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"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

Conectividade Genética, Filogeografia e Conservação de Tubarões Pelágicos no Oceano Atlântico Ocidental

RODRIGO RODRIGUES DOMINGUES

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Zoologia)

Rio Claro-SP
Novembro - 2016

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Orientador: Prof. Dr. Otto Bismarck Fazzano Gadig

Co-orientador: Prof. Dr. Alexandre Wagner Silva Hilsdorf

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE RODRIGO RODRIGUES DOMINGUES, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA), DO INSTITUTO DE BIOCIÊNCIAS.

Aos 12 dias do mês de dezembro do ano de 2016, às 14:00 horas, no(a) Instituto de Biociências - Campus Litoral Paulista, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Prof. Dr. OTTO BISMARCK FAZZANO GADIG - Orientador(a) do(a) Campus Litoral Paulista / UNESP - Instituto de Biociências - São Vicente/SP, Prof. Dr. FERNANDO FERNANDES MENDONÇA do(a) Departamento de Ciências do Mar / Universidade Federal de São Paulo - Santos/SP, Prof. Dr. DANILLO PINHAL do(a) Departamento de Genética / Instituto de Biociências de Botucatu - SP, Prof. Dr. ANDREY LEONARDO FAGUNDES DE CASTRO do(a) Departamento de Ciências Naturais / Universidade Federal de São João Del-Rei - MG, Prof. Dr. FABIO DOS SANTOS MOTTA do(a) Departamento de Ciências do Mar / Universidade Federal de São Paulo - Santos/SP, sob a presidência do primeiro, a fim de proceder a arguição pública da TESE DE DOUTORADO de RODRIGO RODRIGUES DOMINGUES, intitulada **Conectividade Genética, Filogeografia e Conservação de Tubarões Pelágicos no Oceano Atlântico Ocidental**. Após a exposição, o discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final: APROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof. Dr. OTTO BISMARCK FAZZANO GADIG

Prof. Dr. FERNANDO FERNANDES MENDONÇA

Prof. Dr. DANILLO PINHAL

Prof. Dr. ANDREY LEONARDO FAGUNDES DE CASTRO

Prof. Dr. FABIO DOS SANTOS MOTTA


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DEDICATÓRIA

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RESUMO

As espécies *Carcharhinus falciformis* e *Carcharhinus signatus*, são tubarões oceânico-pelágicos, caracterizados por habitarem o ambiente epipelágico de águas quentes e temperados de todo o mundo. Devido ao alto valor da suas nadadeiras, estas espécies são demasiadamente capturadas pela pesca de espinhel pelágico, direcionado para a captura de atuns e espadarte. Em decorrência dessa severa pressão pesqueira, estima-se que estas espécies tiveram reduções populacionais em torno de 75%, e estão atualmente listadas como próximo de ameaçada (*C. falciformis*) e vulnerável (*C. signatus*) na lista vermelha da *Internation union for Conservation of Nature* (IUCN). Por serem espécies migratórias e por habitarem águas internacionais, as ações de conservação exigem esforços conjuntos de vários países, tarefa que no Oceano Atlântico compete à *International Commission for the Conservation of Atlantic Tunas* – ICCAT. A presente tese avaliou os aspectos genéticos populacionais e filogeográficos de ambas espécies, utilizando marcadores moleculares mitocondriais (*C. falciformis* e *C. signatus*) e microssatélites (*C. signatus*). Especificamente, foram avaliados os níveis de diversidade genética, testada a hipótese nula de panmixia e inferidos os cenários filogeográficos responsáveis pelas relações filogenéticas intraespecíficas, e aspectos demográficos populacionais. Além disso, a diversidade genética da subclasse elasmobrânquios foi avaliada e discutida a sua utilização nas avaliações do estado de conservação de espécies e políticas afins. A diversidade genética total para ambas as espécies, utilizando a região controle do DNA mitocondrial foi alta (h = 0,88±0,012 e π = 0,005±0,003 – *C. falciformis*; h = 0,74±0,027 e π = 0,0034±0,0019 – *C. signatus*), No entanto, *C. signatus* apresentou baixos valores para o Atlântico Sul Ocidental (h = 0,545±0,077 e π = 0,0013±0,0009) e para 9 *loci* microssatélites (*Ho* = 0,40). A hipótese nula de panmixia foi rejeitada para *C. falciformis* (Φ_{ST} 0.058, P < 0.0001) e *C. signatus*, ambas espécies

apresentando estrutura genética populacional entre localidades do Hemisfério norte e Hemisfério sul. *Carcharhinus signatus* apresentou uma dispersão baseada em sexo, com machos panmíticos e fêmeas estruturadas (Φ_{ST} = 0,443; P < 0,01), um típico padrão de filopatria reprodutiva, corroborado pelo isolamento-por-distância (r = 0,65, p = 0,03). Duas linhagens matrilineais foram verificadas para ambas espécies, formadas há 485, 571 Kya (*C. falciformis*) e 166, 598 Kya (*C. signatus*), correspondendo ao Pleistoceno. A hipótese de expansão populacional não pode ser rejeitada para ambas espécies, iniciando durante o último máximo glacial – UMG para *C. falciformis*, enquanto que *C. signatus* passou por um efeito gargalo no UMG, seguido de uma expansão populacional no Holoceno. Uma exaustiva revisão bibliográfica, revelou um aumento considerável nos últimos anos dos estudos genéticos populacionais que descrevem a diversidade genética de tubarões e raias. Além disso, as métricas de diversidade genética revelaram que, em geral, os elasmobrânquios possuem baixa variabilidade genética, sobretudo para os marcadores mitocondriais. Por fim, foi discutida a inclusão dessas métricas nas avaliações do estado de conservação de espécies e políticas afins.

ABSTRACT

The shark species, *Carcharhinus falciformis* and *Carcharhinus signatus*, are oceanic-pelagic species that inhabit tropical, subtropical and temperate waters worldwide. The high value of their fins associated with the by-catch these species by longline fisheries, which targeting tuna and swordfish, has led to a population reduction around 75%. Currently, these species are listed on the IUCN Red list as Near Threatened (*C. falciformis*) and Vulnerable (*C. signatus*). Due to their high vagility and migratory behavior, inhabiting internation waters, management and conservation plans require joint efforts from the many country, a task that in the Atlantic Ocean is entrusted to the International Commission for the Conservation of Atlantic Tunas – ICCAT. This Thesis assessed the main aspects of population genetics and phylogeography in both species. Using mitochondrial marker (*C. falciformis* and *C. signatus*) and nine loci microsatellites (*C. signatus*). Especifically, the levels of genetic diversity were assessed, panmixia null hypothesis was tested and the phylogeography scenarios responsible for intraspecific phylogenetic relationships and populations demography were inferred. Furthermore, the genetic diversity of subclass Elamosbranchii and its usefullness to assess conservation policies were discussed. The overall genetic diversity at CR mtDNA in both species was high (h = 0.88±0.012 and π = 0.005±0.003 – *C. falciformis*; h = 0.74±0.027 and π = 0.0034±0.0019 – *C. signatus*). However, *C. signatus* showed low genetic diversity values in the Southwestern Atlantic (h = 0.545±0.077 and π = 0.0013±0.0009) and at 9 *loci* microsatellites (*Ho* = 0.40). The null hypothesis of panmixia was rejected for *C. falciformis* (Φ_{ST} 0.058, P < 0.0001) and *C. signatus*, with both species showing population structured along the western Atlantic Ocean. *Carcharhinus signatus* showed sex-based dispersion, with male roving and female no roving (Φ_{ST} = 0.443; P < 0.01), indicating reproductive phylopatry, supported by isolation-by-distance (r = 0.65, P = 0,03). The Maximum likelihood and Bayesian tree presented two matrilineal lineages to both species, with split started 485,

571 Kya (*C. falciformis*) and 166, 598 Kya (*C. signatus*), corresponding to Pleistocene. The hypothesis of population expansion cannot be rejected to both species, starting during the last glacial maximum – LGM for *C. falciformis*, whereas *C. signatus* passed through a bottleneck effect, followed of population expansion in the Holecene. An exhaustive bibliography revision revelead a considerable increase of genetic population studies of sharks and rays. Moreover, metanalysis of genetic diversity metrics revealed that shark and rays, in general, have low genetic diversity, mainly for mitochondrial markers. Finally, the inclusion of genetic diversity metrics in species assessent and conservation policies was discussed.

CAPÍTULO 1
INTRODUÇÃO GERAL

1 - Aspectos gerais dos Elasmobrânquios e as espécies alvo desta tese

Os Chondrichthyes (peixes cartilaginosos) dividem-se em duas subclasses: Elasmobranchii, composta por tubarões e raias, e Holocephali, composta pelas quimeras (EBERT et al., 2013). Atualmente são conhecidas aproximadamente 1188 espécies de Chondrichthyes, sendo 509 de tubarões, 630 de raias e 49 de quimeras, representando 3.5% da fauna dos chamados peixes atualmente viventes (WEIGMANN, 2016).

A subclasse Elasmobranchii compreende vertebrados com longa história evolutiva, com mais de 455 milhões de anos (GROGAN et al., 2012; EBERT et al., 2013). Durante este período, ocorreram pelo menos cinco severos eventos catastróficos que alteraram o ambiente marinho e provocaram extinções em massa nos oceanos (GROGAN et al., 2012). No entanto, a alta resiliência frente as estes eventos e a variedade de formas e funções desses animais proporcionaram a capacidade de habitarem os mais diferentes ecossistemas marinhos e dúlcicolas, antes ocupados por seus possíveis predadores (EBERT et al., 2013).

Inicialmente, os tubarões foram pequenos consumidores costeiros e ao longo de sua história evolutiva, a seleção favoreceu o aumento do corpo, crescimento contínuo, maturação gonadal tardia, características que os classificam como espécies k-estrategistas (GROGAN et al., 2012).

Atualmente, os tubarões pelágicos, incluindo espécies oceânicas e semi-oceânicas, representam uma pequena parcela da fauna mundial de Chondrichthyes (COMPAGNO, 2008), com cerca de 6% da fauna total de Chondrichthyes. A maioria delas, por exemplo o *Prionce glauca* (Linnaeus, 1758), *Isurus oxyrinchus* Rafinesque, 1810, e *Carcharhinus longimanus* (Poey, 1861) vive a maior parte da sua vida no ambiente oceânico-pelágico, em uma ou mais das três zonas do habitat oceânico: zona epipelágica (até 200m de profundidade), zona mesopelágica (200 – 1000m de profundidade) e zona batipelágica [(1000m – 6000m de profundidade) (CAMHI et al., 2009; PIKITCH et al., 2009)]. Estes

tubarões desempenham papel fundamental nos ecossistemas marinhos, pois estão posicionados na porção apical da cadeia trófica, em função, principalmente, de serem predadores de grande porte que se alimentam de amplo espectro de espécies, variando desde pequenos e médios peixes, assim como grande teleósteos (como atuns), tartarugas marinhas e mamíferos marinhos, o que auxilia na manutenção do equilíbrio da densidade populacional de suas presas, sobretudo os tropicais e subtropicais (CAMHI et al., 2008; PIKITCH et al., 2009; FERRETTI et al., 2010).

As espécies alvo desta Tese aqui apresentada, a saber, o tubarão lombo-preto *Carcharhinus falciformis* (Müller & Henle, 1839) e o tubarão toninha *Carcharhinus signatus* (Poey, 1868), integram essa comunidade de tubarões predadores dos ambientes oceânico-pelágicos, com distribuição em águas subtropicais, tropicais e temperadas. *Carcharhinus signatus* é uma espécie endêmica do Oceano Atlântico, encontrada principalmente na área insular e fora da plataforma continental, em profundidades menores que 100 m, com ocorrência desde a Flórida até a Argentina (EBERT et al., 2013). Já *C. falciformis* é uma espécie cosmopolita e no Oceano Atlantico ocidental ocorre desde Massachussets - EUA até o sul do Brasil, em águas de até 500 m de profundidade (EBERT et al., 2013). Ambas possuem alto valor comercial, principalmente devido aos elevados preços obtidos por suas nadadeiras no mercado internacional (CLARKE et al., 2006).

Em função disso, são espécies cujo monitoramento pesqueiro é bastante complexo, principalmente por causa da dificuldade na identificação espécie-específica, devido a natural similaridade morfológica, e por realizarem extensas migrações, ocorrendo em várias áreas oceânicas e diferentes latitudes (CAMHI et al., 2008), além de serem capturadas durante todo o ano por frotas pesqueiras de vários países (BONFIL, 2008).

2 - Pesca de tubarões pelágicos

Várias populações marinhas de ambientes costeiros e oceânicos vêm sofrendo reduções significativas em sua abundância, principalmente devido à forte pressão exercida pela pesca, chegando, em alguns casos, à ameaça de extinção para algumas espécies. Os maiores impactos, geralmente, ocorrem sobre os recursos pesqueiros com maiores valores econômicos, porém, com a sua sobreexplotação, há direcionamento para a captura de espécies de menor valor comercial (PAULY, 1998; WARD, 2000; MYERS et al., 2007; FERRETI et al., 2010). Este cenário caracteriza um ambiente em desequilíbrio, ocasionando séria ameaça à sustentabilidade dos recursos pesqueiros. Tal situação agrava-se ainda mais para a maioria das espécies de tubarões, devido suas características de organismos k-estrategista, como crescimento lento, alta longevidade e maturação gonadal tardia (CAMHI et al., 2008; CORTÉS, 2000).

Os tubarões pelágicos são capturados em todos os oceanos do mundo, principalmente pela pesca de espinhel pelágico direcionada aos atuns e espadarte, porém, os registros de captura dessas espécies não é realizado de forma adequada, são subnotificadas e não documentados (CAMHI et al., 2009). Essa exploração em escala mundial tem esgotado expressivamente as comunidades de grandes peixes predadores, incluindo várias espécies de tubarões pelágicos de todo o mundo, em taxas superiores a 90% nas últimas décadas (BAUM et al., 2003, 2004; Myers & WORM, 2007; FERRETI et al., 2010). Exemplos de populações colapsadas de tubarões de diferentes zonas marinhas têm sido registrados ao longo das últimas décadas, tais como a espécie *Galeorhinus galeus* (Linnaeus, 1758) na Califórnia (EUA) e na Austrália (RIPLEY, 1946; OLSEN, 1959), *Cetorhinus maximus* [(Gunnerus, 1765) (PARKER & SCOTT, 1965)], *Squalus acanthias* Linnaeus, 1758 (Hoff & Musick, 1990) e *Lamna nasus* (Bonnaterre, 1788) no Atlântico Norte (ANDERSON, 1990; CAMPANA et al., 2001). Mais recentemente, BAUM et al., (2003) registraram decréscimos

acentuados das populações de *Alopias superciliosus*, *Prionace glauca*, *Isurus oxirynchus* e *Carcharhinus longimanus* no Atlântico Norte. Embora os resultados desse estudo sejam motivos de amplos debates (BURGESS et al., 2005; BURGESS et al., 2005b).

As avaliações mais recentes revelam que as populações de tubarões pelágicos continuam declinando em várias regiões do mundo (CLARKE et al., 2013; BARRETO, 2016). Por exemplo, em 2000, a captura de tubarões alcançou picos de 1.44 milhões de toneladas métricas, com redução inexpressiva da captura uma década depois, com taxa de captura de 1.41 milhões de toneladas métricas em 2010 (WORM et al., 2013). No Oceano Atlântico, as estimativas mais recentes têm mostrado redução populacional significativa para as espécies *C. falciformis*, *Sphyrna* spp (tubarões-martelo), *Isurus oxyrinchus* e a categoria "outros tubarões", os quais incluem as espécies do gênero *Carcharhinus*, entre elas as espécies-alvo deste estudo (BARRETO et al., 2016).

Essa intensa exploração pesqueira de tubarões é motivada principalmente pelo alto valor comercial de suas nadadeiras, as quais são utilizadas na culinária asiática como uma iguaria (STEVENS et al., 2000; CLARKE et al., 2006; DULVY et al., 2014). Como uma forma de combater este comércio, a CITES (Convention on International Trade in Endangered Species) votou a inclusão de oito espécies de tubarões, *Rhincodon typus* Smith, 1828, *Cetorhinus maximus*, *Carcharodon carcharias* (Linnaeus, 1758), *Carcharhinus longimanus*, *Sphyrna lewini* (Griffith & Smith, 1834), *Sphyrna zygaena* (Linnaeus, 1758) e *Sphyrna mokarran* (Rüppell, 1837) no apêndice II, a qual inclui espécies ameaçadas de extinção que pode ser afetada pelo comércio internacional. Mais recentemente, em Outubro de 2016, a CITES incluiu nesse mesmo apêndice, outras espécies de elasmobrânquios, como *Carcharhinus falciformis*, *Alopias* spp (tubarões-raposa) e todas as raias-manta (família Mobulidae).

Os parâmetros populacionais que caracterizam a história de vida dos Chondrichthyes,

associados à sobrepesca, perda de habitat e mudanças climáticas, contribuíram para que 25% das espécies conhecidas fossem categorizadas como “ameaçada de extinção” na lista vermelha da *International Union for Conservation of Nature* - IUCN (DULVY et al., 2014). Desta forma, estudos que visam estimar as atuais condições populacionais destas espécies são de suma importância para um efetivo manejo pesqueiro e conservação das espécies.

3 - Importância da aplicação das ferramentas moleculares no manejo e conservação de tubarões

Muitos métodos genéticos têm sido utilizados para responder a uma gama de perguntas sobre a biologia e ecologia de várias espécies de tubarões. Para isto, são empregados vários marcadores moleculares que permitem a interpretação de complexos padrões da variância molecular, bem como uma rápida e acurada reconstrução do perfil molecular de um indivíduo (PORTNOY, 2010). Esta também é uma ferramenta extremamente útil para elucidar questões sobre diversidade genética, distribuição geográfica, relações filogenéticas, identificação taxonômica e análises de parentescos (e.g. FELDHEIM et al., 2001; SHIVJI et al., 2002; CHAPMAN et al., 2007; MENDONÇA et al., 2011; QUATTRO et al., 2013; CARDENÕSA et al., 2014).

Em estudos populacionais, a escolha do marcador molecular é um ponto importante a se considerar. Sua escolha depende de alguns fatores, tais como o táxon analisado e a escala temporal e geográfica. Além disso, deve ser considerada a rapidez e o custo (PORTNOY & HEIST, 2012). Nos últimos anos, uma grande quantidade de marcadores se tornou disponível e seu uso em estudos populacionais com tubarões segue a mesma tendência da evolução dos marcadores moleculares introduzidos na biologia molecular (SCHLÖTTERER, 2004). Além disso, eficientes programas computacionais têm sido desenvolvidos, acompanhando a evolução dos marcadores moleculares, sendo cruciais para a interpretação correta dos

resultados (EXCOFFIER & HECKEL, 2006).

Os primeiros estudos sobre estruturação populacional de tubarões foram realizados utilizando aloenzimas como marcadores (e.g. MACDONALD, 1988). Posteriormente, marcadores moleculares baseados em enzimas de restrição como RFLP (Restriction Fragment Length Polymorphism) passaram a ser utilizados (e.g HEIST et al., 1996, 1996b). Já nos últimos anos, o uso de sequências de DNA mitocondrial e microssatélites foram os marcadores predominantemente utilizados. Mais recentemente, o uso da genômica tem sido utilizado nos estudos genéticos populacionais de elasmobrânquios, no entanto, seu uso ainda é incipiente (DUDGEON et al., 2012; PORTNOY & HEIST, 2012).

Em tempos de exaustiva pressão pesqueira e consequente sobrepesca para a maioria dos recursos marinhos, a preservação da variabilidade genética é de extrema importância para o manejo e conservação das espécies a longo prazo. De acordo com FÉRAL, (2002), o potencial de uma espécie para responder à mudanças ambientais e distúrbios causados por atividades humanas, como a pesca, depende diretamente da extensão da diversidade genética. Neste sentido, a pesca, principal responsável pela diminuição das populações de peixes, não só reduz a população levando-as ao colapso, como também pode afetar a história evolutiva das espécies (HEINO et al., 2015). A ausência de seletividade das capturas atua direcionando mudanças fenotípicas nas populações, como tamanho e idade de primeira maturação (BELGRANO & FOWLER, 2013). Tais mudanças podem ter implicações ecológicas, como a interação entre presa-predador e estrutura trófica de um ambiente, além de consequências evolutivas (BELGRANO & FOWLER, 2013). Desta forma, acessar o nível de variabilidade genética de tubarões, uma vez que há uma evidente redução populacional para a maioria das espécies, é de suma importância para que medidas de manejo sejam implementadas adequadamente, salvaguardando estas espécies em longo prazo.

4- Estrutura genética populacional e filogeografia em tubarões

A estrutura genética de populações pode ser definida como a mudança nas frequências alélicas causadas principalmente pela deriva genética e o fluxo gênico, enquanto a filogeografia visa entender os padrões e processos responsáveis pelo atual padrão de disteibuição de espécies e populações (AVISE, 2000; ALLENDORF et al., 2013). A baixa quantidade de barreiras naturais no ambiente marinho favorece a homogeneização das frequências alélicas nas populações via fluxo gênico (SELKOE et al., 2016). Tal fato, associado à alta capacidade de dispersão larval e alta vagilidade quando adultos, torna as populações de peixes marinhos menos estruturadas quando comparadas com espécies dulcícolas (GRAVES, 1998). No entanto, as populações naturais são dinâmicas, podendo mudar em tamanho, densidade e localização, e ao longo do tempo podem se fragmentar em várias populações (HEY & MACHADO, 2003). O entendimento do padrão e extensão da divergência entre populações é crucial para o delineamento de unidades de manejo e posterior construção de planos de conservação (MORITZ, 1994; PALSBØLL et al., 2006; ALLENDORF et al., 2013).

Para propósitos de conservação, a gestão pesqueira usa o termo estoque para delimitar parcelas de uma população que esta sendo explorada. Numerosos conceitos de estoques têm sido levantados na literatura da ciência pesqueira (BEGG et al., 1999) e de acordo com CARVALHO & HAUSER, (1994), os conceitos dependem de quem os define e para que propósito foram criados. Segundo SPARRE & VENEMA, (1998), uma definição de estoque é praticamente inatingível, embora, o termo mais adequado, segundo os autores, é o definido por GULLAND, (1983), que diz que, para propósitos de gestão pesqueira, desde que não haja indícios de estoques diferentes, é preferível iniciar um trabalho de avaliação de estoque considerando toda a distribuição geográfica da espécie. Conforme se evidencie que os parâmetros de mortalidade e crescimento diferem significativamente nas várias partes da

distribuição da espécie, então, deve-se considerar estoques diferentes, exigindo avaliações separadamente.

No entanto, fica evidente que esse conceito de estoque não leva em consideração parâmetros genéticos, os quais utilizam o isolamento reprodutivo, diferenciação genética de outras populações, especialização ecológica e adaptação local, sendo o uso dos marcadores moleculares uma poderosa ferramenta para determinar diferenças evolutivas entre estoques, enquanto as variações nos parâmetros biológicos explicam melhor as diferenças entre estoques causados pelo ambiente (BEGG et al., 1999).

Informações sobre a genética populacional são de particular importância para entender a estrutura dos estoques, fluxo gênico e a variabilidade genética (BEGG et al., 1999). Esta importância dentro e entre populações pode ser determinada pelas frequências de genes e pelas forças que afetam essas frequências, como migração, mutação, seleção e deriva genética (CARVALHO & HAUSER, 1994).

De acordo com FRANKHAM, (2002), a variabilidade genética é indispensável para os estudos populacionais, pois combinada com estudos ecológicos, comportamentais e demográficos, aumenta o conhecimento sobre a biologia e a história de vida de espécies ameaçadas. Ainda, segundo a IUCN, a diversidade genética é uma das três prioridades globais da conservação da biodiversidade (MCNELLY et al., 1990). Esta preocupação se dá em função das mudanças ambientais serem um processo contínuo, onde a diversidade genética permitir uma maior flexibilidade fenotípica para tais mudanças (FRANKHAM et al., 2002). No entanto, a perda da variabilidade genética geralmente está associada à endogamia (cruzamento entre parentes) e diminuição geral na reprodução, sendo a pesca um dos principais atores da redução populacional e, consequente, redução da variabilidade genética (PINSKY et al., 2014).

Nos últimos anos, o número de artigos abordando os apectos genéticos populacionais

de espécies de tubarões tem aumentando consideravelmente. A grande maioria das espécies de tubarões oceânico-pelágicas têm sido estudadas [(e.g. *Isurus oxyrhincus* – SCHREY & HEIST, (2003); *Alopias pelagicus* Nakamura, 1935 – CARDEÑOSA et al., (2014); *Prionace glauca* – KING et al., 2015; *C. falciformis* – CLARKE et al., (2015); *Pseudokarcharias kamoharai* (Matsubara, 1936) – FERRETE et al., (2015); *Carcharhinus longimanus* – CAMARGO et al., (2016)]. Por outro lado, no Oceano Atlântico, algumas espécies importantes de tubarões pelágicos não possuem estudos genéticos populacionais, como por exemplo, o *Prionace glauca*, a espécie mais abundante entre os tubarões pelágicos, deixando assim, uma lacuna no conhecimento de um aspecto relevante para o gerenciamento correto dos seus estoques.

Carcharhinus falciformis, foi estudado primeiramente por GALVÁN-TIRADO et al., (2011) no Oceano Pacífico e, mais recentemente, por CLARKE et al., (2015), que realizaram um estudo global e amostraram três pontos no Oceano Atlântico Ocidental: costa dos Estado Unidos (n= 62), Golfo do México (n= 40) e Nordeste do Brasil (n=39). Os autores encontraram alta diversidade haplotípica (h = 0.93) e nucleotídica (π = 0.51%) no Oceano Atlântico, e assumiram uma população panmítica. Além disso, encontraram duas linhagens simpátricas, uma com haplótipos exclusivos do Oceano Atlântico e outra com haplótipos do Oceano Atlântico e Índico. Por outro lado, *Carcharhinus signatus* não possui estudos genéticos populacionais até o presente, sendo este estudo o primeiro a abordar este aspectos para a espécie.

OBJETIVOS

Objetivos gerais

Avaliar os níveis de diversidade genética, padrões de estrutura genética populacional e filogeografia de duas espécies de tubarões pelágicos, *Carcharhinus falciformis* e *C. Signatus*, no Oceano Atlântico Ocidental.

Verificar os níveis de variabilidade genética de tubarões e raias e discutir sua utilização nas avaliações do estado de conservação, para os planos de manejo e programas de conservação.

Objetivos específicos

Carcharhinus falciformis

- Caracterizar a diversidade genética de *C. falciformis* ao longo do Oceano Atlântico Ocidental, utilizando a região controle do DNA mitocondrial;
- Testar a hipótese nula de panmixia para *C. falciformis* ao longo do Oceano Atlântico Ocidental, utilizando a região controle do DNA mitocondrial;
- Descrever os processos históricos que modularam o atual padrão de diversidade genética, estrutura genética populacional e demografia histórica de *C. falciformis* ao longo do Oceano Atlântico Ocidental.

Carcharhinus signatus

- Caracterizar a diversidade genética de *C. signatus* ao longo do Oceano Atlântico Ocidental, utilizando a região controle do DNA mitocondrial e 9 *loci* microssatélites;
- Testar a hipótese nula de panmixia para *C. signatus* ao longo do Oceano Atlântico Ocidental, utilizando marcadores mitocondriais e nucleares;

- Descrever os processos históricos que modularam o atual padrão de diversidade genética, estrutura genética populacional e demográfica histórica de *C. signatus* ao longo do Oceano Atlântico Ocidental.

Subsídios da genética ao planejamento sistemático para a conservação das espécies de tubarões e raias

- Descrever a tendência dos estudos que caracterizam a diversidade genética de tubarões e raias nas últimas quatro décadas;
- Quantificar o número de estudos que descrevem a diversidade genética de tubarões e raias em cada categoria da IUCN;
- Descrever os padrões de diversidade genética de tubarões e raias em cada categoria da IUCN;
- Discutir o uso da diversidade genética de tubarões e raias nas avaliações de espécies, planos de manejo e programas de conservação.

Apresentação da Tese

A proposta inicial desta tese foi avaliar aspectos da distribuição, abundância e diversidade genética de *C. falciformis* e *C. signatus* no sudoeste do Oceano Atlântico (Sudeste e Sul do Brasil). No entanto, os dados que seriam utilizados para realização das modealagens não foram satisfatórios. Além disso, a proposta inicial contemplava uma breve caracterização da diversidade genética das duas espécies no Atlântico Sul Ocidental, porém, durante a realização de um estágio no laboratório do Dr. Mahmood Shivji, na Universidade Nova Southeastern University – Flórida (USA), surgiu a possibilidade de estudar ambas espécies quanto aos seus aspectos genéticos populacionais ao longo de todo Oceano Atlântico

Ocidental, permitindo responder perguntas muito mais abrangentes e robustas do que as perguntas da proposta inicial contemplava.

Esse novo foco de estudo permitiu que fossem analisados os aspectos não só da diversidade genética, mas também de estruturação populacional e filogeografia. Além disso, ao longo do doutorado, lendo artigos relacionados ao tema e interessado na conservação das espécies de elasmobrânquios, notou-se que os estudos genéticos populacionais que descrevem os níveis de diversidade genética não são considerados nas avaliações do estado de conservação das espécies. Isso tornou-se um incentivo a escrever um artigo provocativo, levantando justamente a questão da necessidade de incluir a diversidade genética de tubarões e raias no delineamento das políticas de conservação.

Desta forma, a presente tese foi estruturada em dois capítulos gerais e outros três capítulos na forma de artigos e editados de acordo com as normas das revistas cientificas onde os mesmos foram ou serão submetidos. Os capítulos 2 e 4 desta tese encontram-se submetidos em dois períodicos científicos e estão atualmente em fase de revisão (*round 2*).

Capítulo 1 **- Introdução Geral**

Conforme previamente descrito, neste capítulo foram abordados aspectos gerais dos elasmobrânquios, como história evolutiva, importância ecológica, pesca e conservação.

Capítulo 2 **- Effects of Pleistocene on the population genetic structure, demographic history and sympatric lineages of the silky shark (*Carcharhinus falciformis*) along the western Atlantic Ocean**

Neste capítulo foi estudada a diversidade genética, a estruturação populacional e os aspectos demográficos históricos de *C. falciformis* no Oceano Atlântico ocidental, utilizando a região controle do DNA mitondrial.

Capítulo 3 – **Sex-biased dispersion, low genetic diversity and insights into the evolutionary history of the night shark (*Carcharhinus signatus*) along the western Atlantic Ocean**

Neste capítulo, semelhantemente ao capítulo acima, foram estudados os aspectos genéticos populacinais e filogeográficos de *C. signatus* ao longo do Oceano Atlântico ocidental, porém utilizando a região controle do DNA mitocondrial e nove *loci* nucleares. Desta forma, as análises deste artigo foram mais conclusivas quanto ao comportamento ecológico e aos aspectos filogeográficos de *C. signatus*.

Capítulo 4 **- Do shark and rays conservation policies need to consider population genetic diversity?**

Neste capítulo, aborda-se as questões sobre o uso da diversidade genética de tubarões e raias nas políticas de conservação, apresentando-se a evolução dos estudos genéticos que descrevem as métricas de diversidade genética para diferentes marcadores moleculares, a quantidade de estudos realizados por status de conservação da IUCN, bem como é discutido porque preservar a variabilidade genética dos elasmobrânquios é importante para a sua conservação e as futuras direções que devem ser tomadas pelos gestores de conservação desse grupo.

Capítulo 5 **– Conclusões**

Por fim, nesse capítulo, as principais conclusões deste estudo foram sumarizadas.

CAPÍTULO 2

Effects of Pleistocene on the population genetic structure, demographic history and sympatric lineages of the silky shark (*Carcharhinus falciformis*) along the western Atlantic Ocean

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ABSTRACT

The silky shark, *Carcharhinus falciformis*, is a large-bodied, oceanic-coastal, epipelagic species found worldwide in tropical and subtropical waters. It is one of the most frequently caught shark species in pelagic longline fisheries. Despite its commercial importance and likely ecological significance as an upper trophic-level predator, understanding of its biology and ecology is still unavailable for large parts of its distribution. We investigated the genetic diversity, population structure and demographic history of the silky shark along the western Atlantic Ocean based on the use of 707 bp of the mitochondrial DNA control region. A total of 211 silky shark individuals were sampled originating from the five areas along the western Atlantic Ocean: USA east coast - USA (43), Gulf of Mexico - GM (42), Pará State, Northern Brazil - PA (16); Saint Paul and Saint Peter Archipelago, Northeast Brazil - ASPSP (48) and Santa Catarina State, Southern Brazil – SC (62). The mitochondrial sequences revealed 40 haplotypes, with haplotype and nucleotide diversities of 0.88 (±0.012) and 0.005 (±0.003), respectively. Overall population structure was significant (Φ_{ST} 0.058, $P < 0.0001$) among the five western Atlantic Ocean regions examined. In general, demographic history test support a population expansion to northwest (USA and GM) and southwest (PA, ASPSP and SC) Atlantic groups and two lineages in the western Atlantic, originated during the Pleistocene Epoch. Although our results showed that silky sharks have high genetic diversity, the current level of overexploitation demanding management efforts in a long term. Therefore, we recommend that fisheries management plans be done for the two evolutionary significant units separately.

ADDITIONAL KEYWORDS: phylogeography - genetic diversity - marine connectivity - fisheries management.

INTRODUCTION

Current spatial patterns of genetic diversity and degree of isolation or connectivity of a population may reflect historical events such as vicariance and migrations, caused mainly during Pleistocene Epoch (Avise, 2000; Gilman *et al*., 2010; Grant, 2015). In marine systems, climate changes during the last glacial maximum (LGM) are among the most influential factors affecting species distribution and demographic patterns, and the genealogies of most species coalesce during this period (O'Brien, Gallucci & Hauser, 2013; Grant, 2015). The understanding of demographic history is profoundly relevant to explain phylogeographic patterns over macroevolutionary time scales due to their inevitable impact on the structure of gene genealogies (Avise, 2000). Thus, elucidating past evolutionary processes, facilitates understanding the mechanisms that shape current genetic differentiation patterns in a given species.

There are relatively few phylogeographic studies on elasmobranch compared to teleosts (Beheregaray, 2008). Encouragingly, the number of phylogeographic studies of sharks has increased in the last decade (Dudgeon et al. 2012; Portnoy & Heist 2012), but there remain large gaps in species examined and scale of geographic coverage for the many shark species with global distributions. Phylogeography studies particularly of exploited shark species are important not only to shed light on evolutionary mechanisms of diversity and population structure, but also for informing management and conservation efforts (Rocha, Craig & Bowen, 2007).

The silky shark, *Carcharhinus falciformis*, is large-bodied, highly exploited, oceanic and epipelagic species found worldwide in tropical seas. It is most commonly found in waters less than 200 m near continental and insular shelf edges, and also occasionally inshore to 18 m (Ebert, Fowler & Compagno, 2013). The silky shark has been intensively caught worldwide as bycatch (Bonfil *et al*., 2008, Ebert, Fowler & Compagno, 2013) leading to

severe population declines (Barreto et al. 2016). Furthermore, silky shark captures are stimulated by high shark fin prices, which make this species one of the three most important shark species in the global shark fin trade (Clarke *et al*., 2006).

Various aspects of silky shark biology have been assessed across its distribution, including reproduction (e.g. Branstetter *et al*., 1987; Hazin, Oliveira & Macena, 2007), movements and migration (e.g. Kohler, Casey & Turner, 1998), fisheries (e.g Beerkircher, Shivji & Cortés, 2003, Domingues, Caltabellotta & Amorim, 2016), age and growth (e.g. Branstetter *et al*., 1987; Sánchez-de Ita *et al*., 2011) and feeding habits (e.g. Cabrera-Chávez-Costa, Galván-Magaña & Escobar-Sánchez, 2010). Population genetic aspects of this species have been recently reported, but remain in an early state of understanding particularly with respect to geographic distribution of this widespread species (Galván-Tirado *et al*., 2013). Clarke *et al*. (2015) examined silky shark phylogeography and population genetics on a global scale, finding strong phylogeographic partitioning with two highly divergent, matrilineal evolutionary lineages corresponding to the western Atlantic and Indo-Pacific Oceans, but panmitic populations along the western Atlantic Ocean. However, since sampling representation from the western South Atlantic was limited in their study and silky sharks are a significant fishery in this region, we asked the question if inclusion of more geographically widespread samples might provide a more detailed perspective.

Here, we expand the phylogeographic and population genetics assessment of silky sharks by Clarke *et al.* (2015) by increasing the samples sizes and distribution of sharks from western South Atlantic. Furthermore, we also conducted additional phylogeographic analyses using this expanded dataset. Specifically, we tested the hypothesis of matrilineal genetic panmixia along the western Atlantic Ocean, and examined the impact of glacial cycles during the Pleistocene on historical population demographic patterns of the silky shark. Finally, the possible role of geographic barriers, oceanic currents and ecological behaviors on shaping

silky shark phylogeographic structure were discussed, along with their implications for the fisheries management of this highly exploited species.

MATERIALS AND METHODS

Sampling sites and DNA extraction

A total of 211 silky sharks were sampled from five western Atlantic Ocean sites: USA east coast - USA (43), Gulf of Mexico - GM (42), Pará State, Northern Brazil - PA (16); Saint Paul and Saint Peter Archipelago, Northeast Brazil - ASPSP (48) and Santa Catarina State, Southern Brazil - SC [(62) (Fig. 1)].

Samples of muscle and/or fin clips were taken from both landed catch and on board by observers in commercial pelagic tuna longliners. All tissue were stored in 95% ethanol at -20°C until genomic DNA extraction. Total genomic DNA was extracted from approximately 20 mg of tissue samples using the DNAQIAmp Tissue kit (Qiagen Inc., Valencia, CA, USA). To avoid morphological misidentification of species, PCR-multiplex approach was performed according to Domingues, Amorim & Hilsdorf (2013).

Amplification and sequencing

The partial mtDNA control region (mtDNA CR) was amplified by polymerase chain reaction (PCR) using two external primers, Pro-L (5´- AGGGRAAGGAGGGTCAAACT - ´3) (Keeney & Heist, 2006) and Prol-Falc2 (5´- CCCGGGGTGATCCTAATAAT - ´3) developed in this study. Amplification was performed in 25µl reaction, containing 2 µL of DNA *template*, 10.0 mM of each primer, 1X Buffer, 2.5 mM dNTP´s and 2.0 unit of Taq polymerase (Fermentas, *Life Science*©) and ultrapure water. The PCR cycling profile consisted of 15 min at 95°C, followed by 35 cycles at 94°C at 1 min, 62°C at 30 sec, and 72°C at 30 sec and a final extension of 72°C at 7 min. The PCR products were purified using QIAquick PCR purification (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. Cycle sequencing was performed using the Applied Biosystems BigDye

Terminator v3.1 kit (Life Technologies, Carlsbad, CA, USA), following manufacturer's directions. The amplicon was sequenced on an Applied Biosystems 3130 Genetic Analyzer and bases called using Applied Biosystems Sequencing Analysis Software version 5.2. All partial mtDNA CR sequences were sequenced two times to avoid errors and edited manually when necessary using CodonCode Aligner (v. 5.1.5, Codon Code Corporation).

Genetic diversity and population structure

The parameters of genetic diversity such as nucleotide composition, number of transitions and transversions, haplotype (*h*) and nucleotide (π) diversities, number of haplotypes (H), variable sites (S) and polymorphism distribution were estimated using Arlequin 3.5 (Excoffier & Lischer, 2010). To tests the null hypothesis of panmixia, the genetic structure of the population was estimated among all localities using an analogue Wright's F_{ST} (Φ_{ST}) with the statistical significance tested with 16,000 permutations and α = 0.05 (Cockerham & Weir 1993). The estimated probabilities were corrected using Holm-Bonferroni sequential adjustments for multiple tests (Holm, 1979). The global population structure and proportional variance distributed among sites, considering all five sites as belonging to one population, were tested by the analysis of molecular variance- AMOVA (Excoffier, Smouse & Quattro, 1992) as implemented in Arlequin 3.5.

A Mantel test was performed to determine whether there is a significant isolation by distance (IBD) among populations by testing for correlation between genetics distance and geographic distance (minimal nautical distance between localities) using R program (R Core Team, 2015). The statistical significance was tested with 10,000 permutations.

Phylogenetics and demographic history

Phylogenetic CR mtDNA haplotype relationships of silky sharks were reconstructed using Maximum-Likelihood and Bayesian approach. In order, to assess phylogenetic relationships between Atlantic and Indo-Pacific Ocean, we used haplotypes from the Clarke *et*

al. (2015). Both trees were built under the HKY+I model based on Akaike Information Criterion (AIC) as implemented in jModelTest v. 2.1.6 (Darriba *et al*., 2012). Mega 5 was used to generate the Maximum-Likelihood tree with 1,000 bootstrap replicates.

We conducted a dated Bayesian phylogenetic in BEAST v 1.8.2 (Drummond *et al*., 2012) under a Yule tree prior and strict clock to estimate the divergence time and credibility interval between lineages identified in the ML tree. According to Drummond and Bouckaert (2015) for intraspecific phylogeography, a strict clock is usually preferred than relaxed clock. Due to a lack of a species-specific mutation rate for silky shark and to avoid errors, the mutation rate of congeneric species *Carcharhinus limbatus* (0,43% Myr^{-1}, Keeney & Heist 2006) was assumed. The posterior distribution of parameters was estimated using MCMC with 10 million generations and the first 10% discarded as burn-in times. The convergence of model and the effective sample size (ESS) was checked using TRACER v. 1.6 (Rambaut *et al*., 2014). TREEANNOTATOR 1.8.2 (Drummond *et al*., 2012) was used to summarize the information of tree produced by BEAST and FIGTREE v 1.4.2 (Rambaut, 2008) to view the phylogenies. The relationship among haplotypes and their geographic distribution was assessed using Median-joining algorithm in NETWORK 4.6.1.1 (www.fluxus-engineering.com).

Different approaches were applied to infer on population demographic history. The mismatch analysis was tested using two models: sudden demographic expansion (Rogers and Harpending 1992) and geographic expansion population (Ray, Currat & Excoffier, 2003). Theoretical studies have shown that populations at demographic equilibrium have a multimodal distribution, whereas the unimodal distribution is interpreted as demographic expansion (Slatkin & Hudson, 1991, Rogers & Harpending, 1992). The goodness of fit between the observed and expected mismatch distribution for both models was used to test the null hypothesis of demographic expansion, by calculating the Harpending's raggedness index

- H*ri* (Harpending, 1994) and the sum of squared deviations (SSD), as implemented in Arlequin 3.11. In addition, two others neutrality tests, Ramos-Onsis and Rozas's R_2 (Ramos-Onsis & Rozas 2002) and Fu's F*s* (Fu, 1997), were run with the statistical significance of each index tested with 10,000 simulations under coalescent process, as implemented in DNAsp (Librado & Rozas 2009). These tests are more powerful in detecting events of demographic expansion and deviations from neutrality, with R_2 being more robust for small samples sizes, whereas Fu's *Fs* is better for large samples sizes (Ramos-Onsis & Rozas 2002).

Bayesian skyline plots (BSP) were used to infer about female effective populations size and it changes over time using software BEAST v 1.8.2 (Drummond *et al*., 2012). This model generates a posterior probability for female effective population size using Markov Chain Monte Carlo (MCMC). The MCMC was run for 10 million generations and the first 10% were discarded at the burn-in times. We assumed the substitution model (HKY-I), strict molecular clock, a piecewise-constant Bayesian skyline tree prior and a mutation rate of 0.43% Myr^{-1} as mentioned above. We assumed a generation time of 08 years for Atlantic silky sharks, according to Branstetter (1987). The Bayesian skyline plot reconstruction was conducted in TRACER v. 1.6 (Rambaut *et al*., 2014).

RESULTS

Genetic diversity and population structure

A total of 707 bp from mtDNA CR were resolved from 211 specimens of silky shark. Sequences of all haplotypes were deposited in GeneBank under Accession Numbers KU497450 - KU497489. The fragment begins at 119 bp and end at 825 bp when compared with entire mtDNA CR sequences from silky shark (Genbank: KM267626.1). The final sequences resulted in 40 haplotypes, 23 variable sites, 23 mutations (17 transitions, 5 transversions and 1 indel). The nucleotide composition was 30.73% A, 20.38% C, 14.06% G, 34.83% T. Overall haplotype (h) and nucleotide (π) diversities were h = 0.885 (± 0.012) and π

= 0.005 (± 0.003), respectively. The detailed genetic diversity by sites is presented in Table 1. The haplotypes H8 (n=50), H9 (n=37) and H6 (n=26) were the most representative and 22 haplotypes were singletons (see Supporting Information Table S1).

The hypothesis of panmixia of the silky shark along the western Atlantic Ocean was rejected based on haplotype frequencies (overall Φ_{ST} 0.058, $P < 0.0001$), revealing a population genetic structure. The AMOVA including all locations revealed that the higher genetic variation was found within locations (94,2%). The pairwise Φ_{ST} values revealed a matrilineal genetic differentiation mainly between the locations from the Northwest Atlantic – NA group (USA and GM) and the Southwest Atlantic – SA group (SC and PA). Specifically, ASPSP presented break to gene flow only with USA. However, after sequential Bonferroni correction for multiple tests, only SC and USA and SC and GM were statistically significant (Table 2). The greatest difference was observed between SC and USA (Φ_{ST} 0.160, $P < 0.0000$). The Mantel test of pooled samples did not reveal correlation between genetics distance and geographic distance ($r^2 = 0.155$, $P > 0,05$), not rejecting the null hypothesis of absence of IBD.

Phylogenetic and demographic history

The Maximum-Likelihood (not showed) and Bayesian tree (Fig. 2A) involving haplotype for western Atlantic and Indo-Pacific regions (haplotypes downloaded from Clarke et al. 2015) agreed in revealing two lineages, lineage 1 and lineage 2 (hereafter referred as L1 and L2, respectively) with a deep split. The L1 was composed of haplotypes from western Atlantic and Indo-Pacific Oceans, whereas L2 exhibited only haplotypes from western Atlantic Ocean. The divergence date between two clades from the Bayesian method was estimated to have started approximately 485,571 thousand years ago- Kya (317, 313 – 620,578 Kya 95% highest posterior density – HPD, Fig. 2A). The haplotype network also agreed with the results of phylogenetic trees, resulting in two main clades separated by five

mutational-steps, four mutations and one median vector (Fig. 2B). The L1 was composed of the most frequent haplotypes (H6, H8, H9) and linked to many others less frequent haplotypes forming a faint star phylogeny. On the other hand, the L2 presented less frequent haplotype and some singletons haplotypes.

The different demographic tests showed contrasting results. The mismatch distributions to NA group, SA group and pooled samples were bimodal (see Supporting Information Figure S2). The H*ri* index to both demographic models resulted in non-significant low values for all locations, not rejecting the null hypothesis of population expansion, whereas the SSD tests showed significant low values to GM, SA group and overall data, under sudden expansion model and to USA, SA and NA groups under spatial expansion model (Table 3). The R_2 were not significant in any location. On the other hand, Fu's Fs test showed negative values in all locations, but only GM, ASPSP, SA group and overall data gave significant values ($P < 0.02$). USA and NA group, however, presented lows p-values, $P = 0.058$ and $P = 0.034$. Moreover, mismatch distribution parameters showed zero values of θ_0, while θ_1 values tended to infinite (Table 3). The Bayesian skyline plots to track fluctuation of female effective population size to NA group showed a demographic increase starting about 30,000 years ago until about 2,500 years ago, followed by a smooth decrease. The female effective population size was estimated in 173,273 females (Fig. 3A). The SA group presented an abrupt growing about 15.000 years ago until about 2.000 years ago. The female effective population size was estimated in 166,699 females (Fig. 3B)

DISCUSSION

Genetic diversity and population structure

The genetic diversity detected at the CR mtDNA for silky sharks in this study ($h = 0.885\pm0.012$, $\pi = 0.005\pm0.003$, overall samples) is very similar to those found in the Indo-Pacific ($h = 0.86\pm0.02$, $\pi = 0.20\pm0.10\%$) and Atlantic ocean ($h = 0.93\pm0.01$, $\pi = 0.51\pm0.27$)

by Clarke *et al*. (2015), but higher than eastern Pacific (h = 0.439±0.04, π = 0.0008±0.01%) and in the entire Pacific Ocean (h = 0.48±0.03, π = 0.09±0.01%) found by Galvan-Tirado et al. (2013). Also, when compared to others congeneric species, silky sharks have higher genetic diversity (see Supporting Information Table S2), most likely because of its highly mobile behavior. According to Karl *et al*. (2011) pelagic shark species have higher genetic diversity than coastal shark species.

Although silky sharks have great genetic diversity, their populations are currently overfished along the entire Atlantic Ocean (Baum *et al*., 2003; Bonfil *et al*., 2009; Cortés *et al*., 2010; Barreto et al. 2016). Even though, overfishing is responsible for the decrease of genetic variability across a wide range of marine fishes, the genetic signature of a recent abrupt demographic decrease may be not detected in many vertebrates in short term, mainly because long generation times and high connectivity (Allendorf *et al*., 2008, Rodríguez-Zárate, Rocha-Olivares & Beheregaray, 2013; Pinsky & Palumbi, 2014).

The AMOVA (overall Φ_{ST} 0.058, $P < 0.0001$) and pairwise Φ_{ST} values (Table 2) revealed significant matrilineal genetic differentiation, mainly between the NA and SA groups of the silky shark. These results are different of previous findings that did not revealed divergence among different areas along the western Atlantic Ocean for silky shark (Clarke *et al*., 2015). The different results between these two studies are probably due to increase sampling site further in the Southwestern Atlantic. These outcomes indicate the presence of at least two populations, NA and SA groups, with the ASPSP (northeast Brazilian Island) having genetic connectivity to all locations. These findings also raise the following question: what could be the source of such genetic structure pattern in a species with high dispersal capacity? Two likely explanations, or a combination of them, may be invoked to address this question: oceanic currents and reproductive philopatry.

Several authors have suggested that oceanic currents may also affect the population

structure of marine species (Rocha, 2003; Rocha *et al*., 2005; White *et al*., 2010; Han *et al*., 2012; Mendonça *et al*., 2011; 2013). Silky sharks from the ASPSP are connected to all localities. The connectivity between ASPSP and others locations may be correlated with the pattern of oceanic currents in the Atlantic Ocean. The main oceanic current that passing through the ASPSP is the strong South Equatorial Current (SEC), localized between 20° S to 2-3° N, that flows westward toward the Brazilian shelf and bifurcates near 16°S with one branch heading northwards as the North Brazil Current (NBC) and the other, weaker southward branch, as the Brazil Current (BC), connecting ASPSP to PA and SC populations, respectively. Conversely, the SEC meets the NBC flowing to northwestward to form the Guiana Current that reaches the Gulf of Mexico, connecting ASPSP with GM and USA populations. This oceanic current pattern has explained the structure population of two coastal sharks along the western Atlantic Ocean, *Rhizoprionodon porosus* (Mendonça *et al*., 2011) and *Rhizoprionodon lalandii* (Mendonça *et al*., 2013) and the bony fish *Macrodon ancylodon* (Santos *et al*., 2006).

Although the oceanic currents can influence the matrilineal population structure, other factor should be taken into account as reproductive philopatry, behavior and life history. However, there was no relationship between genetic distance and geographic distance (r^2 0.263, $P > 0,05$), the matrilineal population structure of the silky shark can be interpreted as a signature of female reproductive philopatry. Reproductive studies strongly support the philopatry hypothesis of silky shark, because several locations have been used as reproduction grounds along the western Atlantic Ocean, such as Gulf of Mexico (Branstetter *et al*., 1987; Bonfil, Mena & Anda, 1993), around the ASPSP (Hazin *et al*., 2007), however few females pregnant was observed (n=10), and Southern Brazil (Amorim, Arfelli & Fagundes, 1998). Indeed, philopatry is a behavior that can generate population structure in mobile animals and can inhibit great migrations and reproductive mixing (Benavides *et al*., 2011; Chapman *et al*.,

2015). This behavior has been reported indirectly through genetic data in many *Carcharhinus* species (e.g. Keeney *et al*., 2005; Portnoy *et al*., 2010; Benavides *et al*., 2011; Karl *et al*., 2011). However, we recognized the need of use biparentally markers to affirm if this population structure reflect in both sex or only in females of the silky shark.

Phylogenetic and demographic history

The recent deep phylogenetic isolation of the two matrilineal lineages, with the presence of a large clade (L1) with haplotypes from western Atlantic and Indo-Pacific and a second one (L2) with haplotypes restricted to the western Atlantic is a hint to a allopatric divergence between western Atlantic and Indo-Pacific originated by vicariant event, followed by secondary contact (Avise, 2000). Even increasing the collected sites and samples sizes of silky shark in the western Atlantic, our findings corroborate the phylogeographic pattern previously found for silky sharks by Clarke *et al*., (2015) regarding Atlantic and Indo-Pacific divergency. Indeed, this intraspecific phylogenetic pattern between Atlantic and Indo-Pacific oceans is observed to many species with high dispersal potential (Graves, 1998; Theisen *et al*., 2008).

The time estimated for the most recent isolation (485,571 Kya, 317,313 – 620,578 Kya 95% - HPD), corresponds to the major sea level and temperature cycles (100 - 800 Kya) during the Pleistocene (Lambeck, Esat & Potter, 2002). In this period, the Benguela Barrier reduced or may have temporarily ceased the connectivity of tropical species between Indo-Pacific and Atlantic oceans due to the cold current around southern tip of Africa, acting as a permeable barrier (Briggs, 1995; Peeters *et al*., 2004; Ovenden, 2013). Posteriorly (100,000 to 20,000 Kya), the increase of temperature created an avenue of warm water into the south Atlantic, allowing a secondary contact of allopatric populations, which resulted in the present distribution of the clades (Peeters *et al*., 2004; Bowen *et al*., 2016).

Although different approach to tests the hypothesis of demographic expansion yielded

conflicting results, the combination of such results, mainly those from more powerful tests, suggest a sudden demographic and spatial expansion for both SA and NA groups. Even though there are several approaches to determinate the evolutionary drivers acting in a specific gene region, those based on the coalescent theory (Kingman, 1982) are considered to be more powerful (Ramos-Onsins & Rozas 2002; Grant, 2015). According to Ramos-Onsins & Rozas (2002), Fu's *Fs* and R_2 are the more powerful tests, with the R_2 test being the most powerful to small samples sizes (~ n=10), whereas the Fu's *Fs* performs better for large samples (~ n=50). Although, the bimodal distribution is generally interpreted as a signature of population stability; here we inferred the bimodality to both NA and SA groups as a result of the presence of two sympatric lineages, rather than demographic stability (Rogers & Harpending, 1992; Maltagliati *et al*., 2010). Besides that, significant Tajima's D values (not showed) associated with multimodal mismatch distributions are often possible indicators of population bottleneck where the highest negative and significant values of Fu's Fs tests support the evidence that both NA and SA groups experienced a bottleneck followed by a sudden expansion, either spatial or demographic. Moreover, zero value of θ_0 suggests a small population before expansion and θ_1 values tending to infinites is a hint of a large and sudden population expansion (Rogers, 1995).

The demographic history of the silky shark along the western Atlantic Ocean could be attributed to the differences in climate and habitat condition during the Pleistocene. It is consistent with previous findings of some large pelagic fishes (Graves, 1998; Martinez *et al*., 2006; Theisen *et al*., 2008; Bernard *et al*., 2014). During the Pleistocene coastal regions were severely affected by fluctuations of temperature and sea level by up 130 m below present levels (Ludt & Rocha, 2015). However silky sharks are mainly oceanic, their coastal dependence as nursery area and habitat to juveniles (inshore to 18m) may have been affected and caused a bottleneck effect followed by an abrupt population expansion (spatial and

demographic), started before LGM to NA and after to SA groups (Fig. 3AB), when warm water created routes of migrations between south Atlantic and Indo-Pacific oceans (Peeters et al., 2004; Bowen et al., 2016).

Implications for conservation

The outcomes of this silky shark's genetic assessment are an important contribution for future conservation of this species in the Atlantic Ocean. Our results bring new insights about western Atlantic silky shark such as population structure, suggesting at least two populations, NA and SA groups and complex evolutionary history linked with past environmental changes. Furthermore, the female effective population size of silky sharks (173,273 - NA and 166,699 - SA, respectively) is similar to others sharks highly exploited such as *Sphyrna lewini* (140,000; Duncan *et al*., 2006), *Galeorhinus galeus* (198,296; Chabot & Allen, 2009) and *C. leucas* (193,000; Karl *et al*., 2011). The effective population size of a natural population is considerably less than the census population size and the overfishing can reduce it and increase the rate of loss of genetic variation, causing harmful evolutionary and ecological consequences (Ryman, Utter & Laikre, 1995; Allendorf et al. 2008).

These findings have direct impact on fisheries management of silky shark, which is considered as the most vulnerable species to fisheries due to their low productivity and high susceptibility to pelagic longline gear (Cortés *et al*., 2010). As consequence, silky sharks populations is overexploited in entire Atlantic Ocean and is listed globally as near threatened - NT by IUCN (Baum *et al*., 2003, 2004; Bonfil et al., 2009; Barreto *et al*., 2016). Recently, the International Commission for the Conservation of Atlantic Tunas (ICCAT) prohibited their catch and commercialization in the Atlantic Ocean (ICCAT, 2011). Thus, considering their high vulnerability and current level of population declines (Cortés et al., 2010; Barreto et al., 2016), and the present results, we recommend that NA and SA Atlantic, be regarded as two evolutionary significant units – ESUs (Moritz, 1994), ensuring not only the increase of

population size, but also their evolutionary dynamics. Finally, we recognize the needs of addition of nuclear markers (e.g. microsatellites) to elucidate gaps leading for this and past studies and obtained more accurate conclusions.

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Tables:

Table 1. Genetic diversity indices and neutrality tests of the silky shark (*Carcharhinus falciformis*). n: number of individuals; S: polymorphic sites; H: haplotype number; h: haplotype diversity and standard deviation; π: nucleotide diversity and standard deviation.

Collections sites	*n*	S	H	*h*	π
USA	43	15	18	0.925 ± 0.021	0.007 ± 0.004
GM	42	17	19	0.918 ± 0.025	0.006 ± 0.003
PA	16	10	6	0.800 ± 0.069	0.003 ± 0.002
ASPSP	48	16	20	0.904 ± 0.025	0.005 ± 0.003
SC	62	12	11	0.789 ± 0.035	0.003 ± 0.002
NA group	85	18	24	0.924 ± 0.012	0.248 ± 0.137
SA group	126	18	27	0.840 ± 0.028	0.145 ± 0.086
Overall	211	23	40	0.885 ± 0.012	0.005 ± 0.003

Table 2. Pairwise Φ_{ST} values (below diagonal) and *P* values (above diagonal) for Atlantic silky shark. Analysis based on partial-length mitochondrial DNA control region sequences. Significant values ($P < 0.05$) are indicated in bold. Values marked by asterisks are significant after sequential Bonferroni correction for multiple tests.

Collections sites	USA	GM	PA	ASPSP	SC
USA	-	0.270	**0.012**	**0.011**	**0.000***
GM	0.004	-	**0.047**	0.291	**0.004***
PA	**0.126**	**0.067**	-	0.192	0.380
ASPSP	**0.065**	0.003	0.021	-	0.122
SC	**0.160***	**0.072***	0.001	0.013	-

Table 3.Mismatch distribution parameters and neutrality tests of the silky shark. θ0: theta at past time; θ1: theta at present time; SSD: sum of squared deviations; P SSD: p-value of SSD; Hri: Harperding's index; P Hri: p-value of Hri; Fu's Fs: Fu's neutrality test; P *Fs*: p-value of Fu's test; R_2: Ramos-Onsis and Rozas neutrality test; P R_2: p-value of R_2 test.

	Sudden-expansion model						Spatial-expansion model				Neutrality tests			
Collections sites	θ_0	θ_1	SSD	P SSD	*Hri*	P *Hri*	SSD	P SSD	H*ri*	P H*ri*	Fu's *Fs*	P *Fs*	R_2	P R_2
USA	0.000	7.523	0.046	0.151	0.046	0.408	**0.047**	**0.018**	0.046	0.230	-4.446	0.058	0.156	0.921
GM	0.000	∞	**0.240**	**0.001**	0.052	0.960	0.035	0.094	0.052	0.242	**-6.606**	**0.011**	0.128	0.695
PA	0.000	∞	0.016	0.296	0.089	0.422	0.016	0.220	0.089	0.412	-0.569	0.371	0.158	0.591
ASPSP	1.760	∞	0.027	0.227	0.054	0.249	0.027	0.061	0.054	0.253	**-8.673**	**0.001**	0.108	0.554
SC	0.007	∞	0.006	0.172	0.067	0.254	0.006	0.103	0.067	0.281	-2.283	0.168	0.083	0.316
NA group	0.000	5.992	0.046	0.176	0.050	0.366	**0.041**	**0.030**	0.050	0.127	-6.534	0.034	0.122	0.802
SA group	0.000	∞	**0.012**	**0.012**	0.056	0.251	**0.012**	**0.000**	0.056	0.250	**-14.74**	**0.000**	0.072	0.089
Overall	0.000	79.375	**0.031**	**0.013**	0.049	0.325	0.027	0.060	0.049	0.244	**-22.404**	**0.000**	0.079	0.514

Fu's *Fs* significant values (P < 0.02), others tests significant values (p < 0.05).

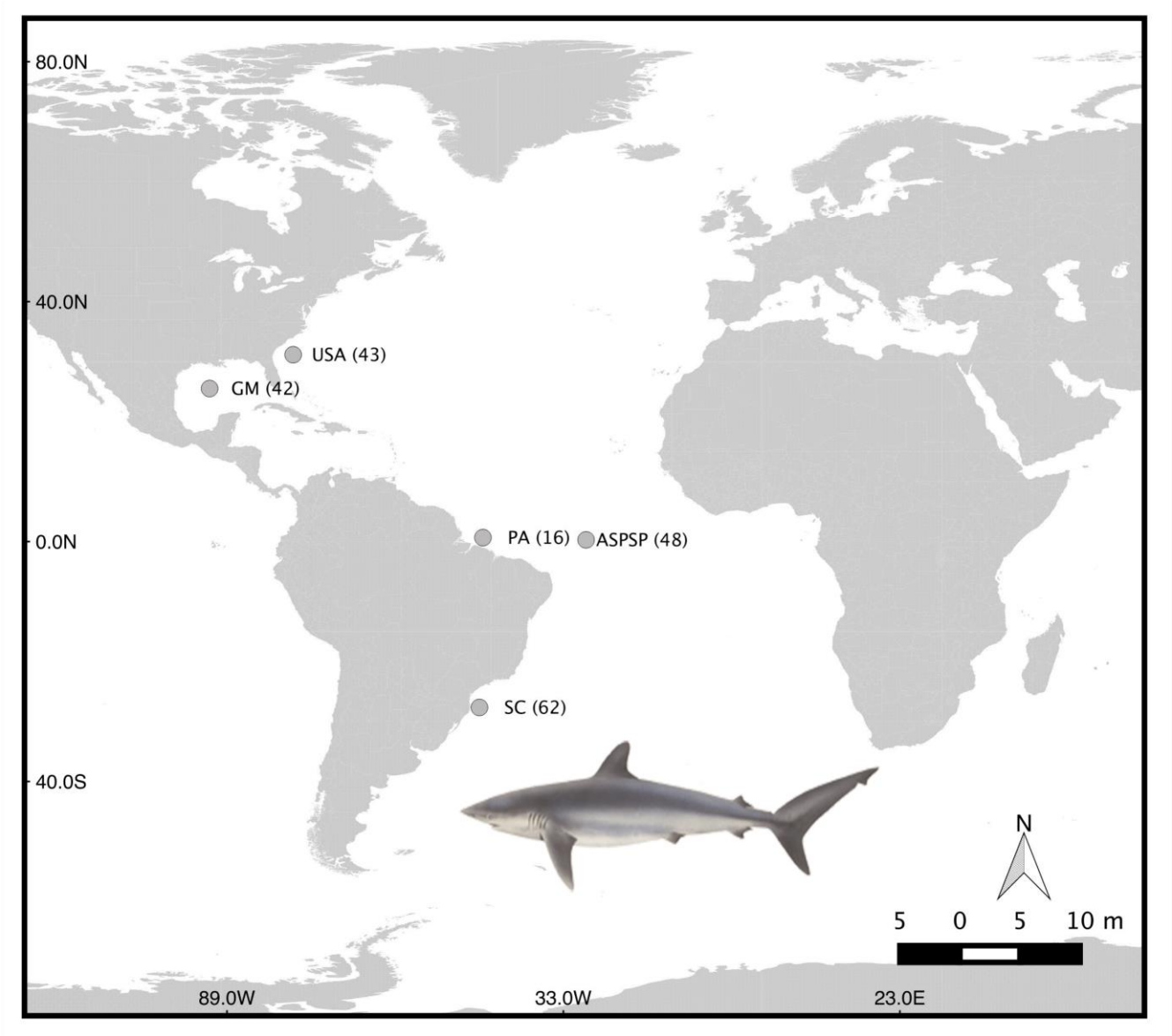

Figure 1. Locations of samples and samples sizes of *Carcharhinus falciformis* along the western Atlantic Ocean (*Carcharhinus falciformis* image source: www.spc.int).

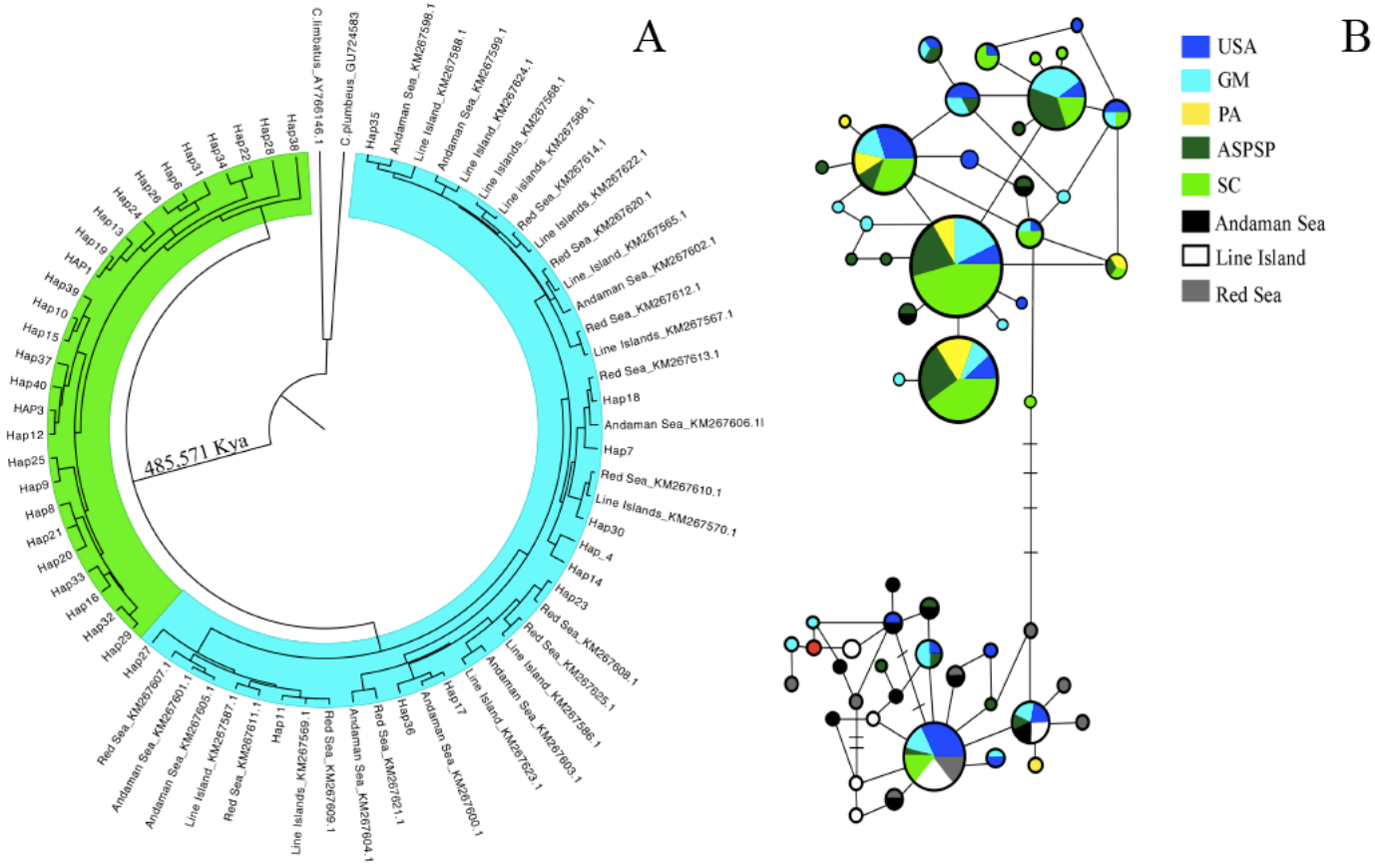

Figure 2. Phylogenetic relationship based on partial length mitochondrial DNA control region of the silky shark (*Carcharhinus falciformis*). (A) Bayesian phylogenetic tree with ages indicated below branches for selected nodes in thousands of years (Kya). Confidence intervals are presented in parenthesis. (B) Median-joining haplotype network based on partial-length mitochondrial DNA control region sequences of silky shark. The circles area is proportional to the haplotype frequency. Branch lengths correspond to one mutational step between haplotypes except where indicated by the number of black bars. Colour code: blue, USA, light blue, GM, black, PA, gray, ASPSP, white, SC and red, median vector.

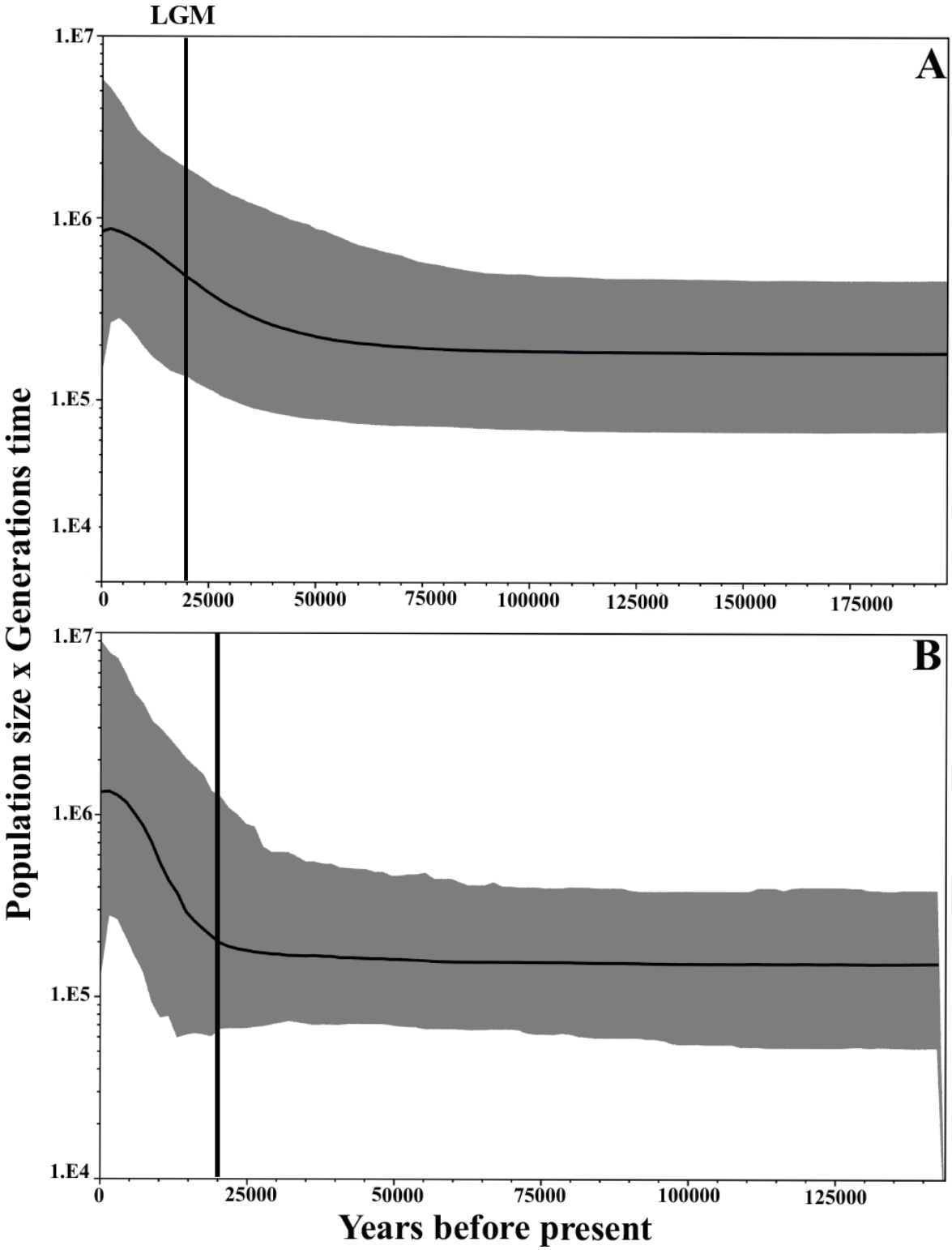

Figure 3. Bayesian Skyline plot showing the historical trends of female effective population sizes. Solid lines represent median estimates and shaded areas represent the 95% HPD limits. (A) Northwest Atlantic group – NA and (B) Southwest Atlantic group – SA.

SUPPORTING INFORMATION

Table S1. Geographical distribution of haplotypes of silky sharks (*Carcharhinus falciformis)* along the western Atlantic Ocean.

Haplotypes	USA (43)	GM (42)	PA (16)	ASPSP (48)	SC (62)
Hap 1	3	1	0	1	0
Hap 2	2	0	0	0	0
Hap 3	2	7	0	7	4
Hap 4	1	2	0	1	0
Hap 5	1	1	0	0	2
Hap 6	8	4	3	3	8
Hap 7	7	3	0	1	3
Hap 8	4	9	4	10	23
Hap 9	4	3	6	9	15
Hap 10	2	1	0	1	0
Hap 11	2	2	0	1	0
Hap 12	1	0	0	0	3
Hap 13	1	1	0	1	0
Hap 14	1	1	0	0	0
Hap 15	1	0	0	0	0
Hap 16	1	0	0	0	0
Hap 17	1	0	0	0	0
Hap 18	1	0	0	0	0
Hap 19	0	1	0	0	0
Hap 20	0	1	0	0	0
Hap 21	0	1	0	0	0
Hap 22	0	1	0	0	0
Hap 23	0	1	0	0	0
Hap 24	0	1	0	0	0
Hap 25	0	1	0	0	0
Hap 26	0	0	1	0	0
Hap 27	0	0	1	0	0
Hap 28	0	0	1	1	1
Hap 29	0	0	0	1	0
Hap 30	0	0	0	1	0
Hap 31	0	0	0	1	0
Hap 32	0	0	0	1	0
Hap 33	0	0	0	2	0
Hap 34	0	0	0	2	0
Hap 35	0	0	0	1	0
Hap 36	0	0	0	2	0
Hap 37	0	0	0	1	0
Hap 38	0	0	0	0	1
Hap 39	0	0	0	0	1
Hap 40	0	0	0	0	1

Table S2. Genetic diversity indices from control region (Dloop) of mitochondrial DNA of sharks of the *Carcharhinus* genus. *h*: *h*: haplotype diversity; π: nucleotide diversity. 1

Species	*h*	π	Citation
Carcharhinus limbatus	0.670 ± 0.20	0.001 ± 0.04	Keeney *et al*., (2003)
Carcharhinus limbatus	0.660 ± 0.17	0.001 ± 0.08	Keeney *et al*., (2005)
Carcharhinus limbatus	0.780 ± 0.04	0.002 ± 0.01	Keeney & Heist (2006)
Carcharhinus sorrah	0.600 ± 0.24	0.003 ± 0.19	Ovenden *et al*., (2009)
Carcharhinus obscurus	0.600 ± 0.29	0.005 ± 0.48	Ovenden *et al*., (2009)
Carcharhinus plumbeus	0.949	0.00475	Portnoy *et al*., (2010)
Carcharhinus leucas	0.760 ± 0.03	0.003 ± 0.0017	Karl *et al*., (2011)
Carcharhinus brachyurus	0.760±0.06	0.001±0.0007	Benavides *et al*., (2011)
Carcharhinus obscurus	0.830±0.03	0.005±0.0005	Benavides *et al*., (2011)
Carcharhinus leucas	0.482± 0.068	0.008±0.05295	Tillet *et al*., (2012)
Carcharhinus limbatus	0.797	0.0021	Sodré *et al.*, (2012)
Carcharhinus amboinensis	0.340±0.26	0.006±0.003	Tillet *et al*., (2012)
Carcharhinus porosus	0.880 ± 0.02	0.004 ± 0.0002	Tavares *et al*., (2013)
Carcharhinus falciformis	0.480±0.03	0.001±0.0001	Galvan-Tirado *et al*., (2013)
Carcharhinus acronotus	0.735	0.001	Portnoy *et al*., (2014)
Carcharhinus melanopterus	0.460	0.001	Vignaud *et al*., (2014)
Carcharhinus sorrah	-	0.0026	Giles *et al*., (2014)
Carcharhinus falciformis	0.930 ± 0.01	0.006 ± 0.32	Clarke *et al*. (2015)
Carcharhinus falciformis	0.885 ± 0.01	0.005 ± 0.003	This study

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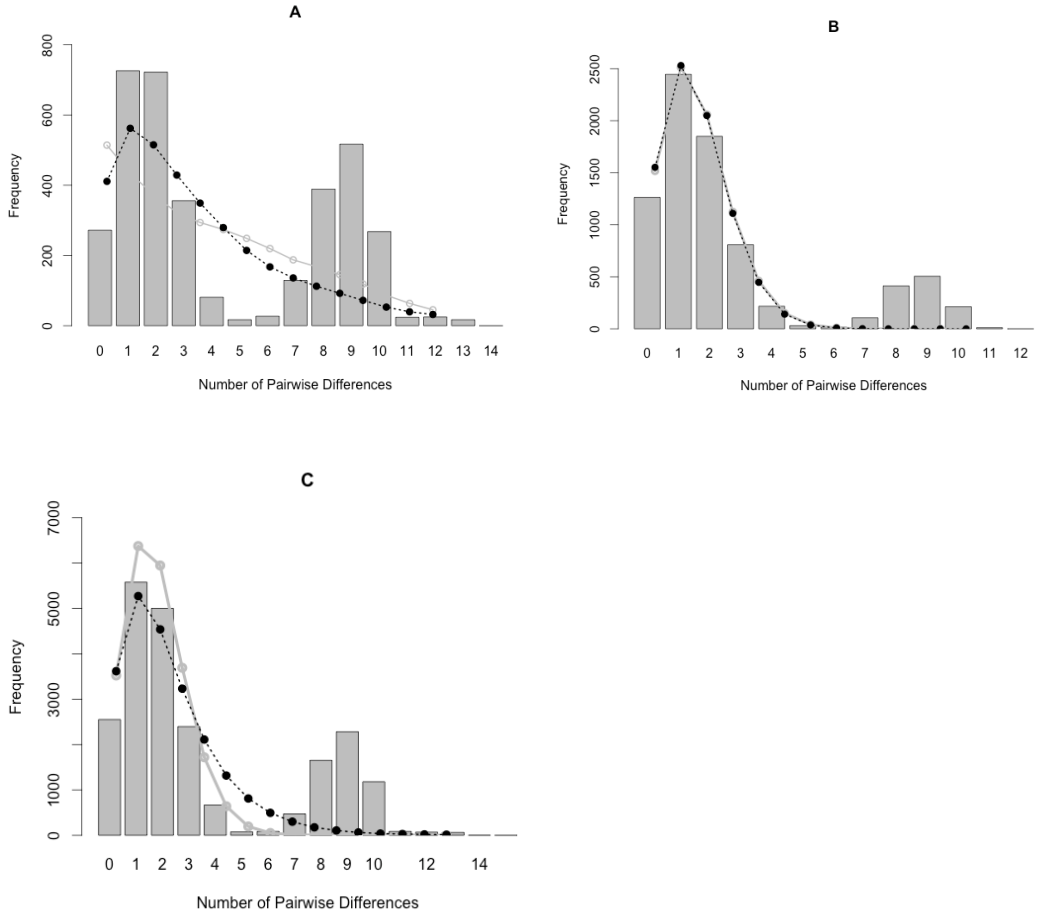
SUPPORTING INFORMATION



Supporting Information Figure S1. Mismatch distribution based on partial-length mitochondrial DNA control regions of silky shark (*Carcharhinus falciformis*). Observed values distribution represented by vertical bars. The line represents the expected distribution under the sudden population expansion model. Solid line represented demographic expansion and dashed line represented geographical expansion. (A) Northwest Atlantic group – NA, (B) Southwest Atlantic group – SA and (C) overall.

CAPÍTULO 3

Sex-biased dispersion, low genetic diversity and insights into the evolutionary history of the night shark (*Carcharhinus signatus*) along the western Atlantic Ocean

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Abstract

The night shark, *Carcharhinus signatus*, is an epipelagic shark species found only in the Atlantic Ocean. It is one of the most frequently caught sharks in pelagic longliner fisheries and is classified as vulnerable by the International Union for the Conservation of Nature (IUCN). Despite their prevalence in commercial fisheries, the population genetic structure of the night shark has not been assessed so far. Therefore, we investigated the genetic diversity, population genetic structure and phylogeography of this species along the western Atlantic Ocean, based on the entire sequence of the mitochondrial DNA control region (CR mtDNA) from 152 individuals. The entire CR mtDNA sequence revealed 19 haplotypes, overall genetic diversity (h = 0.74±0.027 and π = 0.0034±0.0019), and low genetic diversity at 9 *loci* microsatellites (*Ho* = 0.40). A strong signal of sex-biased dispersion was detected based on significant population structure for CR mtDNA (Φ_{ST} = 0.443; P < 0.01) and isolation by distance (r = 0.65, p = 0,03). On the other hand, there was no population structure based at nine microsatellites. The phylogenetics analysis supports the existence of two matrilineal sympatric lineages, separated in the Pleistocene. Demographic analyses indicated a bottleneck effect followed of expansion population sizes during the Holocene. For conservation purposes, we advocated that at least two night sharks populations along the western Atlantic Ocean should considered. Due to low genetic diversity and current overexploitation and vulnerability to fisheries, restrictions to fishing of this shark species along the its geographic distribution should be adopted.

Keywords: Population genetic structure, phylogeography, elasmobranch, conservation.

Introduction

In times of extreme population decline and risk of extinction to several species of sharks (Baum et al. 2003; Dulvy et al. 2008; Ferretti et al. 2010), to shed light on their evolutionary history, biology and population status is pivotal to ensure their conservation. In the last 20 years, genetic data have been incorporated into the management plans of marine species, mostly to determine priority areas for conservation (Palumbi et al. 2003) and evolutionary significant units – ESU (Moritz 1994, Waples 1995). Although, the identification of ESUs is of paramount importance to long-term management, it is still a challenge for marine fish species, due to the high vagility of many fishes associated to the few geographic barriers in the marine environment, which favors their dispersion (Graves 1998; Palsbøll et al. 2007). These factors can lead to two problems: (i) population genetically homogeneous can overlap contiguous management zones and (ii) genetically heterogeneous populations can have overlapping distribution within management zones (Roy et al. 2012). Nevertheless, some factors, such as oceanic currents, sea floor topology, temperature, behavioral traits (e.g. reproductive philopatry) and other variables provide an environmental scenario for population isolation and long-term species differentiation (Avise 1994; Waples 1998).

Many species widely distributed and highly vagile, in general, exhibit little population structure and constitute a single panmictic population (Graves 1998). However, previous studies with sharks have shown significant genetic subdivision at global (e.g. Clarke et al. 2015; Bernard et al. 2016) and regional scales (Karl et al. 2011, 2012). Indeed, sharks have been the focus of many studies on migration and/or site fidelity, using traditional methods such as capture-tagging-recapture and acoustic tracking (e. g. Howey-Jordan et al. 2013; Espinoza et al. 2014; Vaudo et al. 2014). Although these methods can provide various insights about the behavioral traits and ecology of species, they cannot detect whether the

movement has resulted in reproduction, neither reveal the genetic consequences of shark's dispersal (Ovenden 2013; Waples 1998). Furthermore, such methods involve numerous logistical difficulties (Waples 1998). Conversely, molecular markers lend themselves to a large range of statistical analysis about genetic diversity, population connectivity and historical processes that generated such patterns (Frankham et al. 2004).

The night shark, *Carcharhinus signatus* (Poey, 1868) is a oceanic-pelagic shark species, found on tropical and temperate watern in the Atlantic Ocean, it inhabiting outer continental and insular shelves and off upper slopes in depths generally between 50-100 m (Ebert et al. 2013). Age at maturity is about 10 years for female and 8 for male, with 4 to 18 pups per litter (Hazin et al. 2000, Ebert et al. 2013). It is one of the most frequently caught sharks of the *Carcharhinus* genus by the pelagic longline fisheries targeting swordfish and tunas, mainly along the western Atlantic Ocean (Amorim et al. 1998; Carlson et al. 2008; Tavares & Arocha, 2008; Domingues et al. 2013). Even though, this species is well studied about various aspects across their distribution, such as biology and fisheries (Carlson et al. 2008, Domingues et al. 2016), reproduction (Hazin et al. 2000), age and growth (Santana et al. 2004), demographic analysis (Santana et al. 2009) and feeding habits (Vaske Jr. et al. 2009). However, no genetic evaluation of population structure have been done to date. Thus, there is an urgent need to identify the degree of population subdivision of the night shark, and understand what factors are shaping its genetic structure. These information are especially important to fishery management, as failure to detect discrete population can result in overexploitation and erosion of genetic diversity as well as localized extirpation (Roy et al. 2012)

Here, we address these important issues to the genetic conservation of the night shark. Firstly we described the patterns of genetic diversity of the night shark along the western Atlantic Ocean. Secondly, we tested the null hypothesis of panmixia among different putative

population using both mitochondrial DNA and microsatellites. Finally, we investigated the aspects phylogeographic of the night sharks and the mains factors shaping its population structure.

Materials and Methods

Sampling collection

A total of 152 night shark were opportunistically sampled from six different western Atlantic Ocean: USA coast (USA); Gulf of Mexico (GM); Belize (Bze); Venezuela (Vnz); Northeast Brazil - Pernambuco (NBrz) and Southeast Brazil – Santa Catarina [(SBrz) (Fig. 1)]. Samples of muscle and fin tissue were taken from the landed catch, by observer on board of industrial fisheries and during research cruise between 2005 and 2013. All tissue were stored in 95% ethanol at -20°C until extraction. Total genomic DNA was extracted from approximately 20mg of the tissue samples using the DNAQIAmp Tissue kit (Qiagen Inc., Valencia, CA, USA). To confirm the species identification was performed by multiplex PCR as described in Domingues et al. (2013) and used cytochrome oxidase I (COI) region of the DNA mitochondrial, following the methods described by Ward et al. (2005).

DNA mitochondrial and microsatellites essays

The entire mtDNA control region (CR mtDNA) was amplified by polymerase reaction chain (PCR) using initially two external primers CRF6 (5'-AAGCGTCGACCTTGTAAGTC-3') and DasR2 (5'-ACGGTTTGTGGTACATTAC-3'). Amplification was performed out in 50µl reaction containing 50µM of each dNTP, 10pmol of each primer, ~ 20ng of extracted DNA, 1X PCR buffer and 1 unit of HotStar Taq Plus DNA polymerase (Qiagen Inc., Valencia, CA, USA) and ultrapure water. The PCR cycling profile consisted of 15min at 95°C, followed by 34 cycles at 94°C for 1 min, 50°C for 1 min, 72° for 2 min and a final extension 5 min at 72°C. Amplifications were purified using QIAquick PCR purification

(Qiagen Inc., Valencia, CA, USA) following the manufacturer's directions. Cycle sequencing was performed using the Applied Biosystems BigDye Terminator v3.1 kit (Life Technologies, Carlsbad, CA, USA), following manufacturer's directions. The amplicon was sequenced on an Applied Biosystems 3130 Genetic Analyzer and bases were called using Applied Biosystems Sequencing Analysis Software version 5.2.

Nine nuclear microsatellites markers previously published to others shark species were used in this study: blacktip shark (Cli-07, Cli-100, Cli-103, Cli-107, Cli-108) by Keeney and Heist (2003), blue shark (Pgla-13 and Pgla-14) by (Fitzpatrick et al. 2011) and tiger shark (TGR1157 and TGR1238) by Bernard et al. (2014). Amplification to all loci was performed out in 13μl reaction containing 1μl genomic DNA, 1x PCR buffer (0.15 mM $MgCl_2$), 0.2 mM of each dNTP, 1.5mM $MgCl_2$, 0.16μM of the forward microsatellite primer which possessed a 5'- M13 (Schuelke 2000), 0.4μM of the reverse microsatellite primer, 0.4μM of the fluorescently labeled universal M13 primer (Schuelke 2000), and 0.5 U of HotStar Taq DNA Polymerase (QIAGEN Inc.). PCR was performed in a Mastercycler Gradient (Eppendorf Inc.) thermal cycler. The PCR cycling profile consisted of 15 min at 95°C, followed by 35 cycles at 94°C for 1 min, the locus specific annealing temperature (Cli07 – 56°C, Cli100 – 56°C, Cli103 – 53°C, Cli107 – 56°C, Cli108 – 58°C, Pgl13 - 56°C, Pgl14 - 57°C, Tgr1238 - 50°C, Tgr1157 - 57°C), 72° for 1 min and a final extension 5min at 72°C. Microsatellites alleles were resolved on an ABI 3130 genetic analyzer (Applied Biosystems, Inc., Foster City, CA) and scored with LIZ (500) standard using GENEMAPPER 3.7 (Applied Biosystems, Inc., Foster City, CA).

Data analysis

MtDNA Control Region Genetic diversity and phylogenetic relationships

All CR mtDNA sequences were aligned and edited when necessary using Geneious Pro v. 3.5.6 software (Biomatters Ltd. Auckland, NZ). The summary statistical and genetic

diversity such as nucleotide composition, number of transition and transversion, haplotype (*h*) and nucleotide (π) diversities, number of haplotypes (H) and polymorphic sites (S) were estimated using Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). The relationships between haplotypes and their geographic distribution were obtained using Median-joining networks in NETWORK 4.6.1.1 (www.fluxus-engineering.com). The data sets were assessed for the best-fitting model of nucleotide substitution using the Bayesian information Criterion (BIC) as implemented in jModelTest v.2.1.6 (Darriba et al. 2012).

The phylogenetic relationships among haplotypes were constructed using the maximum likehood - ML and Bayesian methods, based on HKY-I nucleotide substitution model (Hasegawa et al. 1985). Mega v.6 (Tamura et al. 2013) generated ML tree with 1,000 bootstrap replicates. A dated Bayesian tree was built in BEAST v. 1.8.2 (Drummond et al. 2012) under Yule tree prior and strict clock to estimate the divergence time between lineages identified in the ML tree. According to Drummond & Bouckaert (2015) for intraspecific phylogeography, a strict clock is usually preferred than relaxed clock. Due to a lack of a species-specific mutation rate for night shark and to avoid errors, was assumed the mutation rate of congeneric species *Carcharhinus limbatus* (0,43% Myr^{-1}, Keeney & Heist 2006). The posterior distribution of parameters was estimated using MCMC with 10 million generations and the first 10% discarded as burn-in times. The convergence of model and the effective sample size (ESS) was checked using TRACER v. 1.6 (Rambaut et al. 2014). TREEANNOTATOR 1.8.2 (Drummond et al. 2012) was used to summarize the information of tree produced by BEAST and FIGTREE v 1.4.2 (Rambaut 2008) to view the phylogenies. The relationship among haplotypes and their geographic distribution was assessed using Median-joining algorithm in NETWORK 4.6.1.1 (www.fluxus-engineering.com).

Population Structure and demographic history

Genetic population structure was estimated using analysis of molecular variance

(AMOVA, Excoffier et al. 1992). The hierarchical AMOVA test was conduced by grouping sampling into three regions along western Atlantic Ocean: North Atlantic (USA and GM); Caribbean (Bze and Vnz) and South Atlantic (NBrz and SBrz), using Arlequin 3.5 (Excoffier et al. 2010). The pairwise population was assessed by analogue Φst and haplotype frequency-based F_{ST} using 10,000 permutations and $\alpha = 0.05$. To test the hypothesis that populations are differentiated because of isolation-by-distance, a Mantel test (Mantel 1967) was performed by testing the correlation between Φst values and geographic distance (minimal nautical distance) using the Adegenet package (Jombart et al. 2008) on R program (R program Core Team 2015). Mantel tests were performed using Pearson correlation with 10,000 permutations.

Different tests were used to infer about population demographic history. Two neutrality tests, Ramos-Onsins and Rozas's R_2 (Ramos-Onsis & Rozas 2002) and Fu's F_S (Fu 1997), were conduced, with the statistical significance tested with 10,000 permutations and $\alpha = 0.05$, under coalescent process, as implemented in DNAsp (Librado and Rozas 2009). According to Ramos Onsis & Rozas (2002) R_2 test is more powerful in detecting demographic events to small sample sizes, whereas Fu's F_S is better for large sample sizes. Bayesian skyline plot (BSP) was used to infer about changes in female effective population size (N*ef*) over time using software Beast (Drummond & Rambaut, 2007). For this, we assumed a strict molecular clock, a piecewise-constant Bayesian skyline tree prior and a mutation rate of 0,43% Myr^{-1} (Keeney & Heist 2006). We assumed a generation time of 10 years (Santana et al. 2009). The posterior distribution of parameters was estimated using MCMC with 10 million generations and the first 10% discarded as burn-in times. The convergence of model and the effective sample size (ESS) was checked using TRACER v. 1.6 (Rambaut et al. 2014) and the Bayesian skyline reconstruction was conducted in Tracer v.1.5 (Drummond & Rambaut 2007).

Microsatellites

The nuclear microsatellite diversity of each population and loci were characterized using the number of alleles (*A*), observed heterozygosity (H_O), expected heterozygosity (H_E), alleles richness (A_R) and inbreeding coefficient (F_{IS}) using the gstudio and Adegenet packages on R (R core Team 2015). The departures of Hardy-Weinberg equilibrium (HWE) for each loci, and populations and linkage disequilibrium (LD) were performed using exact test in Genepop on the web 4.2 (Raymond & Rousset, 1995). The presence of alleles nulls was tested using MICRO-CHECKER 2.2.3 (Van Oosterhout 2004).

The genetic distance between populations was measured across all population using the fixation index G_{ST} (Nei & Chesser 1983), G'_{ST} (Hedrick 2005) and Dest (Jost 2008) using the gstudio package on R. The pairwise comparisons of Dest (Jost 2008) values were tested using DEMEtics package on R. The Structure 2.3.4 (Pritchard et al. 2000) was used to assign individuals to genetic cluster (*K*) and estimate admixture proportions (Q) for each individual. The Structure was run for 1,000,000 steps following a 100,000 step burn-in period and 20 iterations for each *K*-value. To define the number of genetic cluster (K), it was used the method proposed by Evanno (Evanno et al. 2005), as implemented by Structure Harvest 0.6.94. Clumpak (Kopelman 2015) was used to compare all Structure run into a matrix Q of individual's membership coefficient and population ancestry components. Alternatively, was used a multivariate approach, Discriminant Analysis of Principal Components – DAPC (Jombart et al. 2010), to assign individuals to genetic cluster using package Adegenet (Jombart 2008; Jombart et al. 2010). This analysis has been suggested as an alternative to Structure program, mainly because DAPC does not require populations to be in Hardy-Weinberg equilibrium (HWE) or linkage equilibrium (LE) between loci (Jombart et al. 2010).

Results

Mitochondrial DNA

We obtained 1066 bp of the entire CR mtDNA, resolving 18 polymorphic sites, 34 mutations (14 transition and 02 transversion), 16 substitution and 02 indels. The nucleotide composition were 31.88% A, 20,21% C, 13,41% G and 34,50% T. The overall haplotype (*h*) and nucleotide diversity (π) were 0.74 (±0.027) and 0.0034 (±0.002), respectively. The results showed a decrease in haplotype diversity towards the northwest to southwest of the Atlantic regions (Table 1). A total of 19 haplotypes were found, being H7, H1 and H8 the most common, shared by 66, 34 and 20 individuals, respectively. The haplotype H7 was the most common in the South Atlantic individuals, while H1 and H8 more common in North Atlantic individuals (Fig. 2, Table S1).

Significant population structure was observed for night shark along western Atlantic Ocean based on CR mtDNA (Φ_{ST} = 0.443; P < 0.01). The F_{ST} pairwise values ranged from -0,03527 to 0.5975, and Φ_{ST} values from -0.00266 to 0.33914, revealing a strong structure between Northwestern and Southwestern Atlantic. The greatest structure was observed between USA and NBrz (F_{ST} 0.598) and GM and NBrz (Φ_{ST} 0.339) whereas the smallest values between adjacent populations (Table 2). Hierarchical AMOVA resulted in a strong genetic variation among groups (0.482, P < 0.01, 44.61%) and within population (51.78%) but no genetic variation was observed among population within groups. The Mantel test revealed a high correlation (r = 0.65, p = 0,03) between geographic distance and genetic distance mtDNA (Fig. 3).

With regarding to phylogenetics analysis, both Maximum likelihood – ML (not presented) and Bayesian haplotype tree resulted in two sympatric matrilineal lineages, with a deep split estimated to have started approximately 166, 598 thousand years ago – Kya (105,897 – 244,020 Kya 95% highest posterior density – HPD). Haplotype admixture

between both matrilineal lineages was also revealed by the network analysis (Fig. 2). Both *Fs*'s Fu and R_2 neutrality test did not present statistically significant values to any population (Table 1). Conversely, BSP employed to track possible fluctuation on female effective population size showed a demographic increase for NA and SA groups approximately 7,500 and 14,000 years ago, respectively, and for the overall samples 5,000 years ago, whereas Central Atlantic agreed with neutrality tests, showing a smooth decrease (Fig. 4).

Microsatellites

A total of 119 individuals from the five sites (NBrz and SBrz were considered together and named as Brazil - Brz) along the Southwest Atlantic were successfully genotyped at nine microsatellite loci. There was no evidence of genotyping errors, such as stuttering and large allele drop out, but null alleles were present in two loci (Cli-100 and Pgla-13). Only one locus (Cli-100) and two sites (USA and Brz) displayed significant departures from HWE and there were no significant values in the pairwise tests of LD. The number the alleles per locus ranged between 2 (Cli-103) and 22 (Cli-108) and 2 (Vnz) and 6 (USA and Brz) per location, whereas allelic richness ranged from 1.00 (TGR1157) to 3.74 (Cli-108) per locus and 1.68 (Vnz) to 2.01 (Brz) per location (Table 3). The observed and expected heterozygosity per locus ranged from 0.06 (Cli-103) to 0.89 (Cli-108) and 0.08 (Cli-103) to 0.90 (Cli-108), respectively, whereas per population ranged from 0.33 (Vnz) to 0.42 (Brz) and 0.37 (Vnz) to 0.42 (GM), respectively. The inbreeding coefficients showed low values for both locus and population, ranged between -0.049 (TGR1238) and 0.248 (Cli-100) and -0.015 (Bze) and 0.105 (USA), respectively. The whole summary statistical of genetic diversity is presented in Table S1.

The genetic differentiation showed low levels to all index applied: G_{ST} (0.031), G'_{ST} (0.037) and D'Jost (0.05). The pairwise Dest index presented low to moderate values of structure, ranged from -0.015 to 0.058. However, the only significant structure (0.054, *P* =

0.02) was between Brz and Bze (Table 4). Scatter plot of genetic variation in multivariate Dapc presented considerable overlap among populations, not revealing population structure (Fig. 5A). Simulations realized in Structure identified K=3 clusters, but these clusters were not spatially coherent and confirmed the absence of structure among all populations (Fig. 5B). There was no evidence of isolation-by-distance (r = 0.149, P = 0.34) for microsatellite data (Fig. 3).

Discussion

Genetic diversity

Both mitochondrial and nuclear DNA showed low genetic diversity for night shark along the western Atlantic Ocean. For CR mtDNA there was a marked latitudinal gradient of genetic diversity across samples sites, with higher values in the Northwest Atlantic decreasing toward Southwest Atlantic. This variation supports that Northwest Atlantic population (USA, GM) is older than Caribbean (BZE and VNZ) and Southwest Atlantic population (NBrz and SBrz), being considered the center of origin (Rocha et al. 2008). This statement can be supported by higher values of genetic diversity, number of haplotype and two ancient haplotype (H1 and H8) belong to Northwest Atlantic.

Night shark individuals from the Southwest Atlantic showed the lowest genetic diversity among the *Carcharhinus* species (Fig. 6), as opposed to hypothesis that highly mobile species have higher genetic diversity (Karl et al. 2011). This pattern of low genetic diversity to both molecular marker is very similar to others shark species such as *C. limbatus* (Keeney et al. 2005), *Carcharhinus leucas* (Karl et al. 2011) and *Carcharhinus melanopterus*, (Vignaud et al. 2013). Besides, this lower genetic diversity also is congruent with current levels of overexploitation of this species in the western Atlantic Ocean. Nigh sharks have suffered intense overefishing in the last decades and their populations have declined sharply to more than 75% (Baum & Blanchard 2010).

Sex-biased dispersion

Examine the movements of sharks is important to infer about insight into their migration pathways, population structure, nursery areas and vulnerable areas to fisheries and conservation. The molecular tools are very useful to infer about movements, because successful migrants should leave a genetic trail of their movements, offering an indirect means of estimating population connectivity (Ovenden 2013). We reject the null hypothesis that nigh shark are panmictic along the western Atlantic Ocean based on CR mtDNA (Table 2). The flow gene is restricted between Northern Hemisphere vs. Southern Hemisphere for night shark. On the other hand, the null hypothesis of panmixia can not be rejected based on nine loci microsatellite, with exception of Brz and Bze that showed structured based on pairwise Dest test (Table 4), whereas individual-based analysis (DAPC and Structure) not presented any populations difference. The minimum distance to break gene flow of female night sharks was ~3.000 km, between GM and Vnz. A previous study on movements of night shark was performed by NMFS Cooperative where 70 females and 56 males were tagged, and 6 female and 1 male were recaptured, with the a maximum time at liberty of 12.9 year and the maximum distance traveled between 1.000 and 2.000 km in the Northwest Atlantic and Gulf of Mexico (Kohler et al. 1998). Similar patters of populational differences in the western Atlantic Ocean occurred in other large, but more coastal sharks species such as *Sphyrna lewini* (Chapman et al. 2009), *C. leucas* (Karl et al. 2011); *Ginglymostoma cirratum* (Karl et al. 2012) and *Negaprion brevirostris* (Ashe et al. 2015). On the other hand, previous studies with pelagic shark species (*C. falciformis* and *C. longimanus*) do not presented restricted to gene flow in the western Atlantic Ocean (Clarke et al. 2015; Camargo et al. 2016). More recently, Domingues et al (unpublished data) increased the samping effort for C. falciformis and observed similar structuring for southwest and northwest Atlantic. Similar genetic structure might occur for *C. longimanus*, as in the study of Camargo et al. (2016) only few

samples were analyzed from the Southwest Atlantic.

This asymmetric dispersal, females no-roving and males roving, is consistent with male-mediated dispersion and female philopatry (tendency of a individual to return to or to stay in, its home area, natal site or another locality – Mayr 1963). It is supported by isolation-by-distance pattern rather than complete isolation, since intermediate samples site (Bze and Vnz) seem to be an admixture zone, did not presenting break to gene flow with adjacent neighborhood population. Similar finding are reported to others shark species such as *C. melanopterus* (Vignaud et al. 2013) and *Sphyrna tiburo* (Portnoy et al. 2015). As a rule, sex-biased dispersion and female philopatry is a behavior that can generate population structure and have been reported to several mobile marine organisms, such as bony fish, marine mammals and turtle (Rooker et al. 2008; Engelhaupat et al. 2009; Stiebens et al. 2013). Further, evidence of philopatric behavior has been observed in several species of sharks (see review Chapman et al. 2015). For example, six female of lemon sharks (*Negaprion brevirostris*) that were born in Bimini, Bahamas, between 1996 and 1997, have returned to give birth in the same place between 2008 and 2012 (Feldheim et al. 2014). Other evidence direct of female philopatry to sharks is reported to *C. melanopterus* in French Polynesia (Mourier et al. 2013).

Phylogeography and demographic history

The ML (not showed) and Bayesian phylogenetic tree distinguished two matrilineal lineages, indicating a recent deep isolation for approximately 166, 598 Kya (105,897 – 244,020 Kya 95% - HPD), but was unable to assign clustering of haplotypes for any single geographical region. Similarly, *C. leucas* showed two matrilineal lineages for mtDNA in the western Atlantic Ocean (Karl et al. 2011). Even considering the confidence interval, the time of divergence between two lineages corresponds to the major sea level cycles that occur at intervals of ~ 100 Kya over the past ~ 800 Kya, during the Pleistocene (Lambeck et al. 2002).

Although there is a potential geographic barrier (Amazon Barrier) in the western Atlantic, responsible for many vicariant events for different marine species (see review Rocha et al. 2003, 2007), is not reasonable to think that this barrier have created two matrilineal lineages in a highly mobile pelagic shark species. In fact, gene flow was detected between Vnz and NBrz, crossing the AB. However, fluctuations in sea level and temperature during the Pleistocene have been mentioned as a factor influencing the phylogeographic pattern of many pelagic marine fishes (e.g. Alvarado-Bremer et al. 2005, 2005b; Martinez et al. 2006; Portnoy et al. 2014). The presence of two separated sympatric clades with representatives of all populations can be attributed to secondary contact between historical populations that diverged probably caused by sea level and temperature changes (Portnoy et al. 2014).

Even though neutrality tests did not reveal population expansion, haplotype network and BSP agreed with the population expansion after last glacial maximum – LGM to Northwest Atlantic, Southwest Atlantic and overall samples. Many shark species had their population expanded during the LGM influenced by glacial cycles (O'Brien et al. 2013; Portnoy et al. 2014). Indeed, this period is recognized as the main responsible by lineage diversity, population expansion (or contractions) and speciation (Grant 2015). Also, BSP to the overall samples evidenced a bottleneck effect within LGM (15 Kya -5 Kya), followed of an abrupt expansion population in Holocene (Fig. 4D). This notorious populational expansion around 5 Kya also is noted in NA and SA group (Fig. 4A, B). Bottleneck effect in shark population during the Holocene also was evidenced to the Basking shark (Hoelzel et al. 2006).

Implication for Conservation

Our study provides the first assessment about the population genetic structure and phylogeography of a severely overexploited and threat shark species, night shark. These results are important to the conservation of this species along the their most abundance

distribution range. The evidence of low genetic diversity, sex-biased dispersion (male-mediate dispersion and reproductive philopatry) can assist in the future management plans. We advocated that at least two population of night shark should be considered along the western Atlantic Ocean based on CR mtDNA analyses and restrictions to fishing should be adopted based on its low genetic diversity, mainly in the western South Atlantic.

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Table 1. *Carcharhinus signatus* population summary statistics: *n*: sample size; H: number of haplotypes; S: polimorphic sites; *h*: haplotype diversity; π: nucleotide diversity; *F*s Fu: Fu's *F*s test, *P Fs*: P value Fs Fu test and R_2: Ramos-Onsis & Rozas, *P* R_2: *P* value of R_2 test.

	n	H	S	*h*	π	*F*s Fu	*P Fs*	R_2	*P* R_2
USA	62	15	14	0.802 ± 0.037	0.00268 ± 0.00159	-4.519	0.036	0.101	0.508
GoM	9	5	8	0.861 ± 0.872	0.00370 ± 0.00232	0.006	0.497	0.245	0.917
NA group	71	16	14	0.807 ± 0.032	0.00278 ± 0.00164	-4.790	0.028	0.100	0.553
Bze	14	5	8	0.758 ± 0.084	0.00357 ± 0.00214	1.373	0.757	0.227	0.942
Vnz	6	2	7	0.333 ± 0.215	0.00219 ± 0.00160	2.996	0.942	0.373	1.000
CA group	20	5	8	0.695 ± 0.070	0.00348 ± 0.00205	2.133	0.858	0.223	0.982
NBrz	14	3	2	0.275 ± 0.148	0.00038 ± 0.00042	-1.727	0.011	0.125	0.034
SBrz	47	7	12	0.377 ± 0.088	0.00113 ± 0.00082	-2.548	0.844	0.060	0.114
SA group	61	14	13	0.545 ± 0.077	0.00129 ± 0.00089	-4.836	0.005	0.049	0.063
Overall	152	19	18	0.745 ± 0.027	0.00341 ± 0.00192	-4.800	0.078	0.092	0.590

Fs' *Fu* significant values (P < 0.02).

Table 2. Matrix of pairwise F_{ST} values (below diagonal) and Φ_{ST} values (above diagonal) between populations of the night sharks based on mtDNA control region. Significant values ($P < 0.05$) are indicated in bold.

	USA	GM	Bze	Vnz	Nbrz	SBrz
USA	-	-0.008	-0.011	**0.239**	**0.291**	**0.239**
GM	0.002	-	0.002	**0.281**	**0.339**	**0.252**
Bze	0.038	-0.082	-	0.162	**0.256**	**0.177**
Vnz	**0.429**	0.228	0.174	-	-0.063	-0.028
NBrz	**0.598**	**0.565**	**0.475**	0.048	-	-0.003
SBrz	**0.571**	**0.494**	**0.431**	-0.035	0.028	-

Table 3. Characterization of genetic variability in *Carcharhinus signatus* populations along the western Atlantic Ocean based on integrated over all microsatellite loci. *n*: samples sizes, *A*: number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, A_R Allelic richness and F_{IS}: inbreeding coefficient.

Population	*n*	*A*	H_O	H_E	A_R	F_{IS}
USA	43	6	0.420	0.446	2.01	0.105
GM	10	3	0.443	0.418	1.95	-
Bze	14	5	0.430	0.424	1.76	-0.015
Vnz	4	2	0.333	0.368	1.68	-
Brz	48	6	0.417	0.451	1.80	0.083

Table 4. Matrix of population pairwise Dest values (below diagonal) and *P*-values (above diagonal based on nine microsatellite loci.

Population	USA	GM	Bze	Vnz	Brz
USA	-	0.5193	1.000	1.000	0.1314
GM	0.029	-	0.182	1.000	0.3951
Bze	0.016	0.058	-	1.000	0.0189
Vnz	-0.037	-0.023	0.003	-	-
Brz	0.018	0.032	**0.054**	-0.015	-

Significant *P*-values ($P < 0.05$) after Bonferroni correction are highlighted in bold.

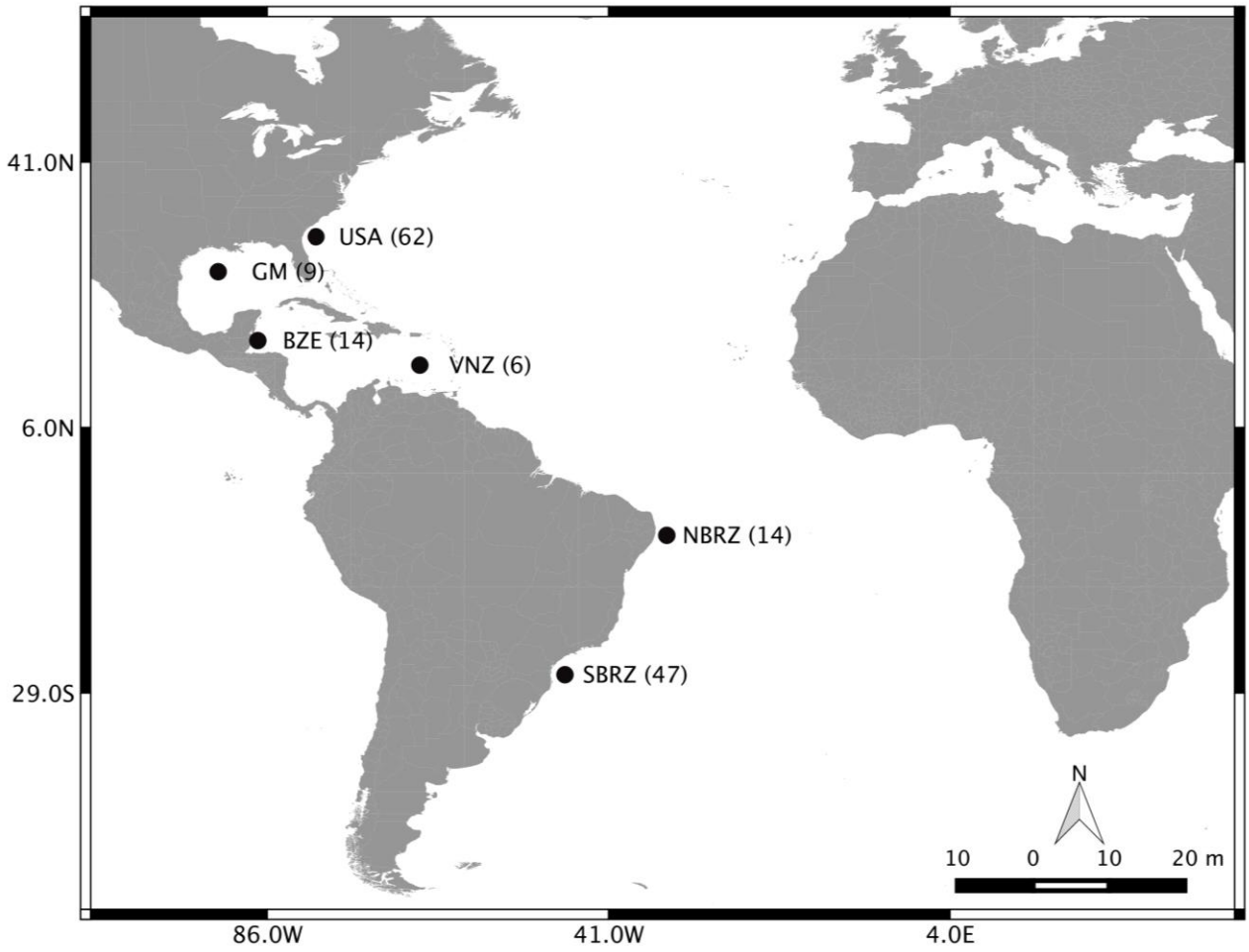

Fig. 1. Locations of samples collections and samples size of night sharks (*Carcharhinus signatus*) along the western Atlantic Ocean.

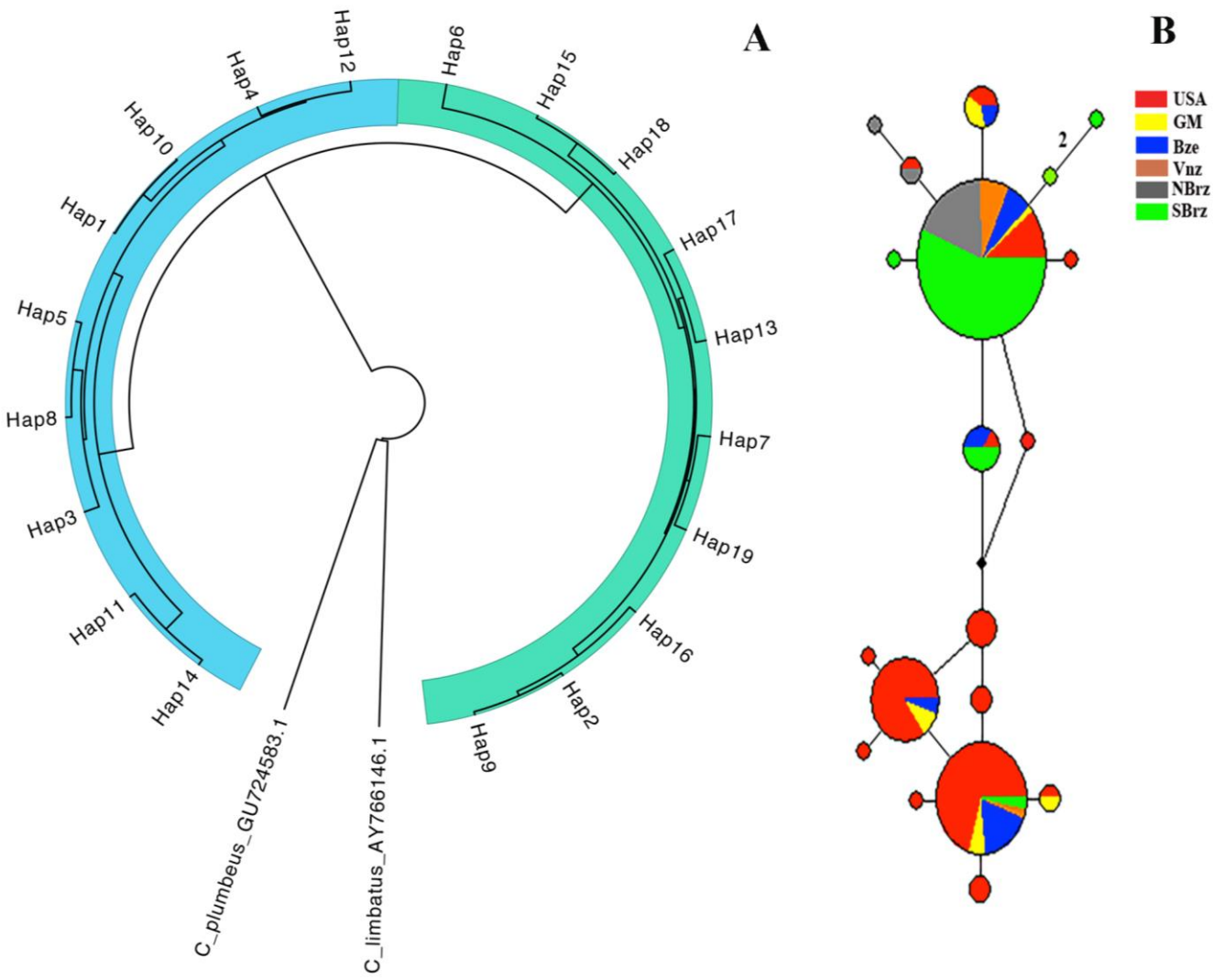

Fig. 2. Phylogenetic relationship based on entire length mitochondrial DNA control region of night shark (*Carcharhinus signatus*). (A) Bayesian phylogenetic tree with age indicated below branches for selected nodes in thousands of years (Kya). (B) Median-joining haplotype network based on entire length mitochondrial DNA control region sequences of night shark. The circles area is proportional to the haplotype. Branch lengths correspond to one mutational step between haplotypes except where indicated by the number. Colour code: red: USA, yellow: Gulf of Mexico, blue: Belize, pumpkin: Venezuela, grey: Northeast Brazil, green: Southeast Brazil, black: median vector.

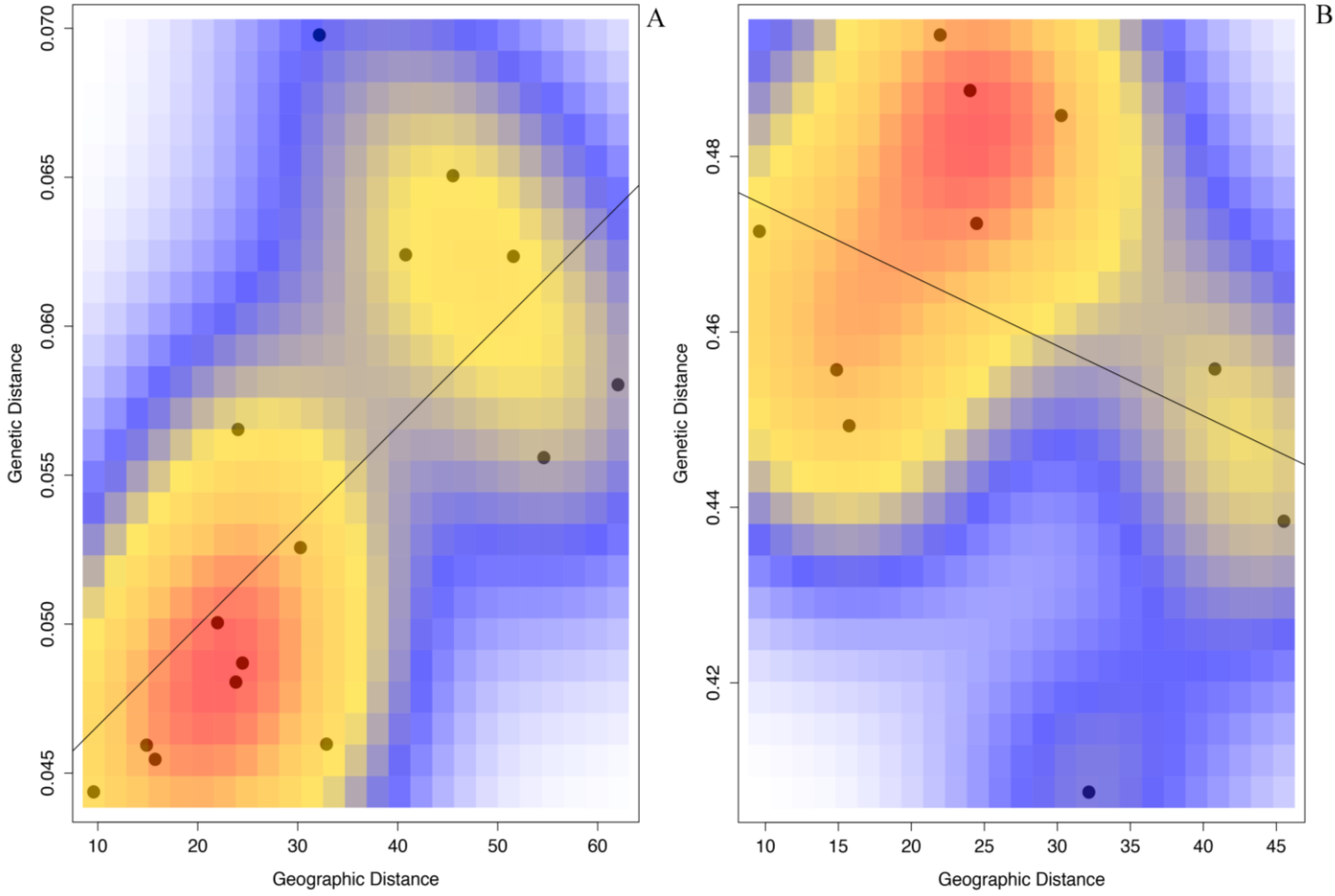

Fig. 3. Isolation by distance relationship represented by genetic distance (pairwise *F*st values) versus geographic distance (minimal nautical distance between localities) between sampled locations of night sharks (*Carcharhinus signatus*) along the western Atlantic Ocean using Pearson's correlation with 10,000 permutations. (A) Control Region mtDNA and (B) microsatellite data.

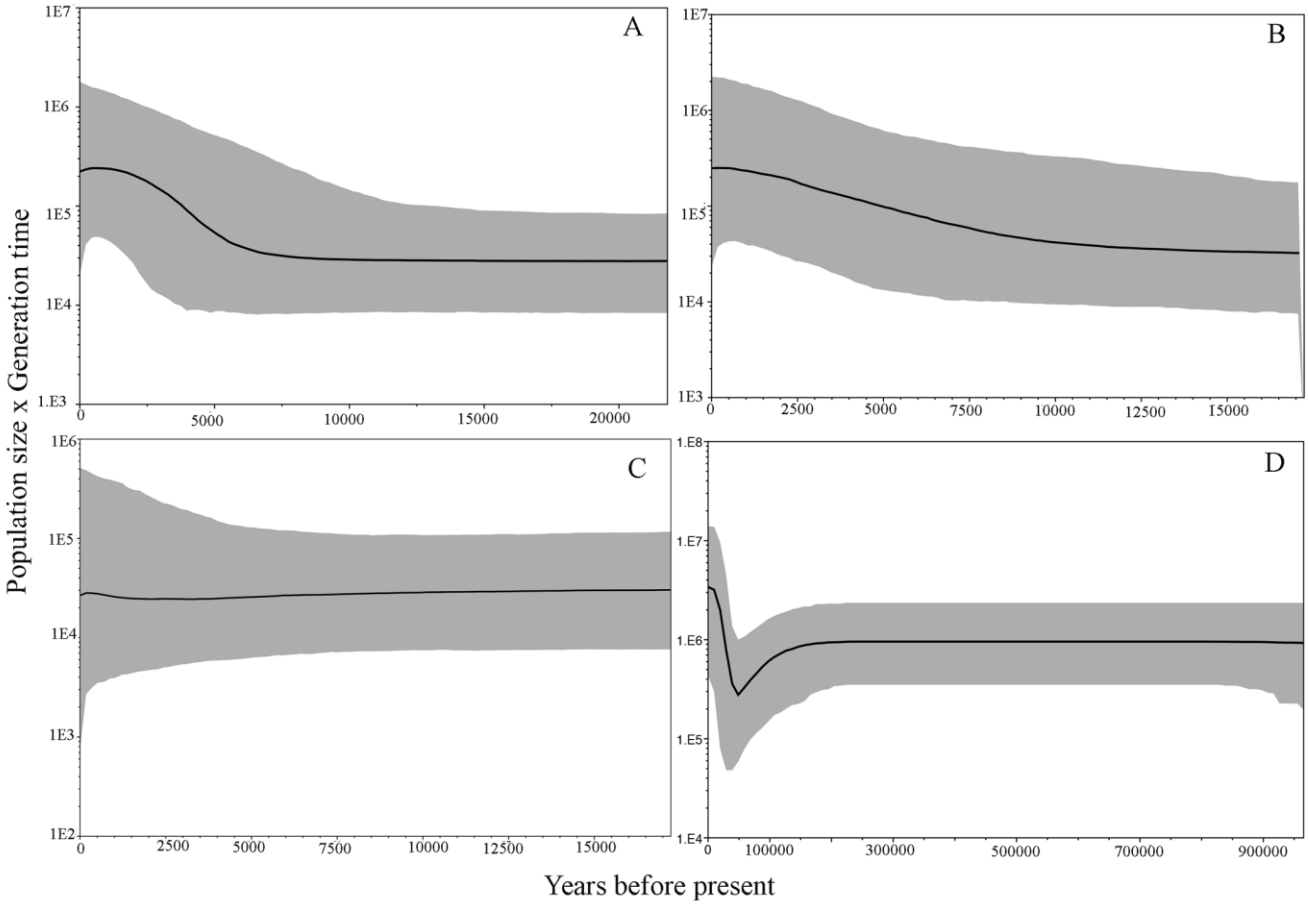

Fig. 4. Bayesian Skyline showing changes in effective population size over time. The solid line is the median estimate, and the blue areas show the 95% HPD limits. (A) Northwest Atlantic Ocean (USA and GM), (B) Southwest Atlantic Ocean (NBrz and SBrz), (C) Central Atlantic Ocean (Bze and Vnz) and (D) overall samples.

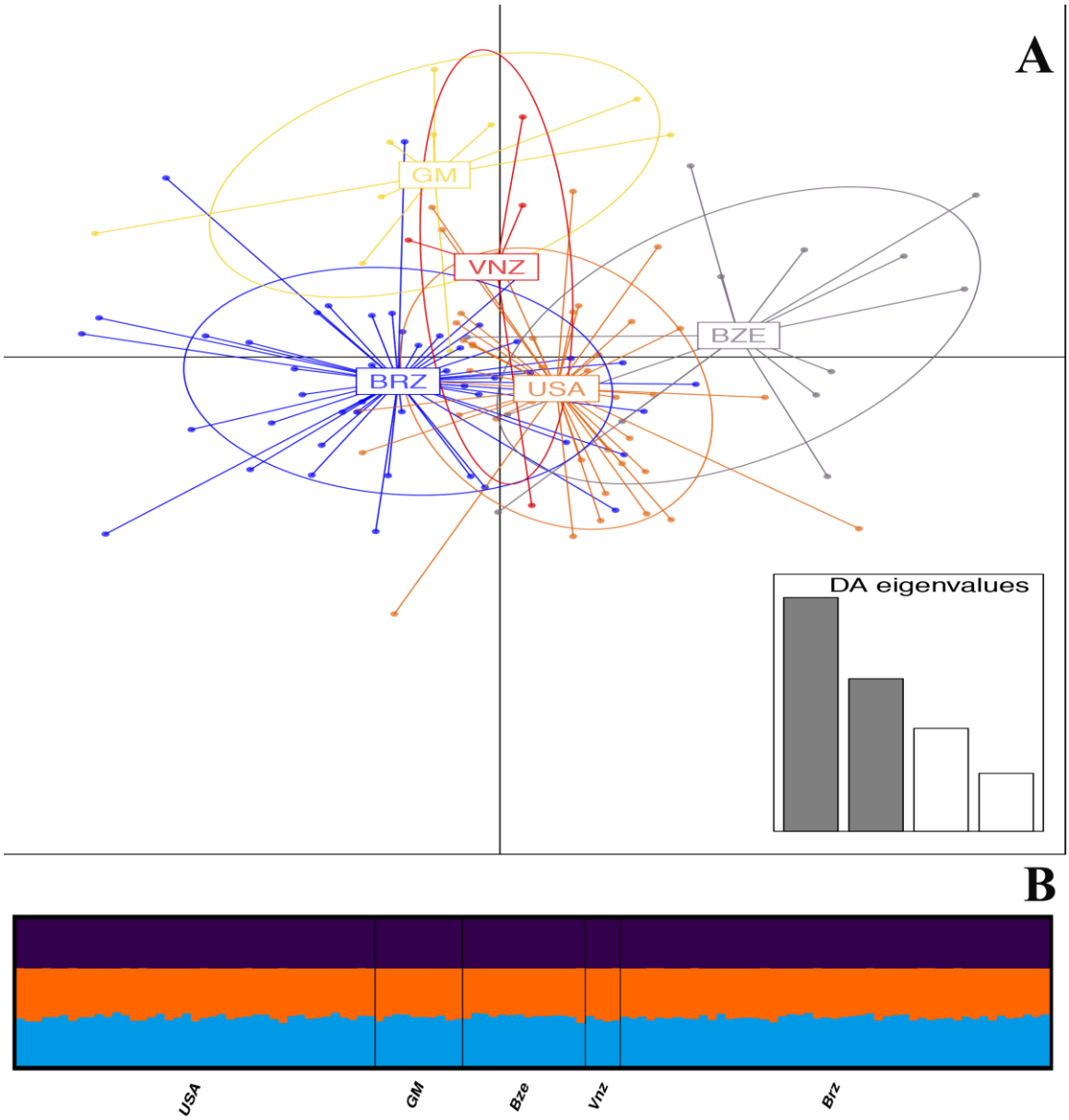

Fig. 5. Model-based clustering for using multilocus genotype data to infer population structure based on nine loci microsatellite genotypes from 120 specimens of the night sharks (*Carcharhinus signatus*) along the western Atlantic Ocean. (A) First and second components of a principal components analysis (DPCA). (B) Population structure test (Structure 2.3.3. software) decipting the number of cluster (*K*=3).

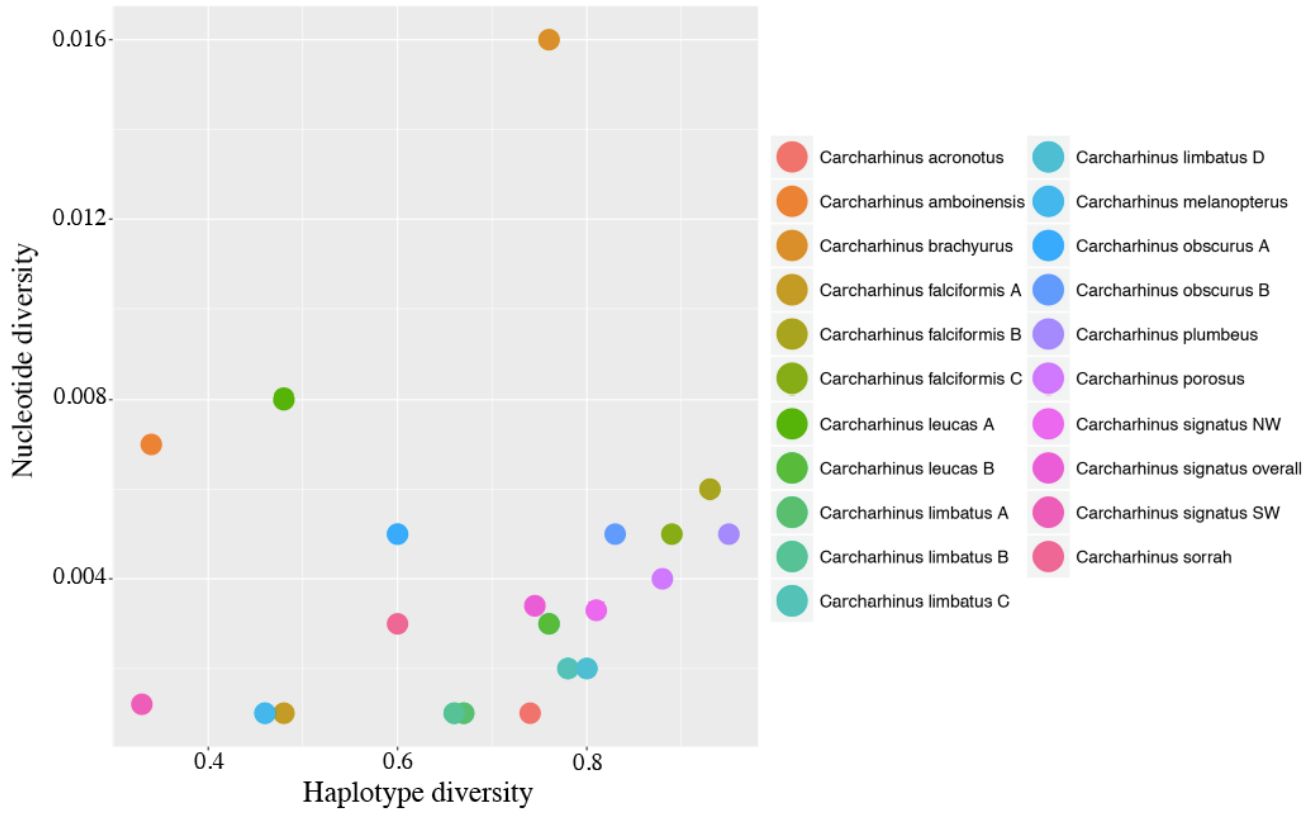

Fig. 6. Scatterplot of genetic diversity (haplotype and nucleotide diversity) of the night shark (*Carcharhinus signatus*) based on Control Region mtDNA.

Table S1. Geographical distribution of haplotypes of the night shark (*Carcharhinus signatus*) along the Western Atlantic Ocean.

Haplotype	USA	GoM	Blz	Vnz	NBrz	SBrz	Total
H1	23	2	6	1	-	2	34
H2	1	-	-	-	-	-	1
H3	1	-	-	-	-	-	1
H4	2	-	-	-	-	-	2
H5	1	-	-	-	-	-	1
H6	1	-	1	-	-	4	6
H7	7	1	4	5	12	37	66
H8	14	3	2	-	-	1	20
H9	2	2	1	-	-	-	5
H10	1	-	-	-	-	-	1
H11	2	-	-	-	-	-	2
H12	1	1	-	-	-	-	2
H13	1	-	-	-	1	-	2
H14	4	-	-	-	-	-	4
H15	-	-	-	-	-	1	1
H16	1	1	-	-	-	-	2
H17	-	-	-	-	1	-	1
H18	-	-	-	-	-	1	1
H19	-	-	-	-	-	1	1

Table S1. Characterization of genetic variability per locus and populations of night sharks (*Carcharhinus signatus*) along the western Atlantic Ocean based on integrated over all microsatellite loci. *n*: samples sizes, *A*: number of alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, A_R Allelic richness and F_{IS}: inbreeding coefficient.

Locus		USA	GM	Bze	Vnz	Brz	Overall
Cli07	*n*	41	9	14	2	44	110
	A	5	3	4	2	4	5
	Ho	0.361	0.444	0.643	0.500	0.455	0.448
	He	0.480	0.500	0.630	0.375	0.449	0.496
	Ar	2.025	2.103	2.414	2.000	1.935	2.043
	Fis	0.252	0.111	-0.020	-0.043	-0.013	0.098
Cli100	*n*	41	10	14	4	45	114
	A	6	4	4	3	9	9
	Ho	0.439	0.500	0.500	0.500	0.522	0.487
	He	0.585	0.595	0.518	0.531	0.709	0.648
	Ar	2.235	2.336	2.110	2.286	2.693	2.449
	Fis	0.252	0.160	0.034	0.059	0.264	0.248
Cli103	*n*	38	10	14	4	48	114
	A	2	2	2	2	2	2
	Ho	0.027	0.200	0.071	0.250	0.041	0.061
	He	0.078	0.180	0.069	0.219	0.040	0.076
	Ar	1.164	1.368	1.143	1.500	1.080	1.152
	Fis	0.471	-0.111	-0.037	-0.143	-0.021	0.190
Cli107	*n*	39	10	14	4	48	115
	A	3	3	3	3	3	3
	Ho	0.744	0.700	0.786	0.500	0.633	0.690
	He	0.654	0.555	0.615	0.625	0.637	0.643
	Ar	2.407	2.213	2.329	2.557	2.345	2.351
	Fis	-0.079	-0.261	-0.278	0.200	0.006	-0.073
Cli108	*n*	37	9	14	4	42	106
	A	22	11	16	4	20	30
	Ho	0.919	0.889	0.929	0.250	0.907	0.888
	He	0.895	0.858	0.921	0.656	0.861	0.899
	Ar	3.525	3.503	3.737	2.771	3.341	3.487
	Fis	-0.020	-0.036	-0.008	0.619	-0.053	0.013
Pgl13	*n*	42	10	14	4	48	118
	A	3	2	2	1	3	3
	Ho	0.167	0.200	0.071	-	0.102	0.126
	He	0.156	0.180	0.069	-	0.151	0.141
	Ar	1.294	1.368	1.143	1.000	1.301	1.273
	Fis	0.042	-0.111	-0.037	-	0.326	0.109
Pgl14	*n*	40	10	14	4	47	115
	A	4	2	3	2	3	4

	Ho	0.425	0.500	0.429	0.500	0.383	0.417
	He	0.513	0.495	0.533	0.500	0.486	0.521
	Ar	1.980	1.906	2.027	1.971	1.882	1.954
	Fis	0.193	-0.010	0.196	-	0.211	0.199
Tgr1157	*n*	37	10	14	4	46	111
	A	7	1	5	3	7	7
	Ho	0.486	0	0.286	0.500	0.404	0.384
	He	0.467	0	0.316	0.406	0.416	0.393
	Ar	2.036	1.000	1.698	2.000	1.913	1.853
	Fis	0.063	NA	0.097	-0.231	0.029	0.023
Tgr1238	*n*	38	9	13	4	48	112
	A	3	2	2	1	3	4
	Ho	0.211	0.556	0.154	-	0.306	0.265
	He	0.191	0.401	0.142	-	0.307	0.253
	Ar	1.397	1.765	1.289	1.000	1.595	1.496
	Fis	0.163	-0.385	-0.083	-	0.001	-0.049

CAPÍTULO 4

Do shark and rays conservation policies need to consider population genetic diversity?

Manuscrito em revisão na revista *Conservation Genetics*

Abstract

Many shark and rays populations are experiencing severe declines, mainly due to overfishing. Furthermore, the high demand for shark fins in Asia, unregulated fisheries, and increases in shark and rays catches arising from the collapse of other fishing resources are also endangering these species. Since many shark and rays species are currently being overexploited, conservation status and management measures have been appraised by international organizations, and policies for conservation have been implemented to promote recovery their shark and rays stocks. Although the effects of population declines of marine fish species on genetic diversity have recently drawn increased attention, the genetic diversity of shark and rays populations has been the subject of few studies so far. We posit that shark and rays conservation polices and management plans need to consider genetic diversity data, since the long-term survival of a species is strongly dependent on the level of genetic diversity within and between populations. In addition, sharks and rays make a particular group of interest for genetics conservation due to some remarkable features of their life history. Therefore, it is imperative that conservation organizations include genetic parameters in future assessments of shark and rays populations.

Keywords Conservation genetics . Elasmobranch conservation . Genetic variability . Fisheries management.

Introduction

Genetic data have helped in conservation research and management, detecting genetically distinct population, measuring genetic connectivity and identifying risks associated demographic change and inbreeding (Allendorf et al. 2013). In the last decades, the emergent discipline of conservation genetics has provided important insights into the dynamics of endangered populations (McMahon et al. 2014). However their effective application on management plans is still incipient, there are classics examples to vertebrates where genetic data have influencing conservation efforts to restore populations, as for example the Pacific Salmon (Waples, 1995) and Florida panther (Johnson et al. 2010).

The effects of population-level declines are of major concern in conservation biology, since small populations suffer from inbreeding and loss of genetic diversity, which is variation of alleles and genotypes within population (Frankham et al. 2002). Furthermore, small populations will lose genetic diversity faster than large populations due to genetic drift. This reduction of genetic diversity have several potential consequences such as compromise the ability of populations to evolve to cope with environmental changes and reduces their chances of long-term persistence (Frankham et al. 2002). Thus, evolutionary biologist and fishery management are interested in elucidation of patterns of genetic and demographic connectivity among different groups of individuals or populations and as genetic variation is distributed within and between populations (Waples and Gaggiotti, 2006; Grant and Cheng, 2012). In fact, these information is an important starting point for development of fisheries management plans and monitoring that aims to detect reductions of gene level variability (Laikre, 2008). As mentioned initially, a good example in which genetic information is considered on fisheries management is the Pacific salmon (*Oncorhynchus spp*), which are renowned for a large number of discrete groups returning to spawn over wide temporal intervals, ecological condition and geographic distances (Altukhov et al., 2000). For example, the concept “Evolutionary Significant Units” – ESUs, which is defined as a population

reproductively isolated and following largely independent trajectory evolutionary (Waples, 1995), was accepted by the U. S. Endangered Species Act (ESA) as equivalent to "species" and deserving of the full force of protection against extinction (Waples, 1995). The most important factor used for defining salmon ESUs was the genetic structure at neutral marker (Waples et al. 2008).

Shark and rays (including stingrays and skates) make up a group of interest to conservation because their ecological importance in marine environmental and current high levels of overexploitation (Dulvy et al. 2014). Currently, there are more than 1139 described species of elasmobranchs in the world (Weigmann, 2016), representing a significant number of mesopredator and occupying top positions on the food chain (Heithaus et al. 2008; Ferretti et al. 2010). Yet, despite their ecological importance, they are one of the most imperiled groups of marine species worldwide (Cortés et al. 2002; Bräutigam et al. 2015), with their life history characteristics of late sexual maturity, lengthy pregnancy, and low fertility making them particularly susceptible to anthropogenic pressures such as fisheries, environmental changes, and pollution (Dulvy et al. 2014). These anthropogenic pressures above-mentioned can cause changes in the genetic diversity (DiBattista, 2008).

Massive population-level declines and extinction risk through heavy harvest occurring over the last decades currently present a significant issue for sharks and rays in all oceans (Ferretti et al. 2010; Worm et al. 2013; Dulvy et al. 2014). The high demand for shark fins in Asia, unregulated fisheries, bycatch and increased shark catch due to the collapse of other fishing resources are among the mains issues that imperil shark and rays species (Musick et al. 2000; Clarke et al. 2006; Herndon et al. 2010; Dulvy et al. 2014; McClenachan et al. 2016). According to a study by Worm et al. (2013), the global catch of sharks from reported and unreported landings, discards, and shark finning was estimate to be around ~ 100 million tons in 2010. Such fishing pressures are more challenging to elasmobranchs because of their

high susceptibility relative to most teleosts, and the several decades sharks and rays need to recover after overfishing (Stevens et al. 2000).

In general, conservation management of sharks and rays species relies on a series of studies about basic biology, life history, and population ecology (Simpfendorfer et al. 2011). However, genetic-dependent shark and rays population differentiation is generally neglected in fisheries management and the possibilities for change appear distant, as many conservation policies currently fail to acknowledge genetic variation (Laikre 2010; Ovenden et al. 2013). Thus, expansion of global population genetic studies to describe the genetic diversity of shark and rays species worldwide is urgently needed to identify genetically distinct populations and preserve their genetic diversity. It is imperative to face the severe factors that jeopardize shark and rays populations.

Here, we did a critical review and discuss the importance of including genetic diversity data in sharks and rays conservation polices and management plans, as well as the need for more genetic studies of these species that assess genetic diversity across geographical ranges. In addition, we suggest future action to minimize the gap between sharks and rays genetic science and conservation policy.

What makes the shark particularly interesting for conservation genetics?

In addition to the ecological importance, sharks and rays make up a particular group of interest to conservation genetics due to some remarkable features, such as (i) evolutionary uniqueness, (ii) effects of overfishing on evolution, (iii) broad geographic distribution, (iv) few studies describing the genetic diversity so far.

Evolutionary uniqueness

Sharks and rays comprise a major vertebrate lineage of evolutionary uniqueness, with approximately 1139 living species, representing a small fraction (<3.0%) of the modern fish

fauna (Nelson et al. 2016; Weigmann, 2016). Compared with marine teleost, sharks and rays presented a low species richness (1139 shark and rays species against 30.000 teleost species). It is highlighted in some orders, which possess only one family and one genus such as: Echinorhiniformes – 2 species, Squatiniformes – 5 species, and Heterodontiformes – 9 species (Ebert et al. 2013). Furthermore, intrinsic factors such as diversity of form and function, as a successful evolutionary resilience contributed to lower historic extinction rate and a high evolutionary adaptability of shark and rays species that allow them to inhabit several marine and freshwater ecosystems (Ferretti et al. 2010; Ebert et al. 2013; Richards et al. 2013). In addition, sharks have survived the mass extinctions in the last 455 million years, which has left ocean waters with far fewer fish (Grogan et al. 2012). Such resilience suggests that sharks have unique genetic properties that relate to their evolutionary adaptability success and, therefore, it must be preserved.

Effects of overfishing on evolution

The overfishing may impact evolutionary processes mainly by changing body size and by early sexual maturity, while also affecting bioeconomic and macroecology (Belgrano and Fowler 2013; Heino et al. 2015). For sharks, only a few studies have shown direct evidence of the influence of fisheries on evolution. Walker et al. (1998) reported changes in the growth rate of gummy sharks (*Mustelus antarcticus*) that was caused by length-selective fishing mortality. Furthermore, Clarke et al. (2013) reported that the median lengths of silky sharks (*Carcharhinus falciformis*) and oceanic whitetip sharks (*Carcharhinus longimanus*) decreased significantly in the Pacific Ocean between 1995 and 2010. These changes may suggest an evolutionary response to overfishing. Recently, Gallagher et al. (2014) suggested that the ecological, behavioral, and physiological adaptations of hammerhead sharks (Sphyrnidae) that once promoted evolutionary success are now maladaptive under the current levels and modes

of exploitation. For example, the disturbance of physiological features that is thought to be to their strategy of prey capture (burst swimming behavior), which support to agility of prey capture, also results in high rate (between 60% - 80%) of at-vessel and post-release mortality (Gallagher et al. 2014). Thus, future management strategies by fisheries should take into account evolutionary traits and unique adaptations, assessing of the different levels of genetic diversity throughout different species across their geographic ranges.

Broad geographic distribution

Many sharks and rays are widely distributed and have high mobility capacity (e.g. *Isurus oxyrinchus*, *Pteroplatytrygon violacea*), which imposes difficulties in sampling enough individuals of different locations that allow identifying discrete populations along their entire distribution. For example at least 150 shark species regularly migrate across national boundaries and ¼ of threatened shark species have a range that includes at least 18 countries (Dulvy et al. 2014). According to Roy et al. (2012), not knowing genetic structure of a species can lead to two problems: first, genetically homogenous populations can overlap contiguous fisheries management zones, which are assessed independently and second, genetically heterogeneous population can have overlapping distributions within fisheries management zones.

Differently from bony fishes and others marine organisms, sharks and rays species do not have a planktonic larval stage with dispersal via ocean currents. Conversely, their dispersal is entirely mediated by the active movement of the adult individuals. In general, large migratory and oceanic species tend to present more homogeneous populations, as for example the blue shark, *Prionace glauca* (Taguchi et al. 2015), whereas smaller more coastal forms such as *Carcharhinus sorrah* commonly present isolated populations (Giles et al. 2014). These coastal sharks and rays tend to find more obstacles such as marine barrier and oceanographic heterogeneity. Thus, an assessment of the extant genetic diversity throughout

their distribution is imperative to avoid local gene pool erosion. For example, the blue shark, *Prionace glauca*, is a worldwide, likely the most wide-ranged species of sharks and the most heavily fished shark species in the world, but few studies and range-limited (e.g. Ovenden et al. 2009; King et al. 2015; Taguchi et al. 2015; Li et al. 2016) have tried to describe the genetic structuring and diversity of this species so far. These studies did not indicate any genetic differentiation into the Pacific and Indo-Pacific regions, attributing this mainly to its higher vagility. Nonetheless, the authors indicated the need for a cooperative fisheries management among different countries, even not having any genetic data available along the broad distribution of blue shark, leaving a considerable gap in obtaining a holistic view of population structure of this species. Thus, it will not be possible to detect negative changes and reductions of genetic diversity unless that major points of their distribution are studied.

Differently than blue shark that does not have global genetic population studies yet, some few widely distribute shark species (e.g. *Sphyrna lewini*, *Rhincodon typus*, *C. taurus*, *Carcharhinus plumbeus*, *Carcharhinus obscurus*, *Carcharhinus brachyurus*, *C. falciformis*) have been studied globally. Although these studies are incipient, they shown that different shark species populations are genetically discrete entities worldwide and may show different level of genetic diversity (Duncan et al. 2006; Castro et al. 2007; Ahonen et al. 2009; Portnoy et al. 2010; Benavides et al. 2011, 2011b; Clarke et al. 2015). Considering that each population should be regarded as ESUs and needs to be managed regionally to reduce the risk of depletion of genetic resources.

Few studies describing the genetic diversity of sharks and rays so far

Despite the increase of the number of genetics studies in the last decade (Fig. 1), at present only ~10% of the sharks and rays species have been investigated regarding their population genetic structure, genetic diversity and demographic history. For example pelagic

stingray *P. violacea,* a cosmopolitan species severely taken as bycatch in pelagic longline fisheries around the world (Forselledo et al. 2008), does not have any population genetic study describing their genetic diversity and identifying discrete population along their distribution range to date. The understanding of the population genetic structure of this species is imperative, given their ecological importance as well as the current populations declines (Forselledo et al. 2008). The same situation occurs in species with narrow geographic distribution and critically endangered such as the daggernose shark, *Isogomphodon oxyrhynchus* (Lessa et al. 2016). Furthermore, the majority of species studied represents the first genetic examination (Dudgeon et al. 2012) with few exception of species re-examined as for example, *Carcharodon carcharhias* and *S. lewini*. This lack of knowledge in the genetic evaluation of many shark species opens a gap between current overexploitation and short and long-term conservation.

The effects of fishing on genetic diversity of shark populations

Despite the negative relationship between overexploited population and genetic diversity, the use of genetics in the management plans of threatened species is still a challenge (Laikre 2010). The effects of population declines in marine fishes in terms of decreased genetic diversity have recently drawn attention and many authors claim the need of application of diversity genetic metrics into conservation plans because of the harmful consequences of loss of diversity genetic, inbreeding, loss of evolutionary potential and change of population structure (Kenchington et al. 2003; Allendorf et al. 2008; Frankham 2010; Laikre et al. 2010; Hoban et al. 2013).

Studies addressed the question about how much fisheries can affect the genetic diversity (mtDNA and microsatellite) of fish and marine mammals stocks have been demonstrated using historical and contemporary samples (Hauser et al. 2000; Pichler and

Baker, 2000). For example, the heterozygosity (microsatellite), number of alleles per locus, and effective population size (N_e) of New Zealand snapper *Pagrus auratus* declined between 1950 and 1988 after beginning of a fishery on this population (Hauser et al. 2002). McCusker and Bentzen (2010) found a positive relationship between genetic diversity (mtDNA and microsatellites) and fish stocks abundance. Meanwhile, Pinsky and Palumbi (2014) compiled data for 140 species of marine fishes across 11,049 loci and clearly showed a reduction in allelic richness in 9 overfished stocks among 12 genera and families. According to Allendorf et al. (2008), uncontrolled harvest may lead to genetic impacts, such as alteration of population subdivision, loss of genetic variation, and selective genetic changes. To date, there is no study assessing directly the relationship between abundance and genetic diversity for sharks and rays species. Nonetheless, many species that are under intense fishing pression have shown low values of genetic diversity, mainly at the nucleotide diversity and heterozygosity observed (Table 1, Fig. 2).

The main goal of most management plans for traditional fisheries mainly seeks to increase the number of individuals from different populations (Hauser and Carvalho, 2008). However, even large populations (census population size - N_c) may face substantial loss of genetic variation because the N_e, which determines the strength of genetic drift in a population, is often much smaller than N_c in overexploited marine fish (Ryman et al. 1995; Allendorf et al. 2008, Hare et al. 2011). For example, millions of individuals may be equivalent to a N_e of only hundreds or thousands (Ryman et al. 1995, Hauser et al. 2002). Thus, as N_e decreases, genetic drift erodes genetic variation, increasing the probability of fixation of deleterious alleles, and reducing the resilience of overfished species (Hare et al. 2011). Although it is one of the most important genetic parameters of wildlife populations, estimative of contemporary N_e are even less available for shark and rays species (Table 2). These studies showed N_e lower values than 500 for some shark and rays species, as for

example *Stegostoma fasciatum* (Dudgeon and Ovenden, 2015) and *Pristis pectinata* [(Chapman et al. 2011) (Table 2)]. Empirical studies suggest that at least N_e of 50 individuals is necessary to prevent genetic erosion on the short term, whereas at least 500 individuals represents the minimal threshold to retain long-term evolutionary potential (Frankham 1995, Franklin and Frankham, 1998). These N_e estimated for elasmobranch populations suggesting the need for a long-term monitoring and can be informative for management decisions. Therefore, we believed that N_e is important genetic parameter to be applied in future fisheries management plans, because their importance as a genetic diversity (Willoughby et al. 2015) and abundance index (Ovenden et al. 2016).

Overlooked and neglected genetic diversity in shark conservation policies

Many shark species are highly migratory and overexploited and thus may require international management efforts, such as bilateral and multilateral fishery management agreements (Musick et al. 2000; Herndon et al. 2010). The conservation status and management measures for many shark species have been evaluated by international organizations, and the policies for conservation are mainly based on retention bans, finning bans and trading bans, intended to promote recovery of shark populations (Tolotti et al. 2015). However, establishing effective international management efforts is challenging because of the wide geographical distribution of shark species, as mentioned above, and the limited data available for most shark species (Herndon et al. 2010; Hoffman et al. 2010; Dulvy et al. 2014). To maintain sustainable fish stocks, these organizations have recommended actions such as prohibiting capture and commercialization of some shark species in their respective jurisdictions (Tolotti et al. 2015). As many other international policy implementations, these organizations does not have any initiative that deals specifically with genetic diversity.

Recently, a consortium among experts representing different organizations - The Global Sharks and Rays Initiative (GSRI) - launched the Global Strategy for the Conservation

of Sharks and Rays (2015-2025), a document that summarizes global priorities for sharks and ray conservation (Bräutigam et al. 2015). This document highlighted the urgent need to prevent the extinction of imperiled coastal sharks and rays in many diversity and endangered hotspots, including coastal waters near Argentina, Australia, Brazil, Colombia, Indonesia, Japan, Madagascar, Mozambique, South African and Uruguay (Lucifora et al. 2011; Bräutigam et al. 2015). Similarly the other conservation initiatives to sharks, the GSRI do not recognize the importance of genetic diversity as a criterion into to management plans. Genetic population inferences such as genetic diversity levels, structure population and demographic history could help the GSRI assess the genetic health of populations in priorities areas for conservation. It could be an excellent match between the use of common genetics tools in management and relevant applications in the policy arena (Hoban et al. 2013).

The same problem extends to the most influential conservation organization in the world, the International Union for Conservation of Species (IUCN). Although the IUCN considers genetic diversity as one of three levels of biodiversity that must be conserved (McNelly et al. 1990), there is no specific genetic criterion listed to categorize sharks and rays or other species into any level of threat (Laikre 2010, Rivers et al. 2014). Even though the IUCN Red Lists take into account a range of quantitative species-specific criteria, such as distribution and number of individuals and decline in abundance, to categorize a given species into Red List categories (IUCN 2001), the genetic diversity is largely ignored in its evaluation criterion, suggesting that any shark and ray species listed on IUCN Red List could be overlooked, mainly in long-term management (Laikre et al. 2008; Willoughby et al. 2015).

According to the Dulvy et al. (2014), 1041 shark and rays species are currently listed on the IUCN Red List. Of these, 181 shark and rays species fall into categories that represent varying degrees of threat (Dulvy et al. 2014). Meanwhile, our Web of Science® search conduced until August 2016 indicated that the number of shark and rays species for which

genetic information is available increased over the last 20 years, with a main focus on sharks than rays (Fig.1). However only 10% of the 1,041 sharks and rays species listed by the IUCN, present some genetic data available (genetic diversity metrics and population genetic structure) and about 25% of the species fall into some threat category (Fig. 2). Even with nearly half of all shark species listed by the IUCN as 'Data Deficient' (DD) due to deficiencies about life history and abundance population dynamics data (Hoffman et al. 2010), some species of this category have genetic data, which could be used in assessment. In fact, the lowest values of observed heterozygosity is to species listed as DD and LC, and for nucleotide diversity in species listed as LC (Fig. 3). For example, artisanal fisheries represent a main threat to nurse sharks (*Ginglymostoma cirratum*), which inhabits coastal waters and are found throughout the Atlantic Ocean (Ebert et al. 2013). Even though the IUCN lists nurse sharks as DD globally (Rosa et al. 2006), this species have its population genetically assessed, which these data could be used to helped their assess. The nurse shark population in the western Atlantic Ocean shows low genetic diversity in the control region of mitochondrial DNA – CR mtDNA (h=48±5%; π=0.08±0.06%) and microsatellites (H_O=0.58). Furthermore, nurse shark populations showed significant and distinct genetic differences between offshore islands and the mainland in the western Atlantic Ocean, and a high degree of genetic variability (78.2%) was found within populations (Karl et al. 2012). These genetic isolation pattern and genetic diversity parameters are comparable to other shark species listed that are categorized by IUCN as threatened, including sand tiger sharks (*Carcharias taurus*) and narrownose sharks [(*Mustelus schmitti*) (Table 1)]. These examples clearly highlight the usefulness of genetic diversity metrics at least as an indicator of the conservation status for certain shark species.

They also imply that the IUCN should include genetic diversity as another criterion for categorizing threats (Frankham 2002; Willoughby et al. 2015). This could be done using a

novel approach for identifying vertebrate species with conservation needs by estimating the expected loss of genetic diversity, using *Ne* as a index (Willoughby et al. 2015). This parameter is very informative for conservation and management of wildlife populations, because provide information on how fast genetic diversity will be lost (Leberg 2005; Dudgeon and Ovenden, 2015). From this prediction is possible to direct conservation efforts to mitigate this loss (Uzans et al. 2015). Thus, given the potential association between N_e and probability of extinction, estimates of N_e might be used to assess the vulnerability of species (Leberg 2005, Willoughby et al. 2015).

Future Challenges

Recently many authors have claimed the use of genetic diversity in forefront of conservation policy and management and not just being a supporting information (Laikre 2010; Hoban et al. 2013). However, we advocate that to make the genetic diversity data more promptly useful for policy makers, it is necessary that some challenges are overcome, either in the scientific or the policies arena, such as describe above:

- Include more conservation genetic scientist into IUCN Shark Specialist Group;
- Improve the communication between scientist and policy makers;
- Prioritize shark and rays species currently overexploited and with narrow geographic distribution;
- Application of genomic into the shark and rays genetic research
-

Include more conservation genetic scientist into international organizations conservation

The importance of a criterion genetic to guarantee population viability and conservation in the long-term is well known within academia. However, outside academia, genetic is still largely overlooked and neglected, in practical management, as well as in

national and international policies, with some exception (Laikre 2010). The main agencies interested in conservation of sharks and rays do not have much geneticist's conservation specialist integrating their team. These organizations have mainly fisheries scientists' membership interested in assess mainly the stock abundance. For example, the IUCN SSC Shark Specialist Group has 139 members, which only few members are effectively genetics experts. This demonstrate the lack of genetic concerns in assessing species, once that the IUCN does not include any direct criteria into assess species (Laikre 2010). Therefore, we recommend the inclusion of more conservation geneticist in the IUCN Shark Specialist Group as well as in others policy conservation agencies. This inclusion would facilitate the insertion of genetics criteria within of shark and rays species assessment and future management plans.

Bridging the gap between genetic science and shark and rays conservation policy

The inclusion of genetics experts among the national and international policy makers could be a good start to push the genetic information into conservation policy. However, it is needed to improve the communication between science and policy makers (Fig. 4). Nowadays, still there is a gap between genetics research that generates knowledge about genetic data (e.g. genetic diversity, population genetic structure and genetic demographic) and conservation organizations that use these data to establish protected measures that aims the recovery of populations (Laikre, 2010; Hoban et al. 2013; Haig et al. 2015). Mostly, policy makers and managers are not geneticists and they have difficulties to interpret genetic data rightly and consequently use in the building of management plans (Hoban et al. 2013, Haig et al. 2015). This could be solved, for example, through providing training to national and international conservation organizations. Workshops, courses and lecture to non-geneticists' researchers, conservation practitioners and decision-makers interested in sharks could be organized to show how the genetic diversity can be effectively used in the management plans.

This would provide an excellent opportunity to show that information of genetic diversity can reveal a wide variety of information for conservation policies. Also, geneticists' research could help in elaboration and review of regional and global sharks reports and proposals of management and research, incorporating genetic data.

Prioritize shark species currently overexploited and with narrow geographic distribution

From the conservation genetic point of view, the worst situation is the existence of an endangered species represented as a single population (Frankham et al. 2002). Frequently small populations are most likely to be affected by the loss of genetic diversity due to overfishing, affecting their evolutionary potential and resulting in elevated extinction risk (Frankham et al. 2002, Allendorf et al. 2008). Thus, obtaining the information on the level of genetic diversity of species either with narrow geographic distribution or isolated population is necessary to indicate the population fragility of species. For example, narrownose shark is an endemic shark species from the Southwest Atlantic Ocean, with narrow geographic distribution, ranging from the Rio de Janeiro – Brazil to Patagonia - Argentina. This shark species has experienced an intense overfishing along the entire geographic range, including its nursery grounds (Massa et al. 2006). Moreover, narrownose shark is among the lowest genetic diversity among sharks (Table 2), becoming highly susceptible to short-term due to overfishing, and long-term due to low genetic diversity. This situation could be extended to others elasmobranch species that have narrow geographic distribution and are currently overfishing, such as the critically endangered elasmobranch species, Daggernose shark, *Isogomphodon oxyrhynchus* (Lessa et al. 2016), Brazilian guitarfish, *Rhinobatos horkelli* (Vooren et al. 2005) and common Angel shark, *Squatina squatina* (Ferretti et al. 2015). On the other hand, shark species widely distributed are rarely panmitic from one end to the other of their distribution area, instead they shown partitioning genetic (Castro et al. 2007; Ahonen

et al. 2009; Clarke et al. 2015). Thus, obtain information regarding the extent of gene flow among populations is imperative to determine whether a species requires the maintenance of genetic diversity through of migration (Frankham, 2002; Allendorf et al. 2013). At the same time, management of genetically depauperate populations must embrace the identification of source founders from genetically diverse populations (Allendorf et al. 2013) and the genetic monitoring through temporal series sampling to assess genetic variations over time (Allendorf et al. 2008; Laikre et al. 2008).

Application of genomic into the elasmobranch genetic research

DNA sequences, mainly control region of mitochondrial DNA, and microsatellites are most widely used markers to elasmobranchs to date (Table 1). However, the availability of new high-resolution molecular markers such as next-generation sequencing - NGS, promises an abrupt advance in genetic studies in the next years, and already there are some applications to shark species (e.g. Feutry et al. 2015; Portnoy et al. 2015; Delser et al. 2016). Differently of other markers, this approach can uncover thousands of polymorphic markers that cover the entire genome in a single step and at minimal cost (Stapley et al. 2010). Furthermore, this approach is more powerful statistically to detect discrete populations in fine-scale, require of relatively small numbers of samples by location, that in turn is an advantage due to levels of threat of shark and rays species, and help in determining which parts of the genomes are responsible for local adaptation even in high gene flow, which will enable the identification of priority area to conservation. However, even with genomic data providing novel insights, their results and utilization remain confined to academic research, and their use in future management plans will continue to be a challenge (Shafer et al. 2015).

Finally, in light of the above considerations, we can assume that the increasing overexploitation of shark populations, mainly by the fisheries will have a long-term impact on

genetic variability and in turn reduce the response to environmental changes and population fitness. Therefore, future assessment of shark populations by conservation organizations should definitely include genetic parameters.

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Table 1. Genetic diversity metrics for shark and rays species. He: expected heterozygosity, Ho: observed heterozygosity and number of alleles in parentheses, Ra: allelic richness, *h*: haplotype diversity and π: nucleotide diversity.

Species	Regions	Molecular Markers	He	Ho	Ra	*h*	*π*	References	IUCN
Sharks									
Squatina argentina	Brazil	A	-	0.2860 (4)	-	-	-	Solé-Cava et al. (1983)	EN
Squatina argentina	Brazil	A	-	0.3670 (4)	-	-	-	Solé-Cava et al. (1983)	EN
Mustelus antarticus	Australia	A	-	0.0057 (32)	-	-	-	MacDonald et al. (1986)	LC
Carcharhinus tilstoni	Australia	A	-	0.037 (13)	-	-	-	Lavery and Shaklee (1989)	LC
Carcharhinus sorrah	Australia	A	-	0.0350 (13)	-	-	-	Lavery and Shaklee (1989)	NT
Carcharhinus plumbeus	NAO, GM	A/RFLP	-	0.0050 (27)	-	-	0.0004	Heist et al. (1995)	VU
Isurus oxyrinchus	Global	RFLP	-	-	-	0.75	0.0035	Heist et al. (1996)	VU
Rhizoprionodon terranovae	NAO, GM	RFLP	-	-	-	0.71	0.0013	Heist et al. (1996b)	LC
Squatina californica	California	A/RFLP	-	0.0056 (7)	-	-	-	Gaida (1997)	NT
Mustelus antarticus	Australia	A/RFLP	-	0.0990 (28)	-	0.53	0.0016	Gardner and Ward (1998)	LC
Carcharodon carcharias	SA, Australia, NZ	CR mtDNA/MS	-	0.68 (5)		-	0.0203	Pardini et al. (2001)	VU
Negaprion brevirostris	WAO	MS	0.79	0.77 (15)	-	-	-	Feldheim et al. (2001)	NT
Carcharhinus limbatus	NAO, GM	CR mtDNA	-	-	-	0.71	0.00106	Keeney et al. (2003)	NT
Isurus oxyrinchus	Global	MS	0.87	0.85 (4)	-	-	-	Schrey and Heist (2003)	VU
Carcharhinus limbatus	NAO, GM, CS	CR mtDNA/MS	0.5	0.50 (8)	-	0.81	0.0021	Keeney et al. (2005)	NT
Carcharhinus limbatus	Global	CR mtDNA	-	-	-	0.84	0.0041	Keeney et al. (2006)	NT
Carcharias taurus	SA, Australia	CR mtDNA/AFLP	-	-	-	0.39	0.0025	Stow et al. (2006)	VU

Sphyrna lewini	Global	CR mtDNA	-	-	-	0.80	0.0013	Duncan et al. (2006)	EN
Cetorhinus maximus	Global	CR mtDNA	-	-	-	0.72	0.0013	Hoelzel et al. (2006)	VU
Rhincodon typus	Global	CR mtDNA	-	-	-	0.97	0.0110	Castro et al. (2007)	VU
Triakis semifasciata	California	CR mtDNA/ISSR	-	-	-	-	0.0067	Lewallen et al. (2007)	LC
Somniosus microcephalus	NPO, SO, NA	Cyt B mtDNA	-	-	-	0.78	0.0022	Murray et al. (2008)	NT
Somniosus pacificus	NPO, SO, NA	Cyt B mtDNA	-	-	-	0.82	0.0037	Murray et al. (2008)	DD
Somniosus antarcticus	NPO, SO, NA	Cyt B mtDNA	-	-	-	0.67	0.0023	Murray et al. (2008)	DD
Negaprion brevirostris	PAO	CR mtDNA/MS	0.81	0.73 (9)	8.5	0.78	0.0059	Schultz et al. (2008)	NT
Negaprion acutidens	IPO	CR mtDNA/MS	0.67	0.58 (9)	2.6	0.28	0.0006	Schultz et al. (2008)	VU
Carcharias taurus	Global	CR mtDNA/MS	0.74	0.65 (6)	3.3	0.73	0.00003	Ahonen et al. (2009)	VU
Galeorhinus galeus	Global	CR mtDNA	-	-	-	0.92	0.0071	Chabot and Allen (2009)	VU
Stegostoma fasciatus	IWPO	ND4 mtDNA/MS	0.73	-	-	0.75	0.0014	Dudgeon et al. (2009)	VU
Carcharodon carcharias	PO	CR mtDNA	-	-	-	0.79	0.0034	Jorgensen et al. (2009)	VU
Prionace glauca	IAA	CR mtDNA	-	-	-	0.92	0.0080	Ovenden et al. (2009)	NT
Carcharhinus sorrah	IAA	CR mtDNA	-	-	-	0.60	0.0030	Ovenden et al. (2009)	NT
Carcharhinus obscurus	IAA	CR mtDNA	-	-	-	0.60	0.0050	Ovenden et al. (2009)	VU
Sphyrna lewini	IAA	CR mtDNA	-	-	-	0.61	0.0098	Ovenden et al. (2009)	EN
Sphyrna lewini	WAO	CR mtDNA	-	-	-	0.38	0.0013	Chapman et al. (2009)	EN
Rhincodon typus	Global	MS	0.68	0.66 (8)	9	-	-	Schmidt et al. (2009)	VU
Carcharhinus plumbeus	Global	CR mtDNA/MS	0.81	0.81 (8)	11.1	0.96	0.0048	Portnoy et al. (2010)	VU
Mustelus schmitti	SAO	Cyt B mtDNA	-	-	-	0.23	0.0015	Pereyra et al. (2010)	EN

Chiloscyllium plagiosum	Japan	Cyt B mtDNA	-	-	-	0.72	0.0025	Fu et al. (2010)	NT
Carcharhinus leucas	WAO	CR mtDNA/MS	0.84	0.83 (5)	-	0.51	0.0012	Karl et al. (2011)	NT
Squalus acanthias	Global	ND2mtDNA/MS	0.60	0.61 (8)	5.6	0.84	0.0086	Veríssimo et al. (2010)	VU
Squalus mitsukurii	HA	CR mtDNA/MS	0.56	0.57 (8)	8.4	0.54	0.0010	Daly-Engel et al. (2010)	DD
Rhizoprionodon porosus	WAO	CR mtDNA	-	-	-	0.88	0.0028	Mendonça et al. (2011)	LC
Sphyrna lewini	EPO	CR mtDNA/MS	0.79	0.77 (15)	-	0.53	0.0011	Nance et al. (2011)	EN
Rhizoprionodon acutus	EA, Indonesian	ND4 mtDNA/MS	0.63	0.48 (6)	-	0.82	0.0034	Ovenden et al. (2011)	LC
Sphyrna lewini	EA, Indonesian	ND4 mtDNA/MS	0.75	0.69 (8)	-	0.34	0.0018	Ovenden et al. (2011)	EN
Carcharhinus brachyurus	Global	CR mtDNA	-	-	-	0.76	0.0160	Benavides et al. (2011)	NT
Carcharhinus obscurus	Global	CR mtDNA	-	-	-	0.83	0.0050	Benavides et al. (2011)	VU
Centroscymnus coelolepis	EAO	CR mtDNA/MS	0.77	0.77 (8)	8.1	0.65	0.0018	Veríssimo et al. (2011)	NT
Carcharhinus leucas	Australia	ND4 mtDNA/MS	0.77	0.77 (3)	-	0.48	0.0791	Tillet et al. (2012)	NT
Carcharhinus limbatus	Brazil	CR mtDNA	-	-	-	0.80	0.0021	Sodré et al. (2012)	NT
Ginglymostoma cirratum	WAO	CR mtDNA/MS	0.58	0.58 (8)	-	0.48	0.0008	Karl et al. (2012)	DD
Carcharodon carcharias	Australia	CR mtDNA/MS	0.68	0.68 (6)	-	0.88	0.0086	Blower et al. (2012)	VU
Sphyrna lewini	MP, GM	CR mtDNA/MS	0.53	0.62 (5)	4.0	0.49	0.0110	Castillo-Olguín et al. (2012)	EN
Carcharhinus amboinensis	Northern Australia	ND/ CR mtDNA	-	-	-	0.78	0.0065	Tillet et al. (2012)	DD
Centrophorus squamosus	Species range	ND2 mtDNA/MS	-	0.74 (6)	12.4	0.57	0.0018	Veríssimo et al. (2012)	VU
Triaenodon obesus	IPO	CR mtDNA	-	-	-	0.55	0.0021	Whitney et al. (2012)	NT
Sphyrna lewini	Global	MS	0.77	0.71 (13)	7.6	-	-	Daly-Engel et al. (2012)	EN
Mustelus antarticus	IPO, Australasia	ND2/ND4/CR mtDNA	-	-	-	0.46	0.0008	Boomer et al. (2012)	LC*

Mustelus lenticulatus	IPO, Australasia	ND2/ND4/CR mtDNA	-	-	-	0.53	0.0009	Boomer et al. (2012)	LC*
Rhizoprionodon terranovae	GM	AFLP	0.32	-	-	-	-	Suarez-Moo et al. (2013)	LC
Carcharhinus brevipinna	Southern IPO	ND4 mtDNA	-	-	-	0.68	0.0013	Geraghty et al. (2013)	NT
Rhizoprionodon lalandii	WAO	CR mtDNA	-	-	-	0.88	0.0028	Mendonça et al. (2013)	DD
Rhizoprionodon porosus	-	CR mtDNA	-	-	-	0.88	0.0041	Tavares et al. (2013)	LC
Carcharhinus porosus	-	CR mtDNA	-	-	-	0.88	0.0044	Tavares et al. (2013)	DD
Carcharhinus limbatus	-	CR mtDNA	-	-	-	0.54	0.0022	Tavares et al. (2013)	NT
Sphyrna tudes	-	CR mtDNA	-	-	-	0.20	0.0005	Tavares et al. (2013)	VU
Carcharhinus falciformis	IPO	CR mtDNA	-	-	-	0.48	0.0009	Galván-Tirado et al. (2013)	NT
Carcharhinus melanopterus	FP	MS	0.58	0.57 (17)	-	-	-	Mourier and Planes (2013)	NT
Negaprion acutidens	FP	MS	0.63	0.62 (16)	-	-	-	Mourier et al. (2013)	VU
Carcharhinus melanopterus	FP	MS	-	0.49 (11)	-	-	-	Vignaud et al. (2013)	NT
Carcharhinus acronotus	US Atlantic, GM	CR mtDNA/MS	0.66	- (23)	9.7	0.85	0.0006	Portnoy et al. (2014)	NT
Carcharhinus melanopterus	FP	CR mtDNA/MS	0.55	0.54 (14)	5.2	0.46	0.0011	Vignaud et al. (2014)	NT
Alopias pelagicus	PO	CR mtDNA/MS	0.64	0.59 (7)	-	0.57	0.0031	Cardeñosa et al. (2014)	VU
Rhincodon typus	IPO	Cyt B CR mtDNA/MS	0.63	0.62 (14)	4.5	0.92	0.0120	Vignaud et al. (2014)	VU
Carcharhinus sorrah	IPO	CR mtDNA	-	-	-	-	0.0025	Giles et al. (2014)	NT
Carcharhinus plumbeus	Australia	ND4 mtDNA	-	-	-	0.28	0.0009	Geraghty et al. (2014)	VU
Carcharhinus obscurus	Australia	ND4 mtDNA	-	-	-	0.52	0.0012	Geraghty et al. (2014)	VU
Pseudocarcharias kamoharai	AO, SIO	CR mtDNA	-	-	-	0.63	0.0017	Ferrette et al. (2015)	NT
Carcharhinus falciformis	Global	CR mtDNA	-	-	-	0.93	0.0032	Clarke et al. (2015)	NT

Mustelus henlei	Northeastern PO	CR mtDNA/MS	0.56	0.45 (6)	4.1	0.77	0.0040	Chabot et al. (2015)	LC
Sphyrna tiburo	NAO	CR mtDNA	-	-	-	0.93	0.0032	Escatel-Luna et al. (2015)	LC
Prionace glauca	North PO	MS	0.61	0.60 (14)	6.7	-	-	King et al. (2015)	NT
Sphyrna lewini	Colombia	CR mtDNA/MS	0.64	0.56 (15)	-	0.58	0.0012	Quintanilha et al. (2015)	EN
Mustelus henlei	GC	CR mtDNA/MS	0.68	0.71 (12)	-	0.84	0.0033	Sandoval-Castillo and Beherengay (2015)	LC
Prionace glauca	IPO	Cyt B mtDNA	-	-	-	0.80	0.0021	Taguchi et al. (2015)	NT
Notorynchus cepedianus	California	MS	0.53	0.41 (7)		-	-	Larson et al. (2015)	DD
Carcharodon carcharias	Northeastern PO	CR mtDNA	-	-	-	0.77	0.0018	Oñate-González et al. (2015)	VU
Triakis semifasciata	California, BJ	CR mtDNA/MS	0.80	0.81 (15)	4.9	-	-	Barker et al. (2015)	LC
Galeorhinus galeus	SA	MS	0.63	0.65 (12)	-	-	-	Bitalo et al. (2015)	VU
Mustelus mustelus	SA	MS	0.53	0.68 (12)	-	-	-	Bitalo et al. (2015)	VU
Carcharodon carcharias	SA	CR mtDNA/MS	0.63	0.67 (14)	-	0.21	0.0027	Andreotti et al. (2015)	VU
Carcharhinus amblyrhynchos	Australia	MS	0.78	0.79 (15)	6.6	-	-	Momigliano et al. (2015)	NT
Carcharhinus limbatus	AP	CR mtDNA/MS	0.75	0.61 (20)	-	0.33	0.0007	Spaet et al. (2015)	NT
Sphyrna lewini	AP	CR mtDNA/MS	0.76	0.72 (20)	-	0.48	0.0001	Spaet et al. (2015)	EN
Carcharhinus sorrah	AP	CR mtDNA/MS	0.65	0.62 (20)	-	0.39	0.0012	Spaet et al. (2015)	NT
Rhizoprionodon acutus	AP	CR mtDNA/MS	0.56	0.53 (20)	-	0.70	0.0013	Spaet et al. (2015)	LC
Galeorhinus galeus	Global	MS	0.43	0.40 (11)	3.9	-	-	Chabot (2015)	VU
Galeorhinus galeus	SPO	CR mtDNA/MS	0.61	0.55 (8)	4.8	0.75	0.0010	Hernandez et al. (2015)	VU
Carcharodon carcharias	Northwest AO, SA	CR mtDNA/MS	0.67	0.56 (14)	8.5	0.74	0.0045	O'Leary et al. (2015)	VU

Scyliorhinus canicula	MS	COI mtDNA/MS	0.59	0.57 (12)	5.3	0.81	0.0032	Kousteni et al. (2015)	LC
Negaprion brevirostris	WAO	CR mtDNA/MS	0.79	0.78 (9)	-	0.83	0.0020	Ashe et al. (2015)	NT
Squatina guggenheim	SAO	Cyt B/ITS2 mtDNA	-	-	-	0.38/0.26	0.0110/0.0070	Garcia et al. (2015)	EN
Centroscymnus coelolepis	Australia, SA, European	CR mtDNA/MS	0.81	(11)	8.3	0.65	0.0018	Catarino et al. (2015)	NT
Sphyrna tiburo	Florida, GM	CR mtDNA/SNPs	-	-	-	0.88	0.0020/0.3129	Portnoy et al. (2015)	VU
Galeocerdo cuvier	Global	CR mtDNA/MS	0.65	0.64 (10)	8.2	0.82	0.0027	Bernard et al. (2016)	NT
Carcharhinus isodon	US waters WAO	CR mtDNA/MS	0.67	(16)	9.0	0.16	0.0002	Portnoy et al. (2016)	LC
Mustelus mustelus	South IO, AO	ND4 mtDNA/MS	0.50	0.50 (8)	2.1	0.47	0.0010	Maduna et al. (2016)	VU
Carcharhinus longimanus	IO, AO	CR mtDNA	-	-	-	0.60	0.0013	Camargo et al. (2016)	VU
Rays									
Rhinobatos productus	GC	CR mtDNA	-	-	-	0.77	0.0119	Sandoval-Castillo et al. (2004)	NT
Raja clavata	NA, MS	CytB mtDNA/MS	0.67	0.65 (5)	-	0.50	0.0060	Chevolot et al. (2006)	NT
Amblyraja radiata	NAO.	CytB mtDNA	-	-	-	0.80	0.0090	Chevolot et al. (2007)	VU
Aetobatus narinari	IPO	CytB/CR mtDNA	-	-	-	0.80/ 0.81	0.0126/ 0.0085	Schluessel et al. (2009)	NT
Urobatis halleri	SC, GCV	MS	0.24	(7)	-	-	-	Plank et al. (2010)	LC
Pristis pectinata	NAO	MS	0.82	0.84 (8)		-	-	Chapman et al. (2011)	CR
Pristis zijsron	Australia	CR mtDNA	-	-	-	0.56	0.0036	Phillips et al. (2011)	CR
Pristis clavata	Australia	CR mtDNA	-	-	-	0.49	0.0040	Phillips et al. (2011)	EN
Pristis microdon	Australia	CR mtDNA	-	-	-	0.65	0.0044	Phillips et al. (2011)	CR
Raja straeleni	Eastern AO, MS, WIO	CR mtDNA	-	-	-	0.67	0.0025	Parsolini et al. (2011)	DD
Rhinoptera steindachneri	BC	ND2 mtDNA	-	-	-	0.08	0.0026	Sandoval-Castillo and Rocha-	NT

								Olivares (2011)	
Raja clavata	Eastern AO, MS, WIO	CR mtDNA	-	-	-	0.55	0.0023	Parsolini et al. (2011)	NT
Dasyatis brevicaudata	IPO	CR mtDNA	-	-	-	0.78	0.0009	Le Port and Lavery (2012)	LC
Paratrygon aiereba	Amazon	ATPase 6	-	-	-	0.99	0.0349	Frederico et al. (2012)	DD
Neotrygon kuhlii	CTR	COI mtDNA	-	-	-	0.76	0.0060	Arlyza et al. (2013)	DD
Dasyatis akajei	PO	AFLP	0.23	-	-	-	-	Li et al. (2013)	NT*
Zapteryx exasperata	NMP	ND2 CR mtDNA	-	-	-	0.76/0.39	0.0013/0.0007	Castillo-Páez et al. (2014)	DD
Aetobatus narinari	GM, CS	CytB mtDNA/MS	0.74	0.73 (10)	9.6	0.60	0.0023	Sellas et al. (2014)	NT
Aetobatus narinari	Florida, GM	MS	0.70	0.66 (8)		-	-	Newby et al. (2014)	NT
Dasyatis akajei	PO	CR mtDNA	-	-	-	0.94	0.0069	Li et al. (2015)	NT
Pristis pristis	Australia	Mitogenomic	-	-	-	0.92	0.0011	Feutry et al. (2015)	CR
Rhynchobatus australiae	IPO	CR mtDNA	-	-	-	0.85	0.0061	Giles et al. (2016)	LC**
Raja polystigma	MS	CR COI 16S mtDNA/MS	0.55	0.51 (7)	2.8	0.94	0.0032	Frodella et al. (2016)	LC**
Raja montagui	MS	CR COI 16S mtDNA/MS	0.65	0.55 (7)	3.3	0.25	0.0002	Frodella et al. (2016)	LC

****** Concatenated data

Geographical regions: SA: South African, NZ: New Zealand, WAO: Western Atlantic Ocean, NAO: Northwest Atlantic Ocean, GM: Gulf of Mexico, CS: Caribbean Sea, NPO: North Pacific Ocean, SO: Southern Ocean, NA: North Atlantic Ocean, PAO: Pacific and Atlantic Ocean, IPO: Indo-Pacific Ocean, IWPO: Indo-West Pacific Ocean, PO: Pacific Ocean, IAA: Indo-Australian Archipelago, SAO: Southwest Atlantic Ocean, HA: Hawaiian Archipelago, EPO: Eastern Pacific Ocean, EA: Eastern Australian, MP: Mexican Pacific, SIO: Southwest Indian Ocean, GC: Gulf of Mexico, AP: Arabian Peninsula, SPO: South Pacific Ocean, MS: Mediterranean Sea, IO: Indian Ocean, BJ: Baja California, WI: Western Indian Ocean, SC: Southern California, CTR: Coral Triangle Region, NMP: Northern Mexican Pacific, FP: French Polynesia.

Molecular Markers: A: Allozymes, RFLP: Restriction Fragment Length Polymorphism, AFLP: Amplified Fragment Length Polymorphism, SNP: Single Nucleotide Polymorphism, MS: Microsatellites, CR mtDNA: Control Region mitochondrial DNA**,** Cyt B mtDNA: Cytochrome b mitochondrial DNA, ND4 mtDNA: NADH dehydrogenase subunit 4 mitochondrial DNA, ND2 mtDNA: NADH dehydrogenase subunit 2 mitochondrial DNA, COI mtDNA: Cytochrome oxidase subunit 1 mitochondrial DNA, 16S mtDNA: 16S RNA ribosomal mitochondrial DNA, ITS2: internal transcribed spacer 2 nuclear DNA.

Table 2. Contemporary effective population size (*Ne*) parameters for shark and rays species.

Species	Regions	Sample size	Loci	Ne (CI 95%)	Ne/Nc	Method	References
Sharks							
Carcharhinus plumbeus	DEL/ES	481/506	8	4890/2709	0.5 (DEL)	LD	Portnoy et al. (2009)
Pristis pectinata	SWFL	137	8	230-250 (142-955)	NA	M-*ratio*	Chapman et al. (2011)
Carcharodon carcharias	Australia	97	6	1512 (122 - ∞)	NA	LD	Blower et al. (2012)
Carcharodon carcharias	NWA/SA	35/131	14	32.2 (25.2–42.6)/346.6 (220.2–728.1)	NA	LD	O'Leary et al. (2014)
Stegostoma fasciatum	Australia	105	14	377 (274-584)	0.82	LD	Dudgeon and Ovenden (2015)
Prionace glauca	NPO	844	14	5468 (2802–52352)	2×10^{-3}t o 10^{-4}	LD	King et al. (2015)
Carcharodon carcharias	SA	302	14	333 (247−487)	0.76	LD	Andreotti et al. (2016)
Carcharhinus isodon	US WAO	345	16	12 798	NA	LD	Portnoy et al. (2016)
Rays							
Raja clavata	IS	363	5	283 (45–857)	$9x10^{-5}/6x10^{-4}$	PL	Chevolot et al. (2008)
Aetobatus narinari	Florida	143	8	2265.7 (243.3–∞)	NA	LD	Newby et al. (2014)

Geographical regions: DEL: Delaware Bay; ES: Eastern Shore of Virginia; SWFL: Southwest Florida; NWA: Northwest Atlantic Ocean; SA: South African; NPO: North Pacific Ocean; US WAO: United States Western Atlantic Ocean, IS: Irish Sea.
Methods: LD: Linkage Disequilibrium; M-*ratio* (Garza and Williamson 2001); PL: Pseudo-maximum likelihood.

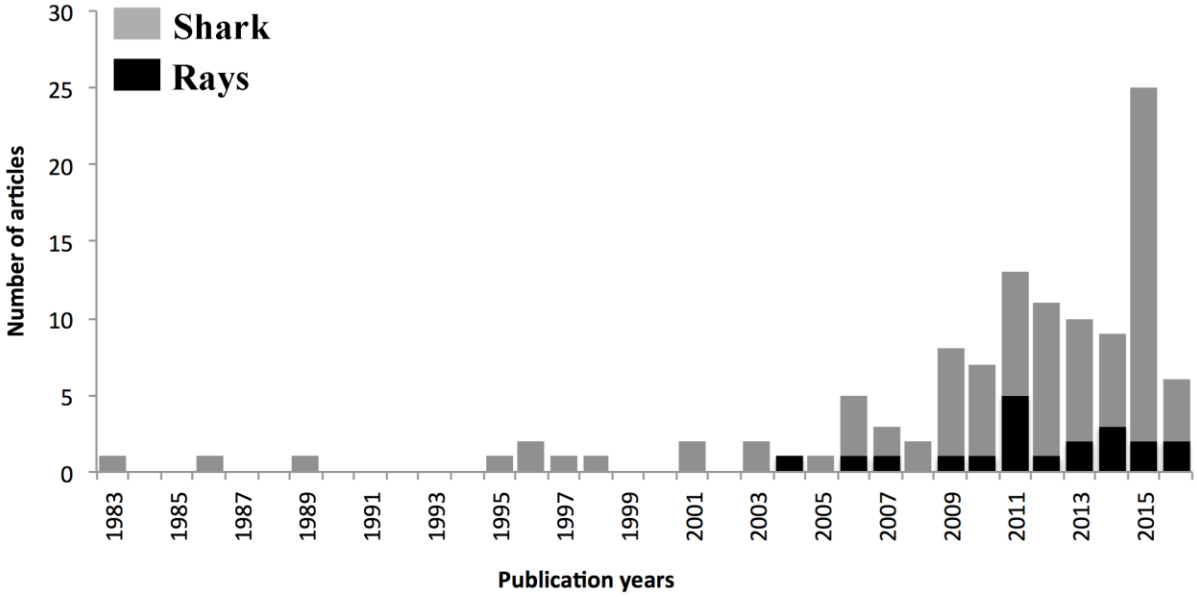

Figure 1. Number of articles published that describe the genetic diversity of shark and rays species between 1983 and 2016

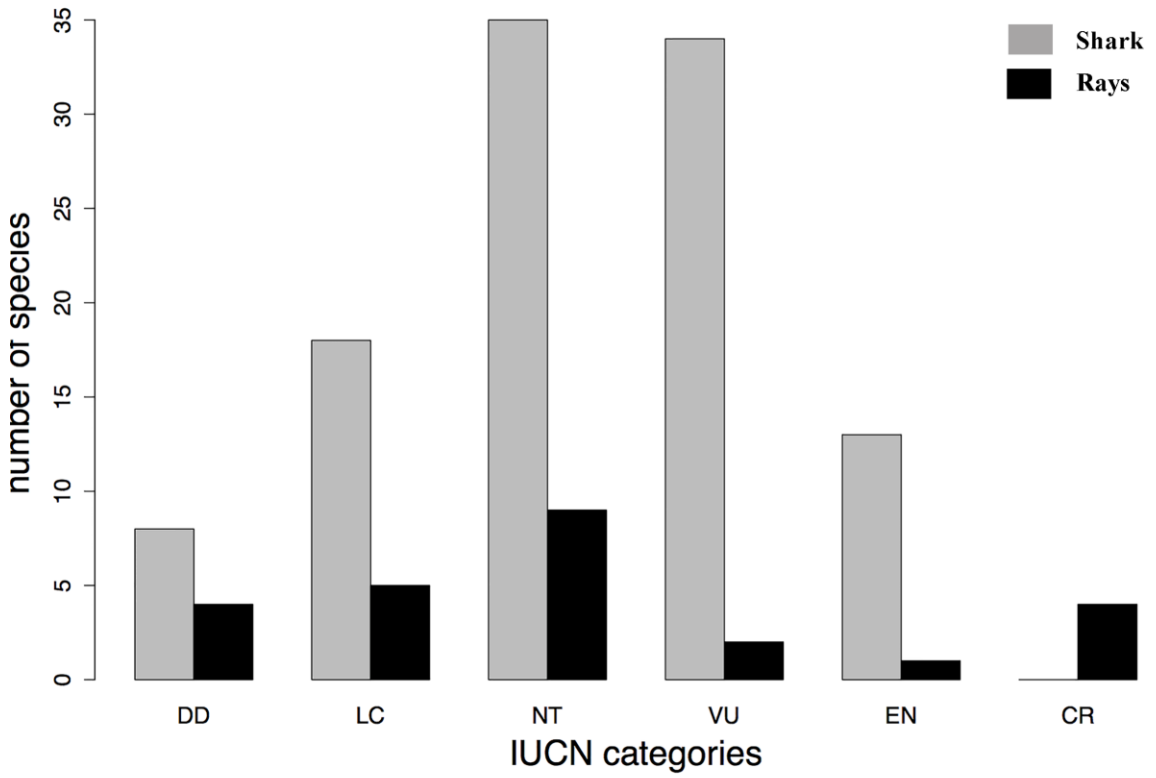

Figure 2. Numbers of articles that describe the genetic diversity of sharks and rays species for each IUCN category. DD: Data Deficient; LC: Least Concern; NT: Near Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered. Grey bars: sharks and black bars: rays.

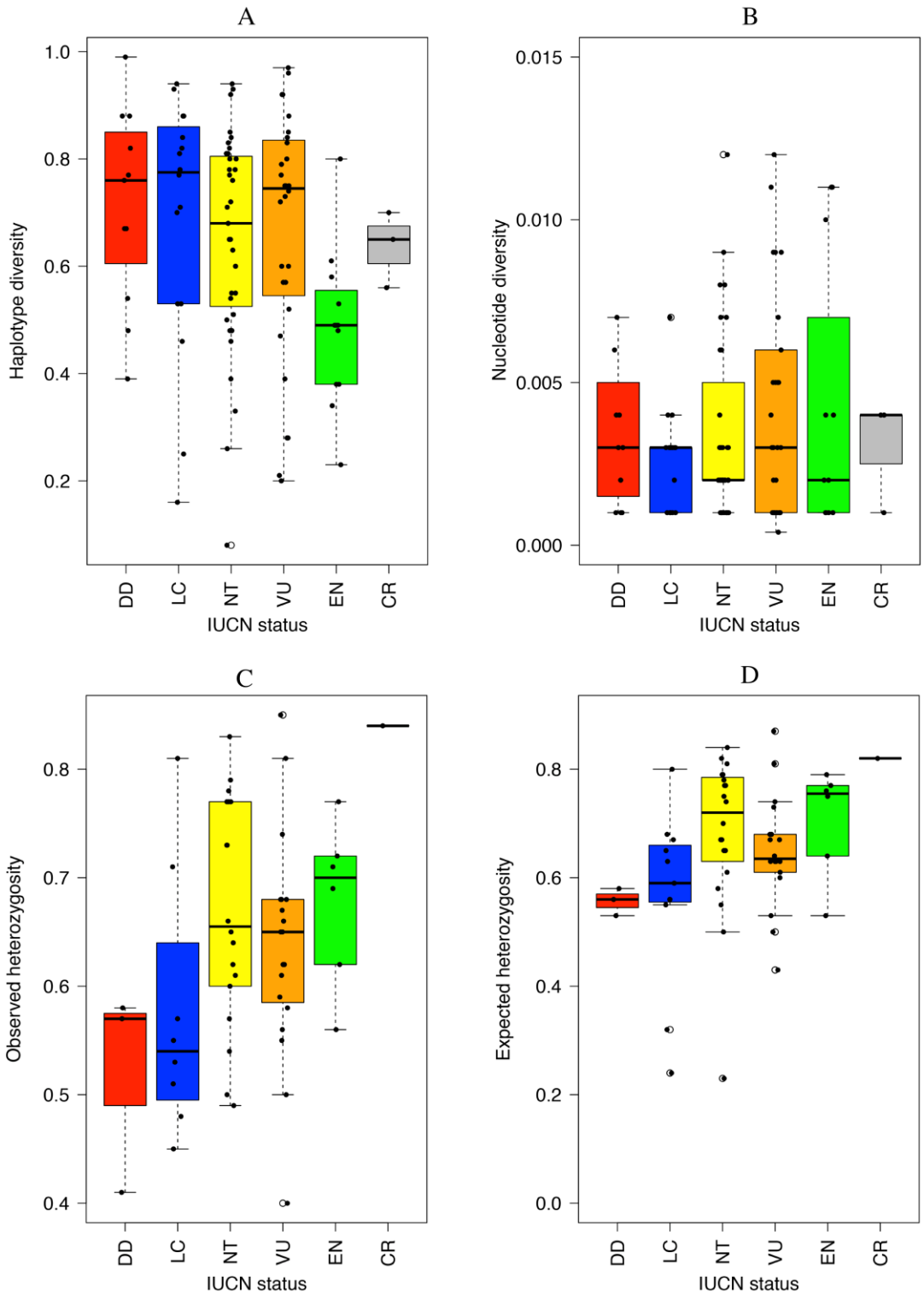

Figure 3. Boxplot of genetic diversity metrics of shark and rays pooled by the IUCN category. (A) Haplotype diversity, (B) Nucleotide diversity, (C) Observed heterozygosity and (D) Expected heterozygosity. DD: Data Deficient; LC: Least Concern; NT: Near Threatened; VU: Vulnerable; EN: Endangered; CR: Critically Endangered.

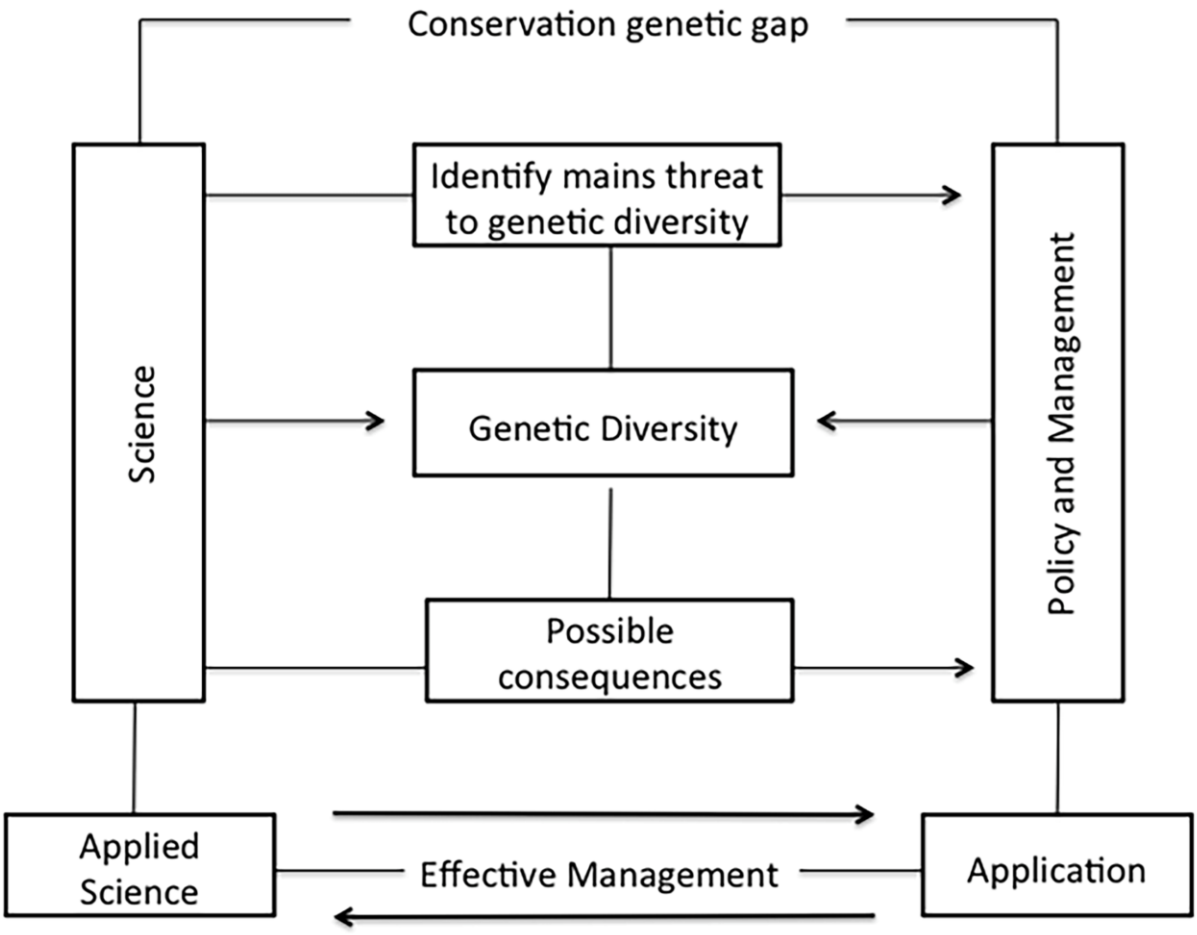

Figure 4. The link between science and conservation policies to preserve the genetic diversity of sharks and rays species. Science must generate knowledgement, identifing the mains threat to genetic diversity and their deleterious effects into populations, whereas policy makers must consider these information given by Science and apply in the policies conservation to effective management.

CAPÍTULO 5

CONSIDERAÇÕES FINAIS

O principal interesse da disciplina da Biologia da Conservação é evitar descréscimos populacionais, devido aos efeitos danosos que isto causa para uma espécie. Assim a máxima da conservação, "*conhecer para preservar*", torna-se essencial para que planos efetivos de manejo que visem a recuperação de uma espécie, possam ser elaborados. Neste sentido, a presente Tese trouxe resultados inéditos sobre a perspectiva da genética populacional de dois tubarões pelágicos altamente afetados pela pesca e inclusos na lista vermelha da IUCN nas condições de próximo de ameaçado (*C. falciformis*) e vulnerável (*C. signatus*). Além disso, por meio de uma exaustiva revisão bibliográfica e metánalise, forneceu uma visão geral sobre os níveis de diversidade genética de ampla gama de espécies de elasmobrânquios e discutiu como isto têm sido usado nos planos de manejo e políticas de conservação.

Ambas espécies constitutem duas populações (hemisfério norte vs hemisério sul) no Oceano Atlântico Ocidental baseado nas análises da região controle do DNA mitochondrial. Por outro lado, as análises baseadas em marcadores biparentais, mostra que machos de *C. signatus* são panmíticos. Mesmo com uma severa pressão pesqueira sobre as populações de *C. falciformis* no Oceano Atlantico ocidental, a diversidade genética da região controle do DNA mitochondrial foi relativamente alta, enquanto que *C. signatus* apresentou baixa diversidade genética para ambos marcadores utilizados, sobretudo para as populações do Atlântico Sul Ocidental.

Ambas as espécies apresentaram duas linhagens matrilineais, formadas durante a época do Pleistoceno. Também, foram constatadas expansões populacionais para ambas espécies durante o final deste período. Especificamente, *C. signatus* passou por um efeito gargalo durante o último máximo glacial, seguido de expansão populacional no Holoceno. Estes resultados mostram que estas espécies, assim como outras relatadas na literatura, tiveram suas histórias demográficas e aspectos filogeográficos afetados durante as ocilações

de temperatura e nível do mar. Estes achados são importantes frente as atuais mudanças climáticas e início da alteração dos níveis dos mares.

De fato, os elasmobrânquios apresentam baixa diversidade genética para diferentes marcadores moleculares, o que demonstra que isto é uma característica do grupo e não de natureza do marcador molecular. Isto vêm sendo constatado para diferentes espécies, por meio de um crescimento considerável dos estudos genéticos populacionais de elasmobrânquios nos últimos anos, sobretudo os tubarões. No entanto, cabe aqui uma pergunta: Estariam estes dados (diversidade genética) sendo considerados de alguma forma nas políticas de conservação dos elasmobrânquios? Embora exista um aumento no número de estudos genéticos das espécies de elasmobrânquios categorizadas em algum status de ameaça da lista vermelha da IUCN, esta reconhecida ONG não lança mão deste conhecimento gerado nas suas avaliações de espécies, o que parece contraditório, pois a mesma afirma que conservar a diversidade genética é um dos principais pilares para a conservação.

Finalmente, para que haja um efetivo manejo dos elasmobrânquios a curto e longo prazo, salvaguardando não somente o aumento populacional, mas também a capacidade de adequação frente as possíveis mudanças ambientais, é necessário que a distância entre a ciência e as políticas de conservação sejam encurtadas, através do diálogo, treinamento e a inserção de geneticistas na construção das políticas de conservação.

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