



Hidden diversity of the olive ridley sea turtle (*Lepidochelys olivacea*) from Angola, West Africa

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Abstract

The olive ridley sea turtle (*Lepidochelys olivacea*) occupies a wide range within the tropical and subtropical areas of the Pacific, Indian, and Southern Atlantic Oceans. Although occurring in large numbers, the IUCN status for this species is "vulnerable" due to existing threats in their nesting sites. We report the first genetic data on *L. olivacea* from Palmeirinhas-Onça beach, Angola, West Africa. Genetic diversity based on a 400 bp-fragment of the mitochondrial control region of 39 individuals indicated low nucleotide and haplotype diversities compared to other Atlantic populations. We detected a new haplotype present in two individuals from Angola, while the remaining specimens shared a haplotype that is distributed in both sides of the Atlantic and in the Mediterranean, suggesting connectivity between these populations. The baseline data we are generating have a broader significance for characterizing intraspecific biodiversity in the olive ridley sea turtle, which is vital for developing effective conservation policies.

Keywords Olive ridley sea turtle · Conservation · *Lepidochelys olivacea* · Angola

Introduction

In an ever-changing world, it is pivotal to assess intraspecific biodiversity to better evaluate the impacts of global change, particularly in species with broad distributions. These species are likely to undergo isolation by distance, creating genetic structure at the population level due to constraints to gene flow. Marine turtles include flagship species that display strong philopatry, and because of this biological feature, are under global conservation concern if their reproduction sites are threatened (Godley et al. 2010).

The genus *Lepidochelys* includes two species: the olive ridley (*L. olivacea*) and the Kemp's ridley (*L. kempi*) marine turtles that occupy coastal habitats as adults. The most

abundant olive ridley has a worldwide distribution in the warm-temperate and tropical oceans. Cold-temperate conditions probably limit both species' distribution (Bowen et al. 1998). Olive ridleys are primarily mainland nesters seldom using oceanic islands or shores with extensive coral platforms to nest (Pritchard 2007). Nesting aggregates of *L. olivacea* have been reported in the east coast of India, Costa Rica, on the Pacific coast of Mexico (Abreu-Grobois and Plotkin 2008), on both sides of the Atlantic Ocean between Guinea-Bissau and Angola (Fretey 2001), and from Florida to Uruguay (Godfrey and Chevalier 2004).

The olive ridley sea turtle is not considered globally endangered; nonetheless, many former abundant areas have significantly been reduced by human activities (Bowen et al. 1998). Understanding the degree of connectivity between breeding and non-breeding areas remains a challenge (Godley et al. 2010). Molecular genetic markers have proven helpful in addressing migration patterns of sea turtles in the absence of direct observations. Genetic segregation and subsequent mtDNA haplotype frequency variation between nesting colonies offer an opportunity to distinguish the origin of adults and migratory juveniles (Bowen et al. 2007). As highly migratory organisms, sea turtles spend part of their life cycle in feeding aggregations, hosting individuals

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from different nesting sites separated by large distances (Costa Jordao et al. 2017).

Phylogenetic studies of the olive ridley turtle using mtDNA revealed four lineages: east Pacific, Indo–west Pacific, Atlantic, and east coast of India (Shanker et al. 2004). Haplotypic diversity of the olive ridley turtle is classified globally between ‘moderate’ and ‘low’ compared to other species (Bowen et al. 1998; Campista León et al. 2019). Sampling coverage of Atlantic nesting populations is low relative to major nesting sites elsewhere globally (Komoroske et al. 2017).

The olive ridley’s geographic distribution and population status along more than 1500 km of the Angola coastline are poorly understood. Nesting sites can be found throughout the coast, from Cabinda to the North of Angola and from the Namibian-Angola border to the South (Weir et al. 2007). In this area, the olive ridley is categorized as “vulnerable” (Abreu-Grobois and Plotkin 2008; Ministério do Ambiente 2018) given the direct and indirect threats: adult turtles are killed for meat consumption, eggs are harvested, wild carnivores and domestic animals predate eggs, and adults are accidentally caught in fishing gear (Carr and Carr 1991). The abundance of this species is declining worldwide and is a priority for conservation in West Africa (Fretey 2001).

Here, we sampled 46 olive ridley turtles from Palmeirinhas-Onça beach, a nesting site in Angola, West Africa, to analyze genetic diversity and evaluate putative connectivity within other Atlantic populations. We sequenced a fragment of the mtDNA control region and compared it with *L. olivacea* sequences available in GenBank. The generated data will provide crucial information on a critical nesting area to better define the IUCN status of this species, globally classified as vulnerable.

Methods

This research was approved by the Sciences department from the University Agostinho Neto, Angola and followed all national and environmental legislation. All genetic analyses were performed at the Sciences department from the University Agostinho Neto, Angola. Between the 2007 and 2011 seasons, 46 samples were collected from olive ridley turtles in the nesting protected beach at Palmeirinhas-Onça beach (– 9.246, 13.092). Samples were obtained directly from nesting females via blood or skin biopsy or tissue from dead embryos. Blood samples were stored in a lysis buffer (100 mM Tris-HCl, pH 8; 100 mM EDTA, pH 8; 10 mM

NaCl; 1.0% sodium dodecyl sulfate) in approximately a 1:10 ratio of blood to buffer, while skin samples were preserved in 96% ethanol or dimethyl sulfoxide. Genomic DNA was isolated using QIAGEN DNeasy Blood and Tissue Kits following the manufacturer’s protocol.

We used primers LCM15382 and H950 (Abreu-Grobois et al. 2006) to amplify an 800 bp-fragment of mtDNA control region using the following PCR conditions: initial denaturation at 94 °C for 3 min, followed by 35 cycles (denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 s) and a final extension at 72 °C for 10 min. The primer LCM15382 was used for the sequencing reaction, and the purified PCR products were sequenced on an ABI 3130XL. PCR volumes of 25 µl included 1 µl of genomic DNA, 0.2 mM (= 0.5 µl) dNTPs (Promega, Madison, WI, USA), 1 U (= 0.2 µl) GoTaq polymerase (Promega, Madison, WI, USA), 5 mM de Gotaq Buffer (5x) (= 5 µl), 1.5 mM (= 1.5 µl) MgCl₂ and 10 pmol (= 0.5 µl) of forward and reverse primers. PCR products were purified by ethanol precipitation.

We retrieved from GenBank all available 147 mtDNA control region haplotype sequences of *L. olivacea*. Sequences were aligned in Geneious Prime 2020.2.5 (Biomatters, New Zealand, <https://www.geneious.com>) using MUSCLE version 3.8.425 (Edgar 2004) with default values and trimmed to 404 bp to include shorter sequences. Sequences from Angola specimens were collapsed into haplotypes using the web program FaBox (Aarhus, Denmark) (Villesen 2007). The relationships among haplotypes were estimated using a median-joining network (Bandelt et al. 1999), as implemented in PopArt v1.7 (Leigh and Bryan 2015). Standard measures of genetic diversity within the Atlantic were calculated using pegas R-package (Paradis 2010). Those include the number of haplotypes and private haplotypes, haplotype (h) and nucleotide (π) diversities (Nei 1987) and corresponding standard deviations.

Results and discussion

The mtDNA control region primers amplified a 800 bp-fragment but some of the Angolan samples were not perfectly preserved and consequently the sequence length ranged from 549 to 911 bp (average 671 ± 158). Further, the 400 bp-fragment we used allowed us to include sequences from previous studies in the performed analyses. Of the 46 olive ridley turtle sequences, seven were eliminated from

Table 1 Sampling locations, haplotypes per site and diversity measures for *Lepidochelys olivacea* mitochondrial control region sequences

Location	N	NH	$h \pm SD$	$\pi \pm SD$
Angola (1)	46	2	0.13 ± 0.063	0.0003 ± 0.0002
French Guiana (1)	31	3	0.28 ± 0.090	0.0007 ± 0.0002
Suriname (1)	11	1	0.00 ± 0.000	0.0000 ± 0.0000
Brazil (1)	15	1	0.00 ± 0.000	0.0000 ± 0.0000
Brazil (2)	92	3	0.08 ± 0.040	0.0003 ± 0.0001
Guinea (1)	2	2	1.00 ± 0.500	0.0051 ± 0.0026
Total	82	4	0.21 ± 0.058	0.0006 ± 0.0002

N number of individuals per location, *NH* number of haplotypes, *h*, haplotype diversity, π , nucleotide diversity, *SD* standard deviation

(1) sequences retrieved from GenBank; (2) data from Hahn (2011)

the data set because we used a mitochondrial marker and as such, sequences from females and respective nestlings will be identical. The remaining 39 sequences of Palmeirinhas—Onça beach (Angola) yielded two haplotypes. The haplotype Lo67 is represented in 37 samples, while the new haplotype, Lo106 (GenBank Accession Number MW811373), was found in two specimens (haplotype nomenclature was assigned by Peter H. Dutton). Haplotype diversity from Palmeirinhas—Onça beach ($h = 0.10$) is less than half compared with values reported, e.g., for French Guiana ($h = 0.28$), but it is closer to the Brazilian sample ($N = 92$, $h = 0.08$) (Table 1).

The median-joining network revealed that the two haplotypes found in Angola are clearly within the Atlantic-Mediterranean clade (Fig. 1). Most of the West African samples (Angola and Guinea-Bissau) share the same haplotype with the western Atlantic, Mediterranean and Madagascar, suggesting wide connectivity between both sides of the Atlantic and the Mediterranean (Fig. 2).

Our findings emphasize the importance of extensive sampling to identify genetic diversity at intraspecific level. The lack of marker resolution prevents a test of historical colonization signature versus contemporary demographic exchange and extensive individual movements, but the haplotype sharing between ocean basins (Figs. 1 and 2) reinforce the importance of consolidating international

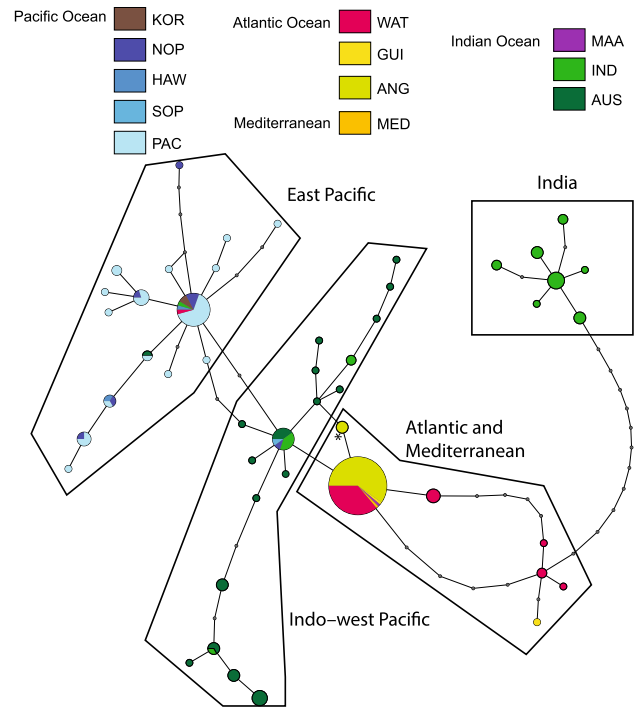


Fig. 1 Haplotype network of *Lepidochelys olivacea* mitochondrial control region considering twelve macro-regions and four clades. The circle size is proportional to the number of individuals sharing the haplotype. The asterisk denotes the new haplotype Lo106 uncovered in Angola. Colours refer to macro-regions and their relative proportions on each haplotype. *NOP* North Pacific, *HAW* Honolulu, *SOP* South Pacific, *WAT* West Atlantic, *MED* Mediterranean, *GUI* Guinea-Bissau, *ANG* Angola, *MAA* Madagascar, *IND* India, *AUS* Australia, *KOR* Korea, *PAC* Pacific

cooperation across regional boundaries to protect a species with broad distributional ranges (Monzón-Argüello et al. 2011). Specifically regarding the olive ridleys, rookeries across the Angolan coastline should be listed as of conservation concern by local or national authorities, and specific funding for monitoring actions should be provided. In tandem, clear environmental education is required to reduce mortality caused by beach trash, egg theft and turtle meat consumption in order to foster a mutually respectful and sustainable ecosystem that will enhance sea turtle conservation (Browne et al. 2009).

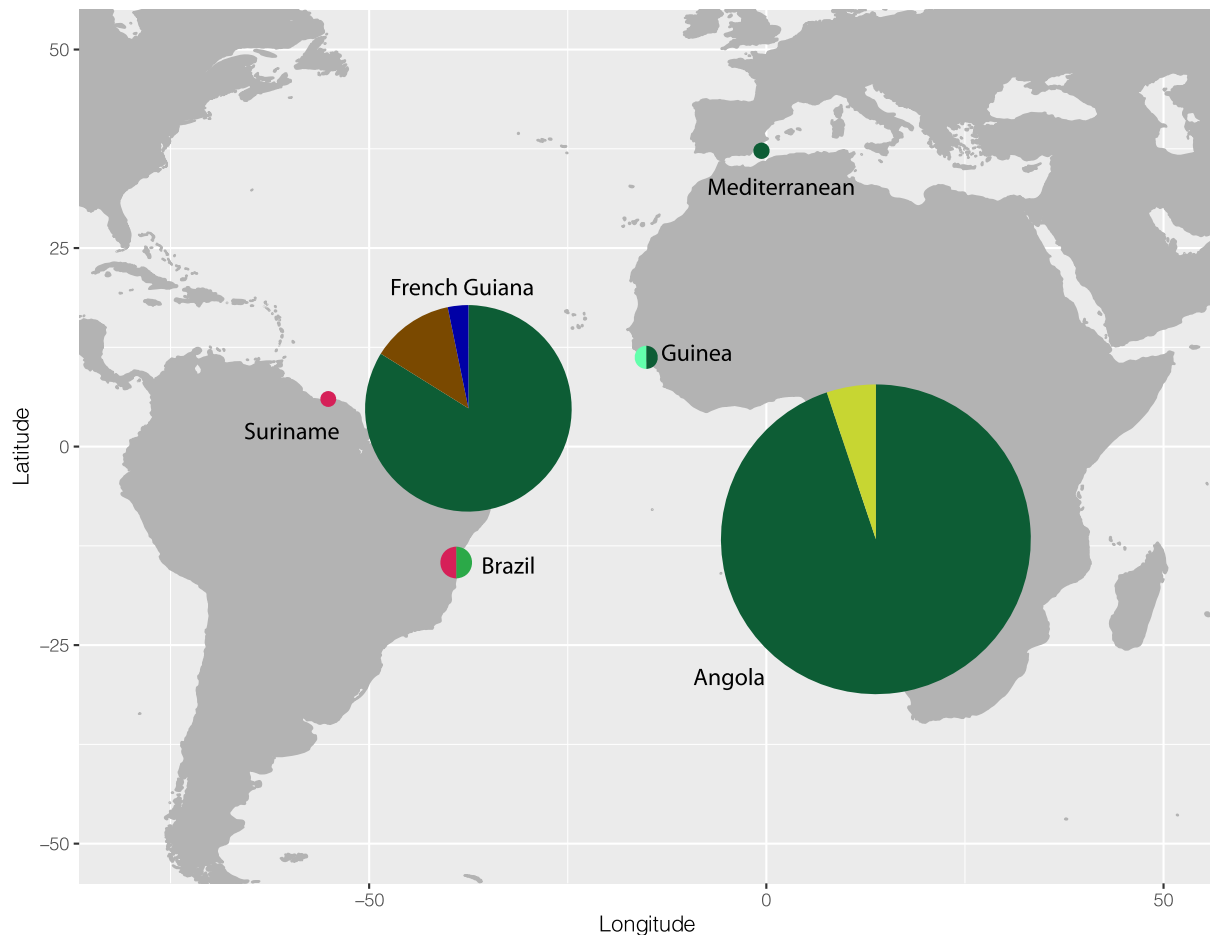


Fig. 2 Geographic distribution of known haplotypes of *Lepidochelys olivacea* from the Atlantic and Mediterranean. The circle size is proportional to the number of individuals sampled in each location. Colours refer to the relative proportions of each haplotype

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10592-021-01422-6>.

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Authors contributions CS, RC and RLC conceived of the study. AC collected specimens for use in this study. AC and RLC performed the molecular work. RC contributed with reagents and materials. RC and FG designed the figures. FG prepared the tables. RC, RLC and FG performed the formal analysis. RLC, RC and FG wrote the original draft. All authors approved the final draft of the manuscript.

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Data availability The new generated haplotype is available in GenBank at <https://www.ncbi.nlm.nih.gov/nucleotide/MW811373>. All haplotypes retrieved from GenBank are available in the Supplementary material. Samples were obtained directly from nesting females via blood or skin biopsy or undeveloped eggshells or tissue from dead embryos. No animals were harmed to obtain the samples.

Declarations

Conflict of interest Authors declare that have no competing or any conflict of interest.

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