range, including Australia–New Zealand ('Australasia', pairwise Φ_{ST} non-significant), South Africa–Namibia ('southern Africa', pairwise Φ_{ST} non-significant) and 'Perú' (Table 5). We, hereafter, refer to these groups as 'management units' (as opposed to 'stocks', because mtCR data may not accurately reflect male-mediated gene flow). Haplotype and nucleotide diversities were highest in southern Africa and Perú management units and were extremely low in Australasian management unit (Table 3).

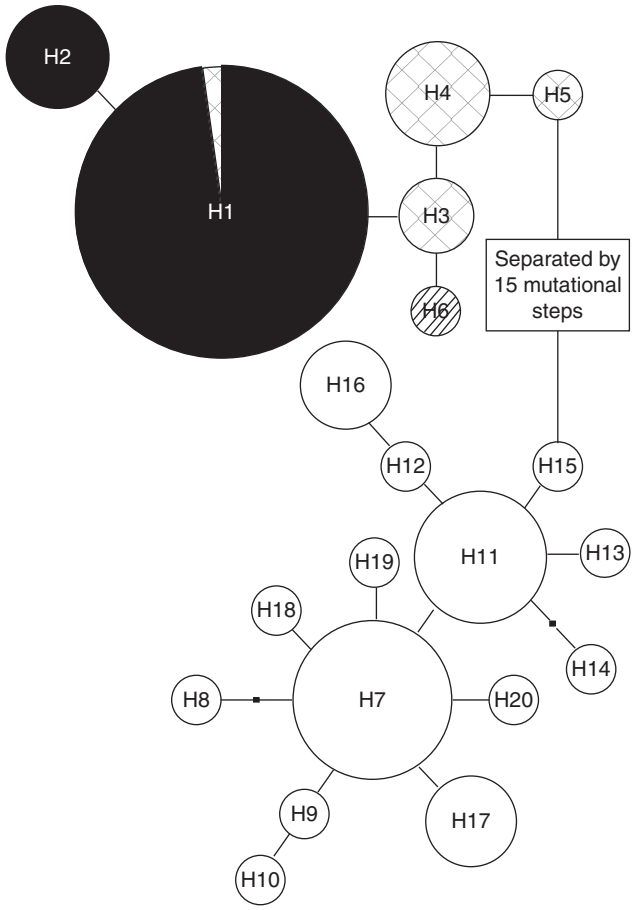

Fig. 2. Copper-shark mitochondrial control region (mtCR) haplotype network, with haplotypes numbered (see Table 1) in circles. Small squares represent inferred mutational steps. Solid circles represent haplotypes observed in Australia–New Zealand, checkered circles or slices are Peruvian haplotypes and Mexico's haplotype is a striped circle; open circles are haplotypes from South Africa and Namibia. Spain and Brazil both displayed Haplotype 7, but are not depicted here.

Historical phylogeography of copper sharks

The analysis of divergence time between southern Africa and Australasia produced an estimate of t of 3.48×10^6 years and the comparison between southern Africa and Perú produced an estimate of 2.36×10^6 years. Both of these had effectively zero migrants per generation. The analysis of the migration rate between Australasia and Perú yielded an estimate of m of 1.47×10^{-6} ($M = 0.12$ migrants per generation). The estimated divergence time (t) for this comparison was 1.61×10^5 years.

Discussion

Population structure and genetic diversity of copper sharks

We reject the null hypothesis that the widely distributed but coastally oriented copper shark is panmictic in the southern hemisphere. Gene flow is highly restricted across deep oceanic habitat, separating the following three regions: Australasia (Australia and New Zealand combined), southern Africa (Namibia and South Africa combined) and Perú, which, at least, means that females are not dispersing among these regions. Females are constrained by the need to return to discrete coastal nursery areas for parturition in many shark species (e.g. Feldheim *et al.* 2004), which probably inhibits transoceanic movement and reproductive mixing. Male sharks do not show the same level of fidelity to these areas for mating (Feldheim *et al.* 2004) and thus male-mediated gene flow often occurs over a wider geographic area and/or persists long after female gene flow is restricted between regions (Schultz *et al.* 2008; Portnoy *et al.* 2010). Analysis of biparentally inherited genetic markers is needed to determine whether the population subdivision we describe in the southern hemisphere reflects dispersal patterns of

Table 2. Copper-shark haplotype frequency distribution for sampling regions

Location	*n*	Haplotype no.																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Australia	37	32	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brazil	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Mexico	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Namibia	26	0	0	0	0	0	0	9	0	1	1	5	1	1	0	1	3	2	0	1	1
New Zealand	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perú	13	4	0	3	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
South Africa	26	0	0	0	0	0	0	10	1	0	0	11	0	0	1	0	0	2	1	0	0
Spain	1	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 copper sharks in all sampling regions of the study

Location	n	No. of haplotypes	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.
Perú	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 copper sharks

Φ-statistics source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	4	563.064	6.21491	94.72
Within populations	112	38.763	0.34610	5.28
Total	116	601.827	6.56101	
Fixation index (Φ_{ST})	0.94725			
$P < 0.000001$				

Table 5. Population differentiation among copper sharks collected in the following five regions: Australia (AUS, $n = 37$), Namibia (NAM, $n = 26$), New Zealand (NZD, $n = 15$), Perú (PER, $n = 13$) and South Africa (SAF, $n = 26$)
Numbers above diagonal show average pairwise nucleotide divergence between populations (Tamura Nei substitution model). Numbers below the diagonal show pairwise Φ_{ST} between populations, with values significantly ($P < 0.000001$) different from 0 in bold

	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**	–

both sexes or just of the females. Nevertheless, the present study has contributed to a growing body of data indicating that oceanic expanses are a fundamental barrier underlying genetic population structure in coastally oriented sharks (Duncan *et al.* 2006; Keeney and Heist 2006; Schultz *et al.* 2008; Ahonen *et al.* 2009; Portnoy *et al.* 2010).

Copper sharks are known to move long distances along continental coastlines (Cliff and Dudley 1992; Compagno *et al.* 2005), which suggests that coastal distance may be a weak barrier to gene flow in this species. We did not detect population genetic structure between South Africa and Namibia, which are separated by ~2700 km of coast. Walter and Ebert (1991) suggested that copper sharks in these two areas may be distinct from one another, given differing seasonal reproductive patterns and a seemingly disjoint distribution. Although our data are inconsistent with this hypothesis, we suggest a sampling strategy that targets newborn sharks that are segregated into their nursery areas is needed to refute it. Our collections from each of these locations could contain an admixture of migratory individuals that might otherwise segregate to breed. We also did not detect population genetic structure between Australia and New Zealand across the Tasman Sea, which is ~2250 km across. There are several potential stepping-stone habitats across this narrow oceanic expanse (e.g. Challenger Plateau, Lord Howe Rise, Norfolk Ridge and seamounts west of these) that potentially facilitate exchange between New Zealand and Australia. However, mtCR diversity is low in Australia and New Zealand (see next paragraph), which limits the resolution of this locus for detecting weak population subdivision.

Global nucleotide-diversity estimates for copper sharks were among the highest observed in any large widely distributed shark species (Duncan *et al.* 2006; Keeney and Heist

2006; Castro *et al.* 2007; Ahonen *et al.* 2009; Portnoy *et al.* 2010), which is due to the presence of two highly divergent mtCR clades in this species. Lemon sharks diverged into distinct Indo-Pacific and Atlantic species (*Negaprion acutidens* and *N. brevirostris*, respectively) after the Tethys Sea closure ~20 million years ago. Although one copper-shark clade was found only in the Atlantic and western Indian Ocean and the other was found only in the eastern Indian and Pacific Oceans, the amount of divergence between these two clades is too low for them to have originated at the Tethys Sea closure. The divergence time between these two clades is estimated to be ~2.4–3.5 million years, which is consistent with vicariant isolation by the rise of the Isthmus of Panama. There is also evidence of a more recent (~160 000 years ago) separation of the South Pacific clade into distinct western (Australasian) and eastern (Peruvian) groups. Notably, genetic diversity in Australasia was substantially lower than either in Perú or southern Africa and is extremely low when compared with other shark populations (Duncan *et al.* 2006; Keeney and Heist 2006; Castro *et al.* 2007; Ahonen *et al.* 2009; Portnoy *et al.* 2010), which suggests either a founder or recent bottleneck event.

Proposed historical phylogeography of copper sharks

We propose a hypothesis for the historical radiation of copper sharks on the basis of the distribution of genetic diversity, the evolutionary relationships among mtCR haplotypes and the fossil record. Although the fossil record for sharks is limited, copper-shark teeth are found in Miocene and Pleistocene deposits in the north-western Atlantic (where the species is now rare or absent, Garrick 1982), Mediterranean and eastern Pacific (Heim and Bourdon 1998; Long 1993; Marsili 2008). When this is coupled with the observation that genetic diversity is highest in the southern African and Peruvian collections, we propose an eastern Pacific and Atlantic centre-of-origin for the species that predated the separation of these basins. Under this model, copper sharks were separated by the rise of the Isthmus of Panama ~3 million years ago, which initiated the divergence of these clades. Notably, the amount of sequence divergence we documented between these copper-shark clades is nearly identical to the amount of sequence divergence observed between eastern Pacific and western Atlantic lemon sharks (*Negaprion brevirostris*), which were uncontroversially separated by this geologic event (Schultz *et al.* 2008).

After the separation of the Atlantic and Pacific, we propose that copper sharks radiated westward from the eastern Pacific to colonise Australasia. Our estimates of divergence time between Australasia and Perú indicate that the founding of Australasian population(s) was relatively recent (~160 000 years ago). The founding population also probably consisted of relatively few females, which would account for the extremely low genetic diversity in Australasia. This model is also supported by the genealogy of the mtCR haplotypes, because all of the eastern Pacific haplotypes are centrally located within the global haplotype network, whereas the two Australasian haplotypes are derived. We cannot rule out that a recent genetic bottleneck is responsible for the low level of genetic diversity in Australasia, which needs to be investigated with additional genetic markers.

How does the phylogeography of the copper shark compare with that of other large-bodied carcharhiniform sharks? Copper sharks fundamentally differ from many of the other large carcharhiniforms in that they use temperate nursery areas (Lucifora *et al.* 2005) and the absence of nursery habitat in the tropics may inhibit migration across these regions. In addition, cool thermal barriers that restrict gene flow in subtropical and tropical carcharhiniforms are less likely to structure copper-shark populations. For example, in scalloped hammerhead (*Sphyrna lewini*) and blacktip sharks (*Carcharhinus limbatus*), the cold Benguela upwelling along the south-western coast of Africa restricts contemporary female-mediated gene flow between Atlantic and Indo-Pacific populations of these subtropical species (Duncan *et al.* 2006; Keeney and Heist 2006; Chapman *et al.* 2009). In contrast, the Benguela upwelling appears not to be a barrier to copper sharks, as evidenced by a lack of genetic differentiation between Namibia and South Africa and shared haplotypes occurring between these collections and single individuals captured in the eastern and western Atlantic (e.g. Spain and Argentina). Conversely, scalloped hammerheads and blacktips exhibit evidence of a recent contact across the tropical Indian Ocean (Duncan *et al.* 2006; Keeney and Heist 2006), whereas there has been an absence of gene flow across the Indian Ocean for ~3 million years in copper sharks. Whereas scalloped hammerhead and blacktip nursery areas exhibit a semi-continuous distribution along the Indian ocean coasts of Africa and Asia, there are no 'stepping stone' temperate nursery habitats for copper sharks across this range. Moreover, the Indian Ocean itself is both wide and warm, which are both likely to inhibit gene flow directly across the pelagic environment in this region in copper sharks. All three species have historically dispersed across the Pacific Ocean, although we propose they have done so from opposite directions (i.e. east to west in the copper shark, west to east in the scalloped hammerheads and blacktips; Duncan *et al.* 2006; Keeney and Heist 2006). This supports the hypothesis that Pacific islands serve as stepping-stone habitats for dispersal of some large sharks between the eastern and western Pacific.

Implications for management and trade monitoring

Our findings have immediate application to the management of copper sharks, which are exploited in many locations around the world for their fins and meat. We show that copper sharks inhabiting distinct continental shelves separated by large ocean expanses can comprise distinct 'management units'. Differences in mtCR haplotype frequencies between these and unsampled management units might be able to facilitate future trade-monitoring efforts for internationally traded products such as fins (e.g. Chapman *et al.* 2009). Additional sampling, both in terms of collection locations and individuals within some of our collections, is needed before such methods could be fully evaluated and reliably implemented. At this stage, we cannot rule out male-mediated genetic connectivity between these management units and therefore we stop short of classifying them as fully differentiated 'stocks' in the classic fisheries sense.

Even if male-mediated gene flow is high, each management unit defined by mitochondrial DNA still represents a discrete pool of breeding females that segregate to reproduce in the sampled area (Moritz 1994). Our results show that connectivity between the continental shelves we sampled, at least in terms of female movements and reproductive mixing, occurs on evolutionary rather than ecological timescales. Thus, copper-shark populations primarily rely on the slow processes of local reproduction and recruitment for replenishment and should therefore be carefully monitored and managed as populations associated with specific continental-shelf regions.

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