UNIVERSIDADE FEDERAL DO RIO GRANDE
INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

USO COMBINADO DE MARCADORES GENÉTICOS DO DNA MITOCONDRIAL E NUCLEAR NO ESTUDO DE TARTARUGAS MARINHAS HÍBRIDAS IMATURAS AO LONGO DO ATLÂNTICO SUL OCIDENTAL

CÍNTIA BRITO PRUDENTE DA SILVA

Orientadora: Prof.ª Drª Maíra Carneiro Proietti

RIO GRANDE
agosto 2018

UNIVERSIDADE FEDERAL DO RIO GRANDE
INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

USO COMBINADO DE MARCADORES GENÉTICOS DO DNA MITOCONDRIAL E NUCLEAR NO ESTUDO DE TARTARUGAS MARINHAS HÍBRIDAS IMATURAS AO LONGO DO ATLÂNTICO SUL OCIDENTAL

CÍNTIA BRITO PRUDENTE DA SILVA

Dissertação apresentada ao Programa de Pós-graduação em Oceanografia Biológica da Universidade Federal do Rio Grande - FURG, como requisito parcial à obtenção do título de MESTRE.

Orientadora: Prof.ª Drª Maíra Carneiro Proietti

RIO GRANDE
agosto 2018

SUMÁRIO

RESUMO

Eventos de hibridização em tartarugas marinhas geralmente são esporádicos e envolvem relatos de um ou poucos indivíduos. No entanto, as populações brasileiras apresentam índices elevados, o que pode ser preocupante devido ao estado de ameaça mundial destes animais. Na área de desova da Bahia foi reportado que 42% das fêmeas de tartarugas-de-pente *Eretmochelys imbricata* eram híbridas com cabeçudas *Caretta caretta*, e 2% híbridas com olivas *Lepidochelys olivacea*. Os híbridos imaturos originados desta área já foram registrados no Ceará e Rio Grande do Sul, assim como Uruguai e Argentina. O presente trabalho investigou a hibridização em tartarugas-de-pente imaturas no Atlântico Sul Ocidental (ASO) permitindo estudar a população independente de gênero ao contrário dos estudos anteriores que eram voltados apenas a fêmeas em área de desova. Através de marcadores do mtDNA (região controle – D-Loop) e nDNA (RAG1, RAG2 e CMOS) foram analisadas amostras de 270 tartarugas imaturas, do nordeste do Brasil ao Uruguai. Foi possível identificar 22 tartarugas híbridas em 270 analisadas (~8%), sendo que onze delas (50%) ocorreram na Praia do Cassino (RS), no extremo sul do Brasil. Esta área apresenta a maior frequência de híbridos ao longo do ASO (~30%), seguida pelo Uruguai com 25%. Estas áreas são comuns para tartarugas-cabeçudas, porém incomuns para tartarugas-de-pente. Desta forma, os híbridos Ei x Cc podem estar adotando comportamento característico de *C. caretta* apesar de possuírem morfologia predominante de *E. imbricata*. Através de análises de agrupamento dos três marcadores do nDNA foi possível inferir que 50% destes híbridos pertencem à geração F1 e 36% são resultado de retrocruzamentos entre híbridos e *E. imbricata* puras (>F1). Além disso este trabalho reporta, pela primeira vez, híbridos imaturos de *E. imbricata* com *L. olivacea* na região, observados no Ceará (n=2) e Espírito Santo (n=1). Considerando o a elevada frequência de híbridos observada no ASO, um monitoramento contínuo deve ser realizado para avaliar a aptidão, integridade genética e detectar a extensão das mudanças nos patrimônios genéticos das populações envolvidas, para garantir sua conservação.

Palavras-chave: Hibridização, Cheloniidae, Marcadores genéticos, Atlântico Sul Ocidental, Distribuição de híbridos, Conservação.

ABSTRACT

Marine turtle hybridization events are usually sporadic and involve reports of few individuals; however, Brazilian populations have high hybrid rates, which could be concerning due to the endangered status of these animals. At the Bahia state rookery, it was reported that 42% of nesting hawksbill turtles (*Eretmochelys imbricata*) were actually hybridized with loggerheads (*Caretta caretta*), and 2% with olive ridleys (*Lepidochelys olivacea*). Immature hybrids originating from this area have been registered in Ceará and Rio Grande do Sul states, as well as Uruguay and Argentina. The present work investigated hybridization in immature hawksbills along the South Western Atlantic (SWA). Through mitochondrial DNA (control-D-Loop) and nuclear DNA (RAG1, RAG2 and CMOS) markers. Immature turtles (n = 270) from the northeastern Brazilian to Uruguay were sampled and analysed. Based on mitochondrial and nuclear DNA, we identified 22 hybrid turtles (~ 8%) with 11 (50%) of them occurring at the extreme South of Brazil. This area presented highest hybrid frequency at the SWA (~30%) followed by Uruguay with 25%. These are common areas for loggerheads but uncommon for hawksbills; therefore, EixCc hybrids may be adopting the behavior of *C. caretta* despite having predominant morphology of *E. imbricata*. By analyzing three nuclear markers, it was possible to infer that 50% of these hybrids are first generation (F1) and 36% are the result of backcrosses between hybrids and pure *E. imbricata* (> F1). In addition, we report for the first time immature *E. imbricata* x *L. olivacea* hybrids at the region, observed in Ceará (n = 2) and Espírito Santo (n = 1). Considering the high frequency of hybrids at the SWA, continuous monitoring should be performed to assess the fitness, genetic integrity and the extent of changes in the gene pools of involved populations. This is fundamental to evaluate if hybrid management should be considered, and ensure the conservation of SWA marine turtles.

Keywords: Hybridization, Sea turtle, Cheloniidae, Genetic markers, South Western Atlantic, Hybrid distribution, Conservation.

1. INTRODUÇÃO

Das sete espécies existentes de tartarugas marinhas, cinco estão presentes no Brasil: a tartaruga-de-couro (*Dermochelys coriacea*), tartaruga-de-pente (*Eretmochelys imbricata*), tartaruga-cabeçuda (*Caretta caretta*), tartaruga-verde (*Chelonia mydas*) e tartaruga-oliva (*Lepidochelys olivacea*). Estas espécies utilizam a costa brasileira para reprodução, alimentação e desenvolvimento (Santos et al., 2011). De modo geral, o ciclo de vida destes animais envolve extensas migrações e mudanças ontogenéticas de habitat e alimentação: após entrarem no mar, os filhotes iniciam uma fase oceânica, seguida por uma fase nerítica quando recrutam para áreas de alimentação costeiras. Com a maturidade sexual, as tartarugas geralmente realizam migrações de larga-escala entre áreas de alimentação e reprodução (Bolten, 2003).

Considerada a mais tropical das espécies, *E. imbricata* se distribui em regiões costeiras de todos os oceanos, associada principalmente a recifes de coral (León & Bjorndal, 2002; Proietti et al., 2012). No Brasil as tartarugas-de-pente imaturas distribuem-se em todo o litoral Norte-Nordeste e, com menor frequência, no Sul-Sudeste (Marcovaldi et al., 2011). A tartaruga-cabeçuda, por outro lado, apresenta distribuição mais ampla, utilizando áreas costeiras ou oceânicas desde zonas equatoriais a temperadas (Witzell, 2002). No Brasil, a distribuição de *C. caretta* imaturas ao longo da costa não é bem conhecida, mas esta espécie é frequente em águas temperadas da plataforma continental do Rio Grande do Sul (Monteiro et al., 2016), com registros também no litoral do Ceará (Santos et al., 2011).

As áreas de reprodução de *E. imbricata* têm sobreposição espacial e temporal com as de *C. caretta*: nas principais áreas de desova das duas espécies – norte da Bahia e Sergipe – a época reprodutiva de *C. caretta* tem início em setembro e termina em fevereiro (Santos et al., 2011), e a de *E. imbricata* inicia em novembro e se estende até março (Marcovaldi et al., 2011). Nesta região são reportados altos índices de hibridização entre estas duas espécies (Vilaça et al., 2012). A hibridização é a produção de descendentes pelo cruzamento entre populações ou espécies geneticamente diferentes (Harrison, 1990). Considerado um processo natural na evolução, também pode ser gerada por fatores antropogênicos como diminuição de tamanhos populacionais, modificação de habitats e introdução de espécies não-nativas, e pode resultar na extinção de espécies, subespécies e

populações locais (Allendorf et al., 2001). A hibridização natural entre tartarugas marinhas da família Cheloniidae já foi relatada entre diferentes espécies (Tabela 1), e a inexistência de barreiras reprodutivas (Seminoff et al., 2003), juntamente com a compatibilidade cromossômica entre elas, é uma possível causa para este intercruzamento (Karl et al., 1995).

Tabela 1- Trabalhos que reportam hibridização entre tartarugas marinhas da família Cheloniidae, e as respectivas espécies envolvidas.

Espécie A	Espécie B	Referências
Eretmochelys imbricata	*Chelonia mydas*	Wood et al.,1983; Seminoff et al., 2003
Eretmochelys imbricata	*Caretta caretta*	Karl et al., 1995; Vilaça et al., 2012; Proietti et al.,2014a
Eretmochelys imbricata	*Lepidochelys olivacea*	Vilaça et al., 2012
Chelonia mydas	*Caretta caretta*	Karl et al., 1995; James et al., 2004
Caretta caretta	*Lepidochelys olivacea*	Vilaça et al., 2012
Caretta caretta	*Lepidochelys kempii*	Karl et al., 1995

Eventos de hibridização em tartarugas marinhas geralmente são esporádicos e envolvem relatos de um ou poucos indivíduos; no entanto, as populações brasileiras apresentam índices elevados, o que pode ser preocupante devido ao estado de ameaça mundial destes animais (IUCN, 2018). Na área de desova da Bahia, Lara-Ruiz et al. (2006) observou que, das fêmeas de *E. imbricata* analisadas (n = 119), 42% eram na realidade híbridas com *C. caretta* e 2% híbridas com *L. olivacea*. Já em estudo realizado por Reis et al. (2010) com 204 fêmeas de *C. caretta* em quatro locais de desova (Rio de Janeiro, Espirito Santo, Bahia e Sergipe), foi observado que das 51 fêmeas amostradas no Sergipe 14 eram híbridas de *C. caretta* e *L. olivacea*, enquanto nas demais áreas nenhum híbrido foi encontrado. A avaliação de híbridos imaturos é mais desafiadora devido à logística envolvida para a amostragem de indivíduos nesta fase de vida, mas é de extrema importância para verificar a sobrevivência e características da prole resultante deste processo. Além da costa brasileira, no Atlântico Sul Ocidental já foram reportadas ocorrências de *C. caretta* e *E. imbricata* no Uruguai e Argentina (Alvarez-Varas et al., 2016; Prosdocimi et al., 2014). A ocorrência de *E. imbricata* no litoral do Uruguai é baixa, com registro de apenas três indivíduos durante doze anos de monitoramento realizado pela

ONG Karumbé (Vélez-Rubio et al., 2013). No entanto, ao longo destes monitoramentos diversas tartarugas com morfologia inconclusiva na determinação da espécie têm sido observadas (A. Fallabrino, comunicação pessoal).

A causa desta elevada hibridização ainda não é bem compreendida, e seu efeito sobre as populações brasileiras são desconhecidos. Com base nisso, são necessárias maiores investigações quanto à ocorrência de tartarugas híbridas no Brasil, para avaliar se esta hibridização afeta sua distribuição, sobrevivência, e ecologia. Estas informações são essenciais para identificar possíveis efeitos deletérios para as populações, e definir se medidas de manejo tartarugas híbridas são requeridas. O uso de ferramentas moleculares facilita a identificação e caracterização do processo de hibridização, e marcadores do DNA mitocondrial (mtDNA) e nuclear (nDNA) têm sido crescentemente utilizados para detectar e estudar tartarugas marinhas híbridas (ver Tabela 1). O mtDNA é herdado apenas maternalmente, e a análise somente deste marcador produz informações limitadas; por exemplo, se um híbrido F1 ou >F1 for morfologicamente similar à espécie representada pelo mtDNA, este marcador não identificará a hibridização. Já o nDNA sofre recombinação dos dois progenitores, e permite identificar a ocorrência de genes de diferentes espécies mesmo quando o híbrido apresenta características morfológicas de apenas uma delas (Vilaça and Santos, 2013). Desta forma, a combinação do uso de marcadores do mtDNA e nDNA auxilia no reconhecimento de indivíduos híbridos, permitindo melhor avaliar sua distribuição, número de gerações (híbridos F1, >F1), e ocorrência de introgressão com espécies parentais puras (Sunnucks, 2000). Identificar o grau de introgressão entre as espécies é de suma importância para avaliar possíveis perdas de adaptações locais e aptidão das populações envolvidas (Allendorf et al., 2001).

Considerando a elevada ocorrência de híbridos na costa do Brasil, e o potencial impacto desta hibridização nas populações brasileiras de tartarugas marinhas, este estudo objetivou melhor entender este fenômeno através da análise da frequência, padrões de hibridização, e distribuição espacial de *E. imbricata* x *C. caretta* híbridas imaturas no Brasil. Através da análise combinada de marcadores do mtDNA (região controle) e nDNA (RAG1, RAG2 e CMOS), avaliamos se: 1) o uso de múltiplos marcadores aumenta a detecção de híbridos imaturos; 2) as tartarugas híbridas possuem tendência espacial na sua distribuição; e 3) os híbridos imaturos identificados são de primeira geração (F1), resultado

do cruzamento entre híbridos (F2), ou de introgressão entre híbridos e espécies parentais puras (>F1).

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar a frequência de ocorrência, distribuição, espécies parentais e número de gerações dos híbridos imaturos de tartarugas-de-pente (*Eretmochelys imbricata*) no Atlântico Sul Ocidental.

2.2. Objetivos específicos

- Identificar os haplótipos da região controle do mtDNA (D-Loop) e de três genes autossomais (RAG1, RAG2 e CMOS) das amostras;
- Reavaliar a frequência de ocorrência de híbridos imaturos na costa brasileira com base no uso combinado de marcadores do mtDNA e nDNA;
- Identificar os padrões de cruzamento entre híbridos e espécies puras;
- Comparar a distribuição espacial dos animais híbridos em relação aos padrões de distribuição das espécies puras.

3. MATERIAL E MÉTODOS

3.1. Amostragem

Neste trabalho foram utilizadas 270 amostras de epiderme e/ou músculo obtidas da nadadeira anterior de tartarugas-de-pente imaturas (comprimento curvilíneo de carapaça – CCC – de 13 a 111 cm) acidentalmente capturadas na pesca, encalhadas na praia ou capturadas intencionalmente em mergulho ou rede, ao longo da costa brasileira e Uruguai. No Brasil, as amostragens ocorreram nos Arquipélagos de São Pedro e São Paulo (ASP) e Abrolhos (AB), no litoral dos estados de Alagoas (AL), Bahia (BA), Ceará (CE), Espírito Santo (ES), Rio de Janeiro (RJ), Santa Catarina (SC), Sergipe (SE), e na praia do Cassino/RS (CA) (Figura 1). Amostras adicionais do litoral do Uruguai (UY) foram cedidas pela ONG Karumbé. Todas as amostras tiveram seu mtDNA caracterizado (sendo

158 descritas em Proietti et al., 2014b). Para o nDNA foram analisadas 141 amostras, priorizando os locais onde os híbridos haviam sido identificados através do mtDNA.

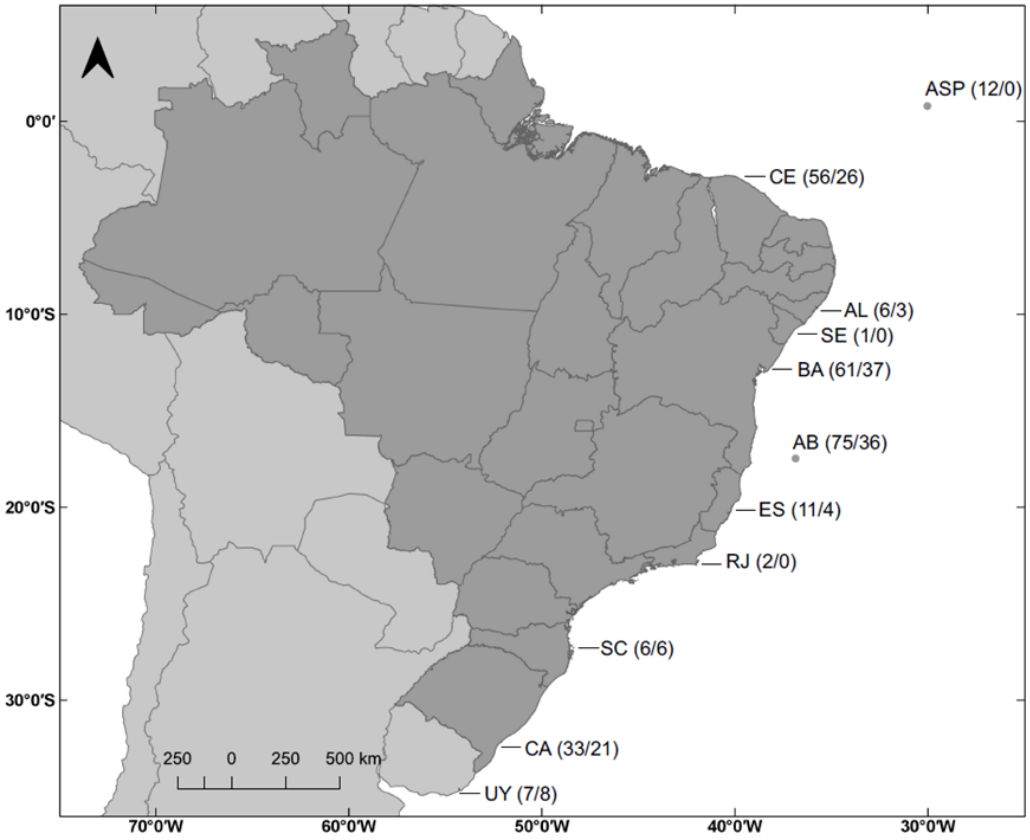

Figura 1. Locais de coleta das amostras no Atlântico Sul Ocidental, indicando o número de indivíduos analisados para mtDNA/nDNA. ASP – Arquipélago de São Pedro e São Paulo, AB – Arquipélago de Abrolhos, AL – Alagoas, CA – praia do Cassino, CE – Ceará, BA – Bahia, ES – Espírito Santo, RJ – Rio de Janeiro, SC – Santa Catarina, SE – Sergipe, UY – Uruguai.

3.2. Análise molecular

O DNA genômico foi extraído através de kits de extração (Qiagen DNEasy Extraction Kit), segundo protocolo do fabricante. Fragmentos da região controle do mtDNA (~850 pb) foram amplificados através de Reação em Cadeia da Polimerase (PCR) utilizando os iniciadores LCM15382 e H950 (Abreu-Grobois et al., 2006), sob as seguintes condições: 5’ de desnaturação a 94°C; 36 ciclos de 30’’ a 94°C, 30’’ a 50°C, 1’ a 72°C; extensão final de 10’ a 72°C. Fragmentos de nDNA foram amplificados usando iniciadores previamente descritos por Vilaça et al. (2012) para três diferentes genes: o fator de maturação do oócito (CMOS – 601 pb e 13 sítios polimórficos), e dois genes ativadores da recombinação somática (RAG1 – 368 pb e dez sítios polimórficos; RAG2 – 620 pb e

oito sítios polimórficos). Estes marcadores foram selecionados por apresentarem melhor diferenciação entre híbridos e não-híbridos entre os já descritos para estudos de hibridização em tartarugas marinhas (Vilaça et al., 2012). As condições da PCR para estes marcadores foram: 5’ de desnaturação a 94°C; 35 ciclos de 30’’ a 94°C, 1’ sob temperatura de anelamento específica (62,5°C para RAG1 e 67°C para RAG2 e CMOS), 1’ a 72°C; e uma extensão final de 10’ a 72°C. Cada marcador utilizado nas análises de nDNA apresentou um número de amostras distinto, uma vez que nem sempre foi possível amplificar os três marcadores para os indivíduos analisados devido à qualidade do DNA extraído.

Após a amplificação, os produtos foram purificados com kits de Purificação (GE Healthcare) e quantificados através de espectrofotometria utilizando um quantificador molecular (BioDrop μLITE). Os produtos purificados foram sequenciados utilizando os serviços da empresa Macrogen (http://dna.macrogen.com/eng/). A análise de qualidade das sequências obtidas, tanto para o nDNA quanto para o mtDNA, foi realizada com o software Chromas 2.6.5 (https://technelysium.com.au). Os fragmentos de mtDNA (740 pb) foram alinhados utilizando a ferramenta Clustal W (Larkin et al., 2007), implementada no programa BioEdit 7.0.9 (Hall, 1999). As sequências obtidas foram classificadas de acordo com o GenBank (https://www.ncbi.nlm.nih.gov/) e banco de dados do Archie Carr Center for Sea Turtle Research (http://accstr.ufl.edu/resources/mtdna-sequences/). Todos os indivíduos foram identificados morfologicamente como *E. Imbricata* (de acordo com Mortimer e Donnelly (2008); os que possuíam mtDNA de outra espécie foram considerados híbridos com base neste marcador. Para o nDNA, a análise das sequências foi feita utilizando a ferramenta PHASE do software DNAsp (Librado and Rozas, 2009), para a identificação do haplótipo de cada alelo dos marcadores analisados. Os haplótipos de nDNA, previamente descritos por Vilaça et al. (2012; Material Suplementar 1) são em sua maioria espécie-específicos, exceto dois haplótipos do RAG1 compartilhados entre *E. imbricata* e *L. olivacea*.

Para caracterizar a hibridização e introgressão, foram utilizadas as considerações apresentadas por Vilaça et al. (2012): “híbridos F1 exibem, para todos os loci, dois alelos derivados de diferentes espécies (e.g. um híbrido F1 de *C. caretta* x *E. imbricata* mostra para todos os loci um alelo de *C. caretta* e um de *E. imbricata*), enquanto a introgressão (>F1) é inferida quando, para uma amostra, um ou mais loci exibem os dois alelos da

mesma espécie (e.g. para o RAG1 o indivíduo apresenta os dois alelos de *C. caretta*, sendo dois alelos do mesmo haplótipo ou haplótipos distintos, desde que ambos sejam exclusivos de *C. caretta*).

3.3. Análise de dados

Para atualizar a frequência de ocorrência e distribuição de híbridos imaturos ao longo da costa brasileira com base no mtDNA, reunimos as sequências do D-Loop das 112 amostras analisadas neste trabalho com os 158 apresentados por Proietti et al. (2014b). Com base no haplótipo característico identificado para cada amostra, foi construída uma rede haplotípica utilizando o software PopArt (Leigh and Bryant, 2015), baseada no método *Median-Joining* (Bandelt et al., 1999).

Métodos de agrupamento com abordagem Bayesiana foram utilizados para detectar o nível de introgressão através dos softwares STRUCTURE (Pritchard et al., 2000) e NewHybrids (Anderson and Thompson, 2002), com base nos marcadores do nDNA. Foi utilizada a base de dados gerada por Vilaça et al. (2012), pois estes programas requerem as informações sobre os haplótipos característicos de cada espécie para inferir sobre os indivíduos analisados. A análise no STRUCTURE foi realizada assumindo frequências alélicas não-correlacionadas no modelo que prevê mistura (admixture model), com burn-in de 100.000 e 1.000.000 randomizações coletadas via Cadeia de Markov (MCMC), e valor de K variando de 1 a 10, com 5 iterações independentes. A variável K equivale ao possível número de populações geneticamente distintas que melhor explica o conjunto de dados, e a escolha do melhor K foi realizada usando a ferramenta online CLUMPAK (Kopelman et al., 2015), segundo o método Evanno (Evanno et al., 2005). Esta ferramenta também fornece uma saída gráfica para cada um dos valores de K obtidos.

A análise no NewHybrids foi implementada considerando seis classes para identificação: duas classes para espécies puras (Ei e Cc), e quatro para os híbridos – primeira geração (F1), segunda geração (F2) e retrocruzamento com espécies puras (F1xEi e F1xCc) – uma vez que nenhum híbrido acima de F2 poderia ser estatisticamente detectado por este método (Anderson and Thompson, 2002). Esta análise foi realizada com burn-in de 10.000 e 1.000.000 randomizações coletadas via Cadeia de Markov (MCMC). Para inferir sobre hibridização, o NewHybrids utiliza um modelo que considera apenas

duas espécies parentais puras envolvidas, portanto neste caso utilizamos as amostras identificadas por Vilaça et al. (2012) como sendo de *E. imbricata* e *C. caretta*, sendo excluída da análise as demais espécies do banco de dados utilizado. Desta forma, as amostras "CE32" e "CE43" não foram consideradas nesta análise, por possuírem mtDNA de *L. olivacea.*

4. SÍNTESE DOS RESULTADOS

4.1. mtDNA

Nós analisamos o mtDNA de um total de 270 indivíduos distribuídos ao longo dos 11 locais de coleta, com base nos fragmentos de 740 pb da região controle (D-Loop). Foram identificados 15 haplótipos, sendo onze típicos de *E. imbricata* e quatro de outras espécies. Dos haplótipos de outras espécies, três são específicos de *C. caretta* (CCA4.2 – 5.6%, CCA4.1 – 0.7% e CCA24.1 – 0.4%), e um de *L. olivacea* (Haplótipo F – 1.1%), sendo todos endêmicos de áreas de desova do Brasil (Bowen et al., 1998; Shamblin et al., 2014).

Observamos um predomínio do haplótipo EiA01, encontrado em todas as áreas amostradas, com frequência de ocorrência total de 78%. Haplótipos puros menos frequentes foram EiA62 (6%), EiA32 (4%), EiA09 (1%), e haplótipos raros – com apenas um registro de ocorrência cada – foram EiA11, EiA23, EiA24, EiA28, EiA61, EiA76 e EiA92. Duas tartarugas-de-pente híbridas com *L. olivacea* foram encontradas no Espírito Santo e uma no Ceará, todas apresentando o haplótipo F. Ao todo 17 híbridas com *C. caretta* foram observadas em cinco locais, sendo dez no Cassino, uma em Alagoas e duas em cada um dos demais locais – Bahia, Ceará, e Uruguai. Os registros apresentam predomínio do haplótipo CCA4.2, sendo um em Alagoas (n total = 6), dois na Bahia (n total = 61), dois no Ceará (n total = 56). O Cassino se mostra como um caso particular, com uma elevada frequência de híbridos com três haplótipos de *C. caretta* distintos – CCA4.2 (21%), CCA4.1 (6%) e CCA24.1 (3%) – e a presença apenas dos haplótipos mais frequentes em *E. imbricata* – EiA01 (64%) e EiA62 (6%) (n total = 33). O Uruguai também apresentou somente dois haplótipos frequentes de *E.*

imbricata – EiA01 (50%) e EiA32 (12.5%) – mas apenas um haplótipo de *C. caretta*, o CCA4.2 (37.5%) (n total = 8).

4.2. nDNA

Foram encontrados seis haplótipos para o marcador RAG1 (n = 126): Hap3, espécie-específico de *E. imbricata*; Hap1 e Hap4, compartilhados entre *E. imbricata* e *L. olivacea*; Hap2, característico de *C. caretta*; Hap8, característico de *C. mydas*; e um haplótipo não identificado anteriormente (denominado Hap10, Apêndice 1 - Material Suplementar 1). Para o RAG2 (n = 89) foram encontrados quatro haplótipos: Hap5, espécie-específico de *E. imbricata*; Hap2, característico de *C. caretta*; Hap6, característico de *C. mydas*; e um haplótipo não identificado anteriormente (denominado Hap7, Apêndice 1 - Material Suplementar 1). Para o CMOS (n = 35) foram encontrados seis haplótipos: Hap3, Hap5, Hap9 e Hap10, espécie-específico de *E. imbricata*; e Hap1 e Hap2, característicos de *C. caretta*.

Na análise de agrupamento do STRUCTURE, o melhor K obtido (K = 4) separou as espécies *E. imbricata* (Ei), *C. caretta* (Cc), *L. olivacea* (Lo) e *C. mydas* (Cm) em diferentes grupos, identificando os híbridos que possuem alelos de duas ou mais espécies distintas. Observando os grupos, todos os híbridos *L. olivacea x C. caretta* (Lo x Cc) possuem componentes proporcionais de cada uma das espécies parentais, indicando serem híbridos F1. Os híbridos de *E. imbricata* x *C. caretta* (Ei x Cc) apresentaram diferentes graus de hibridização. Dentre as amostras analisadas, dois indivíduos (CA23 e PF06), classificados como *E. imbricata* através da análise morfológica e mtDNA, apresentaram alelos de *C. caretta* e por isso aparecem no grupo ‘Ei’ com componentes de ‘Cc’.

Dos três indivíduos previamente identificados como híbridos com *L. olivacea* (Ei x Lo) através do mtDNA, somente em dois foi possível amplificar um marcador do nDNA (RAG1) sendo observado em ambos o haplótipo Hap1, compartilhado entre *E. imbricata* e *L. olivacea*. Portanto, não foi possível inferir sobre o grau de hibridização destes indivíduos, sendo categorizados apenas como híbridos com base na análise do mtDNA. Além dos híbridos de *E. imbricata* x *C. caretta* identificados previamente pelo mtDNA, foi possível identificar dois adicionais (CA23 e PF06), que apresentaram mtDNA de *E. imbricata* e nDNA com um alelo de cada uma das espécies parentais puras, para todos os marcadores amplificados. Estes híbridos têm, portanto, a particularidade de possuírem

mtDNA e morfologia de *E. imbricata*, fato observado em baixa frequência (1%) por Vilaça et al. (2012). Desta forma, estes dois indivíduos podem ser: 1) o resultado do cruzamento entre uma fêmea de *E. imbricata* e um macho de *C. caretta* (ocorrência pouco comum), e portanto geração F1; ou 2) o cruzamento de uma fêmea *E. imbricata* com um macho híbrido Ei x Cc, portanto geração >F1. Com base apenas nos dados disponíveis, não foi possível distinguir entre estas categorias.

Na análise do NewHybrids, todos os indivíduos 'puros' foram corretamente classificados (P > 0.98) como *E. imbricata*. Dos 18 híbridos *E. imbricata* x *C. caretta* identificados através do mtDNA, para cinco não foi possível amplificar os marcadores nucleares utilizados neste trabalho. Sete (AP56, CA24, CA25, CA31, CA36, CE04, CE51) foram incluídos na categoria F1 (p > 0.90) apresentando em cada alelo um haplótipo espécie-específico das espécies parentais e não havendo para estas amostras indício de introgressão. Outros cinco híbridos não foram detectados nesta análise (CA12, CA14, CA22, UY01, UY05) uma vez que possuíam haplótipos espécie-específicos de *E. imbricata* para os dois alelos, apesar de possuírem mtDNA de *C. caretta*, demonstrando assim indícios de introgressão com *E. imbricata.*

5. CONCLUSÃO

Nosso estudo demonstrou a importância do uso conjunto de marcadores do mtDNA e nDNA na avaliação da hibridização entre tartarugas marinhas. Embora a análise do mtDNA seja de suma importância no estudo de híbridos, a análise também do nDNA é fundamental para identificar gerações/espécies parentais, assim como para detectar híbridos nos casos em que não é possível apenas pelo mtDNA (e.g. retrocruzamento entre híbridas e fêmeas puras). Isto é confirmado pela nossa observação de dois híbridos que possuíam morfologia e mtDNA de *E. imbricata*, que só puderam ser identificados como Ei x Cc através da análise de nDNA. Esta observação pode ter sido subestimada devido à limitação de não ter sido possível amplificar os três marcadores de nDNA para todas as amostras. Outra limitação foi que o único marcador nuclear amplificado para todos os indivíduos (RAG1) tem a particularidade de possuir dois haplótipos compartilhados entre *E. imbricata* e *L. olivacea*, não sendo possível inferir conclusivamente sobre a espécie com

a análise do nDNA. Os eventos de hibridização entre *E. imbricata* e *L. olivacea* parecem ser raros, mas estudos adicionais seriam necessários para a identificação de haplótipos espécie-específicos, permitindo determinar a que geração pertencem as tartarugas híbridas resultantes destes cruzamentos, assim como inferir quanto às espécies.

Levando em conta a elevada frequência de híbridos encontrados no Atlântico Sul Ocidental, estudos de comportamento, distribuição, estratégia alimentar, migrações e demografia devem ser realizados para tartarugas híbridas. Para isso, rastreamento por satélite, análise de isótopos estáveis e análises moleculares mais abrangentes como a genômica (e. g. análise de SNPs), são técnicas que auxiliariam no entendimento do processo e permitiriam melhor determinar o papel ecológico destes híbridos e sua influência sobre as populações de tartarugas marinhas. Segundo Bohling (2016), a maior preocupação quanto aos eventos de hibridização é a extinção de unidades genéticas, fenotípicas e/ou evolutivas. Schwartz et al. (2007) sugerem que este processo seja monitorado biológica e ecologicamente, fornecendo uma estrutura para determinar e acompanhar a extensão da hibridização, as tendências ao longo do tempo e as respostas ao manejo.

Atualmente não existem diretrizes para o manejo de zonas híbridas, e os programas de gestão realizados tem como foco áreas onde o evento tem causas antropogênicas como, por exemplo, a alteração de habitats (Crispo et al., 2011) e sobre-exploração (Bohling and Waits, 2015). Para evitar a extinção genética de espécies puras, animais híbridos podem ser eliminados do estoque reprodutivo através de erradicação ou esterilização; este tipo de manejo já foi realizado no controle da hibridização entre os peixes *Cyprinodon variegatus* e *Cyprinodon bovinus*, cujo sucesso foi facilitado pela limitada extensão geográfica do grupo híbrido (Echelle and Echelle, 1997). Considerando o atual estado de ameaça das tartarugas marinhas e a elevada frequência de híbridos na costa do Brasil, este tipo de manejo deve ser avaliado, embora com cautela. De toda forma, um monitoramento contínuo deve ser realizado para avaliar a aptidão, integridade genética e detectar a extensão das mudanças nos patrimônios genéticos das populações envolvidas, para garantir sua conservação.

6. REFERENCIAS

Abreu-Grobois, F.A., Horrocks, J. a., Formia, A., Dutton, P.H., LeRoux, R. a., Velez-Zuazo, X., Soares, L.S., Meylan, A.B., 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis., in: Frick, M., Panagopoulou, A., Rees, A.F., Williams, K. (Eds.), Proceedings of the 26th Annual Symposium on Sea Turtle Biology, Book of Abstracts. p. 179. https://doi.org/10.1007/s10592-005-9102-9

Allendorf, F.W., Leary, R.F., Spruell, P., Wenburg, J.K., 2001. The problems with hybrids: Setting conservation guidelines. Trends Ecol. Evol. 16, 613–622. https://doi.org/10.1016/S0169-5347(01)02290-X

Alvarez-Varas, R., Berzins, R., Bilo, K., Chevalier, J., Chevallier, D., Thoisy, B.D.E., Fallabrino, A., Cruz, M.G., Kelez, S., Lopez-mendilaharsu, M., Marcovaldi, M.A., Mast, R.B., Medrano, C., Miranda, C., Nalovic, M.A., Prosdocimi, L., Rguez-barón, J.M., Santos, A., Soares, L., Thome, J., Vallejo, F., Vélez-rubio, G., 2016. Sea Turtles of South America. SWOT Rep. 14–27.

Anderson, E.C., Thompson, E. a, 2002. A model-based method for identifying species hybrids using multilocus data. Genetics 160, 1217–1229. https://doi.org/test statistics; hybrids

Bandelt, H.-J., Forster, P., Rohl, A., 1999. Median-Joining Networks for Inferring Intraspecific Phylogenies. Mol. Biol. 16, 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bohling, J.H., 2016. Strategies to address the conservation threats posed by hybridization and genetic introgression. Biol. Conserv. 203, 321–327. https://doi.org/10.1016/j.biocon.2016.10.011

Bohling, J.H., Waits, L.P., 2015. Factors influencing red wolf-coyote hybridization in eastern North Carolina, USA. Biol. Conserv. 184, 108–116. https://doi.org/10.1016/j.biocon.2015.01.013

Bolten, a B., 2003. Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. Biol. sea turtles 2, 243–257. https://doi.org/10.1201/9781420040807.ch9

Bowen, B.W., Clark, a M., Abreu-Grobois, F. a, Chaves, a, Reichart, H. a, Ferl, R.J., 1998. Global phylogeography of the ridley sea turtles (Lepidochelys spp.) as inferred from mitochondrial DNA sequences. Genetica 101, 179–189. https://doi.org/10.1023/A:1018382415005

Bowen, B.W., Karl, S.A., 2007. Population genetics and phylogeography of sea turtles. Mol. Ecol. 16, 4886–4907. https://doi.org/10.1111/j.1365-294X.2007.03542.x

Castilhos, J.C. De, Coelho, C.A., Argolo, J.F., Allan, E., 2011. Avaliação do Estado de Conservação da Tartaruga Marinha Lepidochelys olivacea (Eschscholtz, 1829) no Brasil. Biodiversidade Bras. 1, 26–34.

Conceição, M.B., Levy, J.A., Marins, L.F., Marcovaldi, M.A., 1990. Electrophoretic characterization of a hybrid between Eretmochelys imbricata and Caretta caretta (Cheloniidae). Comp. Biochem. Physiol. Part B Comp. Biochem. 97B, 275–278.

https://doi.org/10.1016/0305-0491(90)90280-7

Crispo, E., Moore, J.S., Lee-Yaw, J.A., Gray, S.M., Haller, B.C., 2011. Broken barriers: Human-induced changes to gene flow and introgression in animals: An examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. BioEssays 33, 508–518. https://doi.org/10.1002/bies.201000154

Crochet, P.A., Chen, J.Z., Pons, J.M., Lebreton, J.D., Hebert, P.D.N., Bonhomme, F., 2003. Genetic differentiation at nuclear and mitochondrial loci among large white-headed gulls: Sex-biased interspecific gene flow? Evolution (N. Y). 57, 2865–2878. https://doi.org/10.1111/j.0014-3820.2003.tb01527.x

Crossman, C.A., Taylor, E.B., Barrett-Lennard, L.G., 2016. Hybridization in the Cetacea: Widespread occurrence and associated morphological, behavioral, and ecological factors. Ecol. Evol. 6, 1293–1303. https://doi.org/10.1002/ece3.1913

da Silva, A.C.C.D., de Castilhos, J.C., Lopez, G.G., Barata, P.C.R., 2007. Nesting biology and conservation of the olive ridley sea turtle (Lepidochelys olivacea) in Brazil, 1991/1992 to 2002/2003. J. Mar. Biol. Assoc. UK 87, 1047. https://doi.org/10.1017/S0025315407056378

Echelle, A. a, Echelle, A.F., 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs Pupfish and Sheepshead Minnow. Conserv. Biol. 11, 153–161. https://doi.org/Genetic Considerations in Ecological Restoration

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Garman, S., 1880. On certain species of Chelonioidae. Bull. Museum Comp. Zool. v.6, 123–126.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.

Harrison, R.G., 1990. Hybrid zones: windows on evolutionary process., in: Futuyma, D., Antonovics, J. (Eds.), Oxford Surveys in Evolutionary Biology. Oxford University Press, pp. 69–128.

IUCN, 2018. The IUCN Red List of Threatened Species [WWW Document]. URL www.iucnredlist.org (accessed 2.14.18).

James, M.C., Martin, K., Dutton, P.H., 2004. Loggerhead Turtle , Caretta caretta , and the First Record of a Green Turtle in Atlantic Canada. Can. Field-Naturalist 118, 579–582.

Karl, S.A., Bowen, B.W., Avise, J.C., 1995. Hybridization Among the Ancient Mariners : Characterization of Marine Turtle Hybrids With Molecular Genetic Assays. J. Hered. 86, 262–268.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15, 1179–1191. https://doi.org/10.1111/1755-0998.12387

Lara-Ruiz, P., Lopez, G.G., Santos, F.R., Soares, L.S., 2006. Extensive hybridization in hawksbill turtles (Eretmochelys imbricata) nesting in Brazil revealed by mtDNA analyses.

Conserv. Genet. 7, 773–781. https://doi.org/10.1007/s10592-005-9102-9

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Leigh, J.W., Bryant, D., 2015. POPART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410

León, Y.M., Bjorndal, K.A., 2002. Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. Mar. Ecol. Prog. Ser. 245, 249–258. https://doi.org/10.3354/meps245249

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lima, E.H.S.M., Melo, M.T.D., Godfrey, M.H., Barata, P.C.R., 2013. Sea turtle in the waters of Almofala, Ceará, in Northeastern Brazil, 2001-2010. Mar. Turt. Newsl. 5–9. https://doi.org/0839-7708

Mallet, J., 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20, 229–237. https://doi.org/10.1016/j.tree.2005.02.010

Marcovaldi, M. a., Lopez, G.G., Soares, L.S., Santos, A.J.B., Bellini, C., Barata, P.C.R., 2007. Fifteen Years of Hawksbill Sea Turtle (Eretmochelys imbricata) Nesting in Northern Brazil. Chelonian Conserv. Biol. 6, 223. https://doi.org/10.2744/1071-8443(2007)6[223:FYOHST]2.0.CO;2

Marcovaldi, M., Chaloupka, M., 2007. Conservation status of the loggerhead sea turtle in Brazil: an encouraging outlook. Endanger. Species Res. 3, 133–143. https://doi.org/10.3354/esr003133

Marcovaldi, M., Lopez, G., Soares, L., López-Mendilaharsu, M., 2012. Satellite tracking of hawksbill turtles (Eretmochelys imbricata) nesting in northern Bahia, Brazil: turtle movements and foraging destinations. Endanger. Species Res. 17, 123–132. https://doi.org/10.3354/esr00421

Marcovaldi, M.A., Laurent, A., 1996. A Six Season Study of Marine Turtle Nesting at Praia do Forte , Bahia , Brazil , with Implications for Conservation and Management. Herpetologica 2, 55–59.

Marcovaldi, M.Â., Lopez, G.G., Soares, L.S., Santos, A.J.B., Bellini, C., Santos, A.S. dos, Lopez, M., 2011. Avaliação do estado de conservação da tartaruga marinha Eretmochelys imbricata (Linnaeus, 1766) no Brasil. Biodiversidade Bras. 1, 20–27.

Marcovaldi, M.Â., Vieitas, C.F., Godfrey, M.H., 1999. Nesting and conservation management of hawksbill turtles (Eretmochelyz imbricata) in Northern Bahia, Brazil. Chelonian Conserv. Biol. 3, 301–307.

Monteiro, D.S., Bugoni, L., Estima, S., 2006. Stranding and sea turtle fisheries interactions along the coast of Rio Grande do Sul state, Brazil, in: Book of Abstracts, Twenty-Sixth Annual Symposium on Sea Turtle Biology and Conservation. International Sea Turtle

Society, p. 257.

Monteiro, D.S., Estima, S.C., Gandra, T.B.R., Silva, A.P., Bugoni, L., Swimmer, Y., Seminoff, J.A., Secchi, E.R., 2016. Long-term spatial and temporal patterns of sea turtle strandings in southern Brazil. Mar. Biol. 163. https://doi.org/10.1007/s00227-016-3018-4

Mortimer, J. a, Donnelly, M., 2008. IUCN Red List Status Assessment: Hawksbill Turtle (Eretmochelys imbricata). IUCN Red List Threat. Species 112. https://doi.org/10.2307/1564433

Naro-Maciel, E., Le, M., FitzSimmons, N.N., Amato, G., 2008. Evolutionary relationships of marine turtles: A molecular phylogeny based on nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 49, 659–662. https://doi.org/10.1016/j.ympev.2008.08.004

Payseur, B.A., Rieseberg, L.H., 2016. A genomic perspective on hybridization and speciation. Mol. Ecol. 25, 2337–2360. https://doi.org/10.1111/mec.13557

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Proietti, M.C., Reisser, J., Marins, L.F., Marcovaldi, M. a, Soares, L.S., Monteiro, D.S., Wijeratne, S., Pattiaratchi, C., Secchi, E.R., 2014a. Hawksbill × loggerhead sea turtle hybrids at Bahia, Brazil: where do their offspring go? PeerJ 2, e255. https://doi.org/10.7717/peerj.255

Proietti, M.C., Reisser, J., Marins, L.F., Rodriguez-Zarate, C., Marcovaldi, M. a, Monteiro, D.S., Pattiaratchi, C., Secchi, E.R., 2014b. Genetic structure and natal origins of immature hawksbill turtles (Eretmochelys imbricata) in Brazilian waters. PLoS One 9, e88746. https://doi.org/10.1371/journal.pone.0088746

Proietti, M.C., Reisser, J., Secchi, E.R., 2012. Foraging by Immature Hawksbill Sea Turtles at Brazilian Islands. Mar. Turt. Newsl. 135, 4–8.

Prosdocimi, L., Bruno, I., Diaz, L., Carman, V.G., Albareda, D.A., Remis, M.I., 2014. Southernmost reports of the hawksbill sea turtle, eretmochelys imbricata, in temperate waters of argentina and evidence of a hybrid origin supported by mitochondrial dna analysis. Herpetol. Rev. 45, 1–5.

Reis, E.C., Soares, L.S., Lôbo-Hajdu, G., 2010a. Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conserv. Genet. 11, 1587–1591. https://doi.org/10.1007/s10592-009-9973-2

Reis, E.C., Soares, L.S., Vargas, S.M., Santos, F.R., Young, R.J., Bjorndal, K.A., Bolten, A.B., Lôbo-Hajdu, G., 2010b. Genetic composition, population structure and phylogeography of the loggerhead sea turtle: Colonization hypothesis for the Brazilian rookeries. Conserv. Genet. 11, 1467–1477. https://doi.org/10.1007/s10592-009-9975-0

Rieseberg, L., Wendell, J.F., 1993. Gene flow and its consequences in plants, Hybrid zones and the evolutionary process.

Rosenfield, J.A., Nolasco, S., Lindauer, S., Sandoval, C., Kodric-Brown, A., 2004. The role of hybrid vigor in the replacement of pecos pupfish by its hybrids with sheepshead minnow. Conserv. Biol. 18, 1589–1598. https://doi.org/10.1111/j.1523-1739.2004.00356.x

Sales, G., Giffoni, B.B., Barata, P.C.R., 2008. Incidental catch of sea turtles by the Brazilian

pelagic longline fishery. J. Mar. Biol. Assoc. UK 88, 853–864. https://doi.org/10.1017/S0025315408000441

Sanches, T.M., Bellini, C., 1999. Juvenile Eretmochelys imbricata and Chelonia mydas in the Archipelago of Fernando de Noronha, Brazil. Chelonian Conserv. Biol. 3, 308–311.

Santana, A., Soares, L., Marcovaldi, M.Â., Monteiro, S., 2011. Avaliaçao do estado de conservaçao da tartaruga marinha Caretta caretta Linnaeus, 1758. Biodiversidade Bras. 1, 3–11. https://doi.org/10.1111/j.1439-0426.2009.01342.x

Santos, A.S., Almeida, A.P., Santos, A.J.B., Giffoni, B., Gallo, B., Baptistotte, C., Coelho, C. a., Lima, E.H.S.M., Sales, G., Lopez, G.G., Stahelin, G., Becker, H., Castilhos, J.C., Thomé, J.C. a., Wanderlinde, J., Marcovaldi, M. a. G., Mendilaharsu, M.M.L., Damasceno, M.T., Barata, P.C.R., Sforza, R., 2011. Plano de ação nacional para conservação das tartarugas marinhas.

Schwartz, M.K., Luikart, G., Waples, R.S., 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol. Evol. 22, 25–33. https://doi.org/10.1016/j.tree.2006.08.009

Seminoff, J.A., Karl, S.A., Schwartz, T.S., Resendiz, A., 2003. Hybridization of the green turtle (Chelonia mydas) and hawksbill turtle (Eretmochelys imbricata) in the Pacific Ocean: Indication of an absence of gender bias in the directionality of crosses. Bull. Mar. Sci. 73, 643–652.

Shamblin, B.M., Bolten, A.B., Abreu-Grobois, F.A., Bjorndal, K.A., Cardona, L., Carreras, C., Clusa, M., Monzón-Argüello, C., Nairn, C.J., Nielsen, J.T., Nel, R., Soares, L.S., Stewart, K.R., Vilaça, S.T., Türkozan, O., Yilmaz, C., Dutton, P.H., 2014. Geographic patterns of genetic variation in a broadly distributed marine vertebrate: New insights into loggerhead turtle stock structure from expanded mitochondrial DNA sequences. PLoS One 9, e85956. https://doi.org/10.1371/journal.pone.0085956

Soares, L.S., Bjorndal, K.A., Bolten, A.B., dei Marcovaldi, M.A.G., Luz, P.B., Machado, R., Lo, R., McDaniel, S.F., Payton, A.C., Waltzek, T.B., Wayne, M.L., 2018. Effects of hybridization on sea turtle fitness. Conserv. Genet. 0, 1–12. https://doi.org/10.1007/s10592-018-1101-8

Soares, L.S., Bolten, A.B., Wayne, M.L., Vilaça, S.T., Santos, F.R., dei Marcovaldi, M.A.G., Bjorndal, K.A., 2017. Comparison of reproductive output of hybrid sea turtles and parental species. Mar. Biol. 164. https://doi.org/10.1007/s00227-016-3035-3

Sunnucks, P., 2000. Efficient genetic markers for population biology. Trends Ecol. Evol. 15, 199–203. https://doi.org/10.1016/S0169-5347(00)01825-5

Vélez-Rubio, G.M., Estrades, A., Fallabrino, A., Tomás, J., 2013. Marine turtle threats in Uruguayan waters: Insights from 12 years of stranding data. Mar. Biol. 160, 2797–2811. https://doi.org/10.1007/s00227-013-2272-y

Vilaça, S.T., Santos, F.R. Dos, 2013. Molecular Data for the Sea Turtle Population in Brazil. Dataset Pap. Sci. 2013, 1–7. https://doi.org/10.1155/2013/196492

Vilaça, S.T., Vargas, S.M., Lara-Ruiz, P., Molfetti, É., Reis, E.C., Lôbo-Hajdu, G., Soares, L.S., Santos, F.R., 2012. Nuclear markers reveal a complex introgression pattern among marine turtle species on the Brazilian coast. Mol. Ecol. 21, 4300–4312. https://doi.org/10.1111/j.1365-294X.2012.05685.x

Wallace, B.P., DiMatteo, A.D., Hurley, B.J., Finkbeiner, E.M., Bolten, A.B., Chaloupka, M.Y., Hutchinson, B.J., Alberto Abreu-Grobois, F., Amorocho, D., Bjorndal, K.A., Bourjea, J., Bowen, B.W., Dueñas, R.B., Casale, P., Choudhury, B.C., Costa, A., Dutton, P.H., Fallabrino, A., Girard, A., Girondot, M., Godfrey, M.H., Hamann, M., López-Mendilaharsu, M., Marcovaldi, M.A., Mortimer, J.A., Musick, J.A., Nel, R., Pilcher, N.J., Seminoff, J.A., Troëng, S., Witherington, B., Mast, R.B., 2010. Regional Management Units for Marine Turtles: A Novel Framework for Prioritizing Conservation and Research across Multiple Scales. PLoS One 5, 1–11. https://doi.org/10.1371/journal.pone.0015465

Wayne, R.K., Shaffer, H.B., 2016. Hybridization and endangered species protection in the molecular era. Mol. Ecol. 25, 2680–2689. https://doi.org/10.1111/mec.13642

Whitham, T., Morrow, P., Potts, B., 1991. Conservation of Hybrid Plants. Science (80-.). https://doi.org/doi:10.1126/science.254.5033.779-b

Witzell, W.N., 2002. Immature Atlantic loggerhead turtles (Caretta caretta): suggested changes to the life history model. Herpetol. Rev.

Wood, J., Wood, F., Critchley, K., 1983. Hybridization of Chelonia mydas and Eretmochelys imbricata. Copeia 839–842.

APÊNDICE 1

COMBINED USE OF MITOCHONDRIAL AND NUCLEAR GENETIC MARKERS FURTHER REVEAL IMMATURE MARINE TURTLE HYBRIDS ALONG THE SOUTH WESTERN ATLANTIC

Cíntia Brito[a*], Sibelle Torres Vilaça[b], Ana Luzia Lacerda[a,] Rodrigo Maggioni[c], Maria Ângela Marcovaldi[d], Gabriela Vélez-Rubio[e,f], Maíra Carneiro Proietti[a].

[a]Laboratório de Ecologia Molecular Marinha, Instituto de Oceanografia – IO, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil.

[b]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario K9J 7B8, Canada.

[c]Instituto de Ciências do Mar – LABOMAR, Universidade Federal do Ceará – UFC, Fortaleza, CE, Brazil.

[d]Projeto TAMAR-ICMBio and Fundação Pró-TAMAR, Salvador, BA, Brazil

[e]Asociación Civil Karumbé, Montevideo, Uruguay.

[f]Centro Universitario Regional del Este (CURE), Universidade de la República, Rocha.

Corresponding author: *cintiabrito@gmail.com

Abstract

Marine turtle hybridization is usually sporadic and involves reports of few individuals; however, Brazilian populations have high hybrid rates, which could be concerning due to the endangered status of these animals. The present work investigated hybridization in immature hawksbills along the South Western Atlantic (SWA) through mitochondrial (D-Loop) and nuclear DNA (RAG1, RAG2 and CMOS) markers, allowing us to better understand the patterns and characteristics of hybrid offspring. Immature turtles (n = 270) from northeastern Brazil to Uruguay were sampled and analysed; we identified 22 hybrids (~ 8%), with eleven of them occurring at the extreme South of Brazil. This area presented highest hybrid frequency at the SWA (~30%) followed by Uruguay with 25%. These are common areas for loggerheads but uncommon for hawksbills, and we believe Ei x Cc hybrids may be adopting the behavior of

Caretta caretta. By analyzing nuclear markers, it was possible to infer that 50% of the sampled hybrids are first generation (F1) and 36% are the result of backcrosses between hybrids and pure *Eretmochelys imbricata* (> F1). We also report for the first time immature *E. imbricata* x *Lepidochelys olivacea* hybrids at the Brazilian coast, observed in Ceará and Espírito Santo states. Considering the high frequency of hybrids at the SWA, continuous monitoring should be performed to assess the fitness, genetic integrity and the extent of changes in the gene pools of involved populations. This is fundamental to evaluate if hybrid management should be considered, and ensure the conservation of SWA marine turtles.

Keywords: Hybridization, Sea turtle, Cheloniidae, Genetic markers, South Western Atlantic, Hybrid distribution, Conservation.

Introduction

Hybridization is the production of offspring by the crossbreeding between genetically different populations or species (Harrison, 1990), and at least 25% of plant species and 10% of animal species are involved in hybridization processes and potential introgression (Mallet 2005). According to Rieseberg and Wendell (1993), introgression can be defined as "the incorporation of genes from one set of different populations into another, i.e. the incorporation of external alleles into a new, reproductively integrated population system". These processes are considered natural in evolution, and continuous events of interspecific and intergeneric hybridization may lead to the appearance of new species (e.g. 50-70% of angiosperms; Whitham et al. 1991). However, hybridization can also be generated by anthropogenic factors such as decreasing population sizes, introduction of non-native species and modification of habitats, which may result in the extinction of local species, subspecies and populations (Allendorf et al. 2001).

Hybridization is common in different vertebrate groups, such as birds (Crochet et al. 2003), fish (Rosenfield et al. 2004), and marine mammals – the order Cetacea, for example, has records of hybrids in 20% of species that make up the group (Crossman et al. 2016). In marine turtles, the occurrence of hybrids

has already been reported between several species of the Cheloniidae family, especially the closely related species *Lepidochelys olivacea*, *Caretta caretta* and *Eretmochelys imbricata* (Bowen and Karl 2007; Naro-Maciel et al. 2008). It is possible that this interbreeding occurs due to the lack of reproductive barriers (Seminoff et al. 2003) and a chromosomal compatibility between the different species (Karl et al. 1995).

Table 1. Hybridization events reported between Cheloniidae marine turtles, the respective species, number of observed hybrids and country.

Species A	**Species B**	**#hybrids**	**Country**	**References**
Caretta caretta	*Lepidochelys kempii*	1	USA	Karl et al. 1995
Caretta caretta	*Chelonia mydas*	1	USA	Garman 1880
		4	Brazil	Karl et al. 1995
		1	Canada	James et al. 2004
Eretmochelys imbricata	*Caretta caretta*	2	USA	Karl et al. 1995
Eretmochelys imbricata	*Chelonia mydas*	1	Suriname	Karl et al. 1995
		1	Mexico	Seminoff et al. 2003
Eretmochelys imbricata	*Lepidochelys olivacea*	2	Brazil	Lara-Ruiz et al. 2006

Most data on hybridization in nature comes from morphological evaluation of organisms (Mallet 2005); however, this alone is insufficient to adequately identify and characterize hybrids, since some hybrids do not present mixed morphology. In marine turtles, the first signs of hybridization were observed in animals with intermediate diagnostic characteristics between two species (Wood et al. 1983), and the first molecular observation of hybrids was done in Brazil by Conceição et al. (1990), using isozymes. With the advance of molecular tools, genetic analyses have been increasingly used to increase the detection and understanding of this hybridization process (e.g. Vilaça et al. 2012). Mitochondrial (mtDNA) and nuclear (nDNA) markers may therefore aid in the identification of hybrids even when individuals do not have evidence of hybridization observable through morphology.

In most species, mtDNA is maternally inherited, therefore, relying only on mitochondrial information may be misleading when validating morphological

observations; for example, if a first-generation (F1) or any subsequent generation (>F1) hybrid shows similar morphological characteristics to the species determined through mtDNA, hybridization cannot be detected. In contrast, nDNA is inherited from the two progenitors, allowing the identification of genes of different species even when the hybrid has morphological characteristics and the mtDNA of only one (Vilaça and Santos 2013). Thus, the combined use of mtDNA and nDNA markers assists in hybrid identification, allowing a better evaluating of their distribution and frequency of occurrence. In addition, it is possible to evaluate the number of generations the hybridization has been occurring, as well as the occurrence of introgression (i.e., whether the individual is the result of cross between pure parent species (F1 generation), hybrids (F2) or a backcross between a hybrid and one of the pure parent species) (Sunnucks 2000). Identifying the degree of introgression between species is important in assessing possible losses of local adaptations and fitness of the populations involved (Allendorf et al. 2001). In addition, according to Payseur and Rieseberg (2016), understanding the connection between geographic distribution/gene flow of hybrids and the emergence of new species is fundamental. Considering the hybridization events and species involved, the decision of which individuals and populations should be protected is complex Wayne and Shaffer (2016), and caution is needed when establishing conservation strategies.

Hybridization events in marine turtles are usually sporadic and involve reports of one or a few individuals (see Table 1); however, Brazilian populations have high rates, which may be concerning due to the endangered status of these animals (IUCN 2018). At the Bahia state rookery, Lara-Ruiz et al. (2006) observed through mtDNA that, among *E. imbricata* females analyzed (n = 119), 42% were actually hybrid with *C. caretta* and 2% hybrid with *L. olivacea*. When analysing 204 *C. caretta* females at four rookeries (Rio de Janeiro, Espírito Santo, Bahia and Sergipe states), Reis et al. (2010) observed that 14 out of 51 females from Sergipe were hybrids with *L. olivacea*, with no record for the other rookeries. Occurrences of *C. caretta* and *E. imbricata* immature hybrids have also been reported in Uruguay and Argentina (Alvarez-Varas et al. 2016; Prosdocimi et al. 2014). . Os estudos anteriores foram realizados amostrando fêmeas em áreas de

desova, ao contrário disto a metodologia empregada neste estudo permite analisar a população independente. The occurrence of *E. imbricata* along the coast of Uruguay is low, with only three individuals registered during twelve years of monitoring by the NGO Karumbé (Vélez-Rubio et al. 2013). However, during these surveys several turtles with inconclusive morphology have been observed (A. Fallabrino, personal communication).

The hybridization pattern involving the three species with high occurrence of hybridization in Brazil – *E. imbricata*, *C. caretta* and *L. olivacea* – was investigated by Vilaça et al. (2012) in samples obtained along the coast using 12 nuclear markers. These authors identified the *L. olivacea* hybrids with *C. caretta* and *E. imbricata* as being first generation (F1). In contrast, some *C. caretta* and *E. imbricata* hybrids showed evidence of backcrosses with pure parent species (i.e., introgression), indicating a longer process or longer survival of offspring. This study also suggested that hybridization events at the region has been occurring for at least 40 years (i.e., around two generations), and may be a result of the historical population decline experienced by both species due to exploitation of eggs and female turtles (Santos et al. 2011).

In Brazil, *E. imbricata* reproductive areas overlap spatially and temporally with those of *C. caretta*, with both species reproducing at the Northeast coast and presenting highest nesting concentrations at Bahia and Sergipe states (Marcovaldi et al. 2007; Marcovaldi and Chaloupka 2007). The *C. caretta* reproductive season begins in September and ends in February (Santana et al. 2011), and *E. imbricata* breeding begins in November and extends until March (Marcovaldi et al. 2011). This overlap, together with the population depletion suffered by the species, may contribute to the occurrence of hybridization events. Proietti et al. (2014a) and Vilaça et al. (2012) also indicate that hybridization at the region has a gender bias, and that the encounter of male *E. imbricata* with female *C. caretta* would be favored by the larger population size of loggerhead turtles, in conjunction with a temporal overlap at the peak of reproductive season. *E. imbricata* begin to reproduce near the *C. caretta* reproductive peak (November-December), leading to the encounter of male *E. imbricata* with females of both species. In contrast, since the reproductive peak

of *E. imbricata* is after *C. caretta*, most *C. caretta* have probably left the area when it presents higher numbers of *E. imbricata* females, reducing the probability of their reproduction (Soares et al. 2018).

Immature animals resulting from the hybridization process in Brazil were reported for the first time by Proietti et al. (2014a 2014b), who analyzed 157 *E. imbricata* along the Brazilian coast and identified four individuals at Cassino (Rio Grande do Sul state) with a *C. caretta* haplotype (CCA4.2). This is an unusual area for *E. imbricata*, which occupies preferentially tropical regions of the oceans, associated with coral reefs (León and Bjorndal 2002; Mortimer and Donnelly 2008); on the other hand, *C. caretta* is common at temperate latitudes, occurring frequently at the region (Monteiro et al. 2016). The high frequency of immature hybrids found at Cassino shows that the distribution of these animals may present a spatial tendency, with preference for areas used more by *C. caretta*, despite the predominant *E. imbricata* morphology. Adult hybrids also show differential habitat use: (Marcovaldi et al. 2012) tracked pure and hybrid *E. imbricata x C. caretta* females after nesting in Bahia, and observed that they used distinct areas to feeding. Pure females used areas along the eastern coast of Brazil, while hybrids migrated to the northeast Brazilian coast, including Ceará, which is a feeding area for *C. caretta* (Lima et al. 2013).

The effect of hybridization on marine turtles was investigated for the first time by Soares et al. (2017), who compared factors associated with reproductive success of hybrid and pure *E. imbricata* and *C. caretta* females nesting at Bahia. There was low occurrence of hybrids in females identified as *C. caretta* (1%), while more than half of the females identified as *E. imbricata* presented a *C. caretta* haplotype, reaffirming the prevalence of crosses between *E. imbricata* males and *C. caretta* females. Hybrid females used the same reproductive areas of pure parent species, but with an intermediate peak between *C. caretta* and *E. imbricata*. Based on the analysed reproductive parameters (number of eggs, emergence success, incubation period, number of hatchlings per nest, number of nests per year), hybrid females apparently do not have different reproductive outputs when compared to pure parent species.

Considering the high occurrence of hybrids at the South Western Atlantic (SWA), the potential impacts of this phenomenon on marine turtle populations, and the paucity of studies on the characteristics of hybrid offspring originating from Brazilian populations, the goal of this study was to investigate hybridization in immature turtles at the South Western Atlantic, using molecular methods. Through the combined analysis of mtDNA (control region – D-Loop) and nDNA (RAG1, RAG2 and CMOS) markers, we evaluated if: 1) the use of multiple markers enhances the detection of immature hybrids; 2) immature hybrid turtles present a spatial tendency in their distribution; and (3) the identified immature hybrid turtles are first generation (F1), the result of crossing between hybrids (F2), or of introgression between hybrids and pure parent species (>F1).

Methods

Sampling

A total of 270 skin and/or muscle samples were obtained from the anterior flippers or inguinal area of immature hawksbills (Curved Carapace Length – CCL – from 13 to 111 cm) incidentally captured by fisheries, stranded on the beach, or intentionally caught in dives along the Brazilian coast and Uruguay. In Brazil, sampling was done at the São Pedro and São Paulo (ASP) and Abrolhos (AB) Archipelagos, along the coast of Alagoas (AL), Bahia (BA), Ceará (CE), Espírito Santo (ES), Santa Catarina (SC) and Sergipe (SE) states, and Cassino beach (Rio Grande do Sul state (Figure 1). Projeto Tamar – Fundação Tamar and Centro Tamar-ICMBio, provided samples from BA, CE, ES and SE; Instituto Biota de Conservação provided samples from AL; and Núcleo de Educação e Monitoramento Ambiental (NEMA) provided samples from Cassino beach. The NGO Karumbé provided samples from the coast of Uruguay (UY). All samples had their mtDNA characterized (of which 158 were previously described in Proietti et al. 2014b); for nDNA 141 samples were analyzed, prioritizing sites where hybrids had been identified through mtDNA.

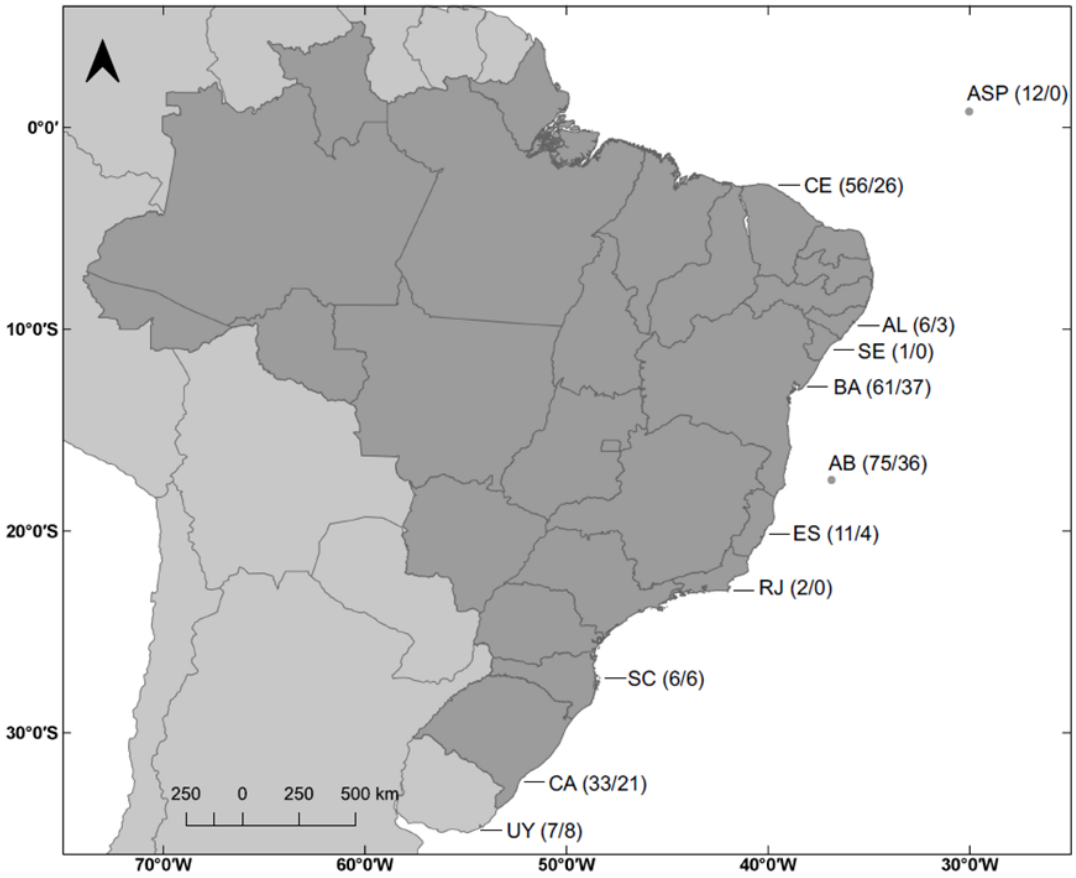

Figure 1. Sampling sites in the South Western Atlantic, indicating the number of individuals analyzed for mtDNA/nDNA. ASP - São Pedro and São Paulo Archipelago; AB - Abrolhos Archipelago; AL - Alagoas; CA - Cassino; CE - Ceará, BA - Bahia; ES – Espírito Santo; RJ - Rio de Janeiro; SC - Santa Catarina; SE - Sergipe; UY - Uruguay.

Molecular analyses

Genomic DNA was extracted using commercial extraction kit (Qiagen DNEasy Extraction Kit), according to the manufacturer's protocol. Fragments of the mtDNA control region (~850 bp) were amplified via Polymerase Chain Reaction (PCR) using primers LCM15382 and H950 (Abreu-Grobois et al. 2006), under the following conditions: 5 ' at 94°C; 36 cycles of 30" at 94°C, 30" at 50°C 1' at 72°C; and a final extension of 10' at 72°C. Fragments of nDNA were amplified using primers previously described by Vilaça et al. (2012), for three different genes: oocyte maturation factor (CMOS – 601 bp and 13 polymorphic sites), and two somatic recombination activating genes (RAG1 – 368 bp and 10 polymorphic sites; RAG2 – 620 bp and 8 polymorphic sites). These markers were chosen due to their ability to differentiate hybrids from non-hybrid turtles, as noted in previous

studies (Vilaça et al. 2012). The PCR conditions for these markers were: 5' denaturation at 94°C; 35 cycles of 30" at 94°C 1' under specific annealing temperatures (62.5°C for RAG1 and 67°C for RAG2 and CMOS) 1' at 72°C; and a final extension of 10' at 72°C. Each nDNA marker was amplified for a distinct number of samples, since it was not possible to amplify the three markers for all analysed individuals.

Amplified products were purified with purification kits (GE Healthcare Illustra GFX Purification kit) and quantified by spectrophotometry using a BioDrop μLITE. The purified products were then sequenced at Macrogen (http://dna.macrogen.com/eng/). Quality analysis of the sequences obtained for both mtDNA and nDNA was performed with Chromas 2.6.5 software (https://technelysium.com.au). The mtDNA fragments were aligned using the Clustal W tool (Larkin et al. 2007) implemented in BioEdit 7.0.9 (Hall 1999). The sequences were cropped to 740bp and classified according to GenBank (https://www.ncbi.nlm.nih.gov/) and the Archie Carr Center for Sea Turtle Research database (http://accstr.ufl.edu/resources/mtdna-sequences/). Since all individuals were morphologically identified as *E. imbricata*, those that had mtDNA of another species were considered hybrids based on this marker. For nDNA, sequence analysis was performed using the PHASE tool of DNAsp software (Librado and Rozas 2009) to identify the haplotype of each allele of the analyzed markers. The nDNA haplotypes, previously described by Vilaça et al. (2012; Supplementary Material 1) are mostly species-specific, except for two RAG1 haplotypes shared between *E. imbricata* and *L. olivacea*.

To characterize hybridization and introgression, we followed the considerations presented by Vilaça et al. (2012): F1 hybrids exhibit for all loci alleles derived from different species, e.g., a *C. caretta* x *E. imbricata* F1 hybrid shows for all loci one *C. caretta* and one *E. imbricata* allele; introgressed animals (>F1) show for one or more loci two alleles of the same species, e.g. for RAG1 the hybrid individual presents two alleles exclusive to *C. caretta*.

Data analyses

To update the frequency of occurrence and distribution of immature hybrids based on mtDNA, we grouped the sequences of the 112 samples analyzed in this work with the 158 presented by Proietti et al. (2014b). Based on the characteristic haplotype identified for each sample, a haplotype network was built using PopArt (Leigh and Bryant 2015), based on the Median-Joining method (Bandelt et al. 1999).

Bayesian clustering methods were used to detect the level of introgression through STRUCTURE(Pritchard et al. 2000) and NewHybrids (Anderson and Thompson 2002), based on the nDNA markers. These programs require information on the characteristic haplotypes of each species (coded as bi-allelic genotypes) to infer the ancestry/admixture of the individuals analyzed, and we therefore used as input the database generated by Vilaça et al. (2012). STRUCTURE analysis was performed assuming non-correlated allele frequencies in the admixture model, with a burn-in of 100,000 and 1,000,000 randomizations collected via Markov Chain Monte Carlo (MCMC), with a K value ranging from 1 to 10, with 5 independent iterations. K is equivalent to the possible number of genetically distinct populations that best explains the data set, and the choice of the best K was performed using the online tool CLUMPAK (Kopelman et al. 2015), according to the Evanno method (Evanno et al. 2005). This tool also provides a graphical output for each of the obtained K values.

The NewHybrids analysis was implemented considering six classes for identification: two classes for pure species (Ei and Cc), and four for hybrids – first generation (F1), second generation (F2), and backcrosses with pure species (F1xEi and F1xCc) – since no hybrid above F2 could be statistically detected by this method (Anderson and Thompson 2002). This analysis was performed with a burn-in of 10,000 and 1,000,000 randomizations collected via the Markov Chain (MCMC). NewHybrids uses a model that considers only two pure parent species, so in this case we used samples identified by Vilaça et al. (2012) as being of *E. imbricata* and *C. caretta*, excluding from the analysis the other species of the database. Thus, samples "CE32" and "CE43" were not considered in this analysis, since they possessed mtDNA of *L. olivacea*.

Results

mtDNA

We analysed mtDNA from a total of 270 individuals distributed along the 11 collection sites, based on the 740pb fragments of the control (D-Loop) region. Fifteen haplotypes were identified (Figure 2), with eleven being characteristic of *E. imbricata* and four of other species. Of the haplotypes of other species, three are specific to *C. caretta* (CC-A4.2 – 5.6%, CC-A4.1 – 0.7% and CC-A24.1 – 0.4%), and one to *L. olivacea* (Haplotype F – 1.1%), all of which are endemic to Brazil's nesting grounds (Bowen et al. 1998; Shamblin et al. 2014).

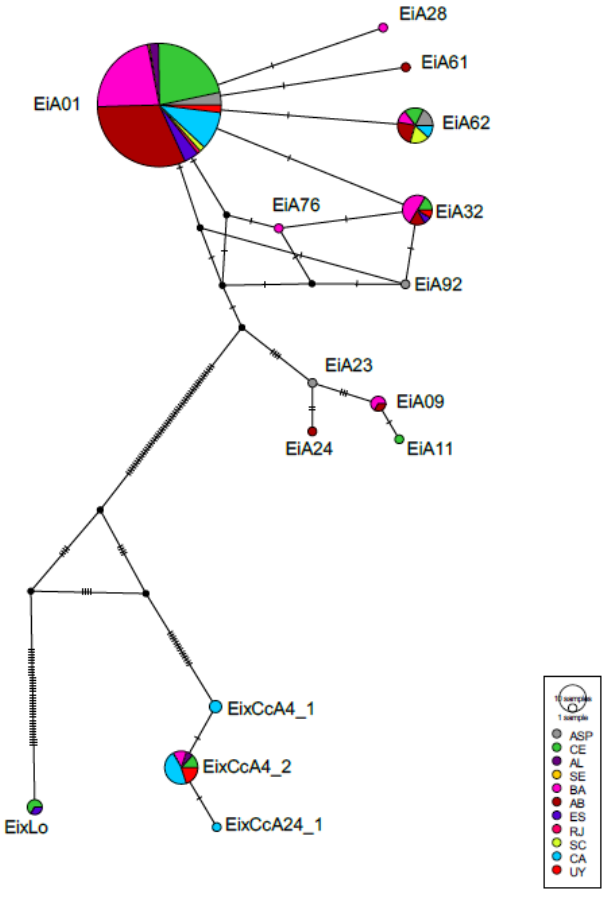

Figure 2. mtDNA (D-Loop) haplotype network constructed based on the Median-Joining method. Circles represent each of the identified haplotypes, size corresponds to the frequency of occurrence of the haplotype, and color represents the sampling location. Dashes between haplotypes represent the number of distinct bases between them.

The haplotype distribution (Figure 3) showed a predominance of haplotype EiA01, found in all areas and with a total frequency of 78%. Less frequent pure haplotypes were EiA62 (6%), EiA32 (4%), EiA09 (1%), and rare haplotypes, with only one occurrence each, were EiA11, EiA23, EiA24, EiA28, EiA61, EiA76 and EiA92. Two hybrid turtles with *L. olivacea* were found in Espírito Santo and one in Ceará, all of which presented the F haplotype. Of the 17 hybrids with *C. caretta*, observed at five sites, ten were found in Cassino, one in Alagoas and two in Bahia, Ceará and Uruguay. These hybrids showed a predominance of the CC-A4.2 haplotype, with one occurring in Alagoas (out of a total of 6), two in Bahia (total n = 61) and two in Ceará (total n = 56). Cassino was a different case, with a high frequency of hybrids with three distinct *C. caretta* haplotypes – CC-A4.2 (21%), CC-A4.1 (6%) and CC-A24.1 (3%) – and the presence of the most frequent *E. imbricata* haplotypes – EiA01 (64%) and EiA62 (6%) (total n = 33). Uruguay presented two *E. imbricata* haplotypes – EiA01 (50%) and EiA32 (12.5%) – and displayed only one haplotype of *C. caretta*, CCA4.2 (37.5%) (total n = 8).

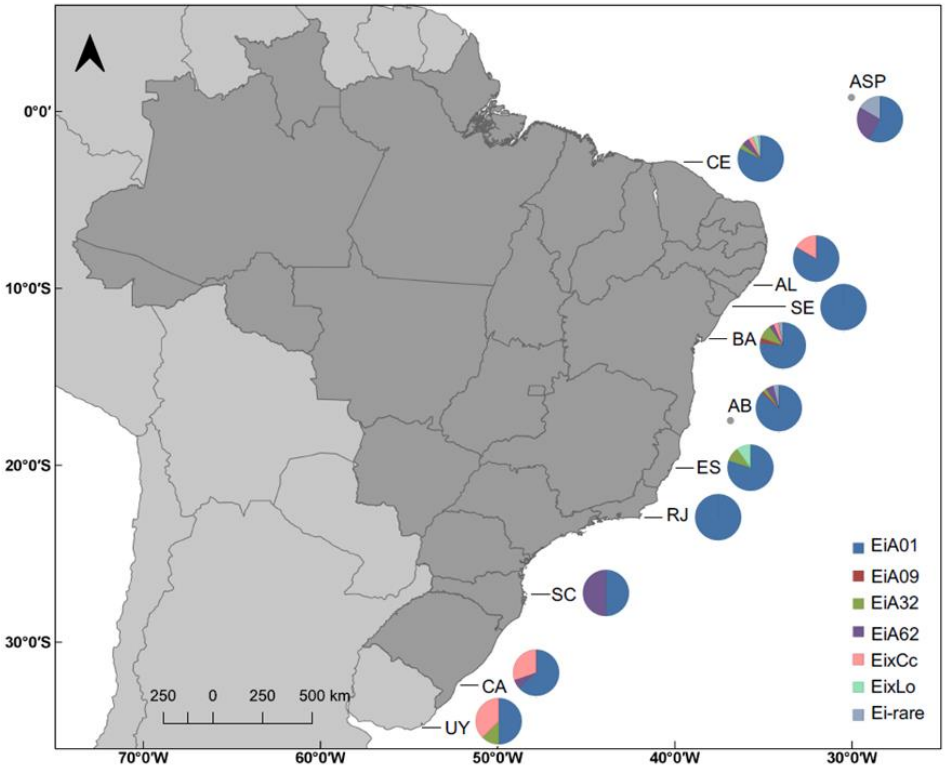

Figure 3. Geographic distribution of D-Loop haplotypes found in the mtDNA analysis along the South Western Atlantic. ASP - São Pedro and São Paulo Archipelago; (n =

12); AB - Abrolhos Archipelago (n = 75); AL – Alagoas (n = 6); CA – Cassino (n = 33); CE – Ceará (n = 56), BA – Bahia (n = 61); ES – Espírito Santo (n = 11); RJ - Rio de Janeiro (n = 2); SC - Santa Catarina (n = 6); SE – Sergipe (n = 1); UY – Uruguay (n = 7).

nDNA

Six haplotypes were found for marker RAG1 (n = 126): Hap3, species-specific for *E. imbricata*; Hap1 and Hap4, shared between *E. imbricata* and *L. olivacea*; Hap2, species-specific of *C. caretta*; Hap8, species-specific of *C. mydas*; and a previously unidentified haplotype (named Hap10, Supplementary Material 1). For RAG2 (n = 89) four haplotypes were found: Hap5, species-specific for *E. imbricata*; Hap2, species-specific of *C. caretta*; Hap6, species-specific of *C. mydas*; and a previously unidentified haplotype (named Hap7, Supplementary Material 1). For CMOS (n = 35) six haplotypes were found: Hap3, Hap5, Hap9 and Hap10, species-specific for *E. imbricata*; and Hap1 and Hap2, species-specific of *C. caretta*.

In general, homozygotes (RAG1 = 83%, RAG2 = 94%, CMOS = 63%) predominated, with heterozygotes represented in most cases by individuals that had alleles of different species (hybrids). The exception was RAG1, in which 11 of the 21 heterozygotes had a haplotype that can be found in both *E. imbricata* and *L. olivacea*, and therefore it was not possible to determine if they were hybrids or pure. The CMOS marker was the only one to identify eleven heterozygotes among the pure individuals, i.e., individuals that presented two distinct but species-specific *E. imbricata* haplotypes in each of the alleles.

In STRUCTURE, the best K obtained (K = 4) separated *E. imbricata* (Ei), *C. caretta* (Cc), *L. olivacea* (Lo) and *C. mydas* (Cm) in different groups (Figure 4), identifying the hybrids that had alleles of two or more distinct species. Observing the clusters, all *L. olivacea* x *C. caretta* (Cc x Lo) hybrids have proportional components of each parent species, indicating that they are F1 hybrids. *E. imbricata* x *C. caretta* (Ei x Cc) hybrids presented different degrees of hybridization. Among the analyzed samples, two individuals (CA23 and PF06), classified as E. imbricata through morphological analysis and mtDNA,

presented alleles of *C. caretta* and therefore appear in the 'Ei' group with 'Cc' components.

Figure 4. Cluster analysis performed in STRUCTURE for three nDNA markers (RAG1, RAG2 and CMOS). X-axis represents each of the individuals analysed, y-axis is the estimated mixture ratio of each of the parent species in the composition of these individuals. Species abbreviation and colors: blue, *E. imbricata* (Ei); orange, *C. caretta* (Cc); purple, *L. olivacea* (Lo); green, *C. mydas* (Cm).

For the three individuals previously identified as hybrids with *L. olivacea* (Ei x Lo) through mtDNA, it was possible to amplify only one nDNA marker (RAG1) in two individuals, and both presented Hap1, shared between *E. imbricata* and *L. olivacea*. Therefore, it was not possible to infer on the degree of hybridization of these individuals, which were therefore categorized as hybrids based only on mtDNA analysis. In addition to the *E. imbric*ata x *C. caretta* hybrids previously identified by mtDNA, it was possible to find two additional hybrids (CA23 and PF06) that presented mtDNA of *E. imbricata* and nDNA with one allele of each of the pure parent species, for all amplified markers. These hybrids have, therefore, the particularity of having mtDNA and morphology of *E. imbricata*, a fact observed at low frequency (1%) by Vilaça et al. (2012). Thus, these two individuals may be: 1) the result of the cross between a female *E. imbricata* and a male *C. caretta* (rare occurrence), and are therefore F1; or 2) a cross between a female *E. imbricata* female and a male Ei x Cc hybrid, hence generation >F1. Based only on the available data, it was not possible to distinguish between these categories.

NewHybrids correctly identified all pure individuals as *E. imbricata* ($P > 0.98$, Figure 5). Of the 17 *E. imbricata* x *C. caretta* hybrids identified through mtDNA, for five samples it was not possible to amplify any of the nuclear markers used in this work. Seven hybrids (AP56, CA24, CA25, CA31, CA36, CE04, CE51) were classified as F1 ($p > 0.90$) presenting for each allele a species-specific

haplotype of the parent species with no evidence of introgression for these samples. Five other hybrids (CA12, CA14, CA22, UY01, and UY05) were not detected in this analysis since they had species-specific *E. imbricata* haplotypes for both alleles, despite having *C. caretta* mtDNA, thus demonstrating signs of introgression with *E. imbricata.* Data on all hybrids identified at the South Western Atlantic, with mtDNA haplotype, parental species involved, and inference on generations, can be seen in Table 2.

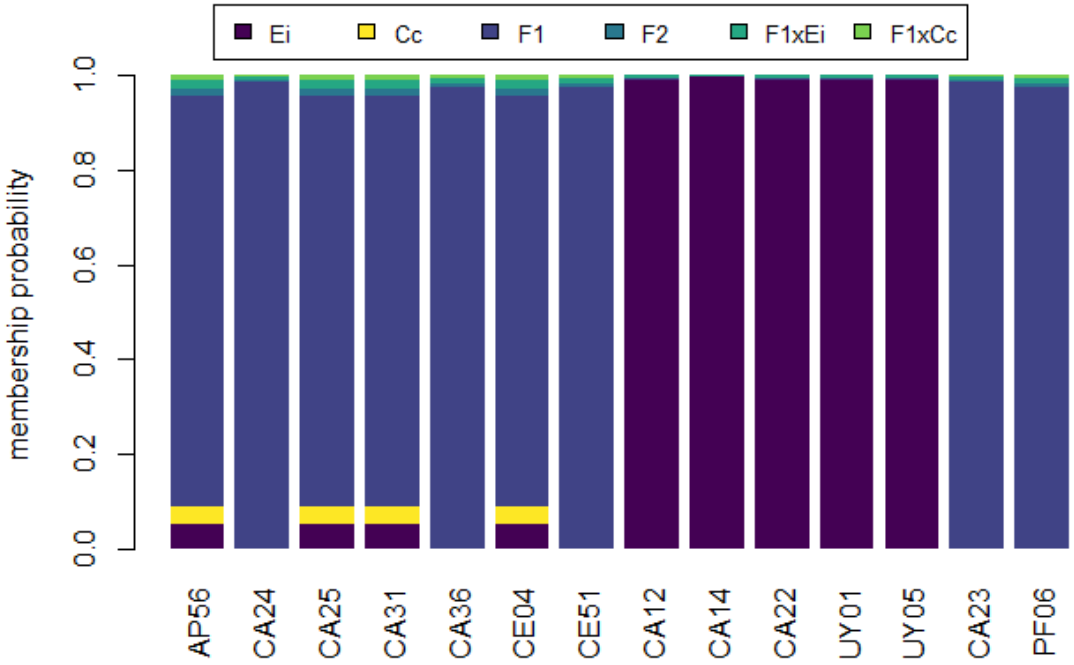

Figure 5. Assignment probability model for the 12 *E. imbricata* x *C. caretta* hybrids identified by mtDNA analysis (AP56 - UY05) and two additional hybrids identified only through nDNA (CA23 and PF06). The y-axis represents the probability that each individual belongs to each of the six categories presented (Ei, Cc, F1, F2, F1xEi e F1xCc).

Table 2. Hybrids identified at the South Western Atlantic, with sampling area, mtDNA haplotype, species involved, and inference on hybrid classes. AL – Alagoas, BA – Bahia, CA – Cassino, CE – Ceará, ES – Espirito Santo, UY – Uruguay.

Sample	Area	Haplotype	mtDNA	nDNA	♀ - ♂	Classes
AL02	AL	CCA4.2	EixCc	-	Cc - Ei	-
AP56	BA	CCA4.2	EixCc	EixCc	Cc - Ei	F1
PF06	BA	EiA01	Ei	EixCc	Ei - Cc or Ei - EixCc	F1 or F1xEi

SA10	BA	CCA4.2	EixCc	-	Cc - Ei	-
CA04	CA	CCA4.2	EixCc	-	Cc - Ei	-
CA12	CA	CCA4.2	EixCc	Ei	EixCc - Ei	F1xEi
CA14	CA	CCA4.2	EixCc	Ei	EixCc - Ei	F1xEi
CA22	CA	CCA4.2	EixCc	Ei	EixCc - Ei	F1xEi
CA23	CA	EiA01	Ei	EixCc	Ei - Cc or Ei - EixCc	F1 or F1xEi
CA24	CA	CCA4.2	EixCc	EixCc	Cc - Ei	F1
CA25	CA	CCA4.2	EixCc	EixCc	Cc - Ei	F1
CA31	CA	CCA4.2	EixCc	EixCc	Cc - Ei	F1
CA32	CA	CCA24.1	EixCc	-	Cc - Ei	-
CA33	CA	CCA4.1	EixCc	-	Cc - Ei	-
CA36	CA	CCA4.1	EixCc	EixCc	Cc - Ei	F1
CE04	CE	CCA4.2	EixCc	EixCc	Cc - Ei	F1
CE32	CE	Hap F	EixLo	Ei/Lo	Lo - Ei	-
CE43	CE	Hap F	EixLo	Ei/Lo	Lo - Ei	-
CE51	CE	CCA4.2	EixCc	EixCc	Cc - Ei	F1
ES05	ES	Hap F	EixLo	-	Lo - Ei	-
UY01	UY	CCA4.2	EixCc	Ei	EixCc - Ei	F1xEi
UY05	UY	CCA4.2	EixCc	Ei	EixCc - Ei	F1xEi

Discussion

In this work, we used mtDNA (D-Loop) and nDNA (RAG1, RAG2 and CMOS) markers to investigate hybridization in immature marine turtles in the South Western Atlantic, and identified 22 hybrid turtles in 270 samples (~ 8%), with 60% of them occurring at the extreme south of their distribution (South Brazil and Uruguay). A previous study conducted along the Brazilian coast with immature turtles identified as *E. imbricata* found four hybrids with *C. caretta* through mtDNA analysis (Proietti et al. 2014b). With the increase in geographical coverage and sample number, we identified 17 hybrid Ei x Cc at the coast of the South Western Atlantic, based on this marker. The highest frequency of these hybrids occurred at Cassino, where 10 out of the 33 turtles analysed (~30%) had *C. caretta* haplotypes, followed by Uruguay, which presented two hybrids in eight samples (25%). Proietti et al. (2014a) hypothesized that the high occurrence of hybrid turtles in temperate areas could be due to the adoption of the behavior of *C. caretta*, which occupies colder regions (Wallace et al. 2010), and not the tropical *E. imbricata* (León and

Bjorndal 2002; Mortimer and Donnelly 2008). Our results corroborate this hypothesis, and we suggest that other methods such as telemetry or diet analysis be used to confirm the differential behavior of hybrids.

Previous Ei x Cc records reported only the occurrence of mtDNA haplotype CCA4.2, the most common haplotype of females at Brazilian nesting grounds (Shamblin et al. 2014). In the present work, two other haplotypes (CCA4.1 and CCA24.1) were identified for the first time in hybrids; these haplotypes are also present only at nesting grounds in Brazil, but at lower frequency (Reis et al. 2010). Based on haplotype frequencies, Shamblin et al. (2014) suggested a possible regionalization of *C. caretta* populations at the Brazilian coast, into two Regional Management Units (RUMs): 1) Sergipe and Bahia 2) and Espírito Santo and Rio de Janeiro. The hybrids we identified presented haplotypes most frequently found in the first RMU, and therefore we can consider that they most likely originate from the Bahia/Sergipe rookeries, reinforcing the observation that this is the main region where loggerheads and hawksbills crossbreed.

We observed three hawksbill x olive ridley hybrids, a type of cross that had not yet been reported for immature marine turtles along the South Western Atlantic. These hybrids presented the F haplotype, described by Bowen et al. (1998) as characteristic of *L. olivacea* and originating from nesting areas of the Atlantic Ocean (in descending order of frequency: Suriname, Brazil and Guinea Bissau). Rookeries for this species in Brazil are concentrated between Bahia and Alagoas, with higher density in Sergipe state (Castilhos et al. 2011). Lara-Ruiz et al. (2006) observed two hybrids with haplotype F when analyzing the mtDNA of 119 adult *E. imbricata* samples from the north coast of Bahia. Vilaça et al. (2012) reported two occurrences of Ei x Lo at the coast of Bahia among 121 female *E. imbricata*, and concluded that all were the result of the crossing between a male *E. imbricata* and a female *L. olivacea* (F1). In addition, the authors state that these individuals showed no signs of introgression; therefore, they may be infertile or generated from rare hybridization events. The rarity of hawksbill and olive ridley hybrids can be explained by both species presenting highest reproductive population densities at different areas: olive ridleys nest mostly at Sergipe state, reducing the probability of crossings between them. Sanches and Bellini (1999)

observed that morphology may also influence this reproduction, since adult *L. olivacea* are smaller (mean CCL 73 cm; Silva et al. 2007) than *E. imbricata* (mean CCL 97 cm; Marcovaldi et al. 1999), which is in turn more similar to *C. caretta* (mean CCL 103 cm; Marcovaldi and Chaloupka 2007).

The analysis of both mtDNA and nDNA increased the number of hybrid turtle detections on the South Western Atlantic coast. Based on mtDNA alone 20 hybrids were identified, and nDNA analysis revealed two additional hybrids not identified by morphology or mtDNA. In addition, with the analysis of three nDNA markers, it was possible to infer that 50% of these hybrids were first generation and 36% were backcrossed between hybrids and pure *E. imbricata* (>F1). The generations of two hybrids (CA23 and PF06) could not be directly determined, since they had morphology and mtDNA of the same species (*E. imbricata*) but nDNA with alleles of two different species (Ei x Cc). With these characteristics the individuals could be: F1, result of the unusual crossing between female *E. imbricata* and male *C. caretta*; or >F1, the result of a backcross between a hybrid male (Ei x Cc) and an *E. imbricata* female.

As mentioned above, most first generation hybrids (F1) are a product of the mating between *C. caretta* females and *E. imbricata* males (Vilaça et al. 2012). Crossings between F1 females and both pure parent species also occur, but crossings of pure females and hybrid males are apparently less frequent. Female *E. imbricata*, *C. caretta* and Ei x Cc hybrids have different nesting peaks, with pure *C. caretta* nesting earlier than hybrids, which nest earlier than pure *E. imbricata* (Soares et al. 2017). Considering this, along with the gender bias of the reproductive groups, it is unlikely that the CA23 and PF06 individuals are the result of a cross between a female *E. imbricata* (pure) and a male *C. caretta* (F1). Since the *E. imbricata* nesting peak occurs when most *C. caretta* males have already left the area, the likelihood of them reproducing is reduced. Indeed, this type of hybrid cross was observed in only one of the 82 females analysed by Soares et al. (2017) at Bahia. This hybrid was also identified as *C. caretta* through morphological analysis, which did not occur in the individuals found in this study. Hybrid males (Ei x Cc) may show an intermediate reproductive period between pure parent species, as observed for hybrid females. In this case, they would

encounter more *E. imbricata* females than the *C. caretta* males. Additionally, considering that backcrosses between Ei x Cc and pure *E. imbricata* have been observed more frequently than introgression with *C. caretta*, it is likely that CA23 and PF06 are a result of the cross between *E. imbricata* and hybrid males.

Conclusions

Our study shows the importance of the combined use of mtDNA and nDNA markers in the evaluation of hybridization among marine turtles. Although mtDNA analysis is of paramount importance in the study of hybrids, nDNA analysis is also crucial for identifying generations/parental species, as well as for detecting hybrids in cases where mtDNA cannot (e.g. hybrid backcrosses with pure *E. imbricata*). This is confirmed by our observation of two hybrids that presented *E. imbricata* morphology and mtDNA, which could only be identified as Ei x Cc through nDNA. This observation may have been underestimated since we were not able to amplify the three nDNA markers for all samples. Another limitation was that the only nuclear marker amplified for all animals (RAG1) presents two shared haplotypes between *E. imbricata* and *L. olivacea*, and therefore it is not possible to infer conclusively about Ei x Lo hybrids with nDNA analysis. The hybridization events between *E. imbricata* and *L. olivacea* seems to be rare, but additional studies are necessary to identify species-specific haplotypes, allowing us to infer on the generation and parental species of these hybrids.

Considering the high frequency of hybrids found in the South Western Atlantic, studies on the behavior, distribution, feeding strategy, migration and demography of hybrid turtles should be performed. Satellite tracking, stable isotope analysis and more comprehensive molecular tools such as genomics (e.g. SNP analysis), are techniques that would aid in understanding the process and better determine the ecological role of these hybrids, as well as their influence on marine turtle populations. According to Bohling (2016), the greatest concern regarding hybridization events is the extinction of genetic, phenotypic and/or evolutionary units due to a continuous hybridization process or hybrid vigor. Schwartz et al. (2007) suggest that this process be biologically and ecologically

monitored continuously, providing a framework for determining and tracking the extent of hybridization, trends over time, and results of management strategies.

There are currently no guidelines for the management of hybrids and hybrid zones in Brazil, and most existing programs are focused on areas where the event has anthropogenic causes such as habitat change (Crispo et al. 2011) and overexploitation (Bohling and Waits 2015). To avoid the genetic extinction of pure species, hybrid animals can be eliminated from the reproductive stock by eradication or sterilization; this type of action has already been carried out to control the hybridization between *Cyprinodon variegatus* and *Cyprinodon bovinus*, which was successful due to the limited geographic extension of the hybrid group (Echelle and Echelle 1997). Considering the current endangered status of marine turtles and the high frequency of hybrids along the South Western Atlantic coast, continuous monitoring should be carried out to assess the fitness, genetic integrity and detect the extent of changes in the gene pools of the involved populations. This is fundamental to evaluate if management of hybrid individuals should be considered, and ensure the conservation of SWA marine turtle populations.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Acknowledgements

C. Brito is a graduate student of the Programa de Pós-graduação em Oceanografia Biológica, and received a scholarship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), which is suffering serious budget cuts by the current Brazilian government. We thank Luis F. Marins and Luiz Felipe Cestari Dumont for suggestions on this manuscript. We thank Projeto Tamar – Fundação Tamar and Centro Tamar-ICMBio, Núcleo de Educação e Monitoramento Ambiental (NEMA), Instituto Biota de Conservação, Associação

Civil Karumbé, Parque Nacional Marinho dos Abrolhos and Reserva Biológica Marinha Arvoredo for providing samples and field assistance. This is a contribution of the research group "ECOMEGA – Ecologia da Megafauna Marinha".

Financing

The Rufford Foundation, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Abreu-Grobois, F.A., Horrocks, J. a., Formia, A., Dutton, P.H., LeRoux, R. a., Velez-Zuazo, X., Soares, L.S., Meylan, A.B., 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis., in: Frick, M., Panagopoulou, A., Rees, A.F., Williams, K. (Eds.), Proceedings of the 26th Annual Symposium on Sea Turtle Biology, Book of Abstracts. p. 179. https://doi.org/10.1007/s10592-005-9102-9

Allendorf, F.W., Leary, R.F., Spruell, P., Wenburg, J.K., 2001. The problems with hybrids: Setting conservation guidelines. Trends Ecol. Evol. 16, 613–622. https://doi.org/10.1016/S0169-5347(01)02290-X

Alvarez-Varas, R., Berzins, R., Bilo, K., Chevalier, J., Chevallier, D., Thoisy, B.D.E., Fallabrino, A., Cruz, M.G., Kelez, S., Lopez-mendilaharsu, M., Marcovaldi, M.A., Mast, R.B., Medrano, C., Miranda, C., Nalovic, M.A., Prosdocimi, L., Rguez-barón, J.M., Santos, A., Soares, L., Thome, J., Vallejo, F., Vélez-rubio, G., 2016. Sea Turtles of South America. SWOT Rep. 14–27.

Anderson, E.C., Thompson, E. a, 2002. A model-based method for identifying species hybrids using multilocus data. Genetics 160, 1217–1229. https://doi.org/test statistics; hybrids

Bandelt, H.-J., Forster, P., Rohl, A., 1999. Median-Joining Networks for Inferring Intraspecific Phylogenies. Mol. Biol. 16, 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bohling, J.H., 2016. Strategies to address the conservation threats posed by hybridization and genetic introgression. Biol. Conserv. 203, 321–327. https://doi.org/10.1016/j.biocon.2016.10.011

Bohling, J.H., Waits, L.P., 2015. Factors influencing red wolf-coyote hybridization in eastern North Carolina, USA. Biol. Conserv. 184, 108–116. https://doi.org/10.1016/j.biocon.2015.01.013

Bolten, a B., 2003. Variation in sea turtle life history patterns: neritic vs. oceanic developmental stages. Biol. sea turtles 2, 243–257. https://doi.org/10.1201/9781420040807.ch9

Bowen, B.W., Clark, a M., Abreu-Grobois, F. a, Chaves, a, Reichart, H. a, Ferl, R.J., 1998. Global phylogeography of the ridley sea turtles (Lepidochelys spp.) as inferred from mitochondrial DNA sequences. Genetica 101, 179–189. https://doi.org/10.1023/A:1018382415005

Bowen, B.W., Karl, S.A., 2007. Population genetics and phylogeography of sea turtles. Mol. Ecol. 16, 4886–4907. https://doi.org/10.1111/j.1365-294X.2007.03542.x

Castilhos, J.C. De, Coelho, C.A., Argolo, J.F., Allan, E., 2011. Avaliação do Estado de Conservação da Tartaruga Marinha Lepidochelys olivacea (Eschscholtz, 1829) no Brasil. Biodiversidade Bras. 1, 26–34.

Conceição, M.B., Levy, J.A., Marins, L.F., Marcovaldi, M.A., 1990. Electrophoretic characterization of a hybrid between Eretmochelys imbricata and Caretta caretta (Cheloniidae). Comp. Biochem. Physiol. Part B Comp. Biochem. 97B, 275–278. https://doi.org/10.1016/0305-0491(90)90280-7

Crispo, E., Moore, J.S., Lee-Yaw, J.A., Gray, S.M., Haller, B.C., 2011. Broken barriers: Human-induced changes to gene flow and introgression in animals: An examination of the ways in which humans increase genetic exchange among populations and species and the consequences for biodiversity. BioEssays 33, 508–518. https://doi.org/10.1002/bies.201000154

Crochet, P.A., Chen, J.Z., Pons, J.M., Lebreton, J.D., Hebert, P.D.N., Bonhomme, F., 2003. Genetic differentiation at nuclear and mitochondrial

loci among large white-headed gulls: Sex-biased interspecific gene flow? Evolution (N. Y). 57, 2865–2878. https://doi.org/10.1111/j.0014-3820.2003.tb01527.x

Crossman, C.A., Taylor, E.B., Barrett-Lennard, L.G., 2016. Hybridization in the Cetacea: Widespread occurrence and associated morphological, behavioral, and ecological factors. Ecol. Evol. 6, 1293–1303. https://doi.org/10.1002/ece3.1913

da Silva, A.C.C.D., de Castilhos, J.C., Lopez, G.G., Barata, P.C.R., 2007. Nesting biology and conservation of the olive ridley sea turtle (Lepidochelys olivacea) in Brazil, 1991/1992 to 2002/2003. J. Mar. Biol. Assoc. UK 87, 1047. https://doi.org/10.1017/S0025315407056378

Echelle, A. a, Echelle, A.F., 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs Pupfish and Sheepshead Minnow. Conserv. Biol. 11, 153–161. https://doi.org/Genetic Considerations in Ecological Restoration

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol. Ecol. 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Garman, S., 1880. On certain species of Chelonioidae. Bull. Museum Comp. Zool. v.6, 123–126.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.

Harrison, R.G., 1990. Hybrid zones: windows on evolutionary process., in: Futuyma, D., Antonovics, J. (Eds.), Oxford Surveys in Evolutionary Biology. Oxford University Press, pp. 69–128.

IUCN, 2018. The IUCN Red List of Threatened Species [WWW Document]. URL www.iucnredlist.org (accessed 2.14.18).

James, M.C., Martin, K., Dutton, P.H., 2004. Loggerhead Turtle , Caretta caretta , and the First Record of a Green Turtle in Atlantic Canada. Can. Field-Naturalist 118, 579–582.

Karl, S.A., Bowen, B.W., Avise, J.C., 1995. Hybridization Among the Ancient Mariners : Characterization of Marine Turtle Hybrids With Molecular

Genetic Assays. J. Hered. 86, 262–268.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15, 1179–1191. https://doi.org/10.1111/1755-0998.12387

Lara-Ruiz, P., Lopez, G.G., Santos, F.R., Soares, L.S., 2006. Extensive hybridization in hawksbill turtles (Eretmochelys imbricata) nesting in Brazil revealed by mtDNA analyses. Conserv. Genet. 7, 773–781. https://doi.org/10.1007/s10592-005-9102-9

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Leigh, J.W., Bryant, D., 2015. POPART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410

León, Y.M., Bjorndal, K.A., 2002. Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. Mar. Ecol. Prog. Ser. 245, 249–258. https://doi.org/10.3354/meps245249

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lima, E.H.S.M., Melo, M.T.D., Godfrey, M.H., Barata, P.C.R., 2013. Sea turtle in the waters of Almofala, Ceará, in Northeastern Brazil, 2001-2010. Mar. Turt. Newsl. 5–9. https://doi.org/0839-7708

Mallet, J., 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20, 229–237. https://doi.org/10.1016/j.tree.2005.02.010

Marcovaldi, M. a., Lopez, G.G., Soares, L.S., Santos, A.J.B., Bellini, C., Barata, P.C.R., 2007. Fifteen Years of Hawksbill Sea Turtle (Eretmochelys imbricata) Nesting in Northern Brazil. Chelonian Conserv. Biol. 6, 223. https://doi.org/10.2744/1071-8443(2007)6[223:FYOHST]2.0.CO;2

Marcovaldi, M., Chaloupka, M., 2007. Conservation status of the loggerhead sea turtle in Brazil: an encouraging outlook. Endanger. Species Res. 3, 133–143. https://doi.org/10.3354/esr003133

Marcovaldi, M., Lopez, G., Soares, L., López-Mendilaharsu, M., 2012. Satellite tracking of hawksbill turtles (Eretmochelys imbricata) nesting in northern Bahia, Brazil: turtle movements and foraging destinations. Endanger. Species Res. 17, 123–132. https://doi.org/10.3354/esr00421

Marcovaldi, M.A., Laurent, A., 1996. A Six Season Study of Marine Turtle Nesting at Praia do Forte , Bahia , Brazil , with Implications for Conservation and Management. Herpetologica 2, 55–59.

Marcovaldi, M.Â., Lopez, G.G., Soares, L.S., Santos, A.J.B., Bellini, C., Santos, A.S. dos, Lopez, M., 2011. Avaliação do estado de conservação da tartaruga marinha Eretmochelys imbricata (Linnaeus, 1766) no Brasil. Biodiversidade Bras. 1, 20–27.

Marcovaldi, M.Â., Vieitas, C.F., Godfrey, M.H., 1999. Nesting and conservation management of hawksbill turtles (Eretmochelyz imbricata) in Northern Bahia, Brazil. Chelonian Conserv. Biol. 3, 301–307.

Monteiro, D.S., Bugoni, L., Estima, S., 2006. Stranding and sea turtle fisheries interactions along the coast of Rio Grande do Sul state, Brazil, in: Book of Abstracts, Twenty-Sixth Annual Symposium on Sea Turtle Biology and Conservation. International Sea Turtle Society, p. 257.

Monteiro, D.S., Estima, S.C., Gandra, T.B.R., Silva, A.P., Bugoni, L., Swimmer, Y., Seminoff, J.A., Secchi, E.R., 2016. Long-term spatial and temporal patterns of sea turtle strandings in southern Brazil. Mar. Biol. 163. https://doi.org/10.1007/s00227-016-3018-4

Mortimer, J. a, Donnelly, M., 2008. IUCN Red List Status Assessment: Hawksbill Turtle (Eretmochelys imbricata). IUCN Red List Threat. Species 112. https://doi.org/10.2307/1564433

Naro-Maciel, E., Le, M., FitzSimmons, N.N., Amato, G., 2008. Evolutionary relationships of marine turtles: A molecular phylogeny based on nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 49, 659–662. https://doi.org/10.1016/j.ympev.2008.08.004

Payseur, B.A., Rieseberg, L.H., 2016. A genomic perspective on hybridization and speciation. Mol. Ecol. 25, 2337–2360. https://doi.org/10.1111/mec.13557

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Proietti, M.C., Reisser, J., Marins, L.F., Marcovaldi, M. a, Soares, L.S., Monteiro, D.S., Wijeratne, S., Pattiaratchi, C., Secchi, E.R., 2014a. Hawksbill × loggerhead sea turtle hybrids at Bahia, Brazil: where do their offspring go? PeerJ 2, e255. https://doi.org/10.7717/peerj.255

Proietti, M.C., Reisser, J., Marins, L.F., Rodriguez-Zarate, C., Marcovaldi, M. a, Monteiro, D.S., Pattiaratchi, C., Secchi, E.R., 2014b. Genetic structure and natal origins of immature hawksbill turtles (Eretmochelys imbricata) in Brazilian waters. PLoS One 9, e88746. https://doi.org/10.1371/journal.pone.0088746

Proietti, M.C., Reisser, J., Secchi, E.R., 2012. Foraging by Immature Hawksbill Sea Turtles at Brazilian Islands. Mar. Turt. Newsl. 135, 4–8.

Prosdocimi, L., Bruno, I., Diaz, L., Carman, V.G., Albareda, D.A., Remis, M.I., 2014. Southernmost reports of the hawksbill sea turtle, eretmochelys imbricata, in temperate waters of argentina and evidence of a hybrid origin supported by mitochondrial dna analysis. Herpetol. Rev. 45, 1–5.

Reis, E.C., Soares, L.S., Lôbo-Hajdu, G., 2010a. Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conserv. Genet. 11, 1587–1591. https://doi.org/10.1007/s10592-009-9973-2

Reis, E.C., Soares, L.S., Vargas, S.M., Santos, F.R., Young, R.J., Bjorndal, K.A., Bolten, A.B., Lôbo-Hajdu, G., 2010b. Genetic composition, population structure and phylogeography of the loggerhead sea turtle: Colonization hypothesis for the Brazilian rookeries. Conserv. Genet. 11, 1467–1477. https://doi.org/10.1007/s10592-009-9975-0

Rieseberg, L., Wendell, J.F., 1993. Gene flow and its consequences in plants, Hybrid zones and the evolutionary process.

Rosenfield, J.A., Nolasco, S., Lindauer, S., Sandoval, C., Kodric-Brown, A., 2004. The role of hybrid vigor in the replacement of pecos pupfish by its hybrids with sheepshead minnow. Conserv. Biol. 18, 1589–1598. https://doi.org/10.1111/j.1523-1739.2004.00356.x

Sales, G., Giffoni, B.B., Barata, P.C.R., 2008. Incidental catch of sea turtles by the Brazilian pelagic longline fishery. J. Mar. Biol. Assoc. UK 88, 853–864. https://doi.org/10.1017/S0025315408000441

Sanches, T.M., Bellini, C., 1999. Juvenile Eretmochelys imbricata and Chelonia mydas in the Archipelago of Fernando de Noronha, Brazil. Chelonian Conserv. Biol. 3, 308–311.

Santana, A., Soares, L., Marcovaldi, M.Â., Monteiro, S., 2011. Avaliaçao do estado de conservaçao da tartaruga marinha Caretta caretta Linnaeus, 1758. Biodiversidade Bras. 1, 3–11. https://doi.org/10.1111/j.1439-0426.2009.01342.x

Santos, A.S., Almeida, A.P., Santos, A.J.B., Giffoni, B., Gallo, B., Baptistotte, C., Coelho, C. a., Lima, E.H.S.M., Sales, G., Lopez, G.G., Stahelin, G., Becker, H., Castilhos, J.C., Thomé, J.C. a., Wanderlinde, J., Marcovaldi, M. a. G., Mendilaharsu, M.M.L., Damasceno, M.T., Barata, P.C.R., Sforza, R., 2011. Plano de ação nacional para conservação das tartarugas marinhas.

Schwartz, M.K., Luikart, G., Waples, R.S., 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol. Evol. 22, 25–33. https://doi.org/10.1016/j.tree.2006.08.009

Seminoff, J.A., Karl, S.A., Schwartz, T.S., Resendiz, A., 2003. Hybridization of the green turtle (Chelonia mydas) and hawksbill turtle (Eretmochelys imbricata) in the Pacific Ocean: Indication of an absence of gender bias in the directionality of crosses. Bull. Mar. Sci. 73, 643–652.

Shamblin, B.M., Bolten, A.B., Abreu-Grobois, F.A., Bjorndal, K.A., Cardona, L., Carreras, C., Clusa, M., Monzón-Argüello, C., Nairn, C.J., Nielsen, J.T., Nel, R., Soares, L.S., Stewart, K.R., Vilaça, S.T., Türkozan, O., Yilmaz, C., Dutton, P.H., 2014. Geographic patterns of genetic variation in a broadly distributed marine vertebrate: New insights into loggerhead turtle stock structure from expanded mitochondrial DNA sequences. PLoS One 9,

e85956. https://doi.org/10.1371/journal.pone.0085956

Soares, L.S., Bjorndal, K.A., Bolten, A.B., dei Marcovaldi, M.A.G., Luz, P.B., Machado, R., Lo, R., McDaniel, S.F., Payton, A.C., Waltzek, T.B., Wayne, M.L., 2018. Effects of hybridization on sea turtle fitness. Conserv. Genet. 0, 1–12. https://doi.org/10.1007/s10592-018-1101-8

Soares, L.S., Bolten, A.B., Wayne, M.L., Vilaça, S.T., Santos, F.R., dei Marcovaldi, M.A.G., Bjorndal, K.A., 2017. Comparison of reproductive output of hybrid sea turtles and parental species. Mar. Biol. 164. https://doi.org/10.1007/s00227-016-3035-3

Sunnucks, P., 2000. Efficient genetic markers for population biology. Trends Ecol. Evol. 15, 199–203. https://doi.org/10.1016/S0169-5347(00)01825-5

Vélez-Rubio, G.M., Estrades, A., Fallabrino, A., Tomás, J., 2013. Marine turtle threats in Uruguayan waters: Insights from 12 years of stranding data. Mar. Biol. 160, 2797–2811. https://doi.org/10.1007/s00227-013-2272-y

Vilaça, S.T., Santos, F.R. Dos, 2013. Molecular Data for the Sea Turtle Population in Brazil. Dataset Pap. Sci. 2013, 1–7. https://doi.org/10.1155/2013/196492

Vilaça, S.T., Vargas, S.M., Lara-Ruiz, P., Molfetti, É., Reis, E.C., Lôbo-Hajdu, G., Soares, L.S., Santos, F.R., 2012. Nuclear markers reveal a complex introgression pattern among marine turtle species on the Brazilian coast. Mol. Ecol. 21, 4300–4312. https://doi.org/10.1111/j.1365-294X.2012.05685.x

Wallace, B.P., DiMatteo, A.D., Hurley, B.J., Finkbeiner, E.M., Bolten, A.B., Chaloupka, M.Y., Hutchinson, B.J., Alberto Abreu-Grobois, F., Amorocho, D., Bjorndal, K.A., Bourjea, J., Bowen, B.W., Dueñas, R.B., Casale, P., Choudhury, B.C., Costa, A., Dutton, P.H., Fallabrino, A., Girard, A., Girondot, M., Godfrey, M.H., Hamann, M., López-Mendilaharsu, M., Marcovaldi, M.A., Mortimer, J.A., Musick, J.A., Nel, R., Pilcher, N.J., Seminoff, J.A., Troëng, S., Witherington, B., Mast, R.B., 2010. Regional Management Units for Marine Turtles: A Novel Framework for Prioritizing Conservation and Research across Multiple Scales. PLoS One 5, 1–11. https://doi.org/10.1371/journal.pone.0015465

Wayne, R.K., Shaffer, H.B., 2016. Hybridization and endangered species protection in the molecular era. Mol. Ecol. 25, 2680–2689. https://doi.org/10.1111/mec.13642

Whitham, T., Morrow, P., Potts, B., 1991. Conservation of Hybrid Plants. Science (80-.). https://doi.org/doi:10.1126/science.254.5033.779-b

Witzell, W.N., 2002. Immature Atlantic loggerhead turtles (Caretta caretta): suggested changes to the life history model. Herpetol. Rev.

Wood, J., Wood, F., Critchley, K., 1983. Hybridization of Chelonia mydas and Eretmochelys imbricata. Copeia 839–842.

Supplementary material

S1. Nuclear DNA marker haplotypes, as described by Vilaça et al. (2012), and two previously undescribed haplotype (Hap10 – RAG1 and Hap7 – RAG2).

Haplotypes – RAG 1:

>Hap1- GenBank accession nº JF415126
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCc
GCCTGCCaATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATCC
TTCCTGAACATCCTCAATTCCcTGGCTGTGCGATGCCCAGTGAAAGAATGT
GATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAAA
GAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGACC
GAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTGA
GAGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGTG
ATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGAA
ATGAACACAGAACTTTGTTCCTGCTGGCTCTGAGAGCAAGAAATGAACACA
GA

>Hap2- GenBank accession nº JF415120
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCT
GCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATC
CTTCCTGAACATCCTCAATTCCTTGGCTGTGCGATGCCCAGTGAAAGAATG
TGATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAA
AGAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAC
CGAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTG
AGAGAACTTAAGCTTCAAGTCAAAACTTTTGCTGAGAAAGAAGAAGGAGGT

GATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGA
AATGAACACAGA

>Hap3- GenBank accession nº JF415128
TGTGACCATATTCTGGCAGACCCAGTGGAAACAACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTtTGGGCAGCTATTGTCCcG
CCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATCC
TTCCTGAACATCCTCAATTCCcTGGCTGTGCGATGCCCAGTGAAAGAATGT
GATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAAA
GAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGACC
GAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTGA
GAGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGTG
ATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGAA
ATGAACACAGA

>Hap4- GenBank accession nº JF415123
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCc
GCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATC
CTTCCTGAACATCCTCAATTCCcTGGCTGTGCGATGCCCAGTGAAAGAATG
TGATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAA
AGAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAC
CGAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTG
AGAGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGT
GATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGA
AATGAACACAGA

>Hap5-GenBank accession nº JF415124
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCc
GCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATC
CTTCCTGAACATCCTCAATTCCcTGGCTGTGCGATGCCCAGTGAAAGAATG
TGATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAA
AGAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAg
CGAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTG
AGAGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGT
GATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGA
AATGAACACAGA

>Hap6-GenBank accession nº JF415121
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCT

GCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATC
CTTCCTGAACATCCTCAATTCCTTGGCTGTGCGATGCCCAGTGAAAGAATG
TGATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAA
AGAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAg
CGAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTG
AGAGAACTTAAGCTTCAAGTCAAAACTTTTGCTGAGAAAGAAGAAGGAGGT
GATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGA
AATGAACACAGA

>Hap7-GenBank accession nº JF415122
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCc
GCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATC
CTTCCTGAACATCCTCAATTCCTTGGCTGTGCGATGCCCAGTGAAAGAATG
TGATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAA
AGAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAg
CGAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTG
AGAGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGT
GATATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGA
AATGAACACAGA

>Hap8- GenBank accession nº JF415125
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCc
aCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATCC
TTCCTGAACATtCTCAATTCCcTGGCTGTGCGATGCCCtGTGAAAGAATGTG
ATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAAAG
AGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGACCG
AGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTGAG
AGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGTGA
TATAAAGGCTGTGTGCCTAACTTTGTTCCTGCTGGCTCTGAGAGCAAGAAA
TGAACACAGA

>Hap9- GenBank accession nº JF415127
TGTGACCATATTCTGGCAGACCCAGTGGAAACGACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTATGGGCAGCTATTGTCCc
GCCTGCCaATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATCC
TTCCTGAACATCCTCAATTCCcTGGCTGTGCGATGCCCAGTGAAAGAATGT
GATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAAA
GAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAGC
GAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTGA

GAGAACTTAAGCTTCAAGTCAAAgCTTTTGCTGAGAAAGAAGAAGGAGGTG
ATATAAAGGCTGTGTGCCTA

>Hap10
TGTGACCATATTCTGGCAGACCCAGTGGAAACgACATGTAGCCACTTATTC
TGTAGAACCTGCATCCTTAAGTGCCTCAAAGTTaTGGGCAGCTATTGTCCC
GCCTGCCGATACCCTTGCTTCCCTACCGACCTGGTGAGTCCAGTGAAATC
CTTCCTGAACATCCTCAATTCCtTGGCTGTGCGATGCCCAGTGAAAGAATG
TGATGAGGAGGTTTTGCTGGGCAAATACTGCCATCATTTGTCCAGTCACAA
AGAGGTGAAAAGGAAAGAGATTTACACGCACGTAAATAAAGGTGGCCGAC
CGAGGCAACACTTACTCTCATTGACCAGGAGAGCGCAAAAGCATCGTCTG
AGAGAACTTAAGCTTCAAGTCAAAGCTTTTGCTGAGAAAGAAGAAGGAGGT
GATATAAAGGCTGTGTGCCT

Haplotypes – RAG 2:
>Hap1- GenBank accession nº JF415134
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGAGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACAGTTCATAGCCGAGGTAAAAGCATGAGTGTTATATTT
GGGGGAAGGTCATATATCCCTCTTGGACAgAGAACCACAGAAAAATGGAAT
AGTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGAT
GCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCATG
TCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTTG
AAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCAC
TAGGCAGCCCAGCTGTGAGCTGCACCATCTTGCCTGGGGGGATCTCTGTC
TCCAGTGCTCTTGTGACTCAGACTGGCGATAAAGAGTTTGTCATGGTTGGG
GGCTACCA

>Hap2- GenBank accession nº JF415129
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGAGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACAGTTCATAGCCGAGGTAAAAGCATGAGTGTTATATTT
GGGGGAAGGTCATATATCCCTgTTGGACAAAGAACCACAGAAAAATGGAAT
AGTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGAT
GCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCATG
TCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTTG
AAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCAC
TAGGCAGCCCAtCTGTGAGCTGCACCATCTTGCCTGGGGGGATCTCTGTCT

CCAGTGCTCTTGTGACTCAGACTGGCGATAAAGAGTTTGTCATGGTTGGG
GGCTACCA

>Hap3- GenBank accession nº JF415130
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGgGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACAGTTCATAGCCGAGGTAAAAGCATGAGTGTTATATTT
GGGGGGAAGGTCATATATCCCTCTTGGACAAAGAACCACAGAAAAATGGAA
TAGTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGA
TGCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCAT
GTCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTT
GAAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCA
CTAGGCAGCCCAtCTGTGAGCTGCACCATCTTGCCTGGGGGGGATCTCTGT
CTCCAGTGCTCTTGTGACTCAGACTGGCGATAAAGAGTTTGTCATGGTTGG
GGGCTACCA

>Hap4- GenBank accession nº JF415133
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGgGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACAGTTCATAGCCGAGGTAAAAGCATGAGTGTTATATTT
GGGGGAAGGTCATATATCCCTaTTGGACAAAGAACCACAGAAAAATGGAAT
AGTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGAT
GCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCATG
TCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTTG
AAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCAC
TAGGCAGCCCAtCTGTGAGCTGCACCATCTTGCCTGGGGGGGATCTCTGTCT
CCAGTGCTCTTGTGACTCAGACTGGCGATAAAGAGTTTGTCATGGTTGGG
GGCTACCA

>Hap5- GenBank accession nº JF415131
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGgGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACAGTTCATAGCCGAGGTAAAAGCATGAGTGTTATATTT
GGGGGAAGGTCATATATCCCTCTTGGACAgAGAACCACAGAAAAATGGAAT
AGTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGAT
GCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCATG

TCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTTG
AAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCAC
TAGGCAGCCCAGCTGTGAGCTGCACCATCTTGCCTGGGGGGATCTCTGTC
TCCAGTGCTCTTGTGACTCAGACTGGCGATAAAGAGTTTGTCATGGTTGGG
GGCTACCA

>Hap6- GenBank accession nº JF415132
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGgGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACgGTTCATAGCCGAGGTAAAAGtATGAGTGTTATATTTG
GGGGAAGGTCATATATCCCTCTTGGACAAAGAACCACAGAAAAATGGAATA
GTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGAT
GCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCATG
TCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTTG
AAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCAC
TAGGCAGCCCAGCTGTGAGCTGCACCATCTTGCCTGGGGGGATCTCTGTC
TCCAGTGCTCTTGTGACTCAGACTGGCGATAAAGAGTTTGTCATGGTTGGG
GGCTACCA

>Hap7
CTCCGTTACCCTGCTGTCTGCGCTGTCAGGGGCAGAACAGAGTCTGAGAA
ATGCCAGTATGTCATCCATGGTGGGAAAACGCCAAACAATGAACTATCTGA
TAAGATTTATGTTATGGGTATAGTATGCAAGCCTAGCAAGAAAATCACCTTT
AAATGCACTGAGAAAGAGTTAGTTGGGGATGTTCCTGAAGCCAGATATGGT
CATACCATTAACACAGTTCATAGCCGAGGTAAAAGCATGAGTGTTATATTT
GGGGGAAGGTCATATATCCCTCTTGGACAGAGAACCACAGAAAAATGGAA
TAGTGTAGTAGACTGTATGCCCCATGTGTTTTTGGTTGATTTTGAGTTTGGA
TGCTGTACCTCATACATCCTTCCAGAGCTTCAGGATGGACTTTCTTTCCAT
GTCTCCATTGCCAGAAATGATACCATCTACATCCTAGGGGGTCATTCTCTT
GAAAATAACATCAGACCCCCCAACTTATACAGACTAAAAATTGACCTGCCA
CTAGGCAGCCCAGCTGTGAGCTGCACCATCTTGCCTGGGGGGATCTCTGT
CTCCAGTGCTCTTGTGACTCAAACTGGCGATAAAGAGTTTGTCATGGTTGG
GGGCTACCA

Haplotypes – CMOS:
>Hap1- GenBank accession nº JF415097
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT

AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGATAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap2- GenBank accession nº JF415098
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGATAGTGTGTCtCCAGGTCCCCAGCTTTACCAGCA
GGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap3- GenBank accession nº JF415099
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGAcAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap4- GenBank accession nº JF415100
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT

AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGaGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGATAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap5- GenBank accession nº JF415101
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCaGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGAcAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap6- GenBank accession nº JF415102
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCaGAGTCCCTGaGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGAcAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap7- GenBank accession nº JF415103
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCTGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT

AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGGTGATGGGGGTGGATGTGGCAGAG
AACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGAtATTGTGACTG
GtTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTG
CTAATGTcTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGT
GCTCCCAAAAGCTGGAAGAtAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap8- GenBank accession nº JF415104
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATaGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGAtAGTGTGTCtCCAGGTCCCCAGCTTTACCAGCA
GGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap9- GenBank accession nº JF415105
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACgCCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGAcAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap10- GenBank accession nº JF415106
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCCGTCTACAAGGCCACCTACCATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACgCCATGACAATGTGGTACGTGTTGT

AGCTGCCAGCACATGTGCTtCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGATGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGACATTGTGACTGG
CTTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGC
TAATGTGTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTG
CTCCCAAAAGCTGGAAGAcAGTGTGTCCCCAGGTCCCCAGCTTTACCAGC
AGGGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA

>Hap11- GenBank accession nº JF415107
TCGACTGGGATCAATTGTGCCTACTACAGCCCCTGGGCTCTGGGGGCTTT
GGGTCTGTCTACAAGGCCACCTACTATGGGGAGACAGTGGCTGTGAAACA
GGTGAAGAAGTGCAGCAAGAACCGGCTGGCATCACGGCAAAGTTTCTGG
GCTGAGCTGAATGTGGCCCGCCTACACCATGACAATGTGGTACGTGTTGT
AGCTGCCAGCACATGTGCTCCTGCCAGCCAAGACAGCCTGGGCAGCATAA
TAATGGAATATGCGGGCCGCACTACCCTGCACCATGTCATCTATGGCACTA
GCTGCCTGCAGGGAAAGGGAGAGGgTGATGGGGGTGGATGTGGCAGAGA
ACCCCTAAGCATAGCTGAGTCCCTGGGTTATTGCTCTGAtATTGTGACTGGt
TTAGCCTTCCTCCACTCACAGTGTATTGTGCACCTGGACCTGAAGCCTGCT
AATGTcTTAATCACCGAGCAGGGTGTGTGTAAGATTGGAGACTTTGGGTGC
TCCCAAAAGCTGGAAGAtAGTGTGTCCCCAGGTCCCCAGCTTTACCAGCAG
GGGGGCACATATACCCACCGTGCCCCTGAGCTCCTCAAAGGGGAGAA