PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

FILOGEOGRAFIA GLOBAL DA TARTARUGA OLIVA

(*LEPIDOCHELYS OLIVACEA*)

Anelise Torres Hahn

Orientador: Dr. Sandro L. Bonatto

TESE DE DOUTORADO
PORTO ALEGRE – RS – BRASIL
2011

.

Feliz aquele que transfere o que sabe e aprende o que ensina

Cora Coralina

SUMÁRIO

DEDICATÓRIA

Aos meus pais: Valmir e Bete.

AGRADECIMENTOS

Ao CNPq e à CAPES pelas bolsas de doutorado (Processo 142635/2007-3) e PDEE (Processo 4544-08-00) concedidas e pelo apoio aos projetos do Laboratório de Biologia Genômica e Molecular.

À PUCRS pela infra-estrutura cedida.

Ao meu orientador, Dr Sandro L. Bonatto, por sempre indicar o caminho a seguir.

À Nancy FitzSimmons e Michael Jensen pela orientação e amizade durante meu estágio PDEE na Austrália e pela colaboração com este trabalho.

Aos colegas e amigos do Laboratório do Biologia Genômica e Molecular, em especial a Cladinara, Felipe, Nelson, Beta, Déa, Alice, Taia, Ana Lúcia, Manoel, Paola, Talita, Cris, Renata, Tatiana, Henrique, Anelisie, Carla.

Aos amigos que fiz durante o estágio PDEE no Institute of Applied Ecology, especialmente a Carla Eisemberg, Anett Richter e sua família, Nicci Aitken, Dave Wong, Nadav Pezaro, Ian Bell, Anna McDonald, Marion.

A todos do Projeto Tamar, em especial Jaqueline Castilhos e Luciano Soares.

Aos queridos amigos, que mesmo longe estavam sempre no coração, Zé e Jorge.

À minha amiga de fé, irmã camarada Daniela que sempre esteve presente, partilhando todos os momentos deste caminho.

Aos meus “cumpadis” e sua linda família, em especial a Dayse.

Aos meus sogros João Ito e Elizabeth e aos meus cunhados Ana Paula e Fernando.

A minha família, em especial aos meus pais, Valmir e Bete e minha irmã Ana Paula, ao Vitor e ao meu lindo sobrinho Pedro, pelo apoio e incentivo.

Ao meu amor Paulo, por estar sempre ao meu lado, com toda paciência, sempre me incentivando e dando suporte.

A minha “véinha” Zilá - *In Memoriam*.

RESUMO

O capítulo inicial desta tese é o primeiro estudo sobre a diversidade genética e estrutura populacional de *Lepidochelys olivacea* (tartaruga oliva) no Brasil. O litoral de Sergipe e do norte da Bahia correspondem as principais áreas de desova da tartaruga oliva no Brasil. Desde 1992, o número de desovas de tartaruga oliva vem crescendo nestas áreas, indicando um aumento populacional; porém, a espécie continua ameaçada, principalmente devido às atividades de pesca e ao desenvolvimento costeiro desordenado. Neste estudo foram utilizadas sequências do DNA mitocondrial (mtDNA), além de 15 loci de microssatélites (STRs) para avaliar a diversidade genética e a estrutura populacional da tartaruga oliva em sítios de desova no Brasil. Além disso, utilizaram-se sequências previamente publicadas do mtDNA do Suriname para comparações populacionais. Identificou-se baixa diversidade genética no mtDNA da tartaruga oliva, com registro de apenas três haplótipos (F, F1 e F2), sendo o mais comum deles (F) encontrado em quase 95% dos indivíduos amostrados. Por outro lado, os loci de STRs mostraram maior diversidade genética. Os resultados também evidenciaram a falta de diferenciação genética entre as praias de desova na costa do Brasil, tanto para o mtDNA quanto para os STRs, sugerindo assim, a existência de uma única população de desova da tartaruga oliva no Brasil. As análises de diferenciação populacional entre Brasil e Suriname indicaram baixa distinção genética entre estas duas áreas, porém características biológicas sugerem que as duas populações atualmente estejam isoladas. O segundo capítulo estuda a filogeografia global de *L. olivacea*, utilizando um segmento do mtDNA e 15 loci de STRs em 330 e 291 indivíduos amostrados, respectivamente. Foram encontradas quatro clados mitocondriais correspondentes aos oceanos Índico, Indo-Pacífico, Pacífico leste e Atlântico. As idades de separação foram 1,6, 0,6 e 0, 32 milhão de anos atrás para o clado exclusivo do oceano Índico, do Pacífico leste e do Indo-Pacífico e Atlântico, respectivamente, ou ainda, mais recente que isto. Nossos resultados corroboram um modelo de extinção/colonização recorrentes para a maioria dos sítios de desova da espécie. A estruturação genética entre os oceanos foi altamente significativa, bem como entre a maior parte dos diferentes sítios de desova intra-oceânicos. Da mesma forma, a análise dos STRs demonstrou que a diferenciação entre os oceanos é alta, no entanto, dentro dos oceanos esta diferenciação é consideravelmente menor e não significativa entre a maior parte das áreas de desova, indicando os machos como importantes veículos para o fluxo gênico. Nossos resultados indicam que as linhagens atuais se diversificaram há aproximadamente 200 mil anos atrás com uma expansão populacional para a espécie há aproximadamente 15 mil anos, sendo que, este cenário é parcialmente corroborado quando analisamos as linhagens separadamente. Os resultados com STRs indicaram crescimento populacional quando as análises foram realizadas agrupando as populações dentro dos oceanos. Os resultados obtidos neste estudo indicam que as populações de desova de *L. olivacea* dos oceanos Índico, Indo-Pacífico, Pacífico leste e Atlântico são distintas e devem ser manejadas separadamente.

ABSTRACT

The first chapter of this thesis is the first study about the olive ridley turtle's (*Lepidochelys olivacea*) genetic diversity and population structure in Brazil. The olive ridley is the most abundant species of marine turtle and is listed as vulnerable by the IUCN. Olive ridleys had a strong history of harvest in the Atlantic Ocean, with some populations being severely depleted; in Brazil the egg exploitation was intense until before 1982. However, a single study with a very low sample size so far investigated the species´ mtDNA diversity in the region. Herein we characterize the genetic diversity and population structure of the olive ridley nesting populations in the Brazilian coast based on 92 samples sequenced for the mtDNA control region and 67 samples genotyped for fifteen microsatellite loci. Although three mtDNA haplotypes were found, two previously unknown but very rare, the Brazilian nesting population presented one of the lowest mtDNA diversity known for the species. Contrary, our newly described microsatellite data showed moderate to high genetic diversity for olive ridleys from Brazilian nesting sites, similar to the few other nesting populations studied so far, suggesting that the high level of egg harvest in Brazil did not result in a recent genetic bottleneck. mtDNA data indicated a population expansion following a population decline in the past while microsatellite data suggested a scenario of demographic stability, supporting the scenario of colonization of Atlantic Ocean via a founder effect. Since results from both markers present no evidence of significant genetic differences between the studied olive ridleys nesting areas in the Brazilian coast, conservation strategies should consider the Brazilian olive ridleys as a single interbreeding population. The second chapter is a global phylogeographic study of the olive ridley. It was proposed that the ridley turtles diverged after the closure of the Isthmus of Panama during the Pliocene, and then *L. olivacea* has spread from the Pacific Ocean into the Indo-Pacific, Indian and only recently to the Atlantic Ocean. Genetic analyses have been consistent with this scenario although some authors have proposed the Indo-Pacific region as the center of origin for the ridley turtles instead. To address this and other questions on the population structure patterns and demographic changes through time, we used mtDNA sequence and the STRs for 300 samples of ridley turtles across their range. The olive ridley nesting sites are well structured for the mtDNA, while for STRs the population divergences are lower for regional rookeries but highly significant among oceans, suggesting male-mediated gene flow within oceans. Beyond a kemp's clade, we corroborated the existence of four geographic mtDNA clades for the olive ridleys: the K clade only found in Indian Ocean, and the East Pacific, Indo-Pacific and Atlantic clades. The K clade originated around 1.6 Mya, the East Pacific clade about 0.61 Mya, and the split between the Indo-Pacific and Atlantic lineages around 0.36 Mya. These results are mostly consistent with the recent colonization of East Pacific and the Atlantic and suggest a model of recurrent extinction/colonization for most ridley nesting sites that may be explained by the climatic changes, especially during the Pleistocene. Diversification times within all five clades are very similar, ranging between 221 Kya and 342 Kya, suggesting the most recent demographic events for most oceanic regions may have been concurrent. Significant statistics for the STR data and similarly shaped star trees in each of the four major olive ridley clades suggested a population expansion, a scenario partially corroborated by the Bayesian Skyline Plot analysis which is indicating a population expansion for *L. olivacea* after the last glacial maximum.

INTRODUÇÃO

Tartarugas marinhas são animais com complexo ciclo de vida e estrutura populacional, devido às longas migrações entre áreas de desova e alimentação, razão pela qual são difíceis de estudar. Devido a este comportamento, os indivíduos passam a maior parte do tempo no mar, onde dificilmente são diretamente observados. Portanto, a maior parte das pesquisas sobre estes répteis ocorre em áreas de desova onde as fêmeas e filhotes são facilmente observados, marcados e amostrados. Porém, esta é apenas uma fração dos estágios do complexo ciclo de vida destes animais, onde em um deles, os indivíduos podem estar altamente segregados (desova) e em outro estão em estoques mistos (alimentação) (Bowen & Karl 2007). Estudos genéticos em tartarugas marinhas têm sido muito utilizados para ajudar a preencher estas lacunas no conhecimento, proporcionando novas perspectivas na biologia e evolução destes animais, além disso, têm sido utilizados para resolver questões sobre genética de populações, filogeografia, fidelidade ao sítio de desova, estoques mistos em áreas de alimentação, paternidade múltipla, hibridação e filogenética (Naro-maciel *et al.* 2008, Bowen & Karl 2007, Karl *et al.* 1995, Lara-Ruiz *et al.* 2006).

O DNA mitocondrial (mtDNA), principalmente sequências da região controle, são amplamente utilizados no estudo de tartarugas marinhas. A molécula de mtDNA é herdada maternalmente, sendo esta uma das características que a torna interessante para o uso em estudos evolutivos e populacionais, pois se consegue traçar a linhagem materna das espécies e observar a história evolutiva destas linhagens num contexto geográfico (Avise 2000). A região controle (também chamada de *D-loop*) não é um gene codificante para proteínas, e contém o sítio de origem para replicação da molécula de mtDNA. A taxa relativamente rápida de mutação permite uma resolução de fina escala de populações, e por esta razão o sequenciamento da região controle tem se tornado o método de escolha para definição de populações com mtDNA (Bowen & Karl 1997). Atualmente sequências da região controle do mtDNA têm sido o

método mais utilizado no estudo de tartarugas marinhas, pois, devido ao comportamento filopátrico das fêmeas, é possível traçar sua linhagem materna em determinadas áreas de desova e definir locais de origem para animais em áreas de alimentação, encontradas em regiões pelágicas ou capturadas incidentalmente em redes de pesca (Bowen & Karl 2007).

Os marcadores microssatélites (*short tandem repeats* (STRs) ou *simple sequence repeats* (SSRs)) consistem em unidades de repetições em série, as quais contém de um a 10 pares de bases (pb) de comprimento (Ciofi *et al.* 1998). São *loci* altamente polimórficos e estão amplamente dispersos em genomas eucarióticos (Ciofi *et al.* 1998), apresentando altas taxas de mutação, as quais geram alelos de diversos comprimentos (Fitzsimonns *et al.* 1995). Por estas razões, são ideais para estudos de diversidade genética e mapeamentos, em função da sua abundância, alto polimorfismo, codominância e fácil detecção (Aggarwal *et al.* 2004). E, em relação as tartarugas marinhas, o fato de os marcadores microssatélites serem herdados biparentalmente faz com que estes auxiliem na investigação por padrões de fluxo gênico de mediadores machos e fêmeas, sendo assim, eficientes para o entendimento das relações genéticas entre populações, estrutura populacional, filogeografia e aspectos migratórios de uma espécie (FitzSimonns *et al.* 1995, Bowen & Karl 1997, Aggarwal *et al.* 2004). Resultados de trabalhos em tartarugas marinhas utilizando marcadores genéticos herdados biparentalmente revelaram valores de fluxo gênico mais altos do que os observados com mtDNA, implicando os machos como veículo para fluxo gênico entre as colônias (Bowen & Avise 1995). Dentro de espécies, a variação em microssatélites entre diferentes populações foi consistente com resultados anteriores com mtDNA indicando que estes marcadores têm se mostrado eficientes para comparar a mediação de fluxo gênico entre machos e fêmeas, complementando os resultados obtidos com mtDNA (FitzSimmons *et al.* 1995).

Tartarugas marinhas

As tartarugas marinhas são répteis da ordem Chelonia e formam um grupo monofilético da subordem Cryptodira (Bowen & Karl 1997, Meylan & Meylan 1999). Habitam os oceanos desde o Cretáceo, há aproximadamente 100 milhões de anos (Naro-Maciel *et al.* 2008, Hirayama 1998). Hoje são reconhecidas sete espécies de tartarugas marinhas, divididas em duas famílias dentro de Chelonioidea – Dermochelyidae e Cheloniidae – (Meylan & Meylan 1999). *Dermochelys coriacea* (Vandelli, 1971) é a única espécie atual de Dermochelyidea, enquanto que na segunda família, são reconhecidas, geralmente, seis espécies classificadas em cinco gêneros: *Chelonia mydas* (Linnaeus, 1758), *Natator depressus* (Garman, 1880), *Eretmochelys imbricata* (Linnaeus, 1766), *Caretta caretta* (Linnaeus, 1958), *Lepidochelys kempii* (Garman, 1880) e *Lepidochelys olivacea* (Eschscholtz, 1829).

Tartarugas marinhas são animais considerados altamente derivados morfologicamente possuindo diversas adaptações para a vida no mar. Todas as espécies compartilham características tais como membros em forma de nadadeiras, glândulas lacrimais grandes e modificadas para remover o excesso de sal do corpo. A carapaça é caracterizada por reduzida quantidade de ossos e hidrodinâmica, facilitando seu deslocamento durante as migrações (Meylan & Meylan 1999).

São animais que possuem um complexo ciclo de vida caracterizado por migrações de centenas a milhares de quilômetros entre as áreas de alimentação e desova (Bowen 1995), e tanto adultos quanto filhotes e juvenis, parecem utilizar o campo magnético terrestre para guiarem-se durante estas migrações (Lohman 2007). As migrações entre as áreas de alimentação e desova podem ocorrer em um intervalo que varia entre um a quatro anos, conforme a espécie (IUCN Marine Turtle Specialist Group - http://iucn-mtsg.org/about-turtles/species). Em aproximadamente 50-70 dias os filhotes nascem e irão emergir da areia, normalmente à noite, quando a

temperatura está mais amena, guiando-se até o mar através do reflexo do brilho das ondas, e depois são levados para mar aberto. Permanecem em habitat pelágico onde são conduzidos passivamente pelas correntes oceânicas para longe de suas praias de nascimento, só reaparecendo na costa em áreas de alimentação, como juvenis (Bowen 1995, Abreu-Grobois & Plotkin 2008).

Estudos de marcação e recaptura mostraram que as fêmeas maduras retornam com alta fidelidade à mesma praia de desova em sucessivas estações reprodutivas (Allard *et al.* 1994, Bowen & Avise 1995, Bowen 1997, Bowen & Karl 1997). A forte fidelidade ao sítio de desova das fêmeas de tartarugas marinhas levou Carr (1967) a formular a hipótese *natal homing*, onde as fêmeas sempre retornariam à praia de nascimento para desovar. Uma das consequências desta hipótese é que a alta filopatria das fêmeas revelaria padrões de estruturação populacional para o mtDNA nas praias de desova (Allard *et al.* 1994). Hendrickson (1980) propôs outro cenário para tentar explicar a alta fidelidade ao sítio de desova, no qual as fêmeas adolescentes seguiriam as fêmeas maduras até uma praia de nidificação e então se fixariam neste local para futuras desovas (hipótese da facilitação social) (Bowen 1997). Análises com DNA mitocondrial da tartaruga verde (*C. mydas*) e da tartaruga cabeçuda (*C. caretta*) vêm dando suporte a hipótese de *natal homing* (Allard *et al.* 1994; Bowen & Karl 1997, Bowen *et al.* 2004).

No passado as tartarugas marinhas foram muito abundantes, com muitas populações com mais de um milhão de indivíduos (Spotila 2011). Porém, nos últimos 100 anos houve uma grande diminuição no número destes animais, sendo que as principais ameaças às populações são: captura acidental em redes de pesca; coleta direta, desenvolvimento costeiro, poluição e patógenos e, aquecimento global (IUCN Marine Turtle Specialist Group - http://iucn-mtsg.org/about-turtles/hazards/). Hoje, poucas populações de tartarugas marinhas não foram afetadas; a maioria está em declínio e muitas já foram extintas (IUCN 1996).

Todas as espécies de tartarugas marinhas estão listadas em alguma categoria de ameaça pela União Internacional para Conservação da Natureza (IUCN) (Bowen *et al.* 1993, Bowen & Avise 1995, IUCN 2006). A espécie mais ameaçada é *L. kempii*, sendo que na última metade do século XX, o número de fêmeas desovando diminuiu de centenas de milhares para pouco mais de 100 (Bowen *et al.* 1998). Todas as espécies que ocorrem no Brasil são citadas em alguma categoria de ameaça na lista de espécies ameaçadas do Brasil (MMA 2003).

Ainda hoje as relações evolutivas das espécies de tartarugas marinhas são bastante debatidas, principalmente entre Chelonini e Carettini, ou até mesmo a evolução da ecologia alimentar nas espécies (Bowen *et al.* 1993, Naro-Maciel *et al.* 2008). Com a aplicação de técnicas moleculares algumas das incertezas foram resolvidas, como a corrente classificação de *L. kempii* e *L. olivacea* que durante muito tempo confundiu taxônomos (Bowen *et al.* 1991, Pritchard 2007). Naro-Maciel *et al.* (2008), utilizando marcadores nucleares e mitocondriais, sugerem que Chelonini é representada por *C. mydas* e *N. depressus* e Carettini por *C. caretta*, *E. imbricata*, *L. kempii* e *L. olivacea*, e que a separação entre as tribos ocorreu há aproximadamente 63 milhões de anos (figura 1).

Dermochelys coriacea (tartaruga de couro)

A tartaruga de couro distribui-se globalmente em todos os oceanos, desovando principalmente em praias tropicais, entretanto, as áreas de forrageio ocorrem principalmente em regiões oceânicas. É a espécie dentre as tartarugas marinhas que migra as maiores distâncias, podendo ser encontrada do Ártico aos trópicos, e também ao sul da Nova Zelândia (Spotila 2004). É, portanto, o animal com a maior distribuição latitudinal que se conhece. Talvez por esta capacidade migratória, exista

pouca diferença genética entre as linhagens de tartaruga de couro do Indo-Pacífico e Atlântico (Dutton *et al.* 1999, Bowen & Karl 2007).

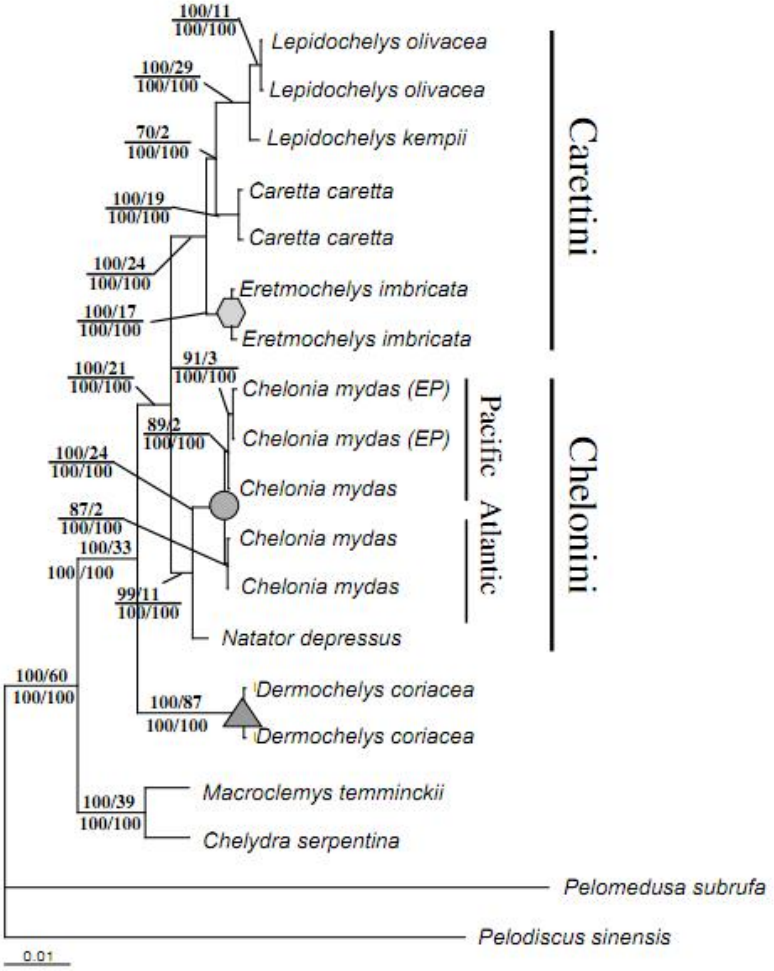


Figura 1: Hipótese para as relações filogenéticas entre as espécies de tartarugas marinhas baseada em marcadores nucleares e mitocondriais.
Reproduzido de Naro-Maciel *et al.* 2008

É a maior espécie de tartaruga existente e um dos maiores répteis viventes, podendo alcançar 1,8m de comprimento de casco e pesar quase 700 kg, sendo que já foi registrado um indivíduo de tartaruga de couro medindo 2 m e 900 kg (Spotila 2004, IUCN Marine Turtle Specialist Group - http://iucn-mtsg.org/about-turtles/leatherback). Também é o réptil de mergulho mais profundo, podendo ir tão ou mais fundo quanto baleias (mais de 1200 m de profundidade). Está classificada como Criticamente

Ameaçada tanto pela IUCN quanto pela lista de espécies ameaçadas no Brasil (Almeida *et al.* 2011a).

A idade média de maturação sexual é aproximadamente entre 13-14 anos (Sarti-Martinez 2000), taxa relativamente alta, comparada às outras espécies, quando se leva em conta seu tamanho e sua dieta, que consiste exclusivamente de zooplancton gelatinoso, por exemplo, mães d'água. Este rápido crescimento se deve às diversas adaptações fisiológicas e anatômicas, tais como: mudanças na taxa metabólica; cartilagens na extremidade óssea repletas de vasos sanguíneos, permitindo a nutrição óssea durante o crescimento do animal; casco formado por uma carapaça não óssea (daí o nome popular); ajustes no fluxo sanguíneo para manter a temperatura corporal estável nas variações de temperatura da água; entre outros (Spotila 2004).

No Brasil a espécie desova principalmente no litoral norte do Espírito Santo, e esta população é considerada extremamente reduzida; ocasionalmente há registros de desovas no Rio Grande do Norte, Bahia, Rio de Janeiro, São Paulo, Paraná e Rio Grande do Sul (Almeida *et al.* 2011a). Indivíduos de diversos estágios de vida são capturados na pesca oceânica, tanto no Brasil como em águas internacionais adjacentes, e animais marcados no Gabão, África, já foram capturados em águas Brasileiras (Almeida *et al.* 2011a).

Chelonia mydas (tartaruga verde)

Assim como outras espécies de tartarugas marinhas, são altamente migratórias, possuindo distribuição global em oceanos tropicais e temperados; apresenta hábitos costeiros incluindo estuários de rios e lagos (Seminoff 2004). Desova nos mais diversos hábitats de oceanos tropicais e subtropicais possuindo o

maior número de sítios de desovas entre todas as espécies (Spotila 2004, Seminoff 2004). Apresenta maturação sexual entre 26 e 40 anos (Almeida *et al.* 2011b).

A tartaruga verde recebe este nome devido à coloração de sua gordura e músculos que, segundo a tradição popular, são deliciosos, e por isso a espécie foi muito visada para uso culinário, como a famosa “sopa de tartaruga” (Spotila 2004). Desde as grandes navegações da Idade Média, os exploradores do Novo Mundo utilizavam as tartarugas verdes como fonte de alimento durante as viagens, e a espécie também auxiliava os navegadores, durante a noite, ao redor das ilhas devido ao “volume” de sua respiração, já que o número de indivíduos era abundante naqueles locais (Spotila 2004, IUCN Marine Turtle Specialist Group – iucn-mtsg.org/ about-turtles/species/green). Hoje o comércio é proibido na maioria dos países, mas mesmo assim, sua carne e também ovos continuam sendo consumidos. Está classificada como ameaçada pela IUCN (Seminoff 2004) e no Brasil é considerada Vulnerável (Almeida *et al.* 2011b).

A alimentação da espécie, durante o estágio juvenil é onívora, consistindo principalmente de invertebrados (Bjorndal 2003). Quando atinge o estágio juvenil (30-40cm) para adultos a dieta passa a consistir principalmente de macro-algas e fanerógamas, podendo também se alimentar de cnidários (IUCN Marine Turtle Specialist Group – iucn-mtsg.org/ about-turtles/species/green).

No Brasil a espécie desova em ilhas oceânicas como a Ilha de Trindade, Fernando de Noronha e Atol das Rocas; há registros de desovas secundárias no litoral norte da Bahia, e esporádicas no Espírito Santo, Sergipe e Rio Grande do Norte (Almeida *et al.* 2011b). Animais juvenis são encontrados em diferentes estágios de vida em toda a costa Brasileira, e há registros de juvenis marcados na nossa costa e recapturados em outros países e também nas ilhas oceânicas (Almeida *et al.* 2011b).

Há uma variação melânica da espécie, encontrada no oceano Pacífico, conhecida como tartaruga negra do Pacífico. Algumas características morfológicas a

distinguem de *C. mydas*, o que leva alguns pesquisadores a nominá-la como *Chelonia agasizii* (ou *Chelonia mydas agasizzi*). Há um caloroso debate sobre a classificação desta variação, porém, até o momento, tanto dados morfológicos quanto moleculares não são suficientes para garantir o status de espécie para estes animais (Karl & Bowen 1999; Chassin-Noria *et al.* 2004).

Natator depressus (flatback turtle)

Esta espécie é a menos estudada entre as tartarugas marinhas. Está restrita a plataforma continental da Austrália, sul da Indonésia e Papua Nova Guiné, desovando apenas na costa norte da Austrália (IUCN Marine Turtle Specialist Group – iucn-mtsg.org/ about-turtles/species/flatback), e, diferente das demais espécies, não apresentam a fase oceânica de desenvolvimento (Spotila 2004).

A espécie foi, por bastante tempo, considerada uma variação de *C. mydas* no Pacífico oeste até ser descrita como nova espécie em 1988 (Limpus *et al.* 1988). Alimenta-se principalmente de invertebrados bentônicos (IUCN Marine Turtle Specialist Group – iucn-mtsg.org/ about-turtles/species/flatback).

Eretmochelys imbricata (tartaruga de pente)

A tartaruga de pente é a mais tropical das espécies de tartarugas marinhas distribuindo-se globalmente em águas costeiras de oceanos tropicais e sub-tropicais. Assim como as outras espécies, realiza migrações entre as áreas de desova e alimentação (Mortimer & Donnelly 2008). A espécie forrageia nos recifes de corais, rochas, áreas de mangues em baías e áreas estuarinas. Os adultos alimentam-se principalmente de esponjas, desempenhando um importante papel na manutenção dos recifes de coral ao redor do mundo (Spotila 2004).

As maiores populações de desova desta espécie encontram-se no oceano Pacífico, principalmente nas áreas da Grande Barreira de Corais da Austrália; no oceano Índico principalmente na costa oeste da Austrália e nas ilhas Seycheles e, no mar do Caribe, principalmente no México. Os indivíduos levam de 20 a 40 anos para atingir a maturidade sexual, dependendo da área geográfica e da disponibilidade de alimento (Mortimer & Donnelly 2008).

No passado, a espécie foi muito caçada devido à beleza de seu casco, que era muito utilizado para manufatura de bijuterias, pentes e diversos outros objetos ornamentais e, por causa disso, a espécie foi quase levada à extinção. Hoje é classificada como criticamente ameaçada pela IUCN, e a comercialização de artefatos produzidos com o casco da espécie está proibida em todos os países (Mortimer & Donnelly 2008). Apesar disso, o tráfico doméstico e internacional permanece como uma das principais ameaças a tartaruga de pente nas Américas, Ásia e em regiões da África (Mortimer & Donnelly 2008).

Caretta caretta (tartaruga cabeçuda)

A tartaruga cabeçuda recebe este nome devido a sua enorme cabeça e a grande e poderosa mandíbula (Spotila 2004). Distribui-se em todos os oceanos tropicais e subtropicais do mundo. É uma espécie altamente migratória, exibindo migrações de desenvolvimento transoceânicas. A maturação sexual se dá entre 20 e 30 anos. São carnívoras, alimentando-se de diversas espécies de invertebrados marinhos como crustáceos e moluscos (IUCN Marine Turtle Specialist Group – iucn-mtsg.org/ about-turtles/species/loggerhead).

As principais colônias de desova da espécie são encontradas na Flórida (EUA), Oman, oeste da Austrália, México e Cabo Verde na África (Spotila 2004). Não há desova no Pacífico leste, porém a área é uma importante área de alimentação para

filhotes que nascem nas áreas de desova do Japão e no leste da Austrália, que retornam ao Pacífico leste como juvenis ou sub-adultos (Bowen *et al.* 1995). No Brasil as principais áreas de desova encontram-se no norte da Bahia, Espírito Santo, norte do Rio de Janeiro e Sergipe (Santos *et al.* 2011). Juvenis podem ser encontrados na costa brasileira desde o Rio Grande do Sul até o Pará. É classificada como Ameaçada pela IUCN, e no Brasil como em perigo (Santos *et al.* 2011).

Lepidochelys olivacea (tartaruga oliva)

A tartaruga oliva tem distribuição global nos oceanos tropicais e subtropicais, onde migram entre as áreas de alimentação e desova. Dentre as tartarugas marinhas são as que apresentam o menor número de pesquisas realizadas no que concerne à dinâmica dos movimentos migratórios. É a espécie de tartaruga marinha mais numerosa (Pritchard 1997; Bowen *et al.* 1998), porém, no Atlântico Ocidental, é a menos abundante (Marcovaldi 2001). Nesta área, distribui-se desde a Flórida, nos EUA, até o sul do Uruguai (Foley *et al.* 2003; Godfrey & Chevalier 2004). Não se sobrepõe geograficamente com *L. kempii*, que ocorre no Golfo do México e norte do Atlântico, e desova exclusivamente no Golfo do México (Bowen *et al.* 1991). *Lepidochelys olivacea* é classificada como vulnerável pela lista vermelha das espécies ameaçadas da IUCN (Abreu-Grobois & Plotkin 2008).

As duas espécies do gênero exibem comportamento de desova sincronizada em massa, conhecido como arribada, onde centenas a milhares de fêmeas emergem para desovar, após vários dias de espera em águas próximas à costa (Bernardo & Plotkin 2007). Surpreendentemente, este comportamento é pouco estudado ou entendido (Plotkin 2007). Porém, a estratégia reprodutiva mais comum na espécie é de desova solitária, podendo também apresentar comportamento de desova misto

(Abreu-Grobois & Plotkin 2008). O tempo de geração para *L. olivacea* é estimado em 20 anos (Abreu-Grobois & Plotkin 2008).

As principais praias de desova de *L. olivacea* estão no Pacífico Leste (México à Colômbia), Atlântico Sul (Guiana ao Brasil e costa oeste da África), Norte do oceano Índico (especialmente Orissa na Índia) e oeste do Pacífico (Spotila 2004). No Atlântico ocidental são conhecidos dois principais sítios de desova da tartaruga oliva: leste do Suriname e Guiana Francesa (Godfrey & Chevalier, 2004); e no Brasil, em Sergipe e norte da Bahia (Silva *et al.* 2007).

Adultos da tartaruga oliva são onívoros; caranguejos, caramujos, mexilhões, cracas, briozoários, algas, peixes entre outros fazem parte da dieta (Spotila 2004). A espécie também realiza mergulhos que podem chegar a mais de 150 metros de profundidade durante o forrageio e migrações (Polovina *et al.* 2004), tornando-as suscetíveis à pesca de profundidade (Spotila 2004).

As principais ameaças às populações são: impactos causados pela pesca, coleta direta, seja dos animais ou ovos, desenvolvimento costeiro, poluição e patógenos, e, aquecimento global (Marcovaldi *et al.* 2003, Thomé *et al.* 2003; Sales *et al.* 2008, IUCN Marine Turtle Specialist Group - http://iucn-mtsg.org/about-turtles/hazards/).

No Brasil, a espécie é citada como ameaçada no livro vermelho de espécies em extinção no Brasil (Martins & Molina 2008). Antes da implementação do Projeto TAMAR em Sergipe, quase todos os ninhos de *L. olivacea* eram coletados para consumo, e moradores do local dizem não ter visto filhotes durante, aproximadamente, 15 anos (Silva *et al.* 2007). A população de desova da tartaruga oliva no Brasil tem demonstrado um aumento de tamanho em torno de 10 vezes desde 1998 (Silva *et al.* 2007).

Na Guiana Francesa também tem se registrado aumento no número de fêmeas desovando (Kelle *et al.* 2009), enquanto que no Suriname, o número de ninhos caiu de

2.800 na década de 60, para em torno de 100-150 ninhos no início dos anos 2000 (Hilterman *et al.* 2008). A maioria das populações do mundo está em declínio, e algumas populações de desova (como na Guiana, Nicarágua, algumas áreas do México, Paquistão e Malásia) foram extintas (Spotila 2004, Cornelius *et al.* 2007).

Lepidochelys kempii (tartaruga de Kempi)

A tartaruga de Kempi distribui-se entre o noroeste do oceano Atlântico, Golfo do México e Caribe e desova apenas no norte do México (Rancho Nuevo) e Texas, no Golfo do México. É a espécie mais ameaçada, sendo que há aproximadamente 50 anos estava à beira da extinção. Já são evidentes os sinais de recuperação populacional, porém a pesca e o desenvolvimento costeiro continuam a ameaçar a espécie.

Filogeografia em tartarugas marinhas

Vicariância, barreiras físicas e distância são as principais forças que geram estruturação populacional (Avise 2000). Espécies com uma grande distribuição geralmente mostram subdivisão populacional, pois o fluxo gênico é restrito devido à distância ou a barreiras que reduzem a migração entre as populações (Frankham *et al.* 2002).

Mecanismos que geram a estrutura populacional em animais marinhos altamente migratórios são pouco conhecidos. Nestes ambientes, as barreiras à distribuição das espécies são menos evidentes do que em ambientes terrestres e algumas delas podem ser as correntes marinhas e as diferenças na temperatura da água (Frankham *et al.* 2002). Em ambientes marinhos tropicais, poucas barreiras tem sido estudas, e as principais são (figura 2): 1- a barreira do velho mundo, que foi formada após o fechamento do mar de Tethys, e separa o Mediterâneo do Oceano

Índico (Briggs 1974; Rocha *et al.* 2007); 2- barreira do Novo Mundo, formada após o fechamento do Istmo do Panamá e separa as faunas do Pacífico leste tropical do mar do Caribe (Knowlton & Weigt 1998); 3- barreira Amazônica, que separa o Brasil do Caribe (Rocha *et al.* 2007); 4- barreira de Benguela, uma ressurgência no sul do oceano Atlântico que separa a fauna tropical do sul dos oceanos Índico e Atlântico (Rocha *et al.* 2005); 5- a plataforma continental rasa entre as ilhas Indonésias (*Sunda Shelf*) expostas durante os níveis oceânicos mais baixos restringindo as trocas entre os oceanos Índico tropical e o Pacífico oeste; 6- a barreira do leste do Pacífico, consiste de uma ampla área de oceano aberto entre o Pacífico leste tropical e as ilhas do Pacífico central (Lessios & Robertson 2006); e 7- a barreira do Atlântico central, onde grandes distâncias oceânicas separam as Américas da África.

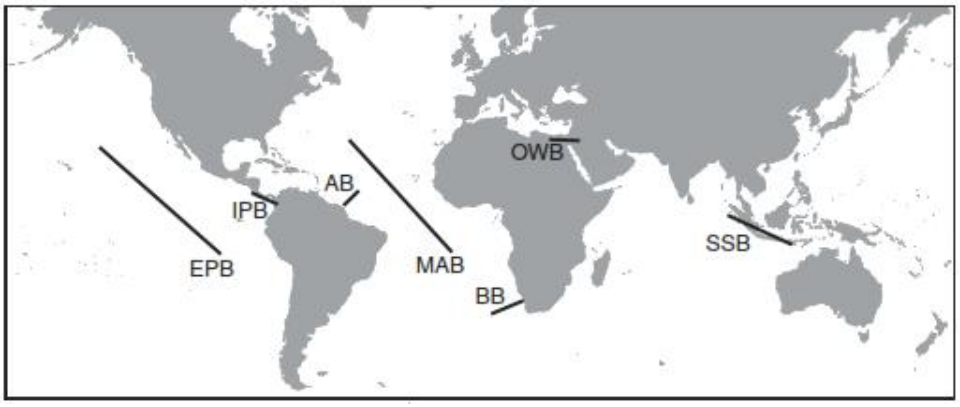


Figura 2: Mapa indicando as sete principais barreiras marinhas biogeográficas. EPB barreira do Pacífico leste, IPB barreira do Istmo do Panamá, AB barreira amazônica, MAB Barreira do Atlântico central, BB barreira de Benguela, OWP barreira do velho mundo, SSB barreira de *Sunda Shelf*. Reproduzido de Rocha *et al.* 2007.

As tartarugas marinhas desovam primariamente nos trópicos, mas há grande variação nos limites latitudinais entre os habitats de desova e alimentação. *Chelonia mydas* e *E. imbricata* são as mais tropicais, enquanto que *L. olivacea* desova principalmente em áreas tropicais, mas pode se alimentar em latitudes mais altas. A

tartaruga cabeçuda pode desovar e alimentar-se em áreas de latitude mais alta do que as espécies citadas até aqui, já a tartaruga de couro desova em áreas tropicais e subtropicais, podendo se alimentar em águas boreais (Bowen & Karl 2007).

Parece haver uma forte influência entre tolerância térmica das espécies de tartarugas marinhas, e o padrão de distribuição e a história evolutiva dos oceanos Atlântico e Indo-Pacífico (Bowen & Karl 2007) (figura 3). *Chelonia. mydas* apresenta duas linhagens distintas, que correspondem aos oceanos Atlântico e Indo-Pacífico, mas uma recente dispersão para o oceano Atlântico através do oceano Índico (Bourjea *et al.* 2007, Bowen & Karl 2007). *Eretmochelys imbricata* também apresenta duas linhagens com o mesmo padrão, mas com uma mais recente no Atlântico leste, que é relacionada as linhagens do Indo-Pacifico. Em *C. caretta* também se observa duas linhagens, porém com menor divisão geográfica. A filogenia mitocondrial mostra duas transmissões matrilineais entre Atlântico e Indo-Pacífico, sendo que uma delas parece ser bastante recente (Bowen & Karl 2007). Em contraste, *D. coriacea* não apresenta segregação de linhagens e possui a filogenia extremamente rasa (Bowen & Karl 2007).

Lepidochelys kempii e *L. olivacea* podem ter sido separadas pela formação do Istmo do Panamá, e dados moleculares são consistentes com esta teoria (Bowen *et al.* 1998). Subsequentemente, *L. olivacea* teria recentemente colonizado o Atlântico através do Indo-Pacífico (Bowen *et al.* 1998), ou uma radiação do final do Pleistoceno do Índico teria colonizado tanto o Atlântico como o leste do Pacífico (Shanker *et al.* 2004). A maioria dos haplótipos da tartaruga oliva apresentam poucos polimorfismos, indicando que a história evolutiva da tartaruga oliva é a mais rasa dentre Cheloniidea.

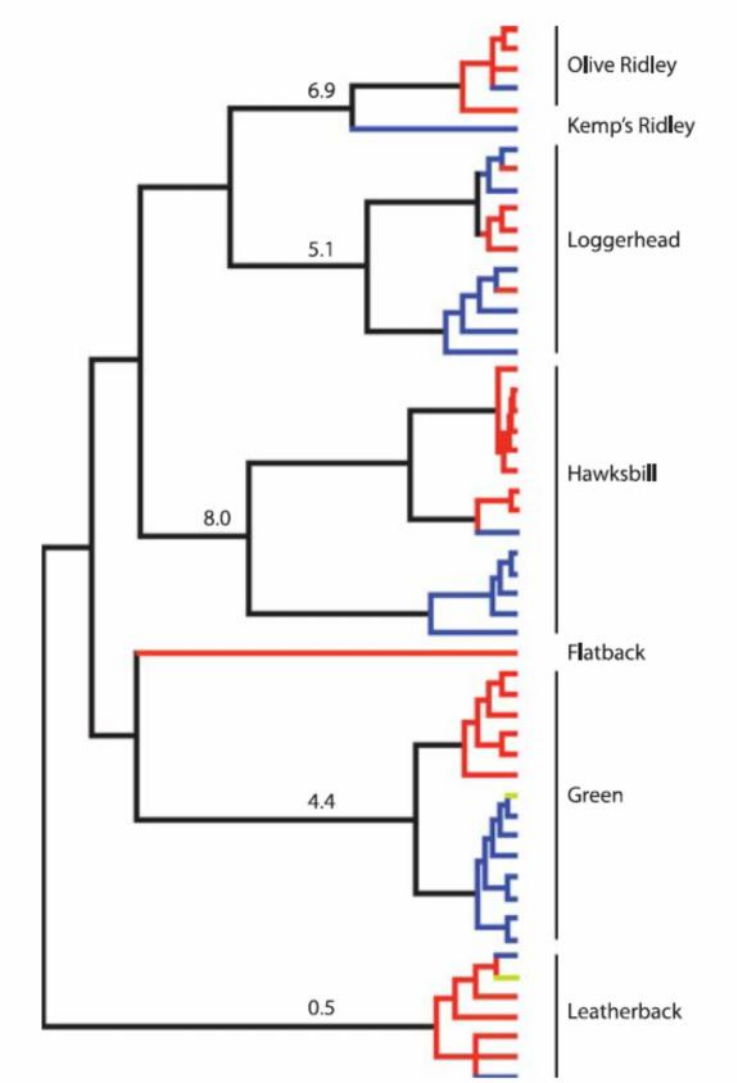


Figura 3: Filogenia matriarcal para as sete espécies de tartarugas marinhas baseada em sequências da região controle do mtDNA. Linhagens do Indo-Pacífico são mostradas em vermelho, linhagens do Atlântico estão em azul, e linhagens observadas em ambos os oceanos estão em amarelo.
Reproduzido de Bowen & Karl (2007).

O padrão de isolamento antigo entre oceanos tropicais e recentes conexões entre espécies que vivem em águas temperadas parece ter uma forte influencia na filogeografia das tartarugas marinhas e, consequentemente, na evolução destes répteis (Bowen & Karl 2007). Os autores concluem que o clima, geografia e oceanografia provavelmente tem maior influência na evolução e na especiação. Em tartarugas marinhas, as antigas divergências entre as espécies não são suficiente para

gerar isolamento reprodutivo e especiação, sendo que indivíduos híbridos são facilmente identificados, seja por morfologia ou através de estudos moleculares (Kamezaki 1983, Frazier 1988, Conceição *et al.* 1990, Wood *et al.* 1983, Karl *et al.* 1995, Barber *et al.* 2003, Seminoff *et al.* 2003, James *et al.* 2004, Lara-Ruiz *et al.* 2006, Reis *et al.* 2009).

Esta tese consiste de dois artigos, um deles é o primeiro estudo sobre diversidade genética e estrutura populacional de *Lepidochelys olivacea* em áreas de desova no Brasil, a ser submetido para a revista *Conservation Genetics*. Neste trabalho foram utilizados os dois marcadores moleculares acima citados, a região controle do mtDNA e 15 loci de STRs.

Já o segundo artigo é um estudo filogeográfico global da tartaruga oliva utilizando os mesmos marcadores moleculares. Neste estudo foram re-sequenciados segmentos mais longos da região controle do mtDNA de amostras da tartaruga oliva previamente publicados por Bowen *et al.* (1998) além da genotipagem dos mesmos indivíduos com 15 loci de STRs; além destas, foram utilizadas amostras adicionais de áreas de desova da tartaruga oliva do Brasil, da Guiana Francesa, de diferentes áreas da Austrália e de áreas do Pacifico leste. Além disso, novas ferramentas de análises para estimativas de mudanças demográficas históricas e migração foram aplicadas.

Devido a sua natureza migratória, as tartarugas marinhas requerem um plano de manejo com cooperação internacional que vise à conservação das populações em áreas de desova e de alimentação distribuídas pelos oceanos e também nas regiões costeiras. Acreditamos que os estudos apresentados aqui contribuirão para o conhecimento da Biologia da Conservação da tartaruga oliva, servindo como subsídio para elaboração de planos de manejo de programas de conservação, assim como para a compreensão dos processos evolutivos que moldaram a distribuição da espécie ao longo do tempo.

Capítulo 1

Genetic diversity and conservation of the olive ridley turtle (*Lepidochelys olivacea*) on the Brazilian coast

(Artigo a ser submetido ao periódico *Conservation Genetics*)

Genetic diversity and conservation of the olive ridley turtle (*Lepidochelys olivacea*) on the Brazilian coast

Anelise T. Hahn[1]*

Felipe G. Graziotin[1]

Luciano S. Soares[3]

Jaqueline C. Castilhos[2]

Luiza B. Fernandez[1]

Sandro L. Bonatto[1*]

[1] *Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Av Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil*

[2] *Fundação Pró-TAMAR, REBIO Santa Isabel s/número, 49190-000 Pirambu, SE, Brazil*

[3] *Fundação Pró-TAMAR, Caixa Postal 2219, 41950-970 Salvador, BA, Brazil*

*Corresponding author: e-mail: anehahn@gmail.com

Telefone number: +55 51 3320 35 00 ext. 4727

Fax number: +55 51 3320.3612

Abstract

The olive ridley is the most abundant species of marine turtle but had a strong history of harvest in the Atlantic Ocean, with some populations being severely depleted. In Brazil egg exploitation was intense before 1982, but population is recovering. However, so far a single study with a very low sample size and exclusively mtDNA investigated the species´ genetic diversity in the region. Here we characterize the olive ridley genetic diversity and population structure in the main nesting areas in the Brazilian coast using 92 mtDNA control region sequences and 67 individuals genotyped for fifteen microsatellite loci. The Brazilian nesting population presented one of the lowest mtDNA diversities known for the species, with only three haplotypes, two previously unknown and very rare. Contrarily, microsatellite data showed relatively high genetic diversity, similar to other few olive ridley nesting populations studied so far, suggesting that the high level of egg harvest in Brazil did not result in a significant genetic bottleneck. MtDNA data indicated an ancient population expansion from a small population, supporting the scenario of colonization of Atlantic Ocean via a founder effect while microsatellite data suggested recent demographic stability. Although we did not detect genetic structure in the Brazilian coast, a marginally significant differentiation between some rookery areas with the microsatellite data in some analyses, together with recent tagging data that indicate high female site fidelity, suggest that genetic differentiation between rookeries promoted by female fidelity may have been constrained by male gene flow.

Keywords: *control region, microsatellites, population genetic structure, demographic history, conservation genetics.*

Introduction

The oceanic *Lepidochelys olivacea* (olive ridley) is one of the smallest and probabaly the most abundant marine turtle, with a global distribution across tropical and sub-tropical oceans (Abreu-Grobois and Plotkin 2008). Olive ridley, as its sister species *Lepidochelys kempii* (Kemp's ridley), are known for an exceptional nesting behavior among marine turtles, the arribada, when hundreds of thousands of females emerge from the sea to lay their eggs at few specific beaches, although they also displays solitary nesting in most part of its distribution (Pritchard 2007). Olive ridley main nesting beaches occur in the east coast of India and Costa Rica and Mexico in the Pacific Ocean (Abreu-Grobois and Plotkin 2008). In the Atlantic Ocean the main nesting areas are Africa, between Guinea-Bissau and Angola (Fretey 2001), Surinam, French Guiana, and northeastern Brazil (Silva et al 2007). In Brazil nesting is concentrated in Sergipe and northern Bahia states, but it can occur in small number in Espírito Santo, and more

sporadically in Ceará (Silva et al 2007), Rio de Janeiro, Rio Grande do Norte (Castilhos et al 2011) and Piauí states (de Santana et al 2009).

Olive ridley is classified as "Vulnerable" by the IUCN Red List of Endangered Species (Abreu-Grobois and Plotkin 2008) and in Brazil as Endangered by the Brazilian Red List of endangered species (Castilhos et al 2011). In the past, olive ridleys in Brazil endured egg overexploitation by the local inhabitants, who have reported they had not seen hatchlings during a period of 15 years, before the implementation of the Projeto Tamar (The Brazilian Sea Turtle Conservation Program) in 1982 (Silva et al 2007). While there is no information about the number of clutches before that period, the nesting population in Sergipe and northern Bahia has been increasing since 1998 (Silva et al 2007); contrasting with nesting sites in other regions where the populations have been decreasing, and some were extirpated (Chaloupka et al 2004; Cornelius et al 2007). Presently, the major threats for the species in Brazil are bycatch in trawl fisheries and in pelagic longline fisheries (Thomé et al 2003; Marcovaldi et al 2006; Sales et al 2008; Castilhos et al 2011).

Olive ridley usually show strong genetic structure between rookeries and low levels of gene flow between most pairs of populations (Bowen et al 1998), although the authors pointed out that Surinam and Brazil in western Atlantic are an exception to this pattern, presenting high levels of gene flow and no evidence of population structure. They found the Atlantic populations presented the lowest diversity for about 400 bp of the mitochondrial DNA (mtDNA) control region, with only two haplotypes: the most common, F, which was found in all three areas studied (Brazil, Surinam and Guinea Bissau) and the haplotype E that was found only in Surinam. This is the single study published so far with genetic data on olive ridley populations in the Atlantic Ocean, having assessed for the Brazilian coast only 15 samples from a single nesting site, and no information exists on bi-parental microsatellite markers.

In the present study we analyzed 92 samples for longer sequences of the mtDNA control region and 67 samples for 15 bi-parental microsatellites loci in nesting females from several sites in Brazil. Our objectives were to characterize the mitochondrial DNA and microsatellite genetic diversity of the main Brazilian nesting populations and their bearing on their population structure and demographic history.

Materials and Methods

Data Collection and DNA extraction

Tissue samples from females found nesting at beaches were collected in Brazil from September to March between 2002 and 2005. For all population analyses the sampled individuals were grouped in three geographically closer rookery areas as follow: **BA** –from nesting beaches from Bahia state; **S-SE** – from Abaís in southern Sergipe; and **N-SE** - from northern Sergipe (Pirambu and Ponta dos Mangues nesting beaches) (Fig. 1). The three samples from the very distant Espirito Santo sites were not included in these analyses given its small sample size. To avoid resampling, all nesting females were double tagged on their front flippers with Inconel tags (National Band and Tag Co. style 681) (Silva et al 2007). Samples were preserved in 70% ethanol and aseptic procedures were used to avoid cross-contamination and protect turtles from infection. Total genomic DNA was extracted with standard fenol:chloroform procedure (Hillis et al 1996).

mtDNA control region

For the mtDNA analyses, samples used were from Ponta dos Mangues (n = 7), Pirambu (n = 44), Aracaju (n = 3) and Abaís (n = 21) in Sergipe; Sítio do Conde (n = 11), Praia do Forte (n = 2) and Arembepe (n = 1) in Bahia, and Espirito Santo (n = 3). We conducted the mtDNA control region amplification by polimerase chain reaction (PCR) using primers LCM 15382 and H950g (Abreu-Grobois et al 2006). PCR reactions of 20 µl included ~40ng of Genomic DNA, 0.1U *Taq Platinum*DNA polymerase (Invitrogen), 100µM of dNTPs, 1X PCR Buffer (Invitrogen), 1.5mM $MgCl_2$ and 0.2µM of each primer. Amplification conditions were initial denaturation at 94^{o}C for 4 minutes, 35 cycles at 94^{o}C for 30 seconds, annealing temperature at 57^{o}C for 1 minute, extension at 72^{o}C for 1 min, and final extension of 72^{o}C for 10 minutes. Products were checked on 1% agarose gel stained with ethidium bromide, purified with shrimp alkaline phosphatase and exonuclease I (GE Healthcare) and sequenced in both directions using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare) in a MegaBACE 1000 automated sequencer (GE Healthcare) following the manufacturer's protocols. Chromatograms were manually checked and the sequences were aligned by the Clustal X algorithm implemented in Geneious 5.1.4 with manual adjustment if necessary. The 14 sequences from Surinam obtained by Bowen et al (1998) were used to estimate its genetic distance to our Brazilian sample.

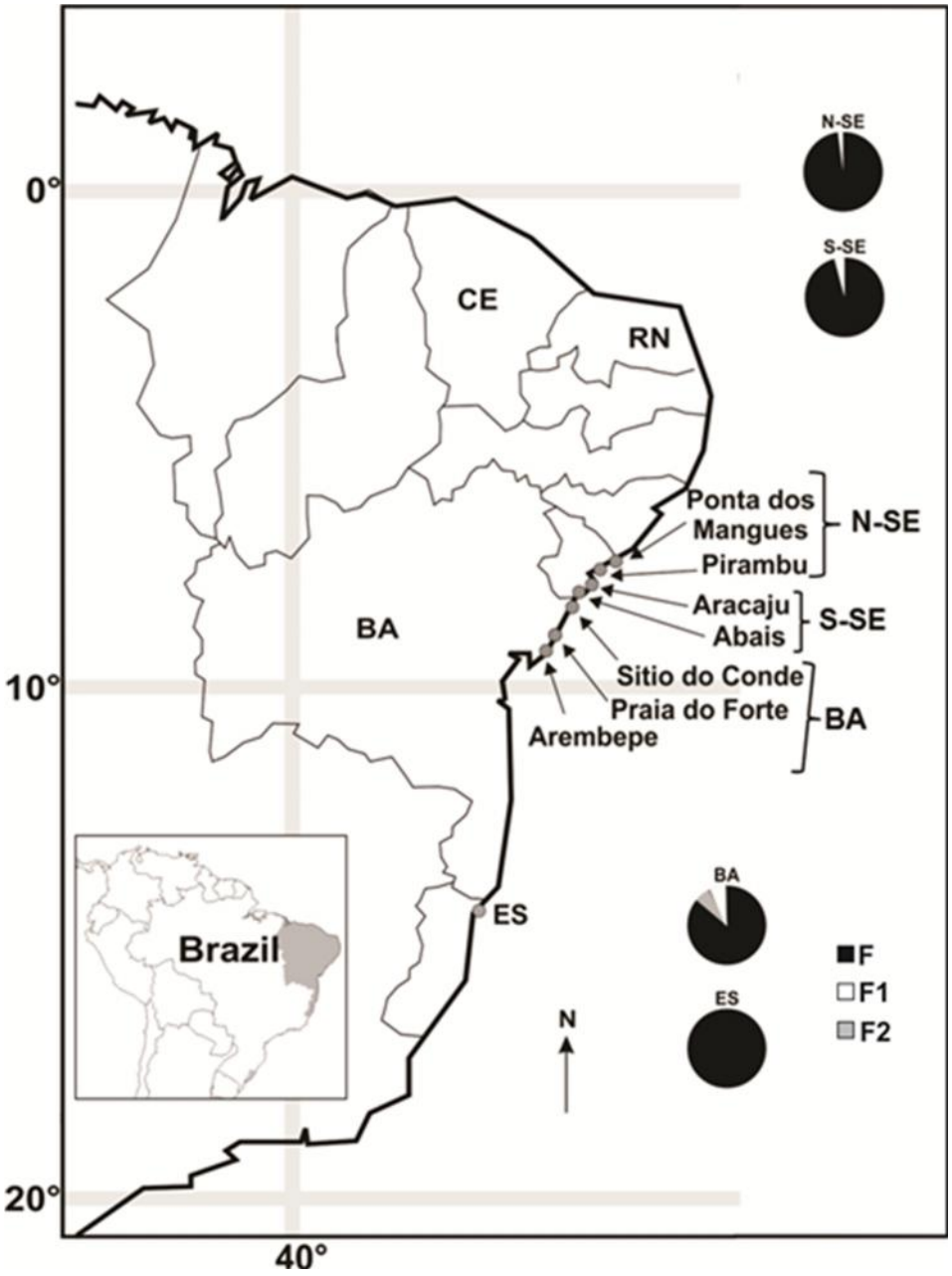


Fig. 1 Distribution of olive ridley nesting beaches in Brazil, location of sampling sites and olive ridley turtle mtDNA control region haplotypes frequencies in the nesting sites. Brazilian states are: ES, Espirito Santo; BA, Bahia; SE, Sergipe (S-SE – South of Sergipe; N-SE – North of Sergipe); AL, Alagoas; PE, Pernambuco; PB, Paraíba; RN, Rio Grande do Norte; CE, Ceará).

Arlequin 3.5 (Excoffier and Lische 2010) was used to estimate haplotype frequency, haplotype (*hd*) and nucleotide diversity (π), and Tajima's D and Fu's Fs neutrality tests. We estimated the population structure among the nesting beaches in Brazil and among nesting sites in western Atlantic (including data from Surinam) through pairwise F_{ST} and Φ_{ST} (Weir and Cokerham 1984) and AMOVA using Arlequin. These analyses were performed considering the three rookery areas as defined above. The analyses that included Surinam sequences were limited to the about 400 bp segment available. The

number of migrants per generation was estimated using the relationship $Nm = 0.5[(1/F_{ST})-1]$ (Takahata and Palumbi 1985).

The demographic history of the olive ridley turtle in Brazil was explored by the mismatch distribution approach implemented in Arlequin. The estimated parameters were validated using the sum of square deviations (SSD) test of goodness of fit that compare observed and expected mismatch distributions using 10000 bootstrap replicates (Schneider and Excoffier 1999).

Microsatellites

Fifteen microsatellite loci were assayed: six characterized by Aggarwall et al (2004) and eight by Aggarwal et al (2008) for the olive ridely, and CM84 and EI8 developed by FitzSimmons et al (1995) (see Table S1 in Supplementary material for details). Forward primers were 5' tailed with the M13 sequence which is used combined with a fluorescent M13 primer (FAM, NED, and HEX) (Boutin-Ganache et al 2001).

Samples used were from Ponta dos Mangues (n = 4), Pirambu (n = 27) and Abaís (n = 14) in Sergipe; Sítio do Conde (n = 18), Praia do Forte (n = 2) and Arembepe (n = 2) in Bahia. PCRs were carried out with the final volume of 10 µl as follow: ~40ng of Genomic DNA, 1 U *taq* DNA polymerase (Invitrogen), 100 µM of dNTPs, 1X PCR Buffer (Invitrogen), 1.5mM $MgCl_2$ 0.0083 µM of tailed forward primer, 0.2 µM of reverse primer and 0.16 µM of M13 fluorescent primer. PCR conditions were: initial denaturation at 94 ^{o}C for 4 minutes, 29 cycles at 94 ^{o}C for 30 seconds, primer-specific annealing temperature for 40 seconds (Table S1, Supplementary material), extension at 72 ^{o}C for 1 minute and 30 seconds, and final extension at 72 ^{o}C for 10 minutes. PCR products were genotyped in MegaBACE 1000 using the ET-ROX 550 size standard (GE Healthcare) and the software Genetic Profiler 2.2 (GE Healthcare).

Genetic diversity statistics such as the number of alleles per locus (K), the mean number of alleles and the observed and expected heterozigosities under Hardy-Weinberg equilibrium (HWE) (Ho and He, respectively) were estimated using Arlequin and the polymorphic information content (PIC) using Cervus 3.0 (Marshal et al 1998). Tests for deviation from HWE and for linkage disequilibrium were calculated using Arlequin, corrected by the sequential Bonferroni method and p = 0.05 (Rice 1989), when appropriate.

Population genetic differentiation between the three rookery areas was assessed by pairwise F_{ST} (Wier and Cokerham 1984) and R_{ST} (Slatkin 1995) and AMOVA calculated in Arlequin. Population structure was assessed with the Bayesian model-based clustering method implemented in STRUCTURE 2.3.3 (Pritchard et al 2000), using both the admixture ancestry model and the independent allele frequencies model. We conducted 10 independent runs for each K (number of clusters) between 1 to 5 and no prior information on number of populations. The length of simulation was set to 1,000,000 steps with the first 10% discarded as burn-in. We used the program CLUMPP (Jakobsson & Rosenberg 2007) to align the multiple outcome generated by Structure and determine the optimal clustering, which was graphically displayed by DISTRUCT (Rosenberg 2004).

BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) sign test and graphical method were used to test if the Brazilian olive ridley nesting population had passed through a recent effective population size reduction. The sign test was performed using the two-phase mutation model, with the single step probability set to 0.95 and the multiple step probability set to 0.05 (as suggested by Piry et al 1999). A population that has gone through a genetic bottleneck will show an excess of heterozygosity (significance was tested with the Wilcoxon sign rank test) relative to a stable one. The graphical method tests for a deficit of rare alleles in a sample of loci; demographically stable populations are expected to show an L shaped allele frequency distribution. Lastly, to detect relatively long-term bottleneck events, the mean ratio of the number of alleles to total range in allele size, the M value of Garza and Williamson (2001) was calculated using the program AGARst (Harley 2001).

Results

mtDNA control region

Sequences with 694 base pairs (bp) were obtained from 92 Brazilian samples. Three haplotypes were found, F, the most frequent and F1 and F2, which differ by an insertion in position 465 and a transition in position 545, respectively, from F (Table 1). When reduced to the ~400 bp reported in the literature, the most frequent is identical to the haplotype F previously reported by Bowen et al (1998) for the Atlantic Ocean. Haplotype and nucleotide diversities for the Brazilian nesting population were very low, 0.08 (±0.04) and 0.0003% (±=0.00012%), respectively. Neutrality tests for the Brazilian olive ridley

were significantly negative for Fu's Fs (-4.34) and Tajima's D (-1.38) ($p < 0.05$), suggesting past population expansion.

Table 1 Absolute control region mtDNA haplotype frequencies for Brazilian nesting sites

Haplotype	ES	BA	S-SE	N-SE	Brazil
F	3	12	23	50	88
F1		1	1	1	3
F2		1			1
Total	3	14	24	51	92

ES – Espirito Santo; BA – Bahia; S-SE – Southern Sergipe; N-SE – Northern Sergipe (Pirambu and Ponta dos Mangues).

Both pairwise F_{ST} and Φ_{ST} between the three Brazilian rookery areas were very small, and consequently the estimated migration rate was very high, suggesting absence of significant genetic differentiation (Table 2). The results of AMOVA analysis revealed that 98% of the variation is found within populations. Genetic divergence between Brazil and Surinam was very low ($F_{ST} = 0.14$), only marginally significant ($P = 0.046$), and consequently the estimated number of migrants was very high (Nm=3.1). There is no differentiation between Brazil and Guinea Bissau since the four known sequences of the latter (Bowen et al 1998) were all from haplotype F.

Table 2 Pairwise comparisons between Brazilian rookeries using mtDNA control region

	BA	S-SE	N-SE
BA	-	0.04 (12)	0.1 (4.5)
S-SE	0.01 (167.2)	-	0 (inf)
N-SE	0.07 (6.64)	0.00 (25.5)	-

Above diagonal F_{ST}, below diagonal Φ_{ST}; between parenthesis, number of migrants (Nm). BA – Bahia; S-SE – Southern Sergipe; N-SE – Northern Sergipe (Pirambu and Ponta dos Mangues).

The mismatch graphics presents an unimodal distribution and its estimated parameters were all compatible with a a population that expanded preceded by a size reduction ($P < 0.05$) (Fig. 2).

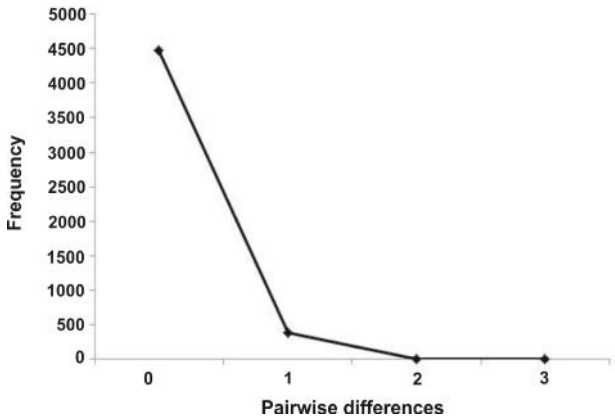


Fig. 2 Mismatch distribution for the mtDNA control region sequences of Brazilian olive ridley

Microsatellites

Sixty seven females were genotyped for at least 12 STR loci, and, in average, 91% of the individuals were genotyped for all 15 loci. Most of the loci were very polymorphic, the number of alleles per locus varied from two to 17 with mean of 9.07 (Table 3). The mean observed and expected heterozigosities for the Brazilian population were 0.55 and 0.67, respectively. The PIC varied from 0.02 to 0.89 with mean of 0.62, showing that all the loci, except by the OR3, were highly informative. No deviation from HWE or linkage disequilibrium after Bofferoni correction for multiple comparisons was found, except for EI8 and OR11 for the total sample.

Table 3 Pairwise F_{ST} and Number of migrants estimates among western Atlantic nesting sites.

	BR	SU
BR	-	3.1
SU	0.14*	-

Above diagonal Nm estimates based in F_{ST}, below diagonal F_{ST} estimates. BR – Brazil, SU – Surinam
*significant (P = 0.046)

None of the pairwise F_{ST} values between the three Brazilian rookery areas were significant, but R_{ST} was significant between BA and N-SE and between S-SE and N-SE, although the values were low (Table 4). Similarly, AMOVA using F_{ST} did not show significant structure among these three areas, with 99.3% of the variation occurring within populations. AMOVA using R_{ST} was marginally significant (p <0.05), with only 7.8% of the variation occurring among populations, indicating low genetic

differentiation among these rookeries. Additionally, STRUCTURE results strongly indicate that the most likely number of cluster (populations) is K = 1 (Fig. 3), as can be visualized in the individual assignment bar plots (Fig. 4).

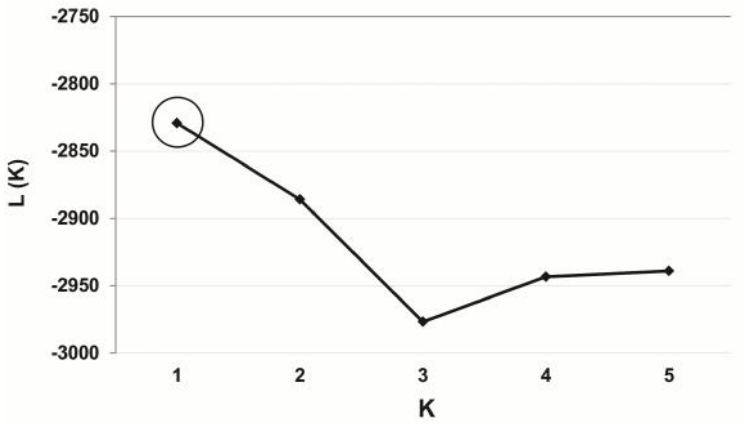


Fig. 3 Mean log-likelihood, L(*K*), for one to five clusters (K) based on ten independent STRUCTURE runs.

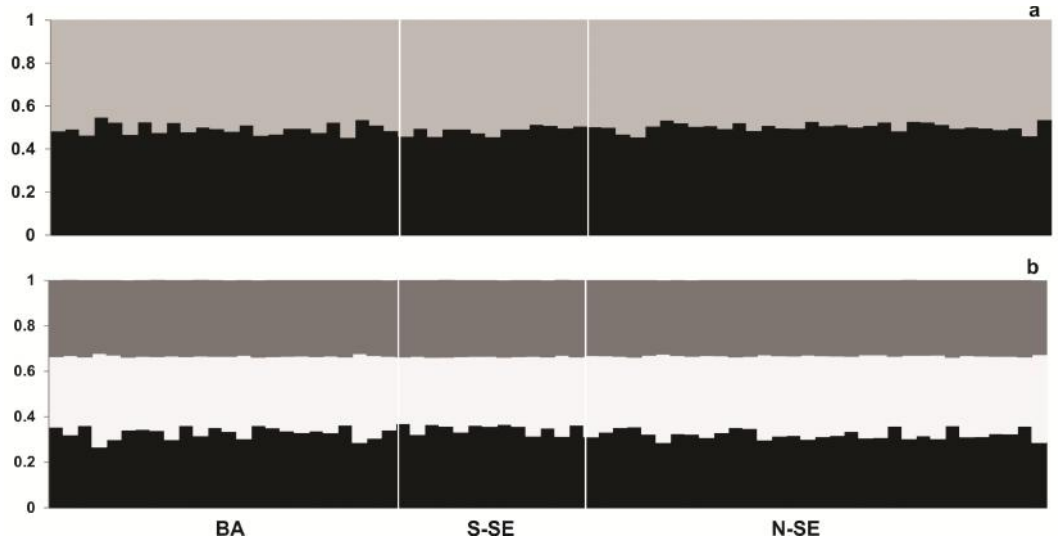


Fig. 4 Proportional membership of each individual of *Lepidochelys olivacea* without use of prior population information. Each individual is represented by a vertical bar, and the length of each bar indicates the probability of membership in each cluster. (a) K = 2; (b) K = 3.

The tests for past demographic changes in the Brazilian population did not found signals of population size reduction or expansion. The test for excess of heterozigosity was not significant (*P* = 0.99) and the distribution of allele frequency was clearly L shaped (Fig. 5). Moreover, the *M* value (*M* = 0.77 ± 0.22) was higher than the critical value suggested by Garza and Williamson (2001), not supporting a scenario of population size reduction.

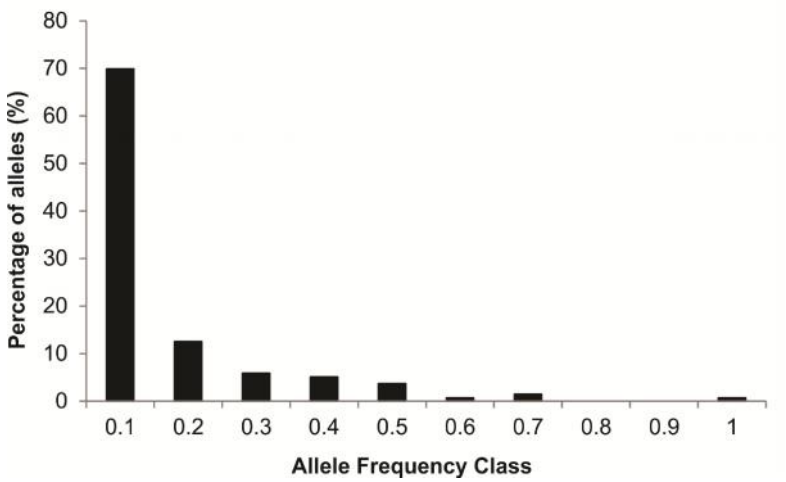


Fig. 5 Allele frequency distribution in the olive ridley Brazilian population. Bars represent the percentage of all alleles detected in each allele frequency class.

Discussion

Population Genetic Structure

With exception of the marginally significant R_{ST} between N-SE and the other two rookery areas, all other microsatellite and all mtDNA results suggest absence or at most a subtle genetic structure between olive ridley rookeries in Brazilian beaches. These results seem to conflict with the evidence that olive ridleys at Sergipe showed high nesting site fidelity (Matos et al, 2012), which should promote genetic differentiation.

The absence of mtDNA differentiation between olive ridley nesting sites in Brazil as distant as 300 km is similar to the absence of differentiation between olive ridleys nesting sites in 2,000 km of the East India coast (Shanker et al 2004). In contrast, López-Castro and Rocha-Olivares (2005) found that females from nesting beaches in South of Baja California, Mexico, are genetically distinct from other nesting beaches (separated from 300 km to over 1,000 km) from Mexico and Costa Rica, although the last two areas, separated over 1,000 km were not distinct. In the case of the Brazilian nesting sites, the apparent disagreement between the lack of differentiation in the maternal mtDNA and the evidence of high female site fidelity may be explained by lack of statistical power in this marker (given the existence of virtually a single haplotype in the Brazilian population) to allow any significant differentiation between areas. As explained below, the extremely low mtDNA diversity in Brazil was probably caused by founder effect, where a single haplotype, most likely F, was brought into the Atlantic and, given the relatively low substitution rate of this region (see above and Encalada et al 1996) several thousands of years would be

needed to generate enough diversity to allow statistical differentiation between maternal lineages isolated in rookeries.

On the other hand, the simplest explanation for the lack of major differentiation in the highly diverse microsatellite loci may be gene flow mediated by males, a common pattern in sea turtles (Bowen and Karl, 2007), that would act as a buffer against significant genetic differentiation between rookeries in these bi-parental loci. Studies with olive ridley turtle using microsatellites and tag data in India's coast, similarly, did not show evidence of population differentiation (Aggarwal et al 2006; Pandav and Choudhury 2000). Additionally, olive ridley is known to be highly vagile and it may be that nesting site fidelity is less important in the species (Bowen and Karl 2007). Furthermore, Matos et al (2012) found that although highly faithful to the nesting site females in Brazil may use more than one beach to nest. No matter how small, gene flow would reinforce the absence of genetic structure. However, given the relatively low sample size from Bahia and Abaís we suggest a more precise measure of this gene flow should be tested with more data from these sites.

With the available mtDNA data a very small but significant differentiation ($F_{ST} = 0.14$) was detected between Brazil and Surinam nesting population, which was expected given the presence of private haplotypes in Brazil (F1 and F2) and Surinam (E). Bowen's et al (1998) found that this comparison was not significant, but their sample size for the Brazilian population was much lower (n=15). The absence of differentiation between Brazil and Guinea Bissau should be taken with much caution given the latter very small sample size (n=4). Moreover, some biological characteristics indicate that nesting populations from western Atlantic are distinct: the nesting season in Brazil and Surinam do not overlap, suggesting adaptation by each population to local conditions. Additionally, none olive ridley tagged in Brazil were found in Surinam or elsewhere and none individual tagged in Surinam was found nesting in Brazil, although some individuals from Surinam were caught in north and northeastern Brazil. Therefore, this very low genetic differentiation based on mtDNA between Brazil and Surinam (and also between Brazil and Guinea Bissau) is likely an effect of the very low diversity of this marker in these populations that was probably a consequence of the recent colonization of the Atlantic by this species (see below).

Genetic diversity and demographic history

Our extended sample and longer control region segment sequenced corroborated the very small mtDNA diversity previously found for the olive ridley nesting in Brazil (Bowen et al 1998), although we have found two additional but rare haplotypes. Actually, considering only the shorter segment (~400 bp) published for other nesting sites of olive ridleys, Brazilian rookeries had the smallest diversity worldwide (not considering the extremely small sample sizes from Guinea Bissau and Malaysia) (Bowen et al 1998, Shanker et al 2004, López-Castro and Rocha-Olivares, 2005).

Contrasting with the mtDNA results, the newly described microsatellite genetic diversity for olive ridleys nesting in Brazil could be considered moderate to high, which is in general, similar or a little lower than the few other olive ridley nesting populations studied for these markers so far. Aggarwal et al (2004) using six and Aggarwal et al (2008) using seven of the loci used here, found the mean observed heterozigosity (Ho) of 0.87 and 0.65 for nesting populations in India, respectively, in comparison with the value of 0.55 found here. In Costa Rica, Jensen et al (2006) found in a paternity study a mean Ho of 0.85 for six of our loci. This moderate to high populational microsatellite variability is, in general, in agreement with studies from other marine turtle species (e.g. FitzSimmons et al 1995; Bowen et al 2005; Roberts et al 2004; Theissinger et al 2009).

Mismatch distribution and the neutrality tests based on mtDNA data support a past population size expansion most probably from a small population. On the other hand, the results of the Bottleneck and Garza and Williamson (2001) methods on the microsatellite data suggest a scenario of recent demographic stability. These differences between mtDNA and microsatellite results may be explained by their different rates of evolution and by the demographic history of the population. The rate of evolution, and therefore the time to increase the genetic diversity of a previously small population (such as one that went through a bottleneck) is much faster in microsatellite loci than in mtDNA sequence data. Interestingly, it has been suggested that olive ridley nesting in Brazil and the Atlantic Ocean in general may have been relatively recently colonized through the Cape of Good Hope (Pritchard 1969, Bowen et al 1998, Shanker 2004, Holder and Holder 2007), probably with a founder effect. A similar scenario was proposed for the origin of green turtle (*Chelonia mydas*) rookeries in Atlantic Ocean, which may have been formed by events of extinction/colonization over evolutionary time as habitat availability varies with climatic changes (Formia et al 2006).

Conservation Implications

Despite most of our results did not find significant genetic differences between the studied olive ridleys nesting areas in the Brazilian coast, the small but significant differentiation in some analyses and the presently high nesting site fidelity found by Matos et al, (2012) suggest a subtle or incipient genetic differentiation between these rookeries that should be considered in conservation strategies. Also, as discussed above, the mostly non-significant differentiation between the mtDNA from the available Atlantic nesting sites (Brazil, Guinea-Bissau and Surinam) may be mainly a consequence of the lack of statistical power of this marker and not a real absence of genetic differentiation between these major areas, and these results should be evaluated with more diverse bi-parental microsatellite markers.

Recent studies identified hybrids between loggerhead and olive ridley (Reis et al 2009b) and less commonly between hawksbill and olive ridley (Lara-Ruiz et al 2006) in Brazilian populations. However, all our 92 olive ridleys present olive ridley mtDNA, supporting Reis et al (2009b) suggestion that population hybridization in Brazilian is occurring unidirectionaly.

Notwithstanding the past intense human exploitation on eggs and the current threats, this study detected evidence of ancient (via founder effect) but not recent (anthropogenic) genetic bottleneck in the Brazilian olive ridley population. This is similar to other marine species that suffered significant recent anthropogenic reductions followed by very recent increase in population size in Brazil, such as humpback whales (Engel et al 2008), in which the reduction was probably not strong enough to left signature of a genetic bottleneck. There is no precise estimate of the number of females nesting in Sergipe and northern Bahia before the implementation of the conservation program (Projeto Tamar) in Brazil, however since 1991/1992 researchers have registered an increasing number of clutches in the area (Silva et al 2007). In the same way, French Guiana has registered population increase, contrary to Surinam which population has been declining from 2,800 nests in the 1960s to around 100-150 nests in the early 2000s (Hilterman et al 2008, Kelle et al 2009).

Acknowledgments

This work was supported by grants and scholarships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo a Pesquisa do Rio Grande do Sul as well as the support from PETROBRÁS/CENPES to Projeto Tamar. We would like to thanks the people from Projeto Tamar who collected the samples and from Laboratório de Biologia Genômica e Molecular-PUCRS for technical assistance, especially to Cladinara Roberts Sarturi. We also thanks F. A. Abreu-Grobois,

Universidad Autonoma de Mexico, Mexico, for providing the sequences for mtDNA primers, and to Paulo E. A. Bergonci for the help with tables and figures.

References

Abreu-Grobois FA, Horrocks JA, Formia A et al (2006) New mtDNA dloop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analyses. Poster presented at the 26th Annual Symposium on Sea Turtle Biology and Conservation, Crete, Greece, 2-8 April 2006. http://lists.iucn-mtsg.org/genetics/meth/primers/ abreu_grobois_ etal_new_dloop_primers .pdf. Accessed 27 April 2012

Abreu-Grobois A, Plotkin P. 2008.*Lepidochelys olivacea*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Accessed 27 April 2012

Aggarwal RK, Velavan TP, Udaykumar D et al (2004) Development and characterization of novel microsatellite markers from the olive ridley sea turtle (*Lepidochelys olivacea*). Mol Ecol notes 4: 77-79

Aggarwal RK, Shanker K, Ramadevi J et al (2006) Genetic analysis of olive ridley (*Lepidochelys olivacea*) populations from the East coast of India using microsatellite markers and mitochondrial d-loop haplotypes. In: Pilcher NJ (Ed) Proceedings of the Twenty-third Annual Symposium on Sea Turtle Biology and Conservation NOAA Technical Memorandum NMFS-SEFSC-536, pp 109

Aggarwal RK, Lalremruata A, Velavan TP, Sowjania AP, Singh L (2008) Development and characterization of ten novel microsatellite markers from olive ridley sea turtle (*Lepidochelys olivacea*). Conserv Genet 9: 981-984

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31:26–28

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Mol Ecol 16: 4886-4907

Bowen BW, Clark AM, Abreu-Grobois AF et al (1998) Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. Genetica 101: 179-189

Bowen BW, Bass AL, Soares L, Toonen J (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle. Mol Ecol 14: 2389-2402

Castilhos JC, Coelho CAC, Argolo JF et al (2011) Avaliação do estado de conservação da tartaruga marinha *Lepidochelys olivacea* (Eschscholtz, 1829) no Brasil. Biodiversidade Brasileira 1 (1):28-36

Chaloupka M, Dutton P, Nakano H (2004) Status of sea turtle stocks in the Pacific. In Papers Presented at the Expert Consultation on Interactions between Sea Turtles and Fisheries within an Ecosystem Context. FAO Fisheries Report no. 738, Supplement. Rome: Food and Agriculture Organization of the United Nations [FAO] 135–164

Cornelius SE, Arauz R, Fretey J, Godfrey MH, Márquez-M R, Shanker K (2007) Effect of land-based harvest of *Lepidochelys*. In: Plotkin PT (Ed) Biology and Conservation of the Ridley Turtles The Johns Hopkins University Press, Baltimore, pp 231-251

Cornuet JM, Luikart G (1996) Description and power analyses of two tests for detecting population bottlenecks for allele frequency data. Genetics 144: 2001-2014

De Santana WM, Silva-Leite RR, Silva KP, Machado RA (2009) Primeiro registro de nidificação de tartarugas marinhas das espécies *Eretmochelys imbricata* (Linnaeus, 1766) e *Lepidochelys olivacea* (Eschscholtz, 1829), na região da Área de Proteção Ambiental Delta do Parnaíba, Piauí, Brasil. PanamJAS 4 (3):369-371

Encalada SE, Lahanas PN, Bjorndal KA et al (1996) Phylogeography and population structure of the green turtle (*Chelonia mydas*) in the Atlantic Ocean and Mediterranean Sea: a mitochondrial DNA control region sequence assessment. Mol Ecol 5: 473–484

Engel MH, Fagundes NJR, Rosenbaum HC et al (2008) Mitochondrial DNA diversity of the southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antarctic feeding areas. Conserv Genet 9: 1253-1262

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Lynux and Windows. Mol Ecol Resources 10: 564-567

FitzSimmons NN, Moritz C, Moore SS (1995) Conservation and dinamics of microsatellite loci over 300 million years of marine turtle evolution. Mol Biol Evol 12 (3): 432-440

Formia A, Godley BJ, Dontaine JF, Brufford MW (2006) Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. Conserv Genet 7: 353-369

Fretey J (2001) Biogeography and conservation of marine turtles of the Atlantic coast of Africa. UNEP/CMS Secretariat. CMS Technical Series Publication, n° 6, Germany

Garza JC, Williamson EG (2001) Detecting of reduction in population size using data from microsatellite loci. Mol Ecol 10: 305–318

Harley EH (2001) AGARst. A program for calculating allele frequencies, GST and RST from microsatellite data. Version 2.0. Cape Town (South Africa): University of Cape Town

Hillis DM, Mable BK, Larson A, et al (1996) Nucleic Acids IV: Sequencing e Cloning. *In*: Hillis D, Moritz C, Mable BK (Eds) Molecular Systematics, 2nd edn. Sinauer Associates Inc. Publishers Sunderland, Massachusetts, pp 321-381

Hilterman ML, Tordoir MT, Goverse E, Reichart HA (2008) Beaches come and beaches go: coastal dynamics in Surinam are affecting important sea turtle rookeries. In Kalb H Rohde AS Gayheart K, Shanker K (ed) Proceedings of the 25th Annual Symposium on Sea Turtle Biology and Conservation, NOAA Technical Memorandum, pp 140–141

Holder KK, Holder MT (2007) Phylogeography and Population Genetics. In: Plotkin PT (Ed) Biology and Conservation of the Ridley Turtles, The Johns Hopkins University Press, Baltimore, pp 107-117 p.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23: 1801-1806

Jensen MP, Abreu-Grobois FA, Frydenberg J, Loeschcke V (2006) Microsatellites provide insight into contrasting mating patterns in arribada vs. non arribada olive ridley sea turtle rookeries. Mol Ecol 15: 2567-2575

Kelle L, Gratiot N, Thoisy B (2009) Olive ridley sea turtle *Lepidochelys olivacea* in French Guiana: back from the brink of regional extirpation? Fauna and Flora International Orix 43 (2): 243-246

Lara-Ruiz P, Lopez GG, Santos FR, Soares L (2006) Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. Conserv Genet 7: 773-781

López-Castro MC, Rocha-Olivares A (2005) The panmixia paradigm of eastern Pacific olive ridley turtles revised: consequences for their conservation and evolutionary biology. Mol Ecol 14: 3325-3334

Marcovaldi MA, Sales G, Thomé JC et al (2006) Sea turtles and fishery interactions in Brazil: identifying and mitigating potential conflicts. Mar Turtle News 112: 4-8

Marshall, TC, Slate, J, Kruuk, LEB, Pemberton, JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7: 639-655

Matos L, Silva ACCD, Castilhos JC et al (2012) Storng site fidelity and longer internesting interval for solitary nesting olive ridley sea turtles in Brazil. Mar Biol 159 (5): 1011-1019

Pandav B, Choudhury BC (2000) Conservation and management of olive ridley sea turtle (Lepidochelys olivacea) in Orissa. Final report: Wildlife Institute of India

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in effective population size from allele frequencies data. J Hered 90: 502-503

Pritchard PCH (1969) Studies of the Systematics and Reproductive Cycles of the Genus *Lepidochelys*. Thesis, University of Florida

Pritchard JK, Stephens M, Donnely P (2000) Inference of a population structure using multilocus genotype data. Genetics 155: 945-959

Pritchard PCH (2007) Arribadas I have known. In*:* Plotkin PT (Ed) Biology and Conservation of the Ridley Turtles. The Johns Hopkins University Press, Baltimore, pp 7-21

Reis EC, Soares LS, Lôbo-Hajdu G (2009b) Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. Conserv Genet 11 (4): 1587-1591

Roberts MA, Schwartz TS, Karl SA (2004) Global population genetic structure and male-mediated gene flow in the green sea turtle (*Chelonia mydas*): analysis of microsatellite loci. Genetics 166: 1857-1870

Rosenberg NA (2004). DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4: 137-138

Rice WR (1989) Analyzing table of statistical tests. Evolution 43: 223-225

Sales G, Giffoni BB, Barata PCR (2008) Incidental catch of sea turtles by the Brazilian pelagic longline fisheries. J Mar Biol Assoc UK 88: 853-864

Shanker K, Ramadev J, Choudury BC et al. (2004) Phylogeography of olive ridley turtles (*Lepidochelys olivacea*) on the east coast of India: implications for conservation theory. Mol Ecol 13: 1899-1909

Schneider S and Excoffier L (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: applications to human mitochondrial DNA. Genetics 152: 1079-1089

Silva ACCD, Castilhos JC, Lopez G, Barata PCR (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: 1047-1056

Slatkin, M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics, 139: 457-462

Takahata N, Palumbi SR (1985) Extranuclear differentiation and gene flow in the finite island model. Genetics 109: 441-457

Theissinger K, FitzSimmons NN, Limpus CJ et al (2009) Mating system, multiple paternity and effective population size in the endemic flatback turtle (*Natator depressus*) in Australia. Conserv Genet 10: 329-346

Thomé JCA, Marcovaldi MA, Marcovaldi GG, et al (2003) An overview of Projeto TAMAR-IBAMA's activities in relation to the incidental capture of sea turtles in Brazilian fisheries. In Seminoff JA (ed) Proceedings of the Twenty-second Annual Symposium on Sea Turtle Biology and Conservation, Technical Memorandum NMFS-SEFSC, pp 119–120

Weir BS and Cokerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370

SUPPLEMENTARY MATERIAL

Table S1 Details of the microsatellite loci used herein.

Locus	Tag[a]	T_a (^oC)	Size Range
OR1	NED	55	150-202
OR2	FAM	55	157-183
OR3	HEX	55	146-148
OR4	NED	55	128-162
OR7	HEX	55	185-201
OR8	FAM	56	144-168
CM84	HEX	58	322-338
EI8	FAM	56	192-262
OR9	FAM	55	158-170
OR11	HEX	55	196-242
OR14	NED	55	161-195
OR16	FAM	55	220-248
OR18	HEX	55	118-148
OR19	FAM	55	148-160
OR22	HEX	57	219-231

[a] Fluorescence label at 5’ end; T_a Locus specific annealing temperature.

Capítulo 2

Global phylogeography of the olive ridley sea turtle

(*Lepidochelys olivacea*)

(Artigo a ser submetido ao periódico *Molecular Ecology*)

Global phylogeography of the olive ridley sea turtle (*Lepidochelys olivacea*)

Anelise Torres Hahn[1]*
Michael Jensen[2]
Brian Bowen[3]
Jaqueline Comin de Castilhos[4]
Alberto Abreu-Grobois[5]
Nancy FitzSimmons[6]
Col Limpus[7]
Scott Whiting[8]
Benoit de Thoisy[9]
Sandro L. Bonatto[1]

[1] Faculdade de Biociências, Laboratório de Biologia Genômica e Molecular (GENOMA), Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS)
[2] NOAA - South West Fisheries Science Center, 3333 N. Torrey Pines Ct, La Jolla, CA 92037, USA
[3] Department of Zoology, University of Hawaii, USA
[4] REBIO Sta Isabel, Fundação Pró-Tamar, Sergipe, Brazil
[5] Instituto de Ciencias del Mar y Limnologia, Universidad Nacional Autonoma del Mexico, Mexico
[6] Institute for Applied Ecology University of Canberra, Australia
[7] Department of Environment and Resource Management, Brisbane, QLD, Australia
[8] Department of Natural Resources, Environment, the Arts and Sport, Palmerston, NT, Australia
[9] Institut Pasteur de la Guyane & Kwata NGO, Cayenne, Guiana Francesa

Keywords: Population structure, mtDNA control region, microsatellites, demographic change, effective population size

* Corresponding author: Av Ipiranga, 6681, Prédio 12C, Sala 172, Partenon, Porto Alegre, RS 90619-900, Brazil. e-mail: anehahn@gmail.com. Telefone number: +55 51 3320 35 00 ext. 4727. Fax number: +55 51 3320.3612

Running title: Phylogeography of the olive ridley

Abstract

The olive ridley (*Lepidochelys olivacea*) is the most abundant sea turtle presenting a circunglobal distribution in tropical and subtropical oceans, while *L. kempii* (Kemp's ridley) is restricted to the North Atlantic. It was proposed that the ridley turtles diverged after the closure of the Isthmus of Panama during the Pliocene, and then *L. olivacea* has spread from the Pacific Ocean into the Indo-Pacific, Indian and only recently to the Atlantic Ocean. Genetic analyses have been consistent with this scenario although some authors have proposed the Indo-Pacific region as the center of origin for the ridley turtles instead. To address this and other questions on the population structure patterns and demographic changes through time, we used mtDNA sequences and genotypes for 15 microsatellites (STRs) loci of about 300 samples of ridley turtles across their range. The olive ridley nesting sites are well structured for the mtDNA, while for STRs the population divergences are lower for regional rookeries but highly significant among oceans, suggesting male-mediated gene flow within oceans. Beyond a kemp's clade, we corroborated the existence of four geographic mtDNA clades for the olive ridleys: the K clade only found in Indian Ocean, and the East Pacific, Indo-Pacific and Atlantic clades. The K clade originated around 1.6 Mya, the East Pacific clade about 0.61 Mya, and the split between the Indo-Pacific and Atlantic lineages around 0.36 Mya. These results are mostly consistent with the recent colonization of East Pacific and the Atlantic and suggest a model of recurrent extinction/colonization for most ridley nesting sites that may be explained by the climatic changes, especially during the Pleistocene. Diversification times within all five clades are very similar, ranging between 221 Kya and 342 Kya, suggesting the most recent demographic events for most oceanic regions may have been concurrent. Significant statistics for the STR data and similarly shaped star trees in each of the four major olive ridley clades suggested a population expansion, a scenario partially corroborated by the Bayesian Skyline Plot analysis which is indicating a population expansion for *L. olivacea* after the last glacial maximum.

Introduction

Phylogeographic studies provide information on how historical processes (e.g. environmental influences, geographical patterns, geological events) interact with aspects of species biology to shape its evolution (Avise 2000). Since the late 1980s, phylogeography has become one of the main subjects of population genetics analyses using a broad range of molecular technics and analytical methodologies to try to understand the history of species including population structure and demographic history (Nielsen & Beaumont 2009). To resolve temporal and spatial components of population structure and to interpret the ecological and evolutionary processes that generate this structure are the main goals of phylogeography (Beheregaray 2008). Furthermore, this knowledge is very important for a better understanding of how the species may response to contemporary threats and use this information for the establishment of more appropriate management and conservation plans. Some of the major

challenges to implementing these plans on threatened species are the correct recognition of the species and the genetic structure among populations.

The Pleistocene climatic changes influenced the patterns of sea levels expansion and retraction, sea surface temperatures distribution as well as the marine currents flow (Gersond *et al.* 2003, Herbert *et al.* 2010), and these should have strongly influenced the genetic structure of the marine turtles (Luschi *et al.* 2003).

The olive ridley sea turtle, *Lepidochelys olivacea* (Eschscholtz, 1829), is distributed worldwide in tropical and subtropical oceans, except in the Gulf of Mexico (Fig. 1) and it is the most abundant sea turtle species (Abreu-Grobois & Plotkin 2008). On the other hand, the Kemp's ridley turtle, *Lepidochelys kempii* (Graman, 1880) is the most threatened sea turtle species, nesting exclusively in the Gulf of Mexico mainly in Rancho Nuevo, Mexico, although the species is also found in other areas of the north Atlantic ocean (Fig. 1) (Morreale *et al*. 1992). The ridley sea turtles are the smallest sea turtle species and both are known by a peculiar mass nesting behavior called *arribada*, where females came ashore for nesting after days of gathering in the nearshore waters, then the females emerge synchronously to lay their eggs (Bernardo & Plotkin 2007). Arribadas occur at few specific beaches in the eastern Pacific, western Atlantic and northern Indian Oceans though solitary nest is the most common form of nesting of the olive ridley turtle and a mixture of these two forms can happen in some areas (Abreu-Grobois & Plotkin 2008). Probably water temperature is a limiting factor to the occurence of these species. As other sea turtle species, adult ridley turtles display a complex life cycle; olive ridley migrate long distances from nesting and breeding areas to epipelagic foraging grounds, while Kemp's ridley feeds primarily on neritic benthic crustaceans and seem to migrate to more moderate distances (Morreale *et al.* 2007). Kemp's and olive ridley are listed in the IUCN Red List of Endangered Species as critically endangered and vulnerable, respectively (Marine Turtle Specialist Group 1996, Abreu-Grobois & Plotkin 2008).

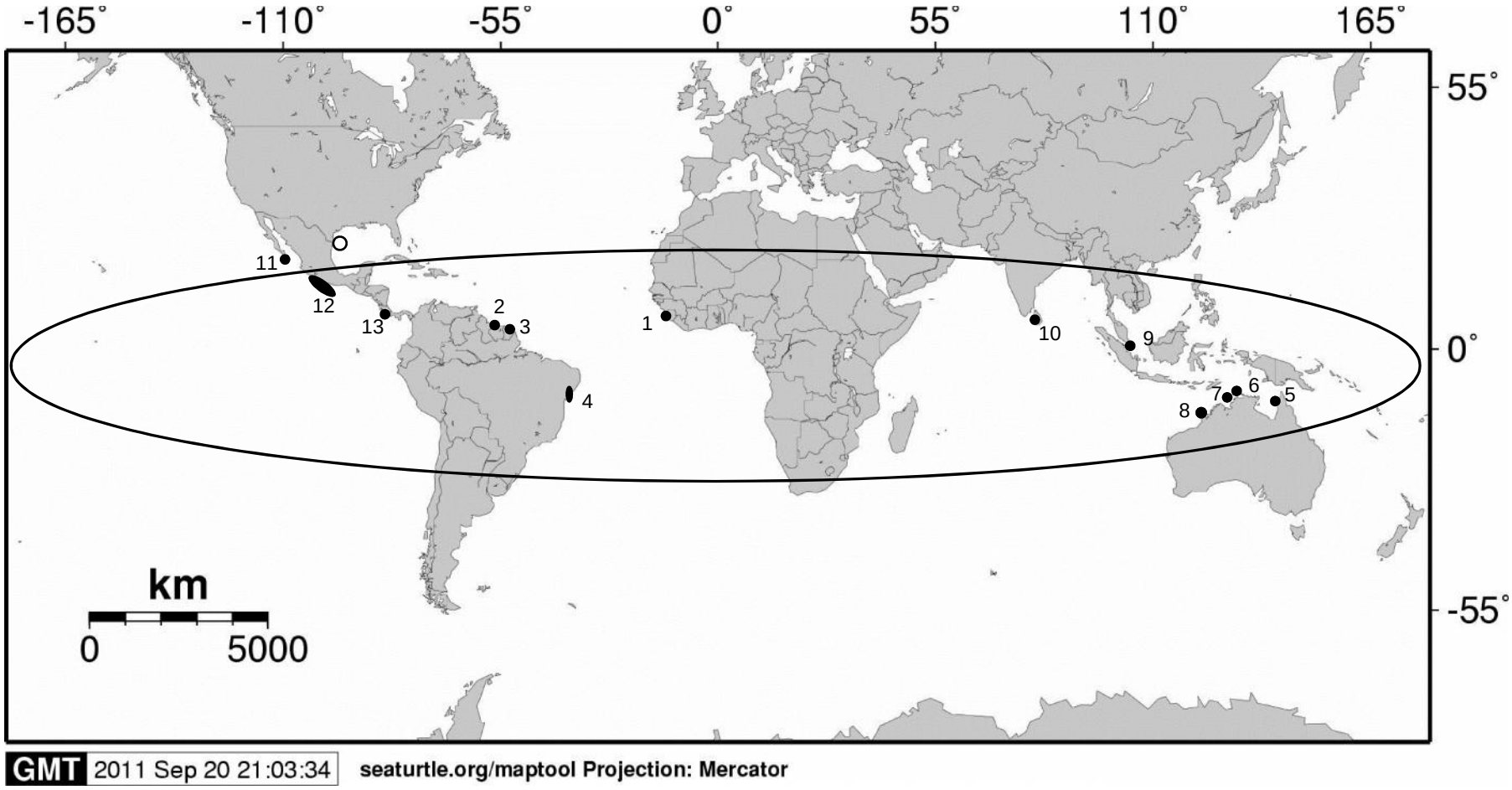


Fig. 1 Approximate geographic range of the olive ridley sea turtle (black line) and sampling sites: black dots are the *Lepidochelys olivacea* and white dot is *Lepidochelys kempii* samples localities used in this paper. Numbers are indicating the mesting sites the samples were collected: 1- Guinea-Bissau; 2-Surinam; 3- French Guiana; 4- Brazil; 5- Flinders Beach; 6- McCluer Group; 7- Tiwi Island; 8- Western Australia; 9- Malaysia;10- Sri Lanka; 11- Baja California; 12- Mexico and 13- Costa Rica. See Matherial and methods for more details.

Both species are very similar morphologically and due to that the taxonomic history of the genus was very confuse (Pritchard 2007). For many years Olive and Kemp´s ridley were considered as the same species, as subspecies or even as members of *Caretta,* and Kemp's ridley was already thought to be a hybrid between green turtle (*Chelonia mydas*) and loggerhead turtle (*Caretta caretta*) (Bowen *et al.* 1991; Pritchard 2007). However, molecular analyses validate the distinct species status of the ridley turtles (Bowen *et al.* 1991, Dutton *et al.* 1996; Naro-Maciel *et al.* 2008).

Pritchard (1969) proposed that Kemp's and olive ridley were isolated by the formation of the Isthmus of Panama 3-4 million years ago (Mya). Based on this idea, *Lepidochelys olivacea* would then spread through the east Pacific into the Indo-Pacific during the late Pliocene and Pleistocene and more recently olive ridley would have colonized the Atlantic from the Indian Ocean (Bowen *et al.* 1998; Bowen & Karl 2007). Analyses with mtDNA are consistent with the split of ridley turtles with the closure of the Isthmus of Panama and with the recent colonization of the Atlantic Ocean by way of the Cape of Good Hope (Bowen *et al.* 1998). Based in the low divergence on the olive ridley control region mtDNA haplotypes (< 0.5%) Shanker *et al.* (2004) proposed that radiation occurred from Indian Ocean into both East Pacific and Atlantic Oceans. Although the recent colonization of the Atlantic Ocean seems very likely we cannot eliminate the possibility that olive ridley existed in Atlantic before this time and were extirpated and then replaced by lineages that migrate from the Indian Ocean (Bowen *et al*. 1998).

The olive ridley show low genetic diversity and shallower evolutionary history compared to other sea turtle species but it presents strong genetic structure among rookeries on a global scale (except by Surinam and Brazil) although the structure is modest within regions apart for more than 300km and sometimes within regions apart by more than 1000km (Bowen *et al*. 1998; Shanker *et al*. 2004; López-Castro & Rocha-Olivares 2005; Holder & Holder 2007; Bowen & Karl 2007). These studies revealed four Kemp's ridley haplotypes and 24 olive ridley haplotypes about 400 bp long. Within olive ridley there appear to be three primary lineages that diverged about 1-1.5 Mya (Bowen *et al*. 1998, Holder & Holder 2007). These studies suggested that all olive ridley haplotypes to have recently derived from either J or K4 haplotypes, the first found only in the Indian-West Pacific and K4 clade only in India and Sri Lanka waters (Holder &

Holder 2007). However, these conclusions are limited since they are based only in a single maternally inherited locus (Holder & Holder 2007). The use of biparentally inherited nuclear DNA such as microsatellites would be essential to clarify the gaps on knowledge about the origin, migration and present day genetic structure of the species. In this work we studied the olive ridley global phylogeography using mitochondrial DNA and fifteen nuclear DNA microsatellites loci.

Materials and methods

Sampling and DNA extraction

Samples from Nancite Beach, Guanacaste Province, Pacific Costa Rica (n = 13); Kijal, Malaysia (n = 2); Southwestern coast of Sri Lanka (n = 16); Orango National Park Guinea-Bissau, Atlantic coast of Africa (n = 6); and Eilanti Beach, Suriman (n = 14) were studied in Bowen et al. (1998) and were re-sequenced here for a longer mtDNA control region segment (see below). In addition, 275 new samples from nesting sites from States of Sergipe (n = 75), Bahia (n = 13), Espírito Santo (n = 3) and Rio Grande do Norte (fishery areas) (n = 8), Brazil, and French Guiana (n=36), in the Atlantic Ocean; Baja California (n = 8) and Escobilla (n = 51) and foraging areas from Pacific Mexico (n = 6) in the Pacific Ocean and, McCluer Island Group (Northwestern Arnhem Land) (n = 11), Tiwi Islands (n = 47); Flinders Beach (Western Cape York Peninsula) (n = 13) Northern Territory, and Western Australia (n = 4), Australia in the Indo-Pacific region, summing up 326 olive ridleys samples and four kemp's ridley samples (Fig. 1). From those, a total of 291 ridleys' samples were also genotyped for 15 STRs loci, being Pacific Costa Rica (n = 19); Escobilla (n = 75), Baja California (n = 8), foraging areas from Pacific Mexico (n = 11), Malaysia (n = 9), Tiwi Island (n = 24), Flinders Beach (n = 13), Sri Lanka (n = 17), Guinea-Bissau (n = 9), Surinam (n = 13), French Guiana (n = 27), Brazil (n = 60) and Kemp's ridley (n = 6). Different methods of collecting and extraction were used in accordance with their place of origin. For samples from Sri Lanka, Australia, Malaysia, Costa Rica, Mexico, Guinea-Bissau and Surinam see Bowen *et al.* (1998). Samples from French Guiana were collected and extracted as Plot *et al.* (2011). For Brazilian samples, see Hahn *et al*. (in prep).

Additional tissue samples from Australia were collected from olive ridleys nesting at the McCluer Island Group (north-western Arnhem Land), at Tiwi Islands (Northern Territory) and at Flinders Beach (Western Cape York Peninsula), and the DNA was extracted from tissues using a salting out method (FitzSimmons *et al.* 1997).

Sequencing and genotyping

Amplifications of the mtDNA control region segment were made by Polymerase Chain Reaction (PCR) using primers LCM 15382 and H950 (Abreu-Grobois *et al.* 2006, Abreu-Grobois personal comm.). The samples from Australia, which are not from Bowen *et al.* (1998) study, were sequenced using the LTEi9 (5'--3') and H950 primers, the LTEi9 segment completely encompass the LCM15382. The PCR setup included: ~40ng of Genomic DNA, 0.1U taq Platinum DNA polymerase (Invitrogen), 100µM of dNTPs, 1X PCR Buffer (Invitrogen), 1.5mM MgCl2 and 0.2µM of each primer in a total volume of 20µl. PCR conditions were: initial denaturation at 94^{o}C for 4 minutes, 35 cycles at 94^{o}C for 30 seconds, annealing temperature at 57^{o}C for 1 minute, extension at 72^{o}C for 1 min, and final extension of 72^{o}C for 10 minutes. To check for contaminations, all PCRs were carried out with negative controls. Products were checked on 1% agarose gel stained with ethidium bromide, enzymatically purified and sequenced in MegaBace 1000 (GE Healthcare). Sequences were checked and aligned (by Clustal X method) in Geneious 5.1.4 (Biomatters LTDA) and manually edited when necessary. Previously available sequences were downloaded from Genbank or obtained from literature, 81 sequences from India (Shanker et al. 2004), 122 sequences from East Pacific Baja California and Mexico (López-Castro 2004; López-Castro & Rocha-Olivares 2005) and the haplotype M and the Kemp's ridley remaining haplotypes (Bowen *et al.* 1998). These sequences were compared with our longer segments, that encompass the shorter sequences amplified in the above studies. Although our longer sequences present a few new polymorphic sites, results from most analyses were very similar between the longer and shorter alignment. Therefore, in order to use sequence information from samples and areas not available here, all analyses were done with the shorter alignment, although some basic statistics were also presented for the longer alignment (supplementary data).

Nuclear DNA variation was assayed using fifteen microsatellite loci, six (OR1, OR2, OR3, OR4, OR7 and OR8) developed by Aggarwal *et al.* (2004), seven (OR9, OR11, OR14, OR16, OR18, OR19 and OR22) by Aggarwal *et al.* (2008), all specific for olive ridley turtles, and, two (CM84 and EI8) developed by FitzSimmons *et al.* (1995). Forward primers were 5' tailed with the M13 sequence which is used combined with a fluorescent M13 primer (FAM, NED, and HEX) (Boutin-Ganache *et al.* 2001). Amplifications were carried out in 10 µl with the following conditions: ~40ng of Genomic DNA, 1 U taq DNA polymerase (Invitrogen), 100 µM of dNTPs, 1X PCR Buffer (Invitrogen), 1.5mM MgCl2 0.0083 µM of tailed forward primer, 0.2 µM of reverse primer and 0.16 µM of M13 fluorescent primer. Thermocycling conditions for the amplification were: initial denaturation at 94 ^{o}C for 4 minutes, 29 cycles at 94 ^{o}C for 30 seconds, primer-specific annealing temperature for 40 seconds (Table S1, supplementary data), extension at 72 ^{o}C for 1 minute and 30 seconds, and final extension at 72 ^{o}C for 10 minutes. PCR products were genotyped in MegaBace 1000 (GE Healthcare) and the allele size number of bases was identified with the software Genetic Profiler (Amersham Biosciences).

Genetic divsersity

Basic mtDNA genetic diversity indices for each population were carried out in Arlequin 3.5 (Excoffier & Lische 2010) including number of haplotypes, nucleotide and haplotype diversities (π and Hd, respectively), polymorphic sites, Fu's Fs (Fu 1997) and Tajima's D (Tajima 1983) neutrality tests. For the microsatellites, diversity statistics were measured as the number of alleles per locus, observed and expected heterozigosity (Ho and He) under Hardy-Weinberg equilibrium in Arlequin, and Polymorphic Information Content (PIC) using Cervus 3.0 (Marshal *et al.* 1998). The loci were tested for deviation of HWE and for linkage disequilibrium also using Arlequin, corrected from deviations using the sequencial Bonferroni method and p = 0.05 (Rice 1989), when appropriate. To plot histograms of allele frequency and private alleles the program GenAlEx 6.2 (Peakal & Smouse 2006) was used.

Phylogeography and population structure

Relationship between the control region mtDNA haplotypes were estimated using the Median joining network implemented in the program Network 4.5 (Bandelt *et al.* 1999). We selected the model of nucleotide substitution for our mtDNA sequences through JModelTest 0.1.1 (Posada 2008). Phylogenetic relationship and divergence times between haplotypes were estimated using the Bayesian approach implemented in Beast 1.6.1 (Drummond & Rambaut 2007) using as calibration point the split between *L. kempii* and *L. olivacea* setting at 5 million years ago (Mya) with 95% confidence interval from 4 to 6 Mya, as estimated by genetic studies (Bowen *et al.* 1991) and by fossil evidence (Dodd & Morgan 1992) and used by Naro-Maciel *et al.* (2008). A strict clock with the HKY model using 6 gamma categories with a mutation rate estimated for marine turtles control region of 1.2% to 2.4% per My (Encalada *et al.* 1996). Each run took 100 million MCMC iterations sampled every 10,000 steps after a 10% initial burn-in.

To assess the pattern of STR genetic variability among the olive ridley individuals we used a principal components analysis (PCA) performed with GenAlEx 6.2 using a genetic distance matrix also obtained with GenAlEx. The mtDNA genetic differentiation among rookeries and oceans were estimated through pairwise F_{ST} (Wier & Cokerham 1984) and Φ_{ST} (Excoffier *et al.* 1992) and AMOVA. Similarly, with the microsatellite data set we estimated for both the pairwise and AMOVA the conventional F-statistics and the summed of squared differences (R_{ST}-like). All F-statistics estimations were performed in Arlequin and the statistical significance were obtained with 10,000 permutations (α=0.05).

In addition, a Bayesian model-based clustering method implemented in Structure 2.3.3 (Pritchard *et al.* 2000) was used to assess the population genetic structure among ridley individuals. This program uses a Markov chain Monte Carlo (MCMC) procedure to estimate the posterior probability that the data fit the hypothesis of K clusters [Pr (X|*K*)]. First, we tested the number of ridleys clusters by performing 10 runs for each *K*, from one to 15 populations, with 100,000 of burn-in and 1,000,000 MCMC iterations with no prior information on sampling location using correlated allele frequencies and assuming the admixture model. We used the program CLUMPP (Jakobsson & Rosenberg 2007) to align the multiple outcome generated by Structure and determine the optimal clustering, which was graphically displayed by DISTRUCT

(Rosenberg 2004). The ad hoc test ΔK (Evanno *et al.* 2005) was used to estimate the most likely number of clusters in these dataset. We also run analyses incorporating prior population information, assuming *K*=3 (the Indo-Pacific, Atlantic and East Pacific oceans) to identify possible migrants, or individuals that have an ancestor from different clusters. Individuals with *q*-values from 0.2 to 0.8 were considered to be potentially admixed, and individuals were considered migrants if $q > 0.2$ (Bergl & Vigilant 2007). Burn-in and run length were the same as described above. A similar analysis was carried out with the program *Structurama* (Huelsenbeck & Andolfatto 2007), that was run 1 million cycles, the first 10% results were discarded as burn-in. This program allow the number of populations to be a random variable following a Dirichlet distribution prior (Pella & Masuda 2006) and uses an efficient variant of MCMC called Gibbs sampling, where each MCMC cycle involves a Gibbs scan of all individuals.

Demographic history and migration

Effective population size (Ne) through time of olive ridley was estimated through Bayesian Skyline Plot (Drummond *et al.* 2005) with the program Beast 1.6.1 (Drummond & Rambaut 2007) using control region mtDNA haplotypes. Four independent runs were performed: 1) for Atlantic lineages; 2) for Indo-Pacific lineages and, 3) for East Pacific lineages and, 4) clade K, from India. The HKY with 6 gamma categories model was used with a strict clock model and substitution rate prior followed a normal distribution with a mean of $1.8E^{-8}$ (per site per year) and a standard deviation of $3.06E^{-9}$. Each analysis were run for 100,000,000 generations with samples drawn every 10.000 generations, with the first 10% of generations discarded as burn-in. Results were checked for convergence using the program Tracer 1.5 (http://beast.bio. ed.ac.uk/) and all parameters had effective sample size (ESS) values >200.

To test for evidence of recent effective population size reduction we used the program BOTTLENECK 1.2.02 (Piry *et al.* 1999) with microsatellites data. Two methods were used: 1) the Two-Phased Model of Mutation (TPM) model of evolution estimated under 100.000 replicates and significance tested with Wilcoxon sign-rank test; 2) the test for a deficit of rare alleles in a sample of loci (the L-shaped graphic model) (Luikart *et al.* 1998). Additionally the

program AGaRst was used to calculate the M-index, where bottlenecked populations would have values of $M < 0.68$ (Garza & Williamson 2001).

The program Lamarc 2.1.5 (Kuhner 2006) was used to estimate effective population sizes and migration rates among pairs of populations of the olive ridley sea turtle. Each dataset was run with the following parameters: a Bayesian analysis with one initial chain of length 50,000 followed by a final chain of length 1,000,000 for mtDNA, sampling trees every 100 steps in each case. For both, short and long chains, 1000 steps were discarded as burn-in and chain temperatures were set (1, 1.1, 3 and 6) to perform multiple simultaneous searches with adaptive heating. The estimated parameters were: theta ($\theta = N_e\mu$ for mtDNA) where N_e is the effective population size and μ is the neutral mutation rate per site per generation; the migration rate ($M = m/\mu$), where m is the chance of a lineage to immigrate per generation. The posterior probability for each parameter was checked using Tracer 1.5 (Drummond & Rambaut 2007). The mean substitution rate was the same used above for mtDNA. The generation time considered for olive ridley sea turtle was of 20 years, as estimated by Abreu-Grobois & Plotkin (2008).

Results

Genetic diversity

mtDNA. The 694 bp long alignment of 330 ridleys sequences presented 61 polymorphic sites that defined 28 haplotypes (Table S2, supplementary data), from these, 27 were olive ridleys haplotypes which are differentiated by 35 polymorphic sites. The frequencies of the haplotypes sequenced here for the ridleys populations are in Table 1. Considering the shorter sequences, 36 ridleys haplotypes from nesting sites were defined by 52 polymorphic sites; four of these were Kemp's ridley haplotypes (Bowen *et al.* 1998) and 32 olive ridley's haplotypes (Bowen *et al.* 1998; Shanker *et al.* 2004; Lopez-Castro & Rocha-Olivares 2005; present study). Only four new sub-haplotypes were found when the longer segments were considered (Table 1 and table S2, supplementary data), suggesting that longer sequences of the mtDNA control region are not very efficient to in evidence new haplotypes, contrasting with results of other sea turtle species (*e.g. Eretmochelys imbricata, Dermochelys coriacea* and *Caretta caretta* - Abreu-

Grobois *et al.* 2006; Velez-Zuazo *et al.* 2008*;* Vargas *et al.* 2008; Monzón-Argüello *et al.* 2010). Therefore, only the shorter but more widely available segment was further analyzed. Basic statistics were presented in Table 2. The olive ridley haplotype diversity varied from 0.76 in Costa Rica to 0.06 in Brazil, with the average of 0.43 (±0.11). The number of haplotypes per rookery varied from 1 in Malaysia to 14 in Mexico (Escobilla). Neutrality tests, both Tajima's D and Fu's F_S were negative for India, Tiwi Island (Australia), all rookeries in East Pacific, and for Atlantic Ocean rookeries (Table 2).

Microsatellites. We genotyped 291 individuals from 11 populations (Table 3) and in average, 95% of the individuals were completely genotyped. Almost all loci were polymorphic in most sampled olive ridleys populations; in Kemp´s ridley three loci were monomorphic (OR3, OR18 and OR19) and CM84 locus did not amplified (Table S3, supplementary data). All populations show visible different allele frequencies in almost all loci as well as private alleles (see Fig S1, supplementary data).The mean number of alleles per locus was 7.9 and varied from 5.5 in Surinam to 13.3 in Escobilla and the mean Ho per locus varied from 0.49 in Surinam to 0.79 in Sri Lanka (Table 3) and was in overall 0.65; PIC varied from 0.59 in Surinam to 0.72 in Sri Lanka and Tiwi Island. For Kemp's ridley, the mean Ho and He was 0.36 and 0.54, respectively, the mean number of alleles was 3.4 and the PIC was 0.45. Detailed information on summary statistics for each locus in each population is in Table S3 (supplementary material). Some loci in few populations were still in deviation from Hardy-Weinberg equilibrium (Table S3, supplementary data) and some pairs of loci showed linkage disequilibrium even after Bonferroni correction for multiple comparisons.

Table 1 Haplotype frequency for *L. kempii* and *L. olivacea* haplotypes sequenced here.

	Indo-West Pacific						East Pacific			Atlantic									
Haplotype	AU				MA	SL	CR	BC	Esc	BR			GF	SU	GB	EP-FG	AT-FG	LK	n
Longer	CY	Arn	Tis	WA						SE	BA	ES							
J	4	7	38	2	2	3	-	-	-	-	-	-	-	-	-	-	-	-	**56**
G	1	3	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**10**
G1**	6	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**8**
H1*	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**2**
H2**	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
J1*	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3**
K	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	**7**
H	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	**2**
I	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	**4**
F	-	-	-	-	-	-	-	-	-	73	11	3	31	12	4	-	8	-	**142**
F2*	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	**1**
E	-	-	-	-	-	-	-	-	-	-	-	-	5	2	-	-	-	-	**7**
F1**	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	**3**
AT1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	**2**
L	-	-	-	-	-	-	2	-	2	-	-	-	-	-	-	-	-	-	**4**
N	-	-	-	-	-	-	4	6	28	-	-	-	-	-	-	4	-	-	**42**
N1*	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
O	-	-	-	-	-	-	4	-	5	-	-	-	-	-	-	2	-	-	**11**
P	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**1**
R	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	**2**
T	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	**2**
U	-	-	-	-	-	-	1	-	6	-	-	-	-	-	-	-	-	-	**7**
V	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	**3**
O1**	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	**1**
U1**	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
N2**	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	**2**
R1**	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	**1**
D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	**4**
n	**13**	**11**	**47**	**4**	**2**	**16**	**13**	**8**	**51**	**75**	**13**	**3**	**36**	**14**	**6**	**6**	**8**	**4**	**330**

*Sub-haplotype which is derived from the shorter segment. ** New haplotype. AU = Australia, CY = Cape York, Arn = Arnhem, TIs = Tiwi Island, WA = Western Australia; MA = Malaysia, SL = Sri Lanka, CR = Costa Rica, BC = Baja California, Esc = Escobilla, BR = Brazil, GF = French Guiana, SU = Surinam, GB = Guinea Bissau, EP-FG = Foraging ground, Esc = Escobilla, AT-FG = Atlantic foraging ground, LK = *L. kempii*

Table 2 Haplotype (Hd) and nucleotide (π) diversities and neutrality tests for olive ridley populations

Sampling sites	N	N Hap	P.S.	Hd	π	Fu's FS	Tajima's D
Indian Ocean	**98**	**10**	**22**	**0.38 (0.06)**	**0.008 (0.004)**	**0.99**	**-0.79**
India	81	8	20	0.27 (0.06)	0.003 (0.002)	-1.59	*-2.05*
Sri Lanka	17	4	14	0.72 (0.08)	0.02 (0.01)	7.02	2.88
West Pacific	**82**	5	5	0.44 (0.06)	0.003 (0.002)	1.28	0.63
Australia	73	5	5	0.46 (0.06)	0.003 (0.002)	1.28	0.63
Tiwi Island	49	5	5	0.36 (0.08)	0.002 (0.001)	-0.38	-0.15
Cape York (Flinders beach)	13	3	4	0.61 (0.08)	0.005 (0.003)	2.67	1.88
Arnhemland (McCluer Group)	11	2	3	0.44 (0.13)	0.003 (0.002)	1.99	1.44
Western Australia	4	3	4	0.83 (0.22)	0.006 (0.005)		
Malaysia	5	1	0	0.00	0.00	0.00	0.00
Total Indo-West Pacific	**180**	**13**	**24**	**0.68 (0.02)**	**0.017 (0.009)**	**5.97**	**1.67**
East Pacific	**215**	**16**	**15**	**0.57 (0.04)**	**0.003 (0.002)**	***-8.53***	**-1.21**
Costa Rica	34	7	7	0.76 (0.04)	0.004 (0.003)	-1.19	-0.82
Escobilla	125	13	13	0.61 (0.05)	0.003 (0.002)	*-5.11*	-0.85
Baja California	56	7	8	0.21 (0.07)	0.0009 (0.0009)	*-6.09*	*-2.16*
Atlantic	**146**	**4**	**4**	**0.15 (0.04)**	**0.0004 (0.0007)**	**-2.53**	**-1.23**
Guinea-Bissau	6	2	2	0.53 (0.17)	0.003 (0.002)	1.72	1.03
Surinam	13	2	1	0.28 (0.14)	0.0007 (0.0009)	0.24	-0.27
French Guiana	36	2	1	0.25 (0.08)	0.0006 (0.0008)	0.50	0.03
Brazil	92	2	1	0.06 (0.03)	0.0002 (0.0004)	-0.96	0.00
East Pacific foraging grounds	**6**	**2**	**1**	**0.53 (0.17)**	**0.001 (0.001)**	**0.63**	**0.00**
Total olive ridley nesting	**541**	**32**	**36**	**0.83 (0.0082)**	**0.014 (0.007)**	**-2.37**	**0.14**

N Hap = haplotype numbers; P.S. = polymorphic sites; Hd = haplotypic diversity, π = nucleotide diversity.
Neutrality test values in bold are significant ($P < 0.05$). Numbers in parenthesis are standard error.

Table 3 Mean number of alleles (K), observed and expected heterozigosity (Ho and He), Polymorphic Information Content (PIC) and the *M*-index for 15 microsatellite loci for olive ridley populations.

	N	K	Ho	He	PIC	*M*
Indo-West Pacific (nesting)	63	13.1	0.70	0.77	0.75	0.80
SriLanka	17	8.7	0.79	0.77	0.72	0.79
Malaysia	9	6.7	0.67	0.79	0.67	0.64
Australia	37	8.3	0.68	0.74	0.72	0.74
Cape York	13	7.2	0.67	0.71	0.65	0.61
Tiwi Island	24	9.3	0.70	0.77	0.72	0.72
Atlantic (nesting)	109	11.2	0.61	0.67	0.63	0.85
Guinea-Bissau	9	5.8	0.61	0.73	0.65	0.64
Surinam	13	5.5	0.49	0.66	0.59	0.58
French Guiana	27	7.0	0.61	0.61	0.56	0.72
Brazil	60	8.7	0.64	0.66	0.62	0.76
East Pacific (nesting)	102	14.4	0.67	0.74	0.71	0.85
Baja California	8	6.6	0.71	0.73	0.65	0.67
Escobilla	75	13.3	0.69	0.73	0.70	0.89
Costa Rica	19	8.5	0.58	0.73	0.68	0.68
East Pacific foraging grounds	11	7.8	0.69	0.75	0.69	-
Lepidochelys kempii	6	3.4	0.36	0.54	0.45	0.58
Overall olive ridley	**285**	**7.9**	**0.65**	**0.72**	**0.66**	**0.87**

N = number of samples

Phylogenetic relationships and phylogeography

The mtDNA Bayesian phylogenetic tree (Fig. 2) support the presence of three very divergent and well supported clades: *L. kempii* haplotypes, the olive ridley K haplotypes found only in Indian and Sri Lanka nesting sites (the K clade) and all other olive ridley haplotypes. The latter is further divided in the following oceanic subclades: East Pacific, Indo-Pacific and Atlantic. The split between the two *Lepidochelys* species (used as a calibration point) was estimated around 4.5 Mya (confidence interval (CI) of 95% between 3.6 to 5.5 Mya). Within the olive ridleys, the K clade split about 1.6 Mya (CI = 0.8 to 2.5 Mya), the East Pacific diverge ~0.61 Mya (CI = 0.27 to 1.0 Mya) and the split between the Indo-Pacific (J clade) and the Atlantic clade was 0.36 Mya (CI = 0.17 to 0.62 Mya). The Time of the Most Common Recent Ancestral (TMRCAs) (diversification times) of the five clades are remarkably similar (Fig. 2): the mode ranging between 221 Kya and 342 Kya. The comntemporary lineages diversified more recently than 200 Kya. The mean substitution rate for the control region mtDNA estimated by this Bayesian analysis was 1.4% per million years (95% HPD between 1.2% and 1.8%), similar to that estimated for the marine turtles control region (Encalada *et al.* 1996).

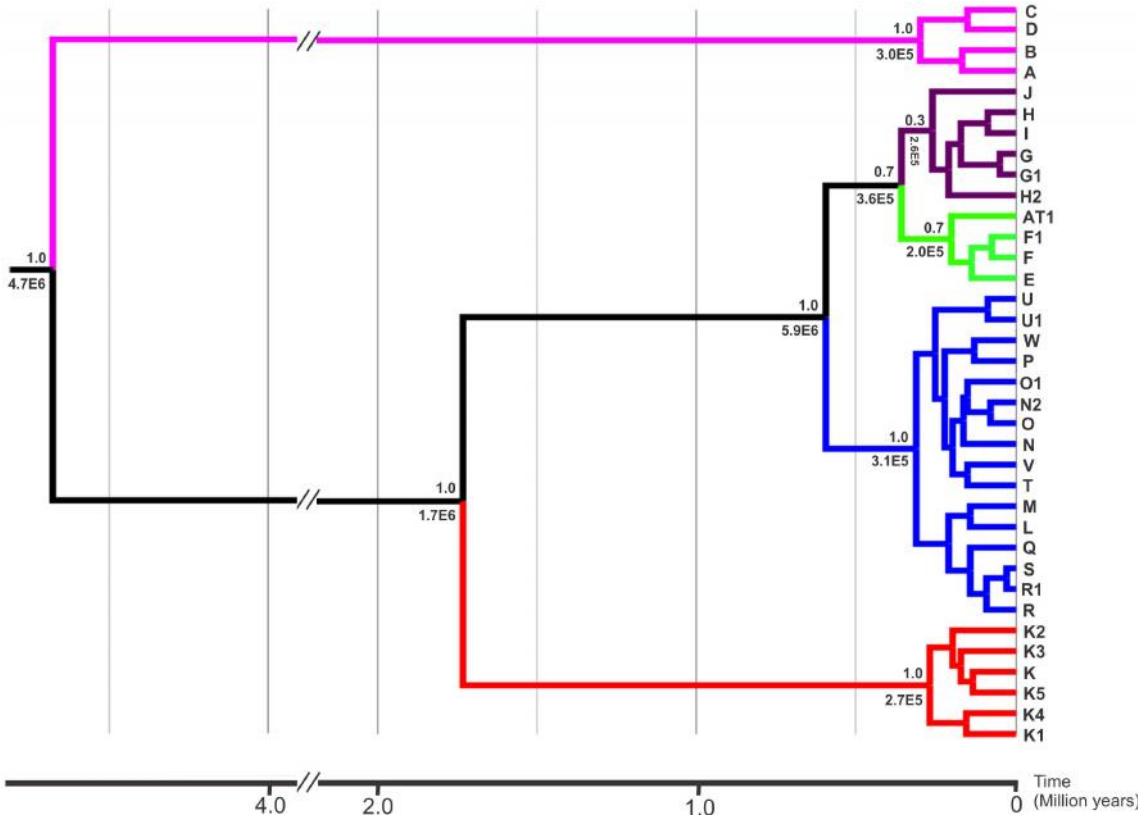


Fig. 2 Bayesian tree evidencing the time for the most recent common ancestor (TMRCA) of ridley turtles, *Lepidochelys kemppi* was used as outgroup. Numbers in red are the posterior probability above 0.7 and number in black are the divergence time estimated. Pink lineages correspond to *L. kempii*, red to the India and Sri Lanka cluster, purple to the Indo-Pacific cluster, green to the Atlantic cluster and in blue to the East Pacific cluster.

The mtDNA MJ haplotype network (Fig. 3) shows a structure very similar to the phylogenetic tree. Kemp´s and Olive ridleys haplotypes are very divergent, separated by 22 mutational steps and olive ridley haplotypes from K clade are separated by 10 substitutions from the other olive ridley haplotypes. The other three clades are also present in the network, although a reticulation exists between the closest Atlantic and Indo-Pacific clades. Also, haplotype J is shared between sites in the Indo-Pacific Ocean, but haplotype H is resolved in two not-shared haplotypes between Australia and Sri Lanka when compared with the longer sequence (Fig. S2, supplementary data). One individual with the haplotype N from East-Pacific was sampled in Indian waters (Shanker *et al.* 2004) (Fig. 3), suggesting a recent migration event. The Atlantic lineages seem to be derivate from the J haplotype first described by Bowen *et al.* (1998).

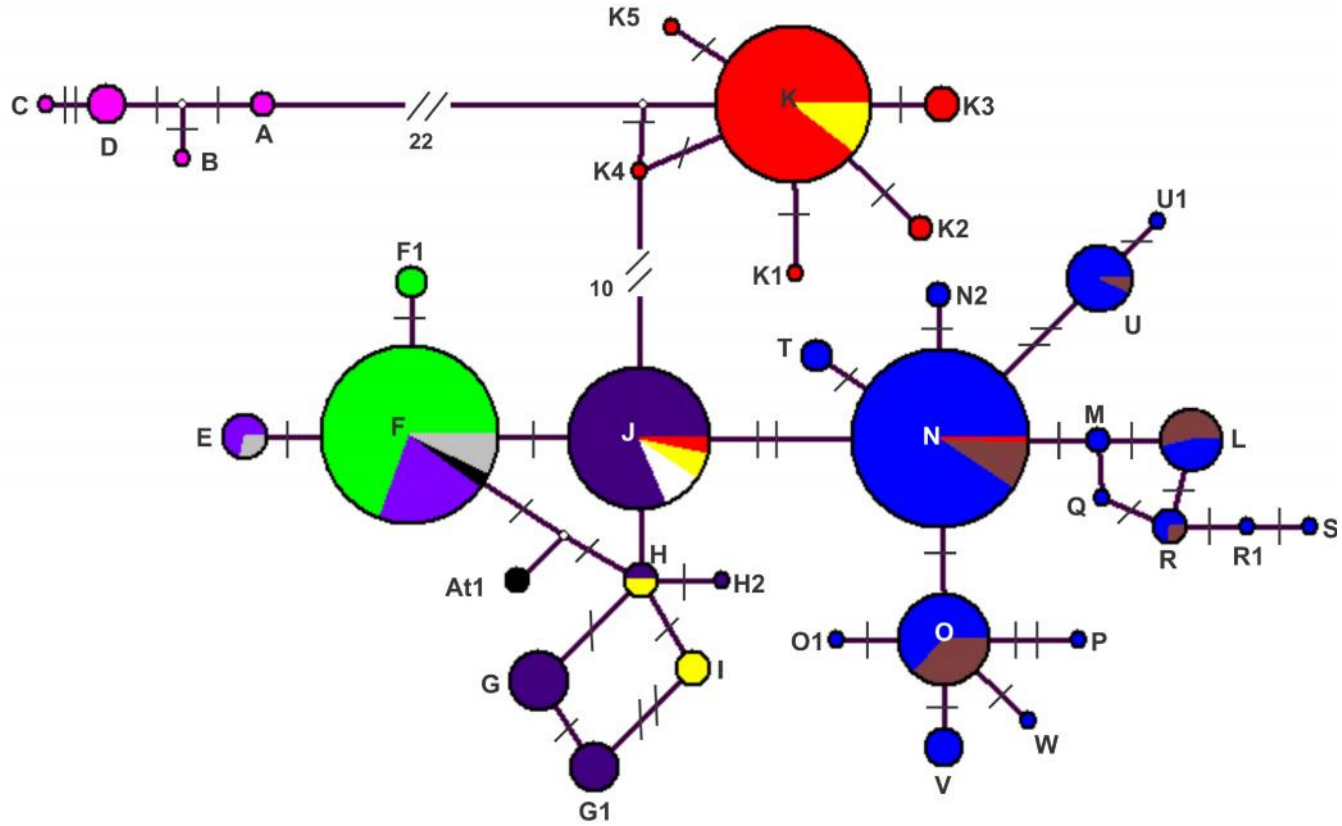


Fig. 3 Median joining network tree showing the relationship among *Lepidochelys kempii* (pink) and *Lepidochelys olivacea* haplotypes (other colors) for mtDNA control region sequences 400 pb long. Nodes are proportional to number of individuals. The white small nodes represent missing haplotypes, slashes represent mutational events.Red represents samples from India, yellow from Sri Lanka, purple represents Australia, white represents Malaysia, green represents Brazil, grey Suriman, violet French Guiana, black Guinea Bissau, blue represents Mexico and brown Costa Rica.

The mtDNA MJ haplotype network with the longer segment has the same pattern (Fig. S2, supplementary data) however, the relation among the haplotypes of "groups" G and H is resolved. Very interestingly, each of the four major Oliva oceanic clades (haplogroups) presents a central and most frequent haplotypes (F, J, K and N, Fig. 3). This pattern suggests each clade may have suffered a bottleneck/founder effect followed by population expansion (see below).

F-statistics

Pairwise mtDNA F_{ST} and Φ_{ST} are presented in Table 4. All F_{ST} and Φ_{ST} values are significantly different between oceans and major nesting areas. As expected, values between within areas/oceans are much lower and most are not significant, although some are significantly different. For example, differences are significant between nesting sites from Australia (Flinders Beach/Tiwi Island and Flinders Beach/Arnhem, but not among Tiwi Island/Arnhem), nesting regions apart approximately by 1000 km. However, these results must be interpreted with caution due to the small sample sizes from both Cape York and Arnhem. Among the nesting sites from AT Ocean, the most different is the population from Brazil, although comparisons with Surinam should be exercised with caution due to its small sample size. Between the EP rookeries the differentiation is significant only for F_{ST} among Baja California/Costa Rica and Baja California/Escobilla. AMOVA results are indicating that olive ridleys are significantly structured between oceans (Indian, Indo-Pacific, Atlantic and East Pacific Oceans), but with low differentiation between nesting sites within oceans (Table 7). The global F_{ST} was 0.64. The same pattern was observed when AMOVA was run with the longer segments (Table S4, supplementary data).

Table 4 Pairwise F_{ST} (below diagonal) and Φ_{ST} (above diagonal) for olive ridley rookeries mtDNA.

	IN	SL	MA	TIs	Arn	CY	GB	SU	GF	BR	BC	ME	CR
IN		***0.58***	***0.9***	***0.92***	***0.9***	***0.91***	***0.91***	***0.92***	***0.93***	***0.96***	***0.94***	***0.92***	***0.92***
SL	**0.24**		0.27	***0.53***	**0.38**	**0.38**	**0.38**	***0.48***	***0.62***	***0.81***	***0.73***	***0.75***	***0.62***
MA	***0.76***	*0.42*		-0.01	0.14	*0.37*	**0.65**	***0.82***	***0.82***	***1***	***0.91***	***0.69***	***0.69***
TIs	***0.69***	***0.42***	-0.02		0.03	*0.25*	***0.62***	***0.66***	***0.7***	***0.85***	***0.83***	***0.71***	***0.73***
Arn	***0.66***	***0.3***	0.09	0.01		0.01	***0.53***	***0.65***	***0.73***	***0.92***	***0.86***	***0.71***	***0.68***
CY	***0.64***	**0.27**	*0.29*	**0.25**	0.12		***0.55***	***0.7***	***0.78***	***0.94***	***0.87***	***0.73***	***0.7***
GB	***0.68***	***0.35***	***0.71***	***0.6***	***0.48***	***0.48***		*0.26*	*0.37*	***0.82***	***0.92***	***0.78***	***0.75***
SU	***0.72***	***0.48***	***0.8***	***0.67***	***0.6***	***0.55***	0.1		-0.05	*0.44*	***0.93***	***0.78***	***0.79***
GF	***0.74***	***0.57***	***0.79***	***0.69***	***0.66***	***0.63***	0.17	-0.05		*0.23*	***0.93***	***0.8***	***0.83***
BR	***0.85***	***0.82***	***0.95***	***0.84***	***0.87***	***0.86***	**0.56**	*0.18*	*0.12*		***0.98***	***0.86***	***0.93***
BC	***0.77***	***0.66***	***0.86***	***0.74***	***0.74***	***0.72***	***0.78***	***0.81***	***0.8***	***0.91***		0.02	0.17
ME	***0.54***	***0.36***	***0.52***	***0.49***	***0.43***	***0.39***	***0.78***	***0.49***	***0.52***	***0.65***	*0.09*		0.07
CR	***0.77***	***0.26***	***0.76***	***0.46***	***0.34***	***0.3***	***0.31***	***0.42***	***0.5***	***0.73***	*0.34*	0.08	

GF = French Guiana, BR = Brazil, GB = Guinea Bissau, SU = Surinam, Tis = Tiwi Island-Australia, CY = Cape York-Australia, Arn = Arnhem-Australia, MA = Malaysia, SL = Sri Lanka, IN = India, CR = Costa Rica, MEX = Mexico, BC = Baja California. Values in italic ($P < 0.05$); values in bold ($P < 0.01$); values in bolds and italic ($P < 0.001$).

Table 5 Pairwise F_{ST} and R_{ST} for ridley turtle populations based on STRs. Below diagonal F_{ST} values, above diagonal R_{ST}.

	SL	Mal	CY	Tis	GB	SU	GF	BR	BC	Esc	CR	EP_FG	LK
SL		0.001	***0.05***	0.01	***0.05***	***0.09***	***0.11***	***0.09***	***0.07***	***0.08***	***0.08***	***0.05***	***0.14***
Mal	0.06		0.02	0	**0.09**	***0.13***	***0.15***	***0.12***	**0.08**	***0.08***	**0.06**	**0.06**	**0.14**
CY	0.02	0.02		*0.02*	***0.16***	***0.20***	***0.22***	***0.19***	***0.16***	***0.15***	***0.15***	***0.13***	***0.21***
Tis	0.01	0.03	-0.01		***0.08***	***0.13***	***0.14***	***0.11***	***0.12***	***0.11***	***0.11***	***0.09***	***0.17***
GB	0.03	0.01	-0.0003	-0.004		*0.05*	**0.06**	**0.04**	**0.06**	***0.07***	***0.07***	**0.05**	***0.19***
SU	**0.10**	0.11	0.05	*0.08*	0.02		*0.04*	0.01	**0.07**	***0.09***	***0.1***	***0.09***	***0.25***
GF	**0.11**	***0.23***	*0.06*	*0.05*	*0.09*	*0.13*		0.01	***0.12***	***0.12***	***0.13***	***0.13***	***0.29***
BR	**0.08**	*0.11*	0.01	0.02	0.01	0.04	0.03		***0.09***	***0.11***	***0.11***	***0.11***	***0.24***
BC	***0.23***	0.07	***0.22***	***0.24***	**0.21**	**0.29**	***0.46***	***0.36***		0.01	0.02	0.01	**0.19**
Esc	***0.30***	***0.14***	***0.29***	***0.32***	***0.28***	***0.35***	***0.45***	***0.40***	-0.01		0.03	0.01	***0.18***
CR	***0.17***	0.02	***0.16***	***0.19***	**0.15**	***0.21***	***0.37***	***0.28***	-0.02	0.02		0.03	***0.17***
EP_FG	***0.27***	*0.12*	***0.30***	***0.28***	**0.27**	***0.37***	***0.51***	***0.42***	0.02	0.04	0.05		***0.18***
LK	***0.41***	***0.33***	***0.41***	***0.38***	***0.38***	***0.40***	***0.53***	***0.45***	***0.31***	***0.34***	***0.28***	***0.36***	

SL = Sri Lanka, Mal = Malaysia, CY = Cape York - Australia, TIs = Tiwi island - Australia, GB = Guinea Bissau, SU = Surinam, GF = French Guiana, BR = Brazil, BC = Baja California-Mexico, Esc = Escobilla-Mexico, CR = Costa Rica, EP_FG = East Pacific foraging ground, LK = *Lepidochelys kempii*. Values in italic ($P < 0.05$); values in bold ($P < 0.01$); values in bolds and italic ($P < 0.001$).

Pairwise microsatellie F_{ST} and R_{ST} are presented in Table 5. Similarly to the mtDNA results, most nesting sites are significantly different, especially between different Oceans while genetic differences were smaller between nesting sites from the same Oceanic basin. However, several within Ocean basin differentiation values were also significant, in special the Fst values for the Atlantic and Indo-Pacific. Among the EP rookeries the differences are not significant and are very small. The EP foraging ground sample was undistinguishable from the EP rookeries . The microsatellite differences Kemp's ridley and olive ridley populations were all very high and significant (Table 5), although the values should be taken with caution since our Kemp´s ridley sample is small. When the F-statistics were estimated between the oceanic basins, all F_{ST} and R_{ST} were highly significant (Table 6). Although agreeing with the mtDNA results indicating that olive ridleys present a clear structure between the Ocean basins, the AMOVA of the microsatellite presented most of the variation within the populations (Table 7).

Table 6 Pairwise F_{ST} (below diagonal) and R_{ST} (above diagonal) based on STRs. All values were significant for P < 0.001.

	IP	**AT**	**EP**	**LK**
IP		0.1	0.08	0.18
AT	0.06		0.1	0.25
EP	0.17	0.22		0.18
LK	0.36	0.42	0.28	

IP = Indo-Pacific, AT = Atlantic, EP = East Pacific and LK = *L. kempii.*

Bayesian clustering analysis

All the genetic clustering analyses including Kemp´s and olive ridleys showed Kemp's ridley grouped in a separated cluster with high probability (not shown). In the further analyses only with olive ridleys, the more likely number of clusters were 5 and 3 (Ln Pr (X|K) =-14759.7 and 14640.5, respectively). The *ad hoc* ΔK method suggested K=3 as the most probable number of cluster (data not shown), a result also supported by the *structurama* analysis (not shown). The analysis with K=3 presented a clear geographic structure, the three clusters mostly consisting of individuals from the three major Ocean basins (AT, EP, IP) (Fig. 4a), with a few exceptions which are consisting of individuals subsequently identified as admixed or migrants

(Fig. 4b). Results of *structurama* showed seven individuals from IP (four from Sri Lanka, two from Malaysia and one from Tiwi Island, Australia) and two individuals from AT (Guinea- Bissau) were assigned as to different populations. Comparing *structurama* results with the test for migrants performed with *structure*, we observed that in most cases when *structurama* assigned one individual as belonging to a different population from that it was sampled, *Structure* assigned it as an admixed individual. All these results are strongly indicating that, for microsatellites, olive ridley turtle shows strong structure among the major Oceans, and lower levels of regional genetic structure.

Table 7 Analysis of molecular variance for control region mtDNA and STRs. For mtDNA the groups considered were Indian, Indo/West Pacific, Atlantic and East Pacific oceans, while for STRs Indo Pacific, Atlantic, and East Pacific oceans each correspond to one group.

Source of variation	Percentage of variation (%)			
	F_{ST}	Φ_{ST}	F_{ST}	R_{ST}
	mtDNA		STR	
Among groups	58.11	82.48	9.17	18.67
Among populations within groups	5.79	5.05	2.23	1.29
Within populations	36.11	12.47	88.6	80.04

The principal component analysis (Fig. 5) based on individual genotypes broadly agrees with the results of the *structure* program and the F-statistics analyses. The individuals from IP, AT and EP comprise three consistent groups although, with some overlapping and with few individuals mixed. In this analysis it could also be observed the high within-population variability and the absence of subgroups constituted by individuals from the same nesting sites.

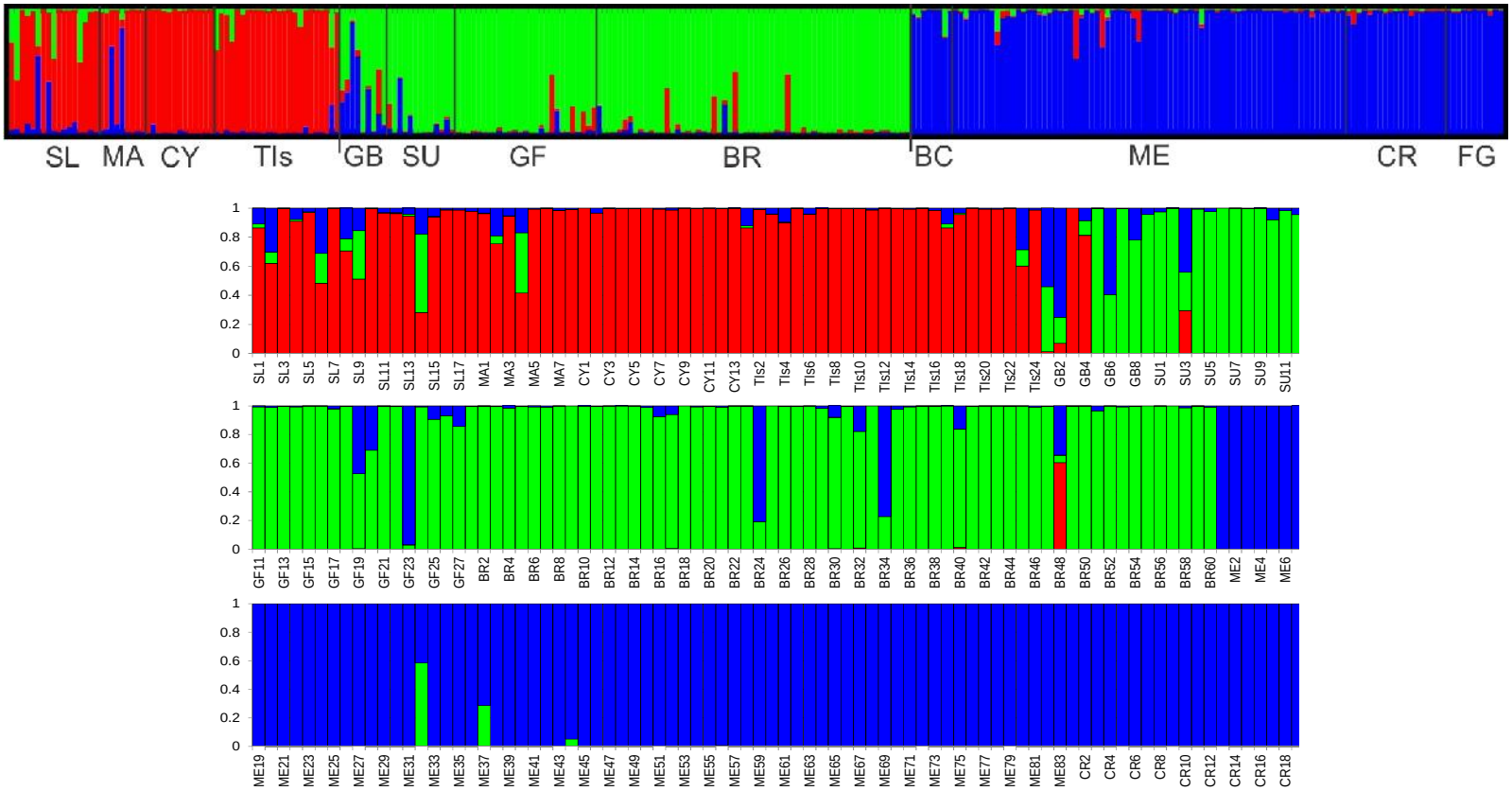


Fig. 4 (a) Proportional membership of each individual of *Lepidochelys olivacea* with K = 3, without use of prior population information. Each individual is represented by a vertical bar, and the length of each bar indicates the probability of membership in each cluster. Indo-Pacific (red), Atlantic (green) and East Pacific (blue). (b) Proportional membership (*q*) of each individual of *Lepidochelys olivacea* inferred by structure using the prior information. Codes used below the horizontal axis are the location where individuals were collected. Colors are the same as in the panel (a). SL = Sri Lanka; MA = Malaysia; CY = Cape York – Australia; TIs = Tiwi Island, Australia; GB = Guinea-Bissau; SU = Surinam; GF = French Guiana; BR = Brazil; BC = Baja California; ME = Mexico; CR = Costa Rica and FG = East Pacific foraging grounds.

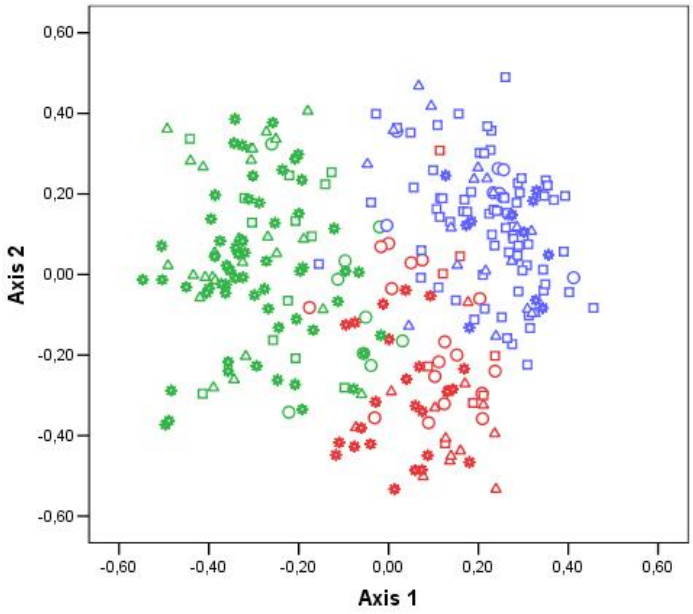


Fig. 5 Pairwise population assignment through PCA suggesting the existence of three clades for *Lepidochelys olivacea*. Red is representing the Indo-Pacific (circle = Sri Lanka, square = Malysia, triangule = Cape York, star = Tiwi ISland), green Atlantic (circle = Guinea Bissau, square = Surinam, triangule = French Guiana, star = Brazil), and blue East Pacific regions (circle = Baja California, square = Mexico and star = East Pacific foraging grounds).

Demographic history and migration

The Bayesian skyline plot for *L. olivacea* mtDNA as a whole showed a signal of a population expansion of about 10 times started around 15 Kya and preceded by a period of constant population size (Fig. 6a). When the major mtDNA clades are analyzed separately, this scenario is partially corroborated. The East Pacific clade present a population expansion scenario very similar to the whole species (Fig. 7 b) only a recent expansion was detected around 15 Kya. For the clade K, in the Indian Ocean, the population expansion may have started between 20 and 30 Kya, although it was weaker and more gradual, making it difficult to precise when it may have started (Fig. 6e). On the other hand, the Indo-Pacific (Australia and Malaysia, Fig. 7c) and the Atlantic clades (Fig. 7d) present no clear signal of population change. The BOTTLENECK results were ambiguous concerning evidence for a recent population declines, since the tests for excess heterozigosity for the TPM model were not significant for both, any of the nesting sites or for the analyses considering the Oceanic basins as populations as well as the distribution of allele frequency was clearly L-shaped for all populations, except for *L. kempii* (Fig. S3, supplementary data). However, the sign-rank test showed significant

heterozigosity deficiency for French Guiana (*P* = 0.0), Brazil (*P* = 0.001, Mexico (*P* = 0.01), and for IP (*P* = 0.02), AT (*P* = 0.0001) and EP (*P* = 0.02), suggesting that some nesting sites populations and each one of the ocean basins have passed through a population expansion (Table S5, supplementary data). On the other hand, the *M*-index showed evidence that Malaysia, Cape York (Australia), Guinea Bissau, Surinam and Baja California has passed through a reductions on effective population size (Table 3), although these results must be interpreted with caution due to the low sample size from these regions. When data are grouped (by ocean or country) the evidence of reduction is not seen (Table 3).

The *lamarc* analyses with the mtDNA sequences were carried out in two ways: 1) with 9 sets of populations: SU, GF, BR, IN, SL, Tis, CY, CR, BC and ME; 2) with 4 sets of populations: Indian Ocean (IN and SL), Indo/West Pacific (Australia and Malaysia), Atlantic Ocean (GB, GF,

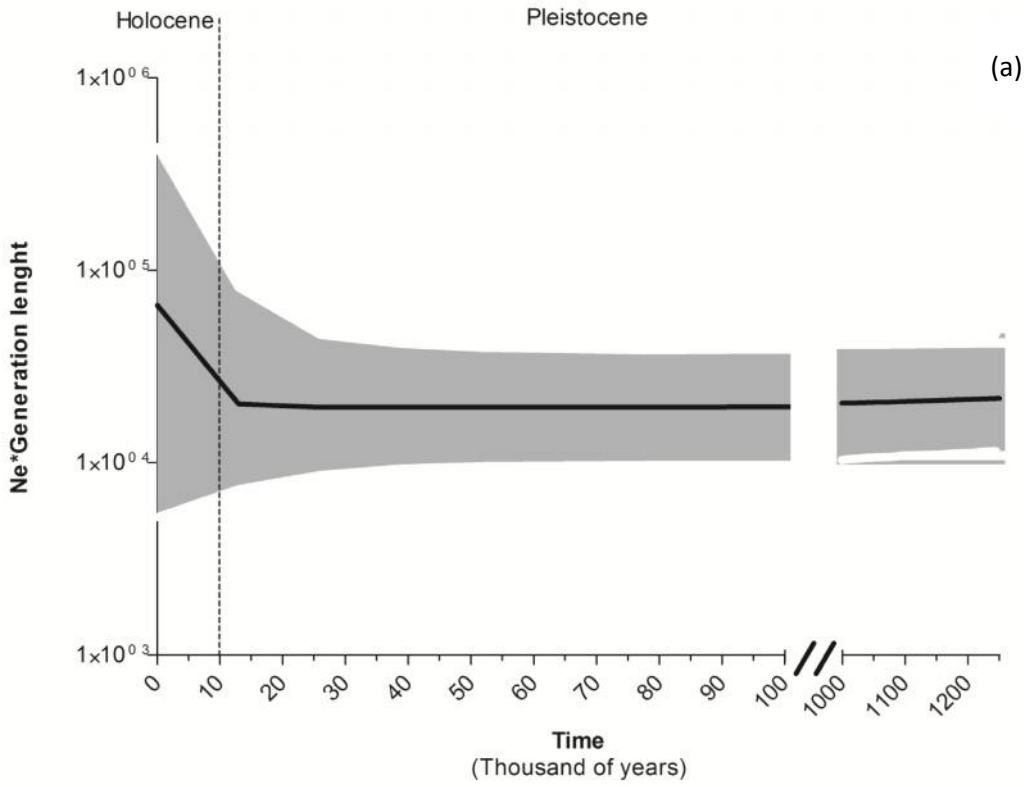
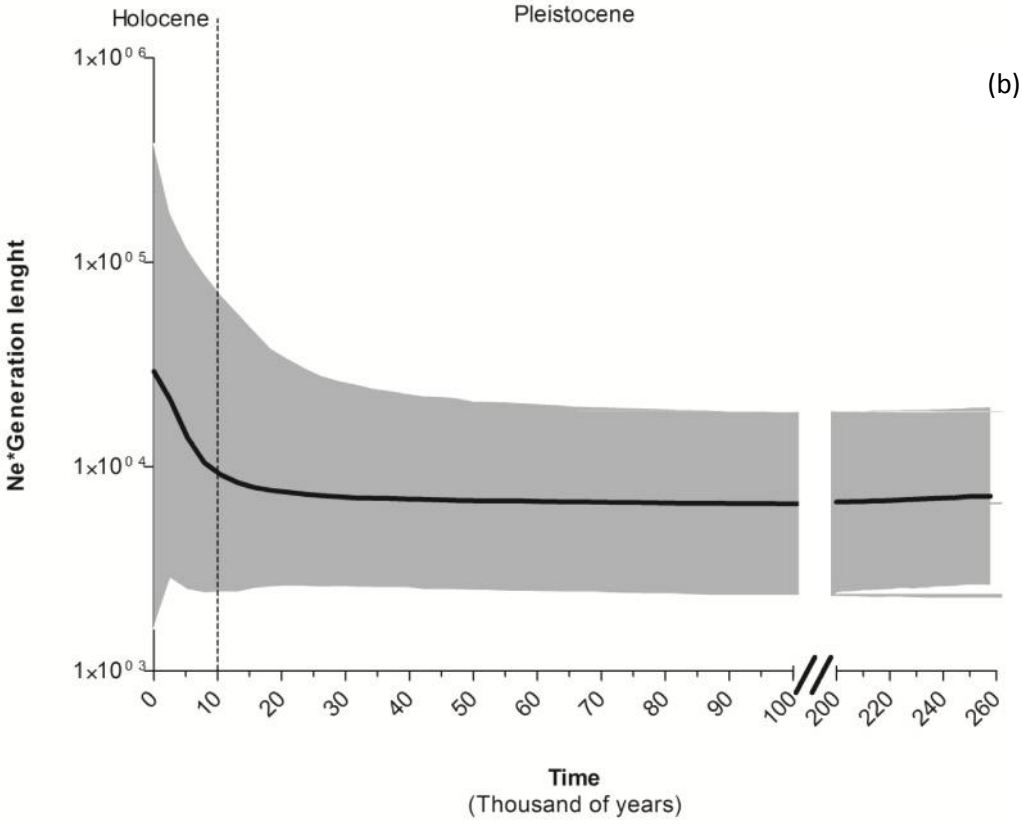


Fig. 6 Bayesian skyline plot showing the effective population size fluctuations through time (a) to olive ridley species; (b) East Pacific clade; (c) to clade J from Indo-Pacific; (d) Atlantic clade; (e) to clade K from Indian Ocean. Solid line = median; grey area = confidence interval.



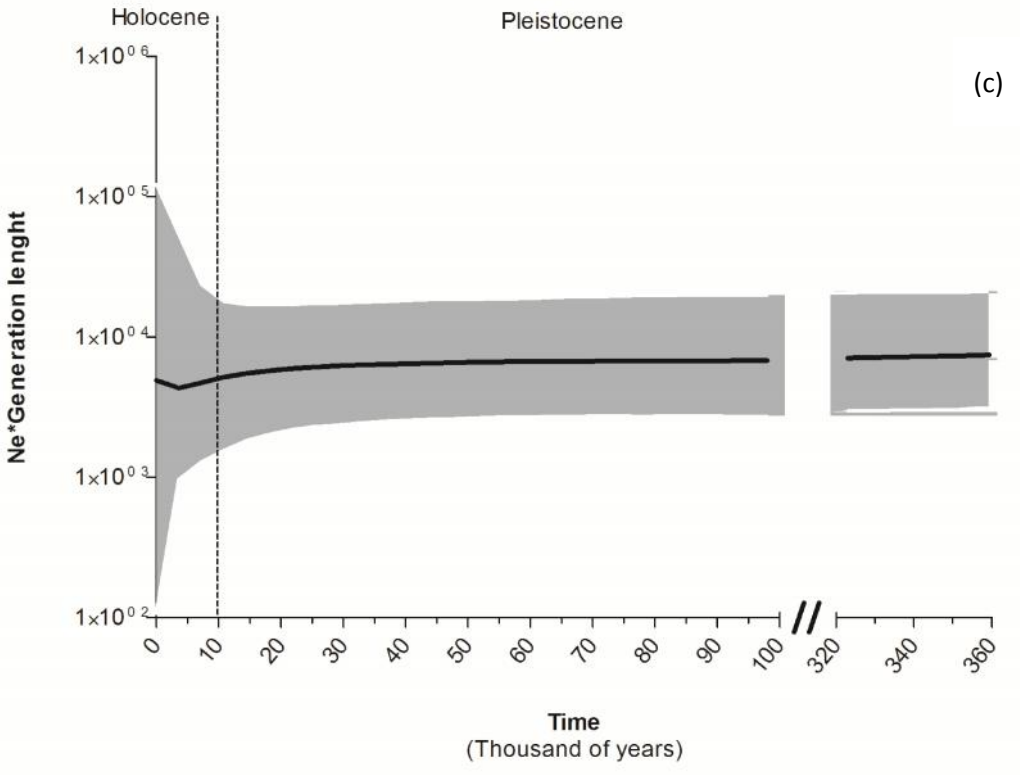
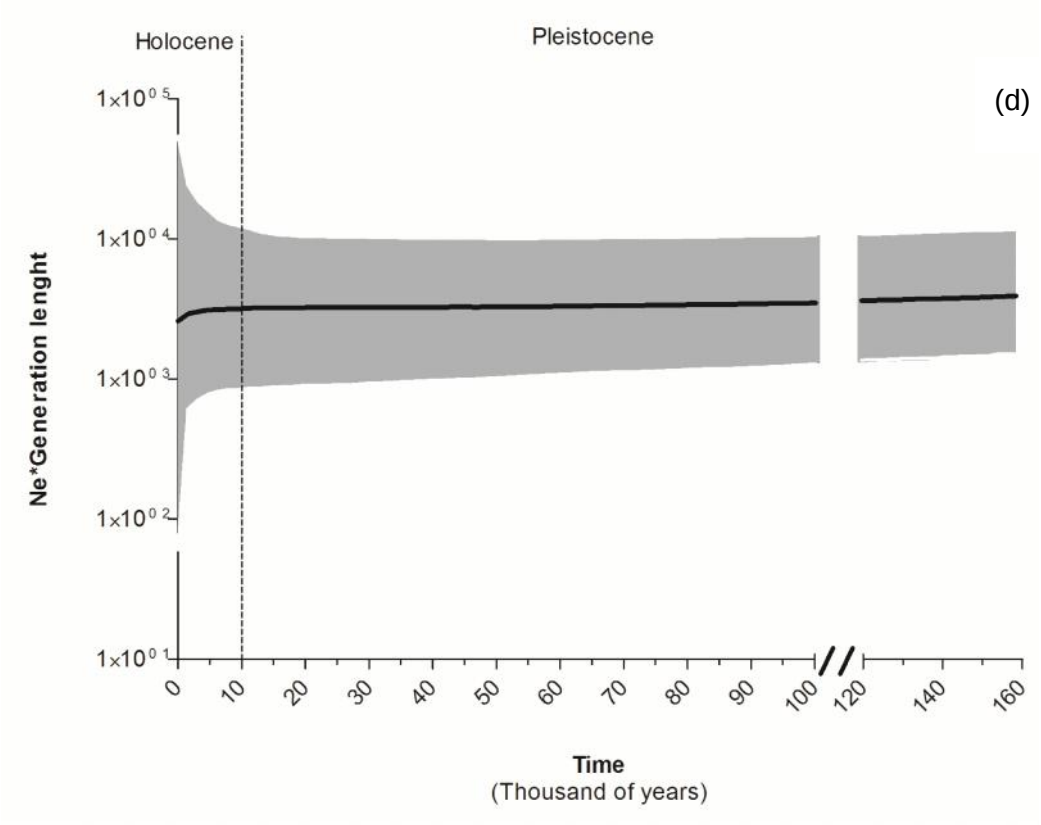


Fig. 6 Continued.



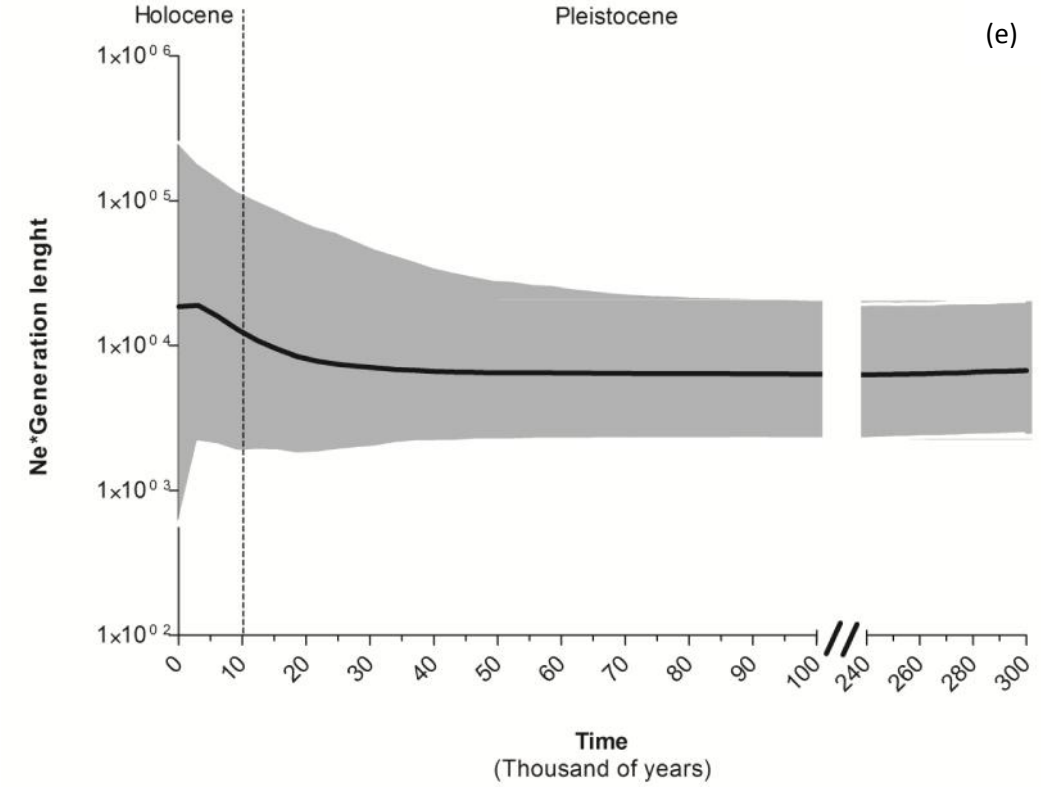


Fig. 6 Continued.

SU and BR) and East Pacific (CR, BC and ME). The results showed that genetic diversity, and consequently the Nef (females effective population size) in olive ridleys varies widely (Table 9), from θ = 0.0063 for IN (Nef ~17,000) to θ = 0.00046 in CY (Australia) (Nef ~1,300) The only pairs of population with Nm values higher than one are those from the East Pacific (ME, CR, and BC) and Sri Lanka into India (Nm = 1.16). All sets of population showed sign of population growth, IN with the higher (G = 921.55) while CY with the smaller signal (G = 521.40) (Table 9).

Table 9 Theta and effective population sizes for mtDNA control region.

	Theta *1000 (mtDNA)	N_{ef}	Growth (mtDNA)
Indian Ocean	**7.06**	**9801 (7351 - 14702)**	**914.26**
India	6.3	17389 (13041 - 26083)	921.55
Sri Lanka	1	2786 (2089 - 4179)	578.79
Indo/West Pacific	**1.58**	**2191 (1643 - 3287)**	**470.76**
Tiwi Island	2.3	6422 (4816 - 9633)	841.91
Cape York	0.47	1266 (950 - 1900)	521.4
Atlantic	**1.72**	**2393 (3589 - 1794)**	**903.29**
Surinam	0.63	1752 (1314 - 2629)	816.08
French Guiana	0.78	2177 (1633 - 3266)	879.16
Brazil	0.55	1536 (1152 - 2304)	706.95
East Pacific	**6.5**	**9030 (13545 - 6772)**	**936.05**
Costa Rica	5.1	14266 (10700 - 21400)	921
Baja California	1.9	5405 (4054 - 8108)	912.01
Escobilla	2	5672 (4254 - 8508)	917.03

Nef = female effective population size; Ne = effective population size

Discussion

Genetic diversity and population structure

Olive ridley has, in general, the smaller genetic diversity compared to other marine turtle species for the mtDNA control region (e.g. Bowen *et al.* 2004, Bowen *et al.* 2005, Formia *et al.* 2006, Bourjea *et al.* 2007, Vargas *et al.* 2008, Reis *et al.* 2009) while for the STRs it is in

general similar to the fewer available estimates (e.g. FitzSimmons *et al*. 1995; Bowen *et al*. 2005; Roberts *et al*. 2004; Theissinger *et al*. 2009). Fluctuation on the population size due to climate changes during the Pleistocene could explain the relative paucity of genetic variation on the olive ridley, which shows the shallower evolutionary history of marine turtle species (see below). The Atlantic olive ridley nesting populations have the lower control region and nDNA genetic diversity when compared to other olive ridley nesting populations. This could be related to the recent colonization of the Atlantic Ocean by the olive ridley along with historical population size fluctuations over evolutionary time (see below). Moreover, as found for olive ridleys nesting in Brazil (Hahn *et al*. in prep.) sequencing a longer segment of the control region dit not uncovered higher levels of genetic diversity or population structure for olive ridleys in our samples, contrasting with results found for other sea turtle species (*e.g. Eretmochelys imbricata, Dermochelys coriacea* and *Caretta caretta* - Abreu-Grobois *et al*. 2006; Velez-Zuazo *et al*. 2008*;* Vargas *et al*. 2008; Monzón-Argüello *et al*. 2010).

Our results shown that olive ridley nesting populations are highly structured, and this structure is related to major Oceanic Basins with modest to high structure among regional nesting sites. F-statistics results were higher for some regional pairs of olive ridley rookeries than previously reported (see Bowen *et al*. 1998, Shanker *et al*. 2004, López-Castro & Rocha-Olivares 2005, Bowen & Karl 2007), besides we found evidences of population structure among Western Atlantic rookeries and among nesting sites in Australia. Olive ridley turtles have the smaller regional levels of genetic structure when compared to other sea turtle species, *e.g. Caretta caretta* which show differentiation between rookeries apart by more than 100 km (Bowen *et al*. 2005), or *Chelonia mydas* which show significant differences on haplotype frequencies in nesting sites apart by 500 km (Dethmers *et al*. 2006). Studying 2000 km of the India East coast for both mtDNA and STRs (Aggarwal & Hyderabad 2004, Shanker et al. 2004) did not find significant differentiation among nesting sites, likewise there are no significant differences among rookeries from East and West coast of India for mtDNA analysis (Shanker personal communication). Besides, Hahn *et al*. (in prep.) did not find differences among nesting areas in Brazil distant about 300 km.

Most studies on sea turtles showed highly structured mtDNA in spite of small differentiation on the nDNA (loggerhead: Schroth *et al*. 1997, Bowen *et al*. 2005, Carreras *et al.* 2007, Monzón-Argüello *et al*. 2010 and green: FitzSimmons *et al.* 1996). The differences between mtDNA and nDNA could be explained by male-mediated gene flow among regional rookeries inside each oceanic basin. A similar pattern has been published for other migratory marine species such as dusky dolphins (Cassens *et al*. 2005) and sperm whale (Lyrholm *et al*. 1998, Lyrholm *et al*. 1999, Engelhaupt *et al*. 2009). However, higher population structure of the mtDNA may be related to its fourfold lower effective population size that accelerates drift (Birky *et al*. 1983).Our microsatellite results did not show strong evidence of population structure among regional nesting sites, although some F_{ST} values were slightly significant for some regional pairs of populations, while among Oceans the population structure is highly significant and this is supported by the results of our Bayesian analyses approaches. One criticism against the use of the F_{ST} is that individual information is lost due to use of allele frequencies of predefined groups of individuals to compute expected heterozigosities that are then compared among groups (Holsinger & Weir 2009). The Bayesian approach uses individual information, thus the use of individual-based approach is potentially more powerful than F_{ST} (Waser & Strobeck 1998). The higher values of F_{ST} than R_{ST} may indicate that genetic drift was more important than mutation on differentiation among populations, corroborating the expansion/retraction scenario.

Low levels of genetic differentiation among regional rookeries could suggest low levels of nesting site fidelity. In Australian waters, olive ridley turtles swim up to 40 km from the nesting beach before re-nest on the same beach (Whitting *et al*. 2007); otherwise in solitary nesting areas of Costa Rica, olive ridley turtles seem to nest in multiple beaches hundreds of kilometers apart during a nesting season in the East Pacific (Kalb 1999, Morreale *et al.* 2007). However, tag data in Brazil suggests that olive ridley are faithful to their nesting beaches (Matos pers. comm.) and, to date, none females tagged in Brazil were caught in the closer Surinam or French Guiana nesting sites (Silva *et al*. 2007). These results suggest some degree of nesting site fidelity in olive ridley turtles although lower than other Cheloniidae. Moreover, olive ridley, like *D. coriacea*, feeds in pelagic environments on oceanic waters (Polovina *et al*. 2004) and

maybe site fidelity is less pronounced in highly vagile pelagic animals (Bowen & Karl, 2007). Another explanation for at least some areas could be there was not enough time for differentiation of regional nesting sites that were very recently re-colonized, due to extinction, from the same source (Holder & Holder 2007).

The mtDNA MJ network shown some haplotypes shared between the oceanic basins, with exception of the Atlantic Ocean. This could suggest recent migration events between Indian/East Pacific, Indian/Indo-West Pacific and no gene flow among Atlantic Ocean and other Oceans; however this should be tested with additional samples from East and West Africa. The results of the structure approach with microsatellites also suggest some recent gene flow among oceans (Fig. 4). Polovina *et al*. (2004) on a satellite tracking study on Hawaii-based longline fisheries indentified six olive ridleys with eastern Pacific origin and three had western Pacific origin indicating that olive ridleys from these oceans are sharing foraging oceanic habitats in central Pacific. Two major geographic barriers separate the tropical marine fauna of the Atlantic and Indo-Pacific: the Isthmus of Panama and Southern Africa, where warm waters from Indian Ocean run over to the Benguela Current in Atlantic Ocean (Bowen & Karl 2007). The Eastern Pacific Barrier is considered the widest marine biogeographic barrier for shallow water marine organisms (Lessios & Robertson 2006), however, it seems not very effective for sea turtles which are known for their extensive migrations (Bolten *et al*. 1998, Moreale *et al*. 2007, Boyle *et al*. 2009).

All results indicate low population structure among nesting sites in East Indian coast (Aggarwal & Hyderabad 2004, Shanker *et al*. 2004) and also between East and West Indian coast (Shanker, personal communication), although the samples from India are significantly different from Sri Lanka, which are separated by only a few kilometers. Nevertheless, we believe these results are inconclusive due to the small sample size from Sri Lanka (N = 17), which probably is not reflecting the haplotype frequencies from that area.

We found population structure between at least two different nesting sites in the Indo-Pacific, even with few samples from Flinders Beach and McCluer Island Group. The high frequency of the haplotype G1 (50%) at Flinders Brach together with its low frequency at Tiwi Island supports the results of genetics distinction of these two rookeries. The nesting site in

Malaysia was not significantly different from the rookeries in Australia, however the low sample size preclude any definitive conclusion, and unfortunately the olive ridley nesting population from Malaysia was extirpated (Cornelius *et al*. 2007) Nevertheless sampling from adjacent nesting populations may help to resolve this issue.

In the East Pacific, low but significant differences was found between rookeries from Baja California and other nesting sites from Mexico and Costa Rica but not between Mexico and Costa Rica, that are more than 1000 km distant (López-Castro & Rocha-Olivares 2005). We found higher population structure between Baja California and adjacent nesting sites but not between the later and Costa Rica.

F-statistics for mDNA data suggest significant levels of population structure among pairs of populations in the western Atlantic rookeries, indicating that rookeries from Brazil are genetically distinct from Surinam and French Guiana. Guinea-Bissau nesting site was also found to be distinct from the western Atlantic sites, despite its low sample size. Despite the predominance of the haplotype F in all Atlantic rookeries (almost 92% of samples) all nesting sites have exclusive haplotypes (*e.g.* E in French Guiana and Surinam; F1 and F2 in Brazil, and AT1 in Guinea-Bissau). This structure is supported by the absence of female tagged in Brazil in samples collected in Surinam or French Guiana, and vice versa (Silva *et al.* 2007).Furthermore, the western Atlantic populations present different nesting season periods which, in Brazil is from October to March while in Surinam and French Guiana is from May to July (Silva *et al*. 2007, Kelle *et al*. 2009, Hilterman *et al*. 2008).

On the other hand, analyses with the STRs do not suggest population structure between the nesting sites from Atlantic, which may indicate male-mediated gene flow. Meanwhile tagging data indicate olive ridley from Brazil and Surinam share feeding grounds in north and northeastern Brazilian shore (Silva *et al*. 2007), where mating could happen among individuals from different nesting sites from Atlantic.

Phylogeography

Classical hypotheses suggested that ridleys species have diverged by vicariance after the Isthmus of Panama closure (Pritchard 1969) and olive ridley colonized the Indian Ocean

being extinct from East Pacific due to climate conditions; only later the species spread worldwide from Indo-Pacific region into East Pacific and more recently into Atlantic Ocean (Bowen *et al*. 1998). However, Shanker *et al*. (2004) hypothesized that ridleys were remnants of a global population which was extinct by the climate conditions pre and after the Isthmus closure; a population would have remained in Indian Ocean and this area would be the olive ridley' source for (re)colonization (Shanker *et al*. 2005). They suggested that the climatic stability of the Indian Ocean throughout the Pleistocene and the unstable conditions of the East Pacific (Lambeck *et al*. 2002, Nishimura 2002) probably contributed to olive ridley survival and then spread worldwide from Indian and West Pacific regions.

Concerning the role of the Isthmus of Panama, Naro-Maciel *et al*. (2008) estimated the upper (more recent) bound of the divergence between Kemp´s and olive ridleys to 4.2 Mya, before the final closure of the Isthmus that is estimated ~2.75 Mya (14 until 2.75 Mya) (Schmittmer *et al.* 2004, Schneider & Schmittmer 2006). These results are compatible with Shanker *et al*. (2004) scenario that these two events were not concurrent, more specifically that the divergence occurred before the closure of the Isthmus.

However, olive ridley have a very shallow evolutionary history (Figs. 2 and 3), with the divergences between clades <600 kya, with the only exception the divergence of the K clade that occurred ~1.6 Mya. Similarly, haplotypes within all clades coalesced between ~220 and 340 kya., These indicate that although the species has more than 4 My of age, the divergence and the origin of most present day oceanic clades are very recent. These results are consistent and extend the hypothesis that olive ridley nesting habitats (with partial exception of the Indian Ocean) are transient (Bowen *et al*. 1998, Shanker *et al*. 2004), suggesting a model of recurrent extinction/colonization for most ridley nesting sites. Our mtDNA data suggested that East Pacific may have been (re)colonized as recently as ~600 kya and the Atlantic around 300 kya from West Pacific or Indian Ocean populations. As suggested by Shanker *et al*. (2004), the presence of clade K, by far the most basal mitochondrial lineage, exclusively in the Indian Ocean is strongly supporting that this is the most ancient of the olive ridley populations that existed today and that this region may be more stable than the others.

It has been estimated that olive ridley has colonized Atlantic Ocean by the Cape of Good Hope (Pritchard 1969) between 0.2-0.4 Ma (Bowen *et al*. 1998, Shanker *et al.* 2004, Holder & Holder 2007). Our data is consistent with this hypothesis, although we could not rule out the existence of ancient populations that went exctinct. The Cape of Good Hope in southern Africa is a known barrier for exchange of tropical organisms between Indian and Atlantic oceans. However, the presence of tropical plankton in sediment cores of southwestern Africa indicate increase on the transportation from Indian Ocean into the Atlantic during inter-glacial periods (Peeters *et al*. 2004).

The transient nature of most olive ridley nesting habitats and the consequent model of recurrent extinction/colonization may be explained by the climatic changes in the last 4 My, induced by the Isthmus of Panama closure and the glacial cycles, especially during the Pleistocene. Thermal physiology of Cheloniidae may have forced the species to nest in warmer shores closer to equator, and only when water temperature was warmer the colonization of areas in higher latitudes was possible. For example, it was supposed that green turtle rookeries in Atlantic Ocean were formed by events of extinction/colonization over evolutionary time as habitat availability varies with climatic changes (Formia *et al*. 2006), and this same pattern of turnover over evolutionary time is likely to have occurred in the olive ridley.

One important consequence of the shallow divergence between mtDNA clades and the model of recurrent extinction/colonization for nesting habitats and whole ocean populations is that, as pointed by Bowen *et al*. (1998), the early history (older than 1.6 Mya) of the olive ridley could not be recovered by the mtDNA data. Therefore, the several hypotheses about where the first divergence between Kemp´s and olive ridleys occurred could not be tested with this mtDNA data.

Concerning the present day oceanic populations, one interesting result is that the diversification times (coalescence age) for all five mtDNA clades (including *L. kempii*) are very similar, the mode ranging between 221 Kya and 342 Kya (Fig. 2). The existence of a similar pattern of diversification in the different oceanic populations could also be seen in the mtDNA network, where all clusters present a star tree shape with a very frequent central haplotype (Fig. 3), which is consistent with demographic expansion. Furthermore, most neutrality tests are in

agreement with the recent expansion scenario since they are indicating population growth (table 2). These results suggest that the most recent demographic events (colonization and population expansion) for most oceanic regions may have been concurrent. Although the TMRCAs estimates are not precise enough to allow to pinpoint an specific event in the past that may have caused these colonizations and expansions, the data suggest that they happened before the last glacial cycle. However, this does not mean that the latter did not have any effect in the olive ridley population size. The Bayesian skyline plot (Fig. 6a) shows that there was a significant population expansion for the whole olive ridley since ~15 kya, after the last glacial maximum. The skyline plots for each clade (Fig.6) shown that this signal is clear in the East Pacific and the K clades, but not in the Atlantic and Indo-Pacific clades.

Conservation genetics

We could identify in ridley turtles five evolutionary significant units (ESUs) with separate management units (MUs) (Moritz 1994) based on the mtDNA relationship and significant divergence in nDNA: 1) Kemp's ridley, in the Gulf of Mexico and North Atlantic; 2) India Ocean with nesting sites in India and Sri Lanka as independent MUs; 3) Indo-Pacific, with three distinct MUs, Malaysia and the two nesting sites in Australia (Tiwi Island and the western Cape York); 4) East Pacific with three MUs, Baja California, mainland nesting sites in Mexico and Costa Rica and, 5) Atlantic, with Surinam/French Guiana, Brazil and Guinea-Bissau as independent MUs.

Human exploitation on olive ridley during centuries and other current threats have severely reduced some populations (Cornelius *et al.* 2007), For example, Surinam population has been declining from 2,800 nests in the 1960s to around 100-150 nests in the early 2000s (Hilterman *et al.* 2008, Kelle *et al.* 2009). Similar problems occurred all over the world, although conservation projects such as the Tamar Project in Brazil reversed this trend (Silva *et al.* 2007). However, this study did not detect any evidence for recent reduction in the genetic diversity of the major olive ridley populations.This suggests that fortunately the population reduction was likely not intense enough to left significant signatures of a genetic bottleneck, similarly to what was observed in several whale populations (e.g. Engel *et al.* 2008, Cypriano-Souza *et al.* 2010).

Acknowledgements

This work was supported by grants and scholarships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS) as well as the support from PETROBRÁS/CENPES to Projeto Tamar. We would like to thank the people from Projeto Tamar who collected the samples and from Laboratório de Biologia Genômica e Molecular-PUCRS for technical assistance, especially to Cladinara Roberts Sarturi. We wish to acknowledge use of the Maptool program for analysis and graphics in this paper. Maptool is a product of SEATURTLE.ORG. (Information is available at www.seaturtle.org)

References

Abreu-Grobois FA, Horrocks JA, Formia A *et al.* (2006) New mtDNA dloop primers which work for a variety of marine turtle species may increase the resolution capacity of mixed stock analyses. *Proceedings of twelveth sixth Annual Symposium on Sea Turtle Biology and Conservation* (eds Compilers Frick M, Panagopoulou A, Rees AF, Williams K), pp. XX-XX, Crete, Greece.

Abreu-Grobois FA, Plotkin (2008) *Lepidochelys olivacea*. IUCN Red listo f threatened species. Available on line: http://www.iucnredlist.org/apps/redlist/details/11534/0.

Aggarwal R, Hyderabad (2004) Genetic analysis of olive ridley (*Lepidochelys olivacea*) populations from the east coast of India using microsatellite markers and haplotyping of mitohhondrial d-loop control region. Available on line: www2.wii.gov.in/ars/2004/ramesha. htm.

Aggarwal RK, Velavan TP, Udaykumar D, *et al.* (2004) Development and characterization of novel microsatellite markers from the olive ridley sea turtle (*Lepidochelys olivacea*). *Molecular Ecology Notes*, **4**: 77-79.

Aggarwal RK, Lalremruata A, Velavan TP, Sowjania AP, Singh L (2008) Development and characterization of ten novel microsatellite markers from olive ridley sea turtle (*Lepidochelys olivacea*). *Conservation Genetics*, **9**: 981-984

Avise, L.C. 2000. *Phylogeography: The history and formation of species*. Harvard University press, Cambridge.

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular and Biological Evolution*, 16: 37-48.

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the southern hemisphere. *Molecular Ecology*, 17: 3754-3774.

Bergl RA, Vigilant L (2007) Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). *Molecular Ecology*, 16: 501–516.

Bernardo J and Plotkin PT (2007) An evolutionary perspective on the arribada phenomenon and reproductive behavioral polymorphism of olive ridley sea turtles (*Lepidochelys olivacea*). In: *Biology and Conservation of the Ridley Turtles* (ed Plotkin PT), 59-87p. The Johns Hopkins University Press.

Birky CWJ, Marayama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplast and some results. *Genetics*, **103**: 513-527.

Bolten AB, Bjorndal KA, Martins HR, *et al.* (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. *Ecological Applications*, **8**(1): 1-7.

Bourjea J, Lapègue S, Gagnevin L *et al.* (2007) Phylogeography of the green turtle, *Chelonia mydas*, in the southwest Indian Ocean. *Molecular Ecology*, **16**: 175-186.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, **31**:26–28.

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. *Molecular Ecology*, 16: 4886-4907.

Bowen BW, Clark AM, Abreu-Grobois AF *et al.* (1998) Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. *Genetica*, 101: 179-189.

Bowen BW, Meylan AB, Avise JC (1991) Evolutionary distinctiveness of the endangered Kemp's ridley sea turtle. *Nature*, **352**: 709-711.

Bowen BW, Bass AL, Soares L, Toonen J (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle. *Molecular Ecology*, **14**: 2389-2402.

Bowen BW, Bass AL, Chow S-M *et al.* (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). *Molecular Ecology*, **13**, 3797–3808

Boyle MC, FitzSimmons NN, Limpus CJ *et al.* (2009) Evidence for transoceanic migrations by loggerhead sea turtles in the southern Pacific Ocean. *Proceedings of the Royal Society. Series B*, *Biological Sciences*, 276: 1993-1999.

Carreras C, Pascual M, Cardona L *et al.* (2007) The genetic structure of loggerhead sea turtle (*Caretta caretta*) in the Mediterranean as revealed by nuclear and mitochondrial DNA and its conservation implications. *Conservation Genetics*, **8**, 761–775.

Cassens I, Waerebeek KV, Best PB *et al.* (2005) Evidence of male dispersal along the coast but no migration in pelagic waters in dusky dolphins (*Lagenorhyncus obscurus*). *Molecular Ecology*, 14: 107-121.

Cornelius SE, Arauz R, Fretey J *et al.* (2007). Effect of land-based harvest of *Lepidochelys*. In: *Biology and Conservation of the Ridley Turtles* (ed Plotkin PT), pp. 231-251. The Johns Hopkins University Press.

Cypriano-Souza AL, Fernández GP, Lima-Rosa *et al.* (2010) Microsatellite characterization of the humpback whale (*Megaptera navaeangliae*) breeding ground off Brazil (breeding stock A). *Journal of Heredity*, **101** (2): 189-200.

Dethmers KEM, Broderick D, Moritz C *et al.* (2006) The genetic structure of Australasian green turtles (*Chelonia mydas*): exploring the geographical scale of genetic exchange. *Molecular Ecology*, **15**, 3931–3946.

Dodd CK, Morgan GS (1992) Fossil sea turtles from the early Pliocene Bone Valley Formation, Central Florida. *Journal of Herpetology*. **26** (1): 1-8.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling 439 trees. *BMC Evolutionary Biology*, **7**, 214.

Drummond AJ, Rambaut A, Saphiro B and Pybus OG (2005). Bayesian Coalescent inference of past population dynamics from molecular sequences. *Molecular and Biological Evolution*, **22** (5): 1185-1192.

Dutton PH, Davis SK, Guerra T and Owens D (1996) Molecular phylogeny for marine turtles based on sequences of the ND4-Leucine and control region of mitochondrial DNA. *Molecular Phylogenetics and Evolution*, 5 (3): 511-521.

Encalada SE, Lahanas PN, Bjorndal KA *et al.* (1996) Phylogeography and population structure of the green turtle (*Chelonia mydas*) in the Atlantic Ocean and Mediterranean Sea: a mitochondrial DNA control region sequence assessment. *Molecular Ecology*, **5**, 473–484.

Engel MH, Fagundes NJR, Rosenbaum HC *et al.* (2008) Mitochondrial DNA diversity of the southwestern Atlantic humpback whale (*Megaptera novaeangliae*) breeding area off Brazil, and the potential connections to Antarctic feeding areas. *Conservation Genetics*, **9**:1253-1262.

Engelhaupt D, Hoelzel AR, Nicholson C *et al.* (2009) Female philopatry in coastal basins and male dispersion across the North Atlantic in a highly mobile marine species, the sperm whale (*Physeter macrocephalus*). *Molecular Ecology*, **18**: 4193-4205.

Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**: 2611-2620.

Excoffier L, Smouse PE, Quattro J (1992) Analysis of molecular variance inferred from metric 57 distances among DNA haplotypes: application to human mitochondrial DNA data. *Genetics*, **58** (131): 479–491.

Excoffier L and Lischer HEL (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Lynux and Windows. *Molecular Ecology Resources*, **10**: 564-567.

FitzSimmons NN, Moritz C, Moore SS (1995) Conservation and dinamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular and Biological Evolution*, **12** (3): 432-440.

FitzSimmons NN, Moritz C, Limpus CJ, Miller JD, Parmenter CJ, Prince R (1996) Comparative genetic Structure of green, loggerhead, and flatback populations in Australia based on variable mtDNA and nDNA regions. In: *Proceedings of the International Symposium on Sea Turtle Conservation Genetics* (eds Bowen BW Witzell WN), pp. 25–32. NOAA Technical Memorandum NMFS-SEFSC-396. National Technical Information Service, Springfield, Virginia.

FitzSimmons NN, Moritz C, Pope L, Limpus CJ (1997) Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and malebiased gene flow. *Genetics*, **147**: 1843–1854.

Formia A, Godley BJ, Dontaine J-F, Bruford MW (2006) Mitochondrial DNA diversity and phylogeography of endangered green turtle (*Chelonia mydas*) populations in Africa. *Conservation Genetics*, **7**,353–369.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915 –925.

Garza JC, Williamson EG (2001) Detecting of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**: 305–318.

Gersonde R, Abelmann A, Brathauer U *et al.* (2003). Last glacial sea surface temperatures and sea ice extent in the Southern Ocean (Atlantic-Indian sector) – A multiproxy approach. *Paleoceanography*, **18** (3): 1061.

Herbert TD, Peterson LC, Lawrence KT, Liu Z (2010) Tropical oceans temperatures over the past 3.5 million years. *Science*, **328**: 1530-1534.

Hilterman ML Tordoir MT Goverse E, Reichart HA (2008) Beaches come and beaches go: coastal dynamics in Surinam are affecting important sea turtle rookeries. In: *Proceedings of the twenty fifth Annual Symposium on Sea Turtle Biology and Conservation* (eds Kalb H, Rohde AS, Gayheart K, Shanker K), pp. 140–141. NOAA Technical Memorandum.

Holder KK, Holder MT (2007) Phylogeography and Population Genetics In: *Biology and Conservation of the Ridley Turtles* (ed Plotkin PT), pp. 107-117 The Johns Hopkins University Press.

Holsinger KE, Weir BS (2009) Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. *Nature Reviews Genetics*, **10**: 639-650.

Huelsenbeck JP, Andolfatto P (2007) Inference of population structure under a Dirichlet process model. *Genetics*, **175**:1787–1802.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801-1806.

Kalb HJ (1999) *Behaviour and physiology of solitary and arribada nesting olive ridley sea turtles (Lepidochelys olivacea) during the interesting period*. PhD Thesys. Texas A&M University, Colege Station.

Kelle L, Gratiot N, Thoisy B (2009) Olive ridley sea turtle *Lepidochelys olivacea* in French Guiana: back from the brink of regional extirpation? *Fauna and Flora International, Orix*, **43** (2): 243-246.

Kuhner MK. 2006. LAMARC 2.0: maximum likelihood and Bayesian estimation of population paramenters. *Genetics and Population Analysis*, **22** (6): 768-770.

Lambeck K, Esat TM, Potter E-K (2002) Links between climate and sea levels for the past three million years. *Nature*, **419**: 199-206.

Lessios HA and Robertson DR. 2006. Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society, Series B*, *Biological Sciences*, **273**: 2201-2208.

López-Castro MC (2004) Caracterización genética de la colonia anidante de Tortuga golfina *Lepidochelys olivácea*, en Baja California Sur, Mexico. Master Thesis.

López-Castro MC, Rocha-Olivares A (2005) The panmixia paradigm of eastern Pacific olive ridley turtles revised: consequences for their conservation and evolutionary biology. *Molecular Ecology*, **14**: 3325-3334.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**: 238-247.

Luschi P, Hays GC, Papi F (2003) A review of long distance movements by marine turtles, and the possible role of ocean currents. *OIKOS*, **103**: 293-302.

Lyrholm T, Gyllensten U (1998) Global matrilineal population structure in sperm whales as indicated by mitochondrial DNA sequences. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **265**, 1679–1684.

Lyrholm T, Leimar O, Johanneson B, Gyllensten U (1999) Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **266**: 347–354.

Marine Turtle Specialist Group (1996) *Lepidochelys kempii*. In: IUCN 2010. *IUCN Red List of Threatened Species*. Version 2010.4. <www.iucnredlist.org>. Downloaded on 06 January 2011

Marshall, TC, Slate, J, Kruuk, LEB & Pemberton, JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**: 639-655.

Monzón-Argüelo C, Rico C, Naro Maciel E *et al.* (2010) Population structure and conservation implications for the loggerhead sea turtle of the Cape Verde Islands. *Conservation Genetics*, **11** (5): 1871-1884.

Morreale SJ, Meylan A, Sadove S, Standora E (1992) Annual occurrence and winter mortality of marine turtles in New York waters. *Journal of Herpetology*, **26**:301-308

Morreale SJ, Plotkin PT, Shaver DJ and Kalb HJ (2007) Adult migration and habitat utilization – ridleys turtles in their element. In: *Biology and Conservation of the Ridley Turtles* (ed Plotkin PT), pp. 213-229 The Johns Hopkins University Press.

Moritz C (1994) Defining evolutionary significant units for conservation. *Trends in Ecology & Evolution*, **9:**373–375.

Naro-Maciel E, Becker HJ, Lima EHSM, Marcovaldi MA, De Salle R (2007) Testing dispersal hypotheses in foraging green sea turtles (*Chelonia mydas*) of Brazil. *Journal of Heredity*, **97**, 29–39.

Nielsen R, Beaumont M (2009) Statistical inferences in phylogeography. *Molecular Ecology*, **18**: 1034-1047.

Nishimura S (2002) Topics on neogene evolution of Pacific Ocean gateways, a summary of the IGCP-355. *Revista Mexicana de Ciencias Geologicas*, **19** (3): 271-273.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6:** 288-295.

Peeters et al. (2004) Vigorous exchange between the Indian and Atlantic Oceans at the end of past five glacial periods. *Nature*, **430**: 661-665.

Pella J, Masuda M (2006) The Gibbs and split-merge sampler for population mixture analysis from genetic data with incomplete baselines. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**: 576–596.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in effective population size from allele frequencies data. *Journal of Heredity*, **90**: 502-503.

Plot V, Thoisy B, Blanc S *et al.* (2011) Reproductive synchrony in a recovering bottlenecked sea turtle population. *Journal of Animal Ecology*: 1-11.

Polovina JJ, Balazs GH, Howell EA, Parker DM, Seki MP, Dutton PH (2004) Foraging and migration habits of loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) in the central North Pacific Ocean. *Fisheries Oceanography*, **13**, 36–51.

Pritchard JK, Stephens M, Donnely P (2000) Inference of a population structure using multilocus genotype data. *Genetics*, **155**: 945-959.

Pritchard PCH (1969) *Studies of the Systematics and Reproductive Cycles of the Genus* Lepidochelys. Phd Thesis University of Florida, Gainesville, FL.

Pritchard PCH (2007) Evolutionary relationships, osteology, morphology, and zoogeography of ridley sea turtles. In: *Biology and Conservation of the Ridley Turtles* (ed Plotkin PT), pp. 45-57. The Johns Hopkins University Press.

Reis EC, Soares LS, Vargas SM *et al.* (2009) Genetic composition, population structure and phylogeography of the loggerhead sea turtle: colonization hypothesis for the Brazilian rookeries. *Conservation Genetics*, **11**: 1467-1477.

Rice WR (1989) Analyzing table of statistical tests. *Evolution*, **43**: 223-225.

Roberts MA, Schwartz TS, Karl SA (2004) Global population genetic structure and male-mediated gene flow in the green sea turtle (*Chelonia mydas*): analysis of microsatellite loci. *Genetics*, **166**: 1857-1870.

Rosenberg NA (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**: 137-138.

Schmittmer A, Sarnthein M, Kinkel H *et al*. (2004) Global impact of the Panamanian seaway closure. *EOS*, **85**: 526-527.

Schneider B, Schmittner A (2006) Simulating the impact of the Panamanian seaway closure on ocean circulation, marine productivity and nutrient cycling. *Earth and Planetary Science Letters*, **246**: 367-380.

Schroth et al. (1996) Evolutionary handicap for turtles. *Nature*, **384**, 521–522.

Shanker K, Ramadev J, Choudury BC *et al.* (2004) Phylogeography of olive ridley turtles (*Lepidochelys olivacea*) on the east coast of India: implications for conservation theory. *Molecular Ecology*, **13**: 1899-1909

Silva ACCD, Castilhos JC, Lopez G, Barata PCR (2007) Nesting biology and conservation of the olive ridley sea turtle (*Lepidochelys olivacea*) in Brazil, 1991/1992 to 2002/2003. *Journal of the Marine Biological Association of the United Kingdom*, **87**: 1047-1056.

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetic*, **105**, 437-460.

Theissinger K, FitzSimmons NN, Limpus CJ *et al.* (2009) Mating system, multiple paternity and effective population size in the endemic flatback turtle (*Natator depressus*) in Australia. *Conservation Genetics*, **10**: 329-346.

Vargas SM, Araújo FCF, Monteiro DS *et al.* (2008) Genetic diversity and origin of leatherback turtles (*Dermochelys coriacea*) from the Brazilian coast. *Journal of Heredity*, **99** (2): 215-220.

Velez-Zuazo X, Ramos WD, Dam RPV *et al.* (2008) Dispersal, recruitment and migratory behavior in a hawksbill sea turtle aggregation. *Molecular Ecology*, **17**: 839-853.

Waser PM, Strobeck C (1998) Genetic signatures of interpopulational dispersal. *Trends in Ecology and Evolution*, **13:** 43-44.

Weir BS and Cokerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**: 1358-1370.

Whitting SD, Long JL, Coyne M (2007) Migration routes and foraging behaviour of olive ridley turtles *Lepidochelys olivacea* in northern Australia. *Endangered Species Research*, **3:** 1-9.

Supplementary data

Tables

Table S1 Anneling temperature and fluorescence label for each one of the microsatellite loci used in this study

Locus	Tag[a]	Ta (^{o}C)
OR1	NED	55
OR2	FAM	55
OR3	HEX	55
OR4	NED	55
OR7	HEX	55
OR8	FAM	56
CM84	HEX	58
EI8	FAM	56
OR9	FAM	55
OR11	HEX	55
OR14	NED	55
OR16	FAM	55
OR18	HEX	55
OR19	FAM	55
OR22	HEX	55

Table S2 mtDNA polymorphic sites and haplotypes designations for *Lepidochelys kempii* and Lepidochelys *olivacea*.

		9	3	4	4	5	7	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	4	4	4	4	4	4
			9	6	8	9	2	0	4	5	6	8	8	9	9	0	0	1	1	2	3	3	4	4	5	8	8	9	0	2	3	5	7	9	9	0	0	0	0	4	4
Haplotypes								7	7	8	6	2	4	6	7	0	2	7	9	4	1	1	5	7	4	3	4	3	8	1	5	7	5	1	4	2	5	6	7	0	1
Longer	Shorter																																							
D	D	-	A	A	A	G	T	T	C	T	G	A	C	A	-	C	A	C	A	A	C	C	G	A	G	C	A	A	C	C	G	C	G	A	C	T	T	A	C	G	T
J	J	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
G	G	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
G1	-	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
H1	H	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
H2	-	-	G	G	-	A	A	C	T	.	A	G	.	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
J1	J	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
K	K	-	G	.	-	A	A	C	T	C	A	G	T	G	A	T	T	.	G	.	.	.	A	.	C	.	.	.	T	T	A	.	A	G	.	C	C	G	A	.	.
H	H	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
I	I	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	.	.	.	T	A	T	A	G	.	C	C	G	A	.	.
L	L	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	T	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
N	N	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
N1	N	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
O	O	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
P	P	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
R	R	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	A	G	T	T	T	A	G	.	T	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
T	T	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	T	C	C	G	A	.	.
U	U	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
V	V	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	.	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
O1	-	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
U1	-	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	C	G	.	C	C	G	A	.	.
N2	-	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	T	T	T	A	G	.	.	.	G	.	T	A	T	A	G	.	C	C	G	A	.	.
R1	-	-	G	.	-	A	A	C	T	.	A	G	T	.	A	.	T	A	G	T	T	T	A	G	.	T	.	G	.	T	A	T	A	G	T	C	C	G	A	.	.
F	F	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	G	.	.	T	A	T	A	G	.	C	C	G	A	.	.
F2	F	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	G	.	.	T	A	T	A	G	.	C	C	G	A	.	.
E	E	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	G	.	.	T	A	T	A	G	T	C	C	G	A	.	.
AT1	-	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	G	.	.	T	A	T	A	G	.	C	C	G	A	.	.
F1	F1	-	G	G	-	A	A	C	T	.	A	G	T	.	A	.	T	.	G	.	T	T	A	G	.	.	G	.	.	T	A	T	A	G	.	C	C	G	A	.	.

Table S2 continued

Haplotypes		4 4 3	4 4 9	4 5 9	4 6 1	4 6 3	4 6 4	4 6 6	4 6 8	4 9 7	5 0 3	5 0 4	5 0 5	5 0 6	5 0 7	5 0 8	5 0 9	5 2 8	5 4 8	5 5 4	5 8 2	5 8 7	6 0 3	6 2 1	6 5 8	6 6 2	6 6 5	6 6 7	6 7 2	6 8 8
Longer	Shorter																													
D	D	T	A	A	T	A	-	G	-	C	-	-	-	-	-	-	-	A	G	T	-	T	A	G	C	A	A	A	A	A
J	J	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
G	G	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	-	G	A	C	-	.	G	A	T	G	G	T	-	G
G1	-	.	G	G	.	G	-	.	C	T	G	C	A	C	G	A	-	G	A	C	-	.	G	A	T	G	G	T	-	G
H1	H	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
H2	-	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
J1	J	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	.	A	T	G	G	T	-	G
K	K	.	G	G	.	.	-	A	-	.	-	-	-	-	-	-	-	G	A	C	-	.	.	A	T	G	G	T	-	G
H	H	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	.
I	I	.	G	G	.	G	-	.	C	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	.
L	L	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
N	N	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
N1	N	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	C	G	A	T	G	G	T	-	G
O	O	.	G	G	.	.	A	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
P	P	C	G	G	.	.	A	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
R	R	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
T	T	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
U	U	.	G	.	C	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
V	V	.	G	G	.	.	A	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
O1	-	.	G	G	.	.	A	.	-	T	G	C	A	C	G	G	T	G	A	C	-	.	G	A	T	G	G	T	-	G
U1	-	.	G	.	C	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
N2	-	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	-	G	A	C	-	.	G	A	T	G	G	T	-	G
R1	-	.	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
F	F	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
F2	F	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	.	C	-	.	G	A	T	G	G	T	-	G
E	E	.	G	G	.	.	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
AT1	-	C	G	G	.	G	-	.	-	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G
F1	F1	.	G	G	.	.	-	.	C	T	G	C	A	C	G	A	T	G	A	C	-	.	G	A	T	G	G	T	-	G

Table S3 Size Range, number of alleles (K), observed and expected heterozigosity (Ho and He) and Polymorphic Information Content (PIC) for 15 micorsatellite loci in all sampled populations.

	Indo/West Pacific			SriLanka					Malaysia					Cape York					Tiwi Sland				
Locus	K	Ho	He	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC
OR1	12	0.85	0.87	158-194 (162)	10	0.82	0.84	0.79	162-186 (162)	7	*0.75*	*0.88*	0.81	158-202 (186)	9	0.83	0.90	0.841	154-202 (186)	12	0.91	0.89	0.85
OR2	14	0.92	0.89	167-187 (179)	9	0.94	0.89	0.85	167-179 (175)	7	0.75	0.83	0.76	155-187 (175)	8	1.00	0.87	0.817	155-189 (173, 175, 177)	13	0.92	0.91	0.88
OR3	3	0.03	0.06	146	1	0.00	0.00	0.00	146	1			0.00	146	1	0.00	0.00	0	140-146 (146)	3	0.09	0.17	0.16
OR4	17	0.74	0.93	124-158 (148)	13	0.59	0.92	0.88	130-162 (136)	9	0.67	0.84	0.77	124-158 (146)	12	0.77	0.92	0.871	124-158 (144, 146)	14	0.89	0.93	0.90
OR7	13	0.83	0.82	187-207 (193, 199)	9	1.00	0.88	0.83	189-215 (189)	6	0.78	0.84	0.77	187-207 (189)	7	0.62	0.66	0.583	187-225 (189)	9	0.83	0.80	0.76
OR8	12	0.80	0.87	148-170 (162)	10	0.82	0.88	0.84	148-166 (154, 162)	8	0.89	0.88	0.81	148-166 (154)	6	0.91	0.84	0.769	148-170 (162)	12	0.71	0.86	0.82
CM84	13	0.61	0.80	322-346 (330)	9	0.59	0.76	0.70	322-352 (330)	8	0.67	0.82	0.75	330-346 (332)	6	0.39	0.79	0.728	328-344 (330)	8	0.75	0.84	0.79
EI8	20	0.75	0.87	190-234 (198)	13	0.82	0.90	0.86	192-220 (200, 210)	8	0.67	0.89	0.82	184-228 (198)	10	0.77	0.82	0.769	184-210 (200)	9	0.73	0.82	0.77
OR9	12	0.78	0.80	158-170 (164)	7	0.77	0.73	0.67	148-170 (164)	7	0.89	0.86	0.79	150-182 (164)	9	0.92	0.85	0.798	154-170 (164)	7	0.67	0.80	0.75
OR11	25	0.82	0.89	198-242 (216)	13	0.94	0.89	0.85	194-240 (226)	11	0.44	0.89	0.83	198-236 (216, 236)	11	1.00	0.88	0.823	184-250 (226)	14	0.79	0.87	0.84
OR14	23	0.82	0.93	159-191 (167)	14	0.88	0.93	0.89	159-189 (177)	10	0.78	0.92	0.86	149-197 (167)	12	0.77	0.90	0.851	147-195 (163)	15	0.81	0.92	0.89
OR16	12	0.68	0.79	222-240 (228)	8	1.00	0.88	0.84	224-252 (224)	7	0.44	0.82	0.75	224-240 (224)	5	0.46	0.59	0.501	222-254 (224)	9	0.65	0.74	0.70
OR18	4	0.57	0.53	118-124 (118)	4	0.75	0.54	0.46	118-120 (118)	2	0.56	0.42	0.32	118-122 (118)	3	0.54	0.58	0.485	118-122 (118)	3	0.48	0.53	0.41
OR19	6	0.59	0.74	148-156 (156)	3	0.88	0.67	0.57	148-156 (156)	3	0.44	0.39	0.34	150-158 (158)	3	0.50	0.49	0.391	148-158 (158)	5	0.48	0.65	0.60
OR22	10	0.77	0.80	219-237 (225)	8	1.00	0.88	0.83	221-249 (229)	6	0.67	0.81	0.73	219-237 (221)	6	0.54	0.63	0.55	219-237 (221)	7	0.77	0.78	0.73
Mean	13.07	0.70	0.77		8.73	0.79	0.77	0.72		6.67	0.67	0.79	0.67		7.20	0.67	0.71	0.65		9.33	0.70	0.77	0.72

Table S3 continued

	Atlantic			Guinea Bissau					Surinam					French Guiana					Brazil				
Locus	K	Ho	He	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC
OR1	11	0.62	0.72	150-186 (162)	7	0.88	0.86	0.78	150-186 (150)	5	0.50	0.64	0.58	150-194 (186)	6	0.42	0.58	0.51	150-202 (186)	10	0.69	0.71	0.66
OR2	13	0.79	0.80	153-183 (183)	5	0.63	0.77	0.68	159-183 (179,181)	8	0.54	0.81	0.75	163-183 (181)	10	0.92	0.82	0.78	157-183 (181)	10	0.81	0.78	0.74
OR3	2	0.01	0.01	146	1	0.00	0.00	0.00	146.00	1	0.00	0.00	0.00	146	1	0.00	0.00	0.00	146-148 (146)	2	0.02	0.02	0.02
OR4	14	0.81	0.87	132-160 (146)	8	0.88	0.90	0.83	128-156 (150)	8	0.78	0.90	0.83	130-162 (154)	10	0.85	0.86	0.82	128-160 (132)	12	0.79	0.81	0.78
OR7	11	0.61	0.62	187-207 (187)	5	0.50	0.81	0.72	187-203 (193)	3	0.42	0.56	0.43	187-199 (193)	5	0.54	0.53	0.46	187-201 (193)	7	0.70	0.62	0.55
OR8	11	0.82	0.83	150-164 (156, 158, 162, 164)	8	1.00	0.91	0.84	146-162 (148)	7	0.55	0.84	0.77	148-164 (162)	8	0.77	0.77	0.73	146-168 (164)	9	0.87	0.83	0.80
CM84	10	0.37	0.59	328-338 (330)	4	0.40	0.78	0.65	330-344 (330)	3	0.17	0.62	0.51	328-340 (330)	7	0.29	0.51	0.48	322-336 (330)	7	0.42	0.59	0.55
EI8	21	0.60	0.88	198-210 (210)	4	0.67	0.73	0.63	194-226 (210)	10	0.50	0.86	0.80	186-232 (194)	14	0.75	0.88	0.84	182-244 (200)	16	*0.57*	*0.86*	0.84
OR9	8	0.58	0.56	158-164 (164)	3	0.11	0.45	0.37	156-166 (164)	5	0.77	0.70	0.63	158-172 (164)	5	0.48	0.41	0.39	158-170 (164)	6	*0.65*	*0.58*	0.53
OR11	20	0.83	0.90	198-240	12	0.78	0.96	0.90	196-236 (236)	11	0.69	0.89	0.84	198-238 (234, 236)	11	0.96	0.88	0.85	196-242 (198)	15	*0.81*	*0.91*	0.89
OR14	18	0.69	0.81	157-193 (165)	12	0.78	0.92	0.86	161-195 (173)	9	0.54	0.87	0.81	161-193 (173)	10	0.55	0.61	0.57	161-195 (173)	16	0.76	0.79	0.77
OR16	12	0.58	0.62	222-242 (222, 228)	6	0.56	0.73	0.63	222-238 (228)	3	0.50	0.57	0.46	222-234 (228)	5	0.52	0.55	0.47	220-248 (228)	8	0.63	0.65	0.59
OR18	4	0.59	0.48	116-120 (118, 120)	3	0.89	0.63	0.51	118-120 (120)	2	0.46	0.37	0.29	116-120 (120)	3	0.70	0.48	0.38	118-122 (120)	3	0.51	0.47	0.38
OR19	6	0.71	0.74	148-156 (152)	4	0.67	0.75	0.66	148-160 (148,156)	5	0.82	0.81	0.74	148-160 (148)	5	0.81	0.76	0.70	148-160 (156)	5	0.66	0.71	0.65
OR22	7	0.55	0.59	219-237 (225)	5	0.50	0.78	0.68	219-225 (225)	2	0.09	0.46	0.34	219-231 (225)	5	0.56	0.54	0.47	219-231 (225)	5	0.64	0.60	0.52
Mean	11.2	0.61	0.67		5.8	0.61	0.73	0.65		5.5	0.49	0.66	0.59		7.0	0.61	0.61	0.56		8.7	0.64	0.66	0.62

Table S3 continued

Table S3 continued

	East Pacific			Baja California					Escobilla					Costa Rica					Foraginf Grounds				
Locus	K	Ho	He	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	K	Ho	He	PIC
OR1	12	0.79	0.87	150-186 (150, 170)	7	0.88	0.87	0.79	150-194 (150)	11	0.82	0.86	0.84	150-194 (154)	10	*0.56*	*0.88*	0.84	150-190 (150, 166)	9	0.91	0.91	0.85
OR2	13	0.70	0.79	153-181 (153)	7	0.63	0.79	0.71	153-187 (153)	11	0.71	0.74	0.70	153-187 (153)	9	0.74	0.87	0.82	153-183 (169)	9	0.64	0.87	0.81
OR3	2	0.01	0.01	146	1	0.00	0.00	0.00	144-146 (146)	2	0.01	0.01	0.01	146	1	0.00	0.00	0.00	146	1	0.00	0.00	0.00
OR4	22	0.77	0.94	132-158	10	0.75	0.95	0.88	124-168 (136)	22	0.83	0.94	0.93	130-156 (132)	11	*0.58*	*0.89*	0.85	124-164 (136)	11	0.73	0.92	0.87
OR7	12	0.76	0.74	193-211 (193)	6	0.75	0.78	0.69	189-211 (193)	12	0.80	0.75	0.72	193-209 (193)	6	0.58	0.64	0.60	193-211 (193)	7	0.82	0.82	0.76
OR8	15	0.79	0.81	148-160 (148)	5	0.88	0.73	0.64	148-170 (154)	12	0.77	0.81	0.78	142-166 (154)	10	0.77	0.78	0.74	148-182 (154)	10	0.91	0.88	0.82
CM84	14	0.72	0.89	324-352 (328)	11	0.75	0.93	0.86	324-352 (328)	14	0.74	0.90	0.88	324-352 (328)	12	0.69	0.89	0.85	328-350 (328)	10	0.64	0.87	0.81
EI8	32	0.74	0.95	186-242 (188, 190)	11	0.63	0.94	0.87	176-258 (200)	29	*0.80*	*0.94*	0.93	176-252 (188)	17	*0.63*	*0.94*	0.91	186-260 (188)	13	0.64	0.95	0.90
OR9	11	0.71	0.63	156-164 (164)	4	0.88	0.64	0.53	154-168 (164)	8	0.73	0.58	0.51	158-192 (158)	6	0.58	0.72	0.65	162-170 (164)	4	0.73	0.54	0.45
OR11	18	0.86	0.92	198-236 (198)	11	1.00	0.95	0.88	196-234 (196)	17	0.85	0.91	0.90	196-232 (222)	12	0.83	0.88	0.84	198-230 (198)	10	0.82	0.89	0.83
OR14	29	0.85	0.95	167-205 (181, 205)	11	0.88	0.94	0.87	151-203 (181)	27	*0.86*	*0.95*	0.95	147-203 (169-173)	15	0.89	0.95	0.91	151-199 (181)	13	0.73	0.91	0.86
OR16	11	0.68	0.77	228-242 (228)	4	0.50	0.59	0.51	224-244 (228)	11	0.74	0.80	0.77	224-242 (228)	7	0.42	0.70	0.65	228-244 (228)	6	0.82	0.77	0.69
OR18	7	0.46	0.51	118-122 (118)	3	0.50	0.58	0.48	112-124 (118)	6	0.42	0.49	0.43	118-124 (118)	3	0.63	0.51	0.40	112-122 (118)	5	0.46	0.58	0.51
OR19	4	0.56	0.53	154-156 (156)	2	0.88	0.53	0.37	148-156 (156)	4	0.54	0.54	0.44	154-156 (156)	2	0.44	0.51	0.37	154, 156	2	0.64	0.52	0.38
OR22	14	0.66	0.77	225-241 (225)	6	0.75	0.73	0.65	221-263 (225)	13	0.70	0.76	0.74	225-241 (225)	7	0.33	0.76	0.70	225-243 (225)	7	0.91	0.83	0.76
Mean	14.40	0.67	0.74		6.60	0.71	0.73	0.65		13.27	0.69	0.73	0.70		8.53	*0.58*	*0.73*	0.68		7.80	0.69	0.75	0.69

Table S3 continued

	L. kempii					Total *L. olivacea*	
Locus	Size Range (pb)	K	Ho	He	PIC	Size Range (pb)	PIC
OR1	146-154 (146)	3	0.00	0.62	0.50	146-202 (150)	0.86
OR2	159-175 (163)	4	0.33	0.64	0.53	153-189 (153)	0.89
OR3	146	1	0.00	0.00	0.00	140-148 (146)	0.02
OR4	142-164 (154)	4	0.40	0.64	0.54	124-168 (156)	0.92
OR7	193-199 (195)	4	0.50	0.77	0.66	187-225 (228)	0.77
OR8	148-170 (164,166, 170)	4	0.60	0.80	0.67	142-182 (154)	0.85
CM84	NA					322-352 (330)	0.81
EI8	180-196 (180)	6	0.80	0.89	0.77	176-260 (200)	0.92
OR9	148-156 (148)	3	0.20	0.38	0.31	148-192 (164)	0.64
OR11	212-250 (250)	6	0.67	0.85	0.75	184-250 (226)	0.93
OR14	163-199 (163)	5	0.67	0.93	0.74	147-205 (173)	0.92
OR16	226-238 (228)	5	0.67	0.83	0.73	220-254 (228)	0.78
OR18	114	1	0.00	0.00	0.00	112-124 (118)	0.49
OR19	154	1	0.00	0.00	0.00	148-160 (156)	0.71
OR22	227-237 (227)	4	0.50	0.71	0.60	219-263 (225)	0.78
Mean		3.40	0.36	0.54	0.45		0.75

Table S4 Analysis of molecular variance for control region mtDNA longer segment. The groups considered were Indian Ocean (Sri Lanka), Indo Pacific, Atlantic, and East Pacific oceans

Source of variation	Percentage of variation (%)	
	F_{ST}	Φ_{ST}
Among groups	57.35	71.16
Among populations within groups	2.14	0.31
Within populations	40.52	28.54

Table S5 Expected heterozygosity and expected heterozygosity under equilibrium for each microsatellite loci for each population and for Oceanic basins

	Indo/West Pacific			**Sri Lanka**			**Malaysia**			**Cape York**			**Tiwi Island**			**Atlantic**			**Guinea Bissau**			**Surinam**		
	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P
OR1	0.87	0.86	0.43	0.84	0.83	0.40	0.88	0.85	0.24	0.90	0.87	0.25	0.89	0.89	0.34	0.72	0.84	0.01	0.86	0.85	0.50	0.64	0.72	0.14
OR2	0.89	0.89	0.51	0.89	0.80	0.01	0.83	0.85	0.26	0.87	0.84	0.30	0.91	0.90	0.31	0.80	0.87	0.03	0.77	0.76	0.57	0.81	0.84	0.17
OR3	0.06	0.44	0.01	0.00		0.11	0			0			0.17	0.48	0.04	0.01	0.20	0.11	0			0		
OR4	0.93	0.91	0.16	0.92	0.89	0.29	0.84	0.90	0.03	0.92	0.92	0.45	0.93	0.92	0.20	0.87	0.88	0.28	0.90	0.88	0.41	0.90	0.87	0.16
OR7	0.82	0.88	0.03	0.88	0.80	0.01	0.84	0.80	0.19	0.66	0.81	0.01	0.80	0.84	0.14	0.62	0.84	0.00	0.81	0.76	0.22	0.56	0.51	0.44
OR8	0.87	0.86	0.50	0.88	0.83	0.01	0.88	0.87	0.50	0.84	0.78	0.14	0.86	0.89	0.09	0.83	0.84	0.30	0.91	0.87	0.06	0.84	0.83	0.46
Cm84	0.80	0.88	0.01	0.76	0.80	0.41	0.82	0.87	0.05	0.79	0.77	0.44	0.84	0.82	0.41	0.59	0.83	0.00	0.78	0.74	0.46	0.62	0.58	0.52
Ei8	0.87	0.92	0.01	0.90	0.89	0.19	0.89	0.87	0.40	0.82	0.89	0.02	0.82	0.84	0.21	0.88	0.92	0.01	0.73	0.66	0.30	0.86	0.89	0.08
OR9	0.80	0.86	0.03	0.73	0.72	0.00	0.86	0.84	0.38	0.85	0.87	0.25	0.80	0.78	0.49	0.56	0.78	0.01	0.45	0.54	0.23	0.70	0.72	0.35
OR11	0.89	0.94	0.01	0.89	0.89	0.33	0.89	0.93	0.03	0.88	0.91	0.08	0.87	0.91	0.03	0.90	0.92	0.15	0.96	0.95	0.09	0.89	0.90	0.17
OR14	0.93	0.94	0.35	0.93	0.90	0.43	0.92	0.92	0.60	0.90	0.92	0.14	0.92	0.92	0.34	0.81	0.91	0.00	0.92	0.95	0.06	0.87	0.87	0.38
OR16	0.79	0.86	0.02	0.88	0.77	0.05	0.82	0.84	0.29	0.59	0.72	P	0.74	0.84	0.02	0.62	0.86	0.00	0.73	0.80	0.09	0.57	0.52	0.40
OR18	0.53	0.57	0.31	0.54	0.53	0.01	0.43	0.32	0.39	0.58	0.51	0.34	0.53	0.48	0.42	0.48	0.55	0.21	0.63	0.54	0.24	0.37	0.29	0.42
OR19	0.74	0.71	0.41	0.67	0.40	0.33	0.39	0.54	0.18	0.49	0.51	0.36	0.65	0.69	0.26	0.74	0.70	0.33	0.75	0.66	0.14	0.81	0.73	0.06
OR22	0.80	0.83	0.18	0.88	0.77	0.00	0.81	0.80	0.55	0.63	0.77	0.03	0.78	0.79	0.35	0.60	0.74	0.03	0.78	0.76	0.48	0.46	0.30	0.29
P_{Hex}	0.98			0.64			0.71			0.92			0.94			0.99			0.13			0.36		
P_{Hd}	0.02			0.38			0.31			0.08			0.07			0.0001			0.88			0.67		

Table S5 continuation

	French Guiana			Brazil			East Pacific			Baja California			Escobilla			Costa Rica			*L. kempii*		
	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P	He	Heq	P
OR1	0.58	0.74	0.03	0.71	0.83	0.01	0.87	0.85	0.40	0.87	0.85	0.51	0.86	0.85	0.38	0.88	0.87	0.47	0.62	0.60	0.55
OR2	0.82	0.86	0.12	0.78	0.83	0.08	0.79	0.87	0.01	0.79	0.85	0.08	0.74	0.85	0.01	0.87	0.85	0.36	0.64	0.71	0.17
OR3	0			0.02	0.22	0.12	0.01	0.20	0.11	0			0.01	0.21	0.12	0			0		
OR4	0.86	0.86	0.46	0.81	0.87	0.05	0.94	0.93	0.29	0.95	0.93	0.05	0.94	0.93	0.26	0.89	0.88	0.43	0.64	0.73	0.19
OR7	0.53	0.68	0.06	0.62	0.76	0.04	0.74	0.85	0.01	0.78	0.81	0.17	0.75	0.86	0.01	0.64	0.75	0.07	0.77	0.71	0.25
OR8	0.77	0.81	0.15	0.83	0.81	0.45	0.81	0.89	0.01	0.73	0.76	0.31	0.81	0.86	0.06	0.78	0.87	0.01	0.80	0.74	0.17
Cm84	0.51	0.78	0.00	0.59	0.76	0.02	0.89	0.88	0.41	0.93	0.95	0.22	0.90	0.89	0.41	0.89	0.91	0.20	0.00		
Ei8	0.88	0.93	0.02	0.86	0.91	0.02	0.95	0.95	0.19	0.94	0.95	0.37	0.94	0.95	0.05	0.94	0.94	0.39	0.89	0.88	0.62
OR9	0.41	0.68	0.01	0.58	0.71	0.07	0.63	0.84	0.00	0.64	0.67	0.31	0.58	0.78	0.00	0.72	0.75	0.24	0.38	0.60	0.10
OR11	0.88	0.87	0.50	0.91	0.90	0.39	0.92	0.91	0.33	0.95	0.95	0.62	0.91	0.91	0.46	0.88	0.90	0.17	0.85	0.85	0.72
OR14	0.61	0.86	0.00	0.79	0.90	0.00	0.95	0.95	0.43	0.94	0.95	0.38	0.95	0.95	0.23	0.95	0.93	0.05	0.93	0.93	1.00
OR16	0.55	0.68	0.08	0.65	0.79	0.02	0.77	0.84	0.05	0.59	0.68	0.17	0.80	0.85	0.09	0.70	0.79	0.05	0.83	0.79	0.30
OR18	0.48	0.47	0.42	0.47	0.45	0.48	0.51	0.74	0.01	0.58	0.55	0.55	0.49	0.71	0.02	0.51	0.50	0.44	0.00		
OR19	0.76	0.68	0.14	0.71	0.65	0.30	0.53	0.55	0.33	0.53	0.33	0.14	0.54	0.57	0.35	0.51	0.28	0.06	0.00		
OR22	0.54	0.68	0.07	0.60	0.65	0.25	0.77	0.88	0.00	0.73	0.81	0.08	0.76	0.87	0.00	0.76	0.80	0.20	0.71	0.71	0.63
P_{Hex}	0.99			0.99			0.98			0.85			0.99			0.85			0.38		
P_{Hd}	0			0.001			0.02			0.16			0.01			0.16			0.65		

Figures

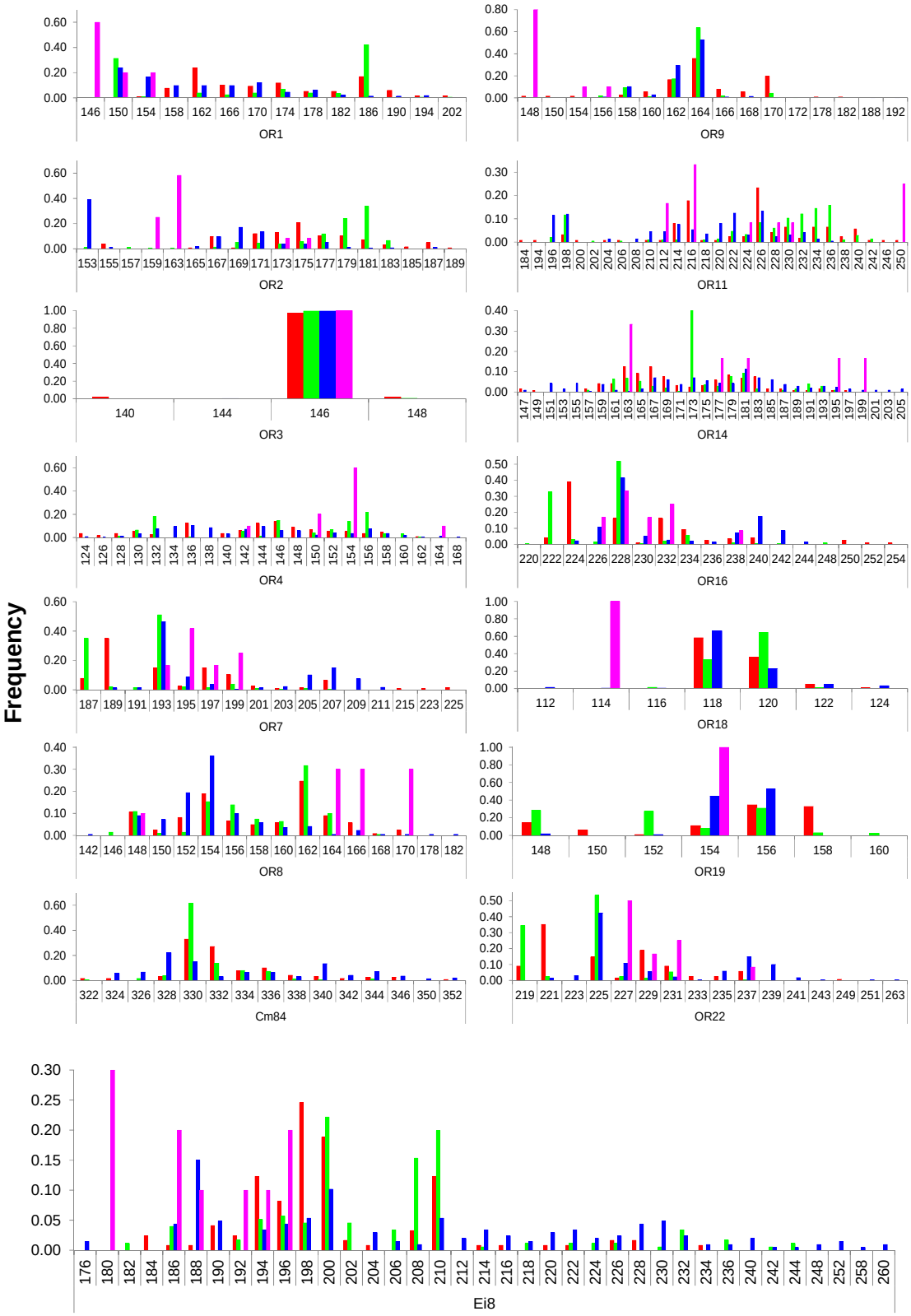

Frequency
OR1
OR9
OR2
OR11
OR3
OR14
OR4
OR16
OR7
OR18
OR8
OR19
Cm84
OR22
Ei8

Fig. S1 Allele frequency for each locus per cluster and for *Lepidochelys kempii*. Red bars represent the frequencies in Indo-Pacific cluster, green in Atlantic, blue in East Pacific and pink in *Lepidochelys klempii* cluster.

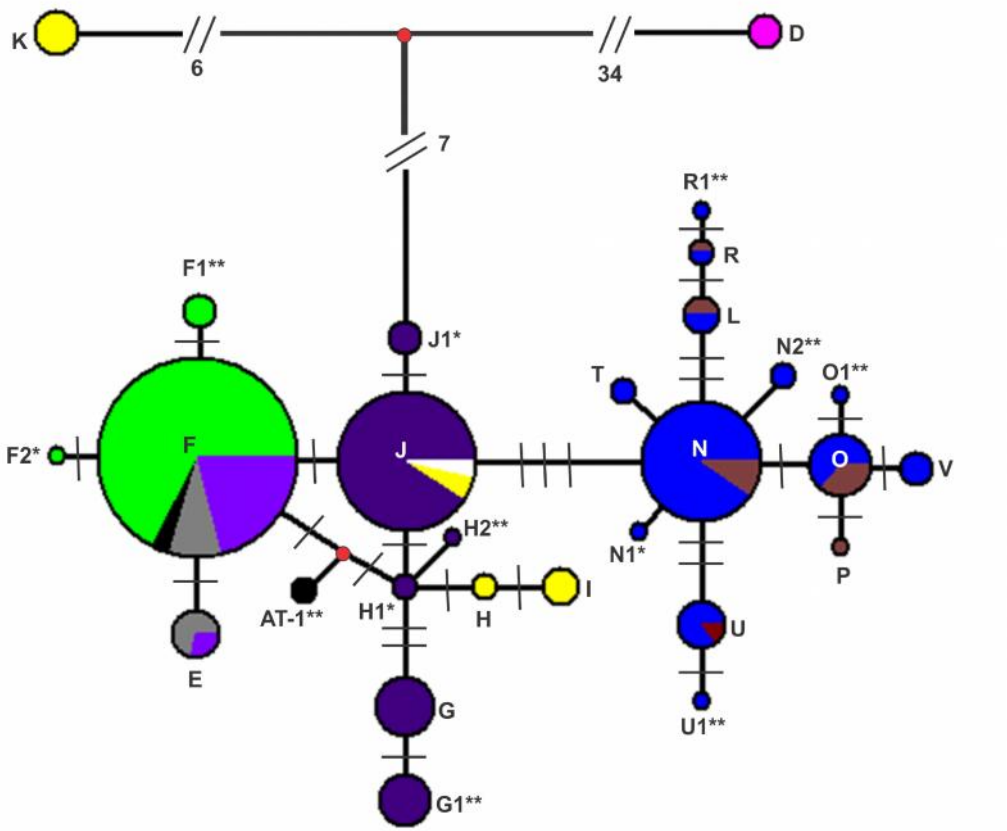


Fig. S2 Median joining network tree showing the relationship among *Lepidochelys kempii* (pink) and *Lepidochelys olivacea* haplotypes (other colors) for mtDNA control region sequences about 700 pb long. Nodes are proportional to number of individuals. The red small nodes represent missing haplotypes, slashes represent mutational events. Red represents samples from India, yellow from Sri Lanka, purple represents Australia, white represents Malaysia, green represents Brazil, grey Suriman, violet French Guiana, black Guinea Bissau, blue represents Mexico and brown Costa Rica.

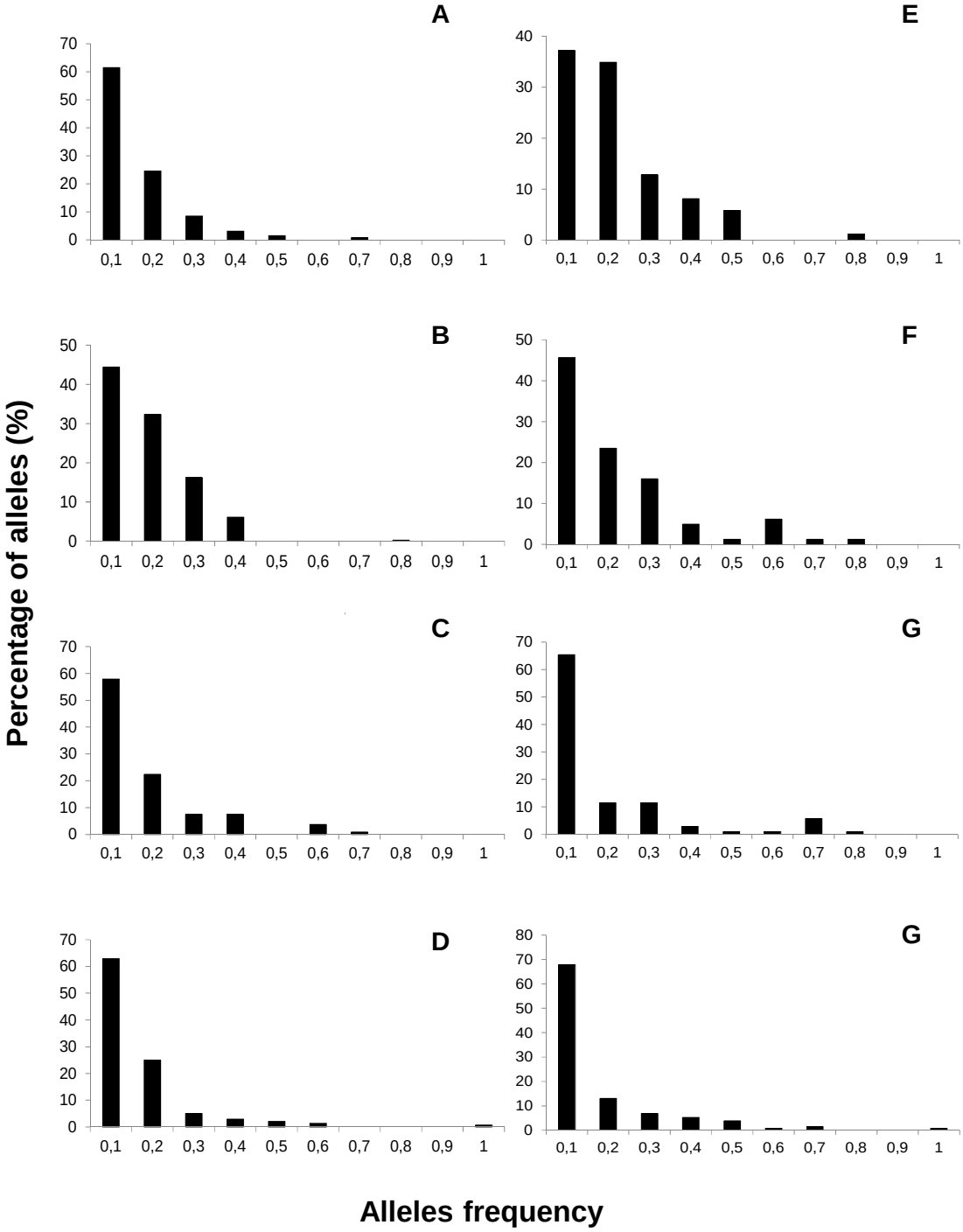


Fig. S3 Allele frequency distribution in the ridley turtles populations. The bars represent the percentage of all alleles detected in each allele frequency class. A- Sri Lanka, B- Malaysia, C- Cape York-Australia, D-Twi Island Australia, E- Guinea-Bissau, F- Surinam, G- Frewnch Guiana, H- Brazil, I- Baja California, J- Escobilla, K- Costa Rica, L- East Pacific – Foraging Ground, M- Indo-Pacific, N- Atlantic, O- East Pacific.

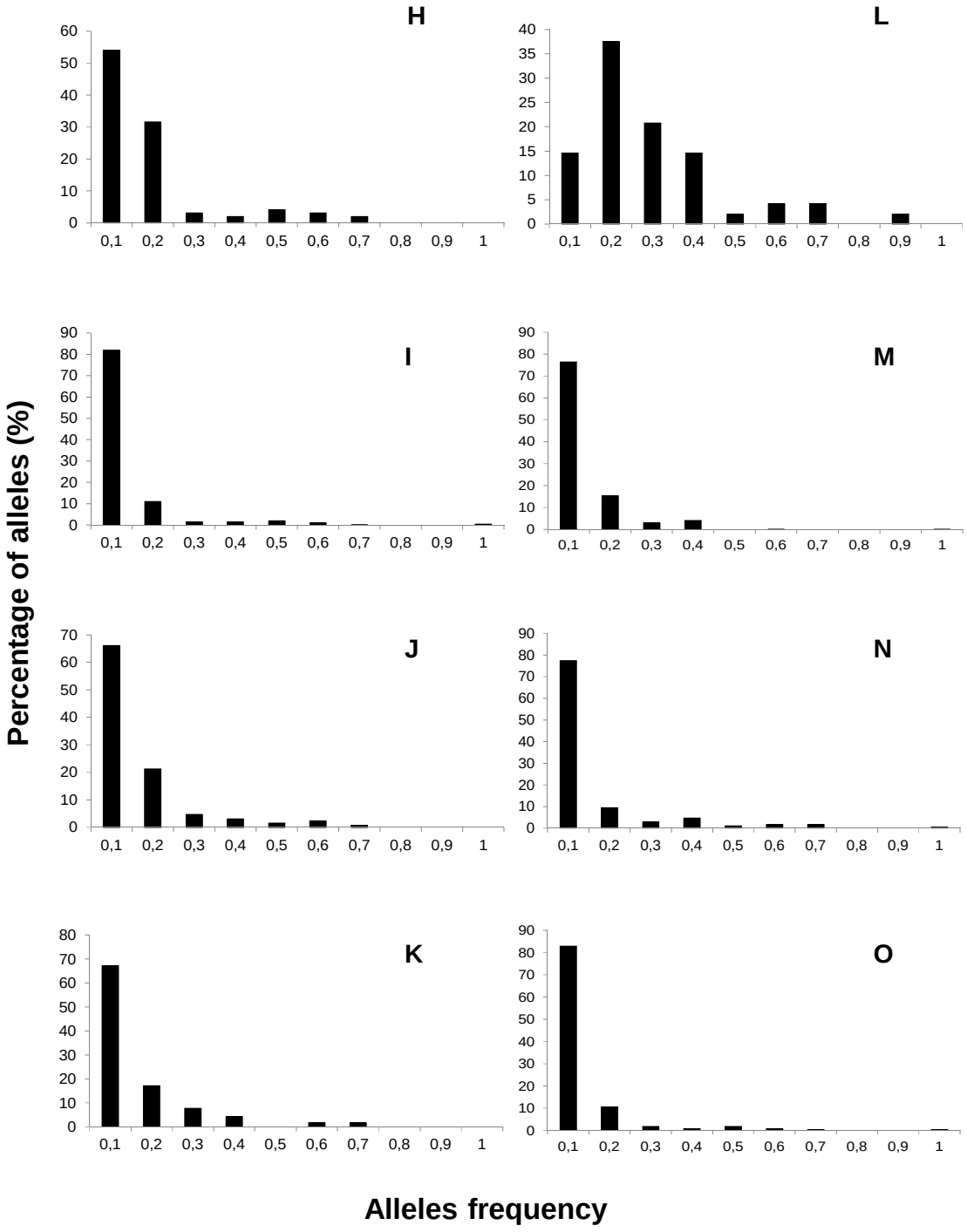


Fig. S3 continued

CONCLUSÕES GERAIS

Os resultados do presente trabalho demonstram a baixa diversidade no mtDNA da tartaruga oliva no Brasil, assim como na maior parte das populações de desova amostradas. A tartaruga oliva, comparada com as demais espécies de Chelonidae, possui a menor diversidade genética para mtDNA. Populações de desova do Atlântico apresentam a menor diversidade genética dentre as demais colônias de desova da espécie. Esta menor diversidade genética e o padrão de estrutura populacional podem ser explicados pela recente colonização do oceano Atlântico em conjunto com um forte efeito fundador. Como a taxa de mutação para o mtDNA em tartarugas é em geral mais lenta comparada a outros vertebrados, não teria havido tempo suficiente para maiores níveis de diferenciação das linhagens no oceano Atlântico.

Nas demais áreas amostradas verificou-se maiores níveis de diversidade genética e de estrutura populacional, porém quando comparadas a outras espécies de Chelonidae, estes níveis podem ser considerados baixos. Este padrão pode ser explicado pela flutuação no tamanho populacional causado por extinções/colonizações devido às mudanças de temperatura causadas durante os períodos glaciais e interglaciais, o que explicaria também a diversificação recente das linhagens atuais de *L. olivacea*. Nossos resultados apontam para um considerável aumento do tamanho populacional para a espécie há aproximadamente 10 mil anos, próximo ao início do atual período interglacial. Portanto, um forte efeito fundador, combinado a baixa taxa evolutiva do mtDNA para a tartaruga oliva pode ser a causa do padrão de diversidade e estrutura populacional da espécie.

Nossos resultados indicam que o padrão filogeográfico das linhagens de *L. olivacea* está relacionado com as mudanças climáticas causadas pelos ciclos de glaciação/interglaciação, gerando grandes flutuações nas temperaturas dos oceanos e mudança nas correntes marinhas, os quais provavelmente mantiveram as populações

restritas a áreas de temperaturas mais elevadas, e isoladas das demais populações durante os períodos glacias; em períodos interglacias, com o aumento da temperatura da água, alguns indivíduos da espécie provavelmente puderam migrar para outras regiões, colonizando novas áreas.

Atualmente, as maiores barreiras oceânicas para a dispersão das espécies parecem não ser efetivas para a tartaruga oliva, já que foi registrada a evidência de migrantes da espécie em todas as bacias oceânicas. Além disso, os machos parecem exercer um importante papel no fluxo gênico da espécie, uma vez que a estrutura populacional entre áreas de desova regionais da tartaruga oliva é menor quando consideramos os STRs.

Os indícios da existência de uma única população de desova no Brasil deverão ser averiguados com base em um maior número de amostragens na Bahia, Abaís e Ponta dos Mangues. Além disso, deve-se investigar a possibilidade de que indivíduos do Suriname e Brasil estajam sobrepondo-se em áreas de forrageio na costa nordeste do Brasil. Em relação ao conhecimento da filogeografia da espécie, é importante aumentar o número de indivíduos amostrados em algumas áreas bem como de novas áreas ainda pouco representadas, como na África, além de diferentes áreas de alimentação. Além disso, a genotipagem de novos e mais marcadores independentes bem como o emprego de novos médodos de análises, tal como a *Aproximation Bayesian computational* (ABC) para testes de cenários demográficos complexos, serão importantes para melhores esclarecimentos a respeito da história evolutiva da tartaruga oliva.

Referências Bibliográficas

Abreu-Grobois FA, Plotkin (2008) *Lepidochelys olivacea*. IUCN Red listo f threatened species. Available on line: http://www.iucnredlist.org/apps/redlist/details/11534/0.

Aggarwal RK, Velavan TP, Udaykumar D, *et al.* (2004) Development and characterization of novel microsatellite markers from the olive ridley sea turtle (*Lepidochelys olivacea*). *Molecular Ecolology Notes*, **4**: 77-79.

Allard MW, Miyamoto MM, Bjorndal KA, Bolten AB, Bowen BW (1994) Support for natal homing in green turtles from mitocondrial DNA sequences. *Copeia, **1994*** (1): 34-41.

Almeida AP, Thomé JCA, Baptistone C *et al.* (2011a) Avaliação do estado de conservação da tartaruga marinha *Dermochelys coriacea* (Vandelli, 1761) no Brasil. *Biodiversidade Brasileira*, **1** (1): 37-44

Almeida AP, Santos AJB, Thomé ACA, *et al.* (2011b) Avaliação do status de conservação da tartaruga marinha *Chelonia mydas* (Linnaeus, 1758) no Brasil. *Biodiversidade Brasileira*, **1** (1): 12-19.

Avise, LC (2000) *Phylogeography: The history and formation of species.* Harvard University press, Cambridge.

Barber RC, Fontaine CT, Flanagan JP and Louis EE jr (2003) Natural hybridization between a Kemp's ridley (*Lepidochelys kempii*) and a loggerhead sea turtle (*Caretta caretta*) confirmed by molecular analysis. *Chelonian Conservation and Biology*, **4**: 701-704.

Bernando and Plotkin (2007) An evolutionary perspective on the arribada phenomenon and reproductive behavioral polymorphism of olive ridley sea turtles (*Lepidochelys olivacea*). In: (Ed Plotkin PT). pp 59-87. Biology and Conservation of Ridley Sea Turtles. The Johns Hopkins University Press.

Bjorndal KA (2003) Foraging ecology and nutrition of sea turtles. In: (Ed Bjorndal KA), *Biology and Conservation of sea turtles*. Smithsonian Press.

Bourjea J, Lapegue S, Gagnevin L et al. *et al.* (2007) Phylogeography of the green turtle, *Chelonia mydas*, in the Southwest Indian Ocean. *Molecular Ecology*, **16**, 175-186.

Bowen BW (1995) Tracking marine turtles with genetic makers – voyages of ancient mariners. *Bioscience***, 45** (8): 528-534.

Bowen BW (1997) Complex population and the conservation genetics of migratory marine mammals: lessons from sea turtles. *Molecular Genetics of Marine Mammals***, 3**:77-84

Bowen WB, Abreu-Grobois FA, Balazs GH, *et al.* (1995) Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. *Proc. Natl. Acad. Sci.*, **92**, 3731-3734

Bowen BW, Avise JC (1995) Conservation genetics of marine turtles. 190-237 p. *In.* Avise JC, Hamrick JL (Ed). *Conservation Genetics: case histories from nature.* New York, Chapman and Hall Press.

Bowen BW, Bass AL, Chow S, M. Bostrom, *et al.* (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). *Molecular Ecology***, 13**: 3797-3808.

Bowen BW, Clark AM, Abreu-Grobois AF et al. (1998) Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. *Genetica*, 101: 179-189.

Bowen BW, Grant WS, Hillis-Starr Z *et al.* (2007) Mixed stock analysis reveals the migrations of juvenile hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea. *Molecular Ecology*, **16**, 49 –60.

Bowen BW, Karl SA (1997) Population genetics, phylogeography and molecular evolution. In: (eds Lutz PL, Musick JA), pp. 29-50.*The Biology of Sea Turtles***.** New York, CRC Press.

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. *Mol Ecol* 16: 4886-4907.

Bowen, BW, Meylan AB, Avise JC (1991) Evolutionary distinctiveness of the endangered Kemp's ridley sea turtle. *Nature***, 352**: 709-711.

Bowen BW, Nelson SW, Avise CJ (1993) A molecular phylogeny for marine turtles: trait mapping, rate assessment and conservation relevance. *Proceedings of the National Academy of Sciences of USA* **90:** 5574–5577

Briggs CJ (1974) Marine Zoogeography. New York: McGrawhill.

Carr A. 1967. *So Excellent a Fishe: A Natural History of Sea Turtles.* Charles Scribner's Sons; New York. vii+ 280 pp.

Chassin-Noria O, Abreu-Grobois A, Dutton PH, Oyama K (2004) Conservation genetics of the East Pacific green turtle (*Chelonia mydas*) in Michoacan, Mexico. *Genetica*, **121**, 195–206.

Ciofi C, Funk SM, Cooter T *et al.* (1998). Genotyping with microsatellite markers. In: (eds Karp A, Isaac PG, Ingram DS), pp 195-201. *Molecular tools for screening biodiversity, plants and animals.* London, Chapman and Hall Press.

Conceição MB, Cornejo DO, Felger RS (1990) Electrophoretic characterization of a hybrid between *Eretmochelys imbricata* and *Caretta caretta* (Cheloniidae). *Comparative Biochemestry and Physiology,* **97**, 275-278.

Cornelius SE, Arauz R, Fretey J, Godfrey MH, Márquez MR, Shanker K (2007). Effect of land-based harvest of *Lepidochelys*. 231-251p. In: Plotkin, PT (Ed) Biology and Conservation of the Ridley Turtles The Johns Hopkins University Press. X + 356 p. il.

Dutton PH, Bowen BW, Owens DW, Barragan A, Davis SK (1999) Global phylogeography of the leatherback turtle (*Dermochelys coriacea*). J Zool., **248**, 397-409.

FitzSimmons NN, Moritz C, Moore SS (1995) Conservation and dinamics of microsatellite loci over 300 million years of marine turtle evolution. *Mol Biol Evol*, 12 (3): 432-440.

Foley, AM, Dutton PH, Singel KE (2003) The first records of olive ridleys in Florida, USA. *Marine Turtle Newsletter***, 101**: 23–25

Frankham R; Ballou JD and Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK, 616p il.

Frazier J (1988) Sea turtles in the land of the dragon. *Sanctuary* (Asia), **8**, 15-23.

Godfrey MH, Chevalier J (2004) The status of olive ridley sea turtles in the west Atlantic. *Report requested by the olive ridley sea turtle assessment group of the Marine Turtle Specialist Group – SSC/IUCN*.

Hendrickson J R (1980) The ecological strategies of sea turtles. *American Zoology***, 20** (3): 597-608

Hilterman ML Tordoir MT Goverse E, Reichart HA (2008) Beaches come and beaches go: coastal dynamics in Surinam are affecting important sea turtle rookeries. In: (eds Kalb H,

Rohde AS, Gayheart K, Shanker K), pp. 140–141. *Proceedings of the 25th Annual Symposium on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum.

Hirayama R (1998) Oldest known sea turtle. *Nature*, **392**, 705-708.

James MC, Martin K, Dutton PH (2004) Hybridiztion between a green turtle, *Chelonia mydas*, and a loggerhead turtle, *Caretta caretta*, and the first record of a green turtle in Canada. *Canadian Field Naturalist*, **118**, 579-582.

Kamezaki N (1983) The possibility of hybridization between the loggerhead turtle, *Caretta caretta*, and the hawksbill turtle, *Eretmochelys imbricata*, in specimens hatched from eggs collected in Chita Peninsula. *Japanese Journal of Herpetology*, **10**, 52-53.

Karl SA, Bowen BW (1999) Evolucionary significants units versus geopolitical taxonomy: molecular sistematics of an endangered sea turtle (genus *Chelonia*). *Conservation Biology***, 13** (5): 990-999.

Karl SA, Bowen BW, Avise JC (1995) Hybridization among the ancient mariners: identification and characterization of marine turtle hybrids with molecular genetics assays. *Journal of Heredity*, **86**, 262-268.

Kelle L, Gratiot N, Thoisy B (2009) Olive ridley sea turtle Lepidochelys olivacea in French Guiana: back from the brink of regional extirpation? Fauna and Flora International, *Orix*, 43 (2): 243-246

Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. *Proc R Soc Lond B*, **265**, 2257–2263.

Lara-Ruiz P, Lopez GG, Santos FR, Soares L (2006) Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. *Conserv Genet* 7: 773-781

Lessios HA and Robertson DR. 2006. Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proc. R. Soc. B*., 273: 2201-2208.

Lohman KJ (2007) Sea turtles: Navigating with magnetism. *Current Biology***, 17**(3): 102-104.

Marcovaldi MA (2001) Status and distribution of the olive ridley turtle, Lepidochelys olivacea, in the western Atlantic Ocean. In: (eds Eckert KL, FA Albreu-Grobois), pp. 52-56. *Proceedings of the Regional Meeting "Marine Turtle Conservation in the Wider Caribbean Region: a Dialogue for Effective Regional Management.* WIDECAST, IUCN-MTSG, WWF, and UNEP-CEP.

Marcovaldi MA, Thomé J, Frazier JG (2003) Marine turtles in latin America and the Caribean: A regional perspective of successes, failures and priorities for the future. *Marine turtles newsletter***, 100:** 38-42

Marine Turtle Specialist Group 1996. *Caretta caretta*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 15 January 2011.

Martins M and Molina FB (2008) Répteis ameaçados de extinção. In: Machado ABM, Drummond GM, Paglia AP (Ed) *Livro Vermelho do Fauna Ameaçada de extinção*. Brasília, DF: MMA; Belo Horizonte, MG: Fundação Biodiversitas, 2v 1420p. Il

Meylan AB, Meylan PA (1999) An introdution to the evolution, life history and biology of sea turtles. 3-5 p. In: *Research and management thechniques for the conservation of sea turtles*. Eckert KL *et al.* (Eds.). IUCN/SSC Marine Turtle Specialist Group Publication n° 4. Internet (www.iucn-mtsg.org/publications) acessado em 20/10/2005

MMA, 2003. Lista das espécies ameaçadas de extinção no Brasil. Disponível em www.mma.gov.br. (acessado em 18/05/2011).

Mortimer JA, Donnelly M (2008) *Eretmochelys imbricata*. In: *IUCN 2010. IUCN Red List of Threatened Species*. Version 2010.4. <www.iucnredlist.org>. Downloaded on 15 January 2011

Naro-Maciel E, Le M, FitzSimmons N, Amato G (2008) Evolutionary relationships of marine turtles: a molecular phylogeny based on nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution*, doi: 10.1016/j.ympev. 2008.08.004

Plotkin PT (2007) Introduction. 3-5p. In: Biology and Conservation of Ridley Sea Turtles. Plotkin, P. T. (Ed.). The Johns Hopkins University Press. X + 356 p. il.

Polovina JJ, Balazs GH, Howell EA, Parker DM, Seki MP, Dutton PH (2004) Forage and migration habitat of loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*)sea turtles in the central North Pacific Ocean. *Fisheries Oceanography***, 13** (1): 36-51

Pritchard PCH (1997) Evolution, phylogeny and current status. *In:* (Eds. Lutz PL, Musick JA, pp. 1-28. *The biology of sea turtles.* New York, CRC Press.

Pritchard PCH (2007) Arribadas I have known. 7-21 p. *In: Biology and Conservation of the Ridley Turtles*. Plotkin, P. T. (Ed.). The Johns Hopkins University Press. X + 356 p. il.

Reis EC, Soares LS, Lôbo-Hajdu G (2009b) Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil. *Conservation Genetics*, 11 (4): 1587-1591.

Rocha LA, Craig MT and Bowen BW. 2007. Phylogeography and the conservation of coral reef fishes. *Coral Reefs*, 26: 501-512.

Rocha LA, Robertson DR, Rocha CR, Tassel JLV, Craig MT and Bowen BW. 2005. Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. *Molecular ecology*, 14: 3921-3928.

Sales G, Giffoni BB, Barata PCR (2008) Incidental catch of sea turtles by the Brazilian pelagic longline fisheries. *J. Mar. Biol. Assoc. UK*, **88**, 853-864.

Sarti Martinez AL (2000) *Dermochelys coriacea*. In: *IUCN 2010. IUCN Red List of Threatened Species*. Version 2010.4. <www.iucnredlist.org>. Downloaded on 15 January 2011.

Seminoff JA, Karl SA, Schwartz T, Resendiz A (2003)Hybridization of the green turtle (*Chelonia mydas*) and the hawksbill turtle (*Eretmochelys imbricata*) in the Pacific Ocean: indications of an absence of gender bias in the directionality of crosses. *Bulletin of Marine Science*, **73**, 643-652.

Seminoff JA (2004) *Chelonia mydas*. In: *IUCN 2010. IUCN Red List of Threatened Species*. Version 2010.4. <www.iucnredlist.org>. Downloaded on 15 January 2011.

Shanker K, Ramadev J, Choudury BC, et al. (2004) Phylogeography of olive ridley turtles (*Lepidochelys olivacea*) on the east coast of India: implications for conservation theory. *Mol Ecol* 13: 1899-1909.

Silva ACCD, Castilhos JC, Lopez G, Barata PCR (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: 1047-1056.

Spotila JR (2004). Sea turtles – a complete guide to their biology, behavior and conservation. The Jond Hopkins University Press. X + 227 p. il.

Spotila JR (2011) Saving sea turtles. The Johns Hopkins University Press. XI + 216 p. il.

Thomé JCA, Marcovaldi MA, Marcovaldi GG, et al. (2003) An overview of Projeto TAMAR-IBAMA's activities in relation to the incidental capture of sea turtles in Brazilian fisheries. *In Proceedings of the Twenty-second Annual Symposium on Sea Turtle Biology and Conservation* (comp. J.A. Seminoff). NOAA Technical Memorandum NMFS-SEFSC, 503, 119–120.

Wood JR, Wood FE, Critchley K (1983) Hybridization of *Chelonia Mydas* and *Eretmochelys imbricata*. *Copeia*, **1983**, 839-842.