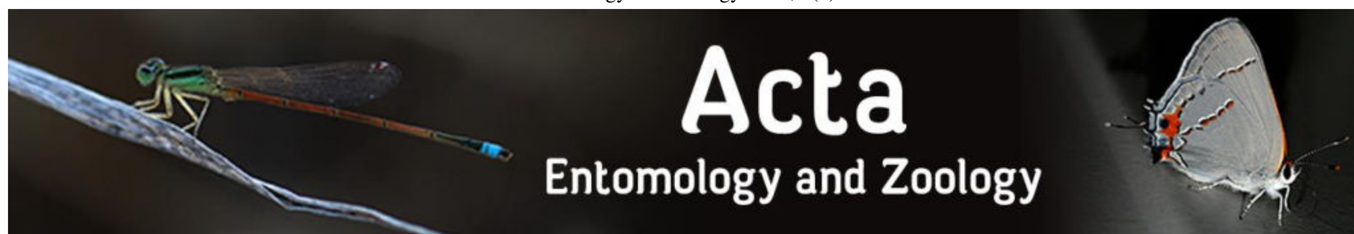

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DNA barcode analysis of the endangered green turtle (*Chelonia mydas*) in the breeding grounds of Badagry, Lagos, Nigeria

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Abstract
The Nigerian coastline is long and heavily populated. It is also the nesting site of a huge numbers of species that are dependent on that ecosystem for their reproduction cycle. Artisanal fishermen and the local populace are dependent on these species as a cheap and alternative source of protein. The Sea Turtles having been classified as threatened by the IUCN are a major part of that ecosystem. Currently, there is however a dearth of research works on the species. The usefulness of the DNA barcoding region (CO1) of the mitochondrial genome is used to analyze populations of *Chelonia mydas* along Badagry beach in Lagos, Nigeria. Seven samples were obtained and CO1 sequences were generated from them. Twenty seven more sequences were harvested from the GenBank of which only five were used in the final analysis either because there were errors (insertions, deletions or stop codons) or because of redundancies. A Six hundred and Eighteen (618bp) index free sequences was obtained. The intraspecific divergence in the Nigerian samples was very low, being between 0.00 and 0.002, while the intraspecific distance when the other sequences were added increased a bit ranging from 0.002 to 0.54. Neighbor- joining phylogenetic tree produced two distinct clade, with the Nigerian samples being clearly mapped into one with bootstrap value of 100% while others mapped into another. The best substitution model is TN93+G. The Tajima index was also very low indicating a population with very low genetic divergence. The average codon usage also followed the pattern A (29.3) > C (28.7) > T (27.3)> and a G (14.8). An expansion of the sampling points and increase number of samples is recommended for a more robust ideal of the relatedness within and without.

Keywords: DNA barcoding, Green Turtle, mtDNA, genetic diversity, intra specific distance

Introduction

Nigeria and most of the tropics are a hotbed of biodiversity. The long coastal length of Nigeria and the adjoining breeding sites especially along the Eight Hundred and fifty kilometers of coastline is a favourable breeding sites for many aquatic species. The occurrence of the green Turtle being a pantropical species have been reported almost all through this coastline. They are important as a source of meat for the local population and the eggs which come in huge numbers Kurniarum (2015) [1]. The consumption of eggs is believe to be the main cause of a severe decline in many sea Turtle populations (Thorbjarnarson *et al.*, 2000) [2]. Apart from the aforementioned, ghost fishing and the destruction of breeding sites have both affected the rate of regeneration in the Green Turtle.
The extensive use of the mitochondrial DNA (mtDNA) barcoding for the identification of species to assessing the phylogenetic relationship in species that have a wide ranged distribution has to a large extent improved the ease with which species are assessed and identified. This technology utilizes the use of a specific portion of the DNA in the mitochondrial genome to distinguish species that may be morphologically difficult to distinguish because they are closely related taxa-wise. Besides being useful in taxonomy, DNA barcoding is of great utility in conservation biology and can be applied where traditional methods are not very efficient. It is important to note that to date, there is no literature DNA barcoding of Nigerian species of *Chelonia mydas*. In this study, we have collected samples of *Chelonia mydas* caught by artisanal fishermen prior to being sold to people who eat them in Badagry town, in Lagos, Nigeria. The genetic profiles of these samples were evaluated using the cytochrome oxidase subunit 1(CO1) marker gene and compared with similar DNA sequences of members of the genus retrieved from the public

database on the GenBank.

Adding value to the amount of genetic information on the *Chelonia mydas* species is important, so is also as an assessment of the utility of DNA barcoding in the identification of these species of importance to science. A construction of the phylogenetic relationship between the Nigerian species and others is also necessary.

Materials and Methods

Tissue sample collection and preservation

A total of seven (7) tissue samples were taken from the front flippers of live animals from different landing locations along the coastline of Badagry in Lagos, Nigeria. These was stored at room temperature in 90% Ethanol and shipped to the Paul Hebert Centre for DNA Barcoding and Biodiversity Studies, Dr. BAM University, in Aurangabad, India. DNA was extracted using the Qiagen kit as recommended by manufacturers. The CO1 mitochondrial gene was amplified as a whole using the metazoan primers LCO1490 and HCO2198 developed by Folmer *et al*. (1994) [5]. The PCR reaction mixes of 15ul included 2ul of genomic DNA, 1 U of Taq polymerase, 200 uM of dNTPs, 1 X Tris-KCI buffer with 1.5mM Mgcl2 and 0.5 uM of each primer. Before sequencing, the PCR products were cleaned by precipitation using 20% polyethylene glycol (Lara-Ruiz *et al*., (2006) [4]. All good PCR products were then sequenced using the ABI 3130 sequencer.

Sequences were first checked for stop codons, aligned and edited in MEGA 7. The consensus sequences subjected to exploratory analysis using DAMBE and the MEGA 7.0. Three major phylogenetic construction methods were used to check the robustness of the obtained with 500 bootstrap replicates.

Results and Discussions

A 618pb CO1 fragment was analyzed in this study. The sequences obtained were aligned and compared with other GenBank CO1 sequences for *Chelonia mydas*. Initially twenty- seven (27) sequences were obtained. However, there were stop codons in some while the software DAMBE collapsed sequences that were the same (redundancies).The Nigerian species were reduced to three (3) while the mined sequences from the GenBank were reduced to five (5). Eight (8) unique sequences were finally used in the analysis. The comparisons revealed no stop, insertion or deletions. The genetic diversity was assessed in DAMBE and the Tajima's Theta (Pi) is 0.0314 with a Tajima's D of 0.43016. The transition/ Trans version plot is indicated in Figure 1.

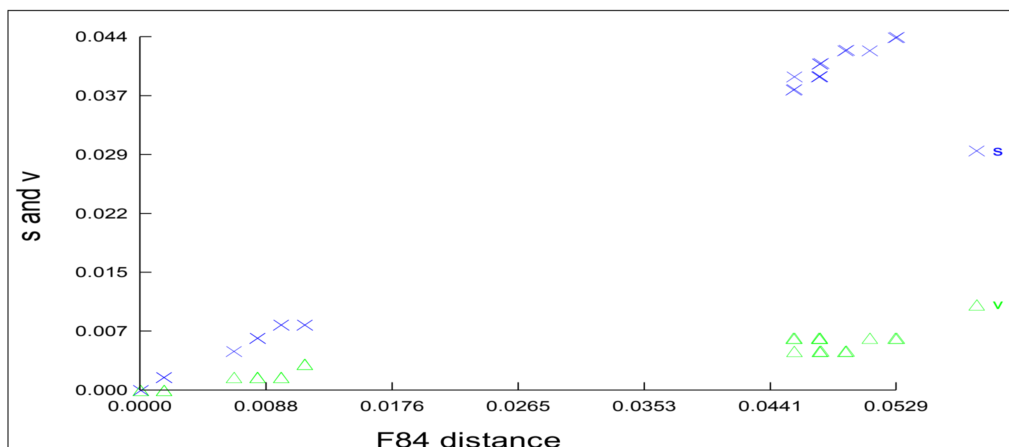

Fig 1: Showing the transition/ Trans version plot.

The presence of phylogenetic signal was also tested by the method of Steel *et al*., (1993) [11] with modifications. Phylogenetic signal is the tendency of related species to resemble each other more than species drawn at random from the same tree. The values obtained indicate a uniform relatedness between the species. Table 1 below indicates the outcome.

Table 1: Descriptive output of the test of the presence of the phylogenetic signal

Seq. ID	Seq. Name	A	C	G	T
0	GQ152878.1_Chelonia_mydas	0.2906	0.2938	0.1477	0.2679
1	GQ152880.1_Chelonia_mydas	0.2938	0.2938	0.1461	0.2662
2	GQ152881.1_Chelonia_mydas	0.2922	0.2906	0.1445	0.2727
3	GQ152877.1_Chelonia_mydas	0.2906	0.2955	0.1477	0.2662
4	MW996713.1_Chelonia_mydas	0.2927	0.2894	0.1447	0.2732
5	ST2	0.2938	0.2808	0.1494	0.2760
6	ST1_ST4	0.2943	0.2780	0.1496	0.2780
8	ST3_ST5_ST6_ST7	0.2938	0.2792	0.1494	0.2776

Mean sequence divergence was low. Intra specific for the Nigerian samples ranged from 0.00 to 0.048, while intraspecific range when the sequences harvested from the GenBank was added ranged from 0.00 to 0.054. We found a 0.00 intraspecific distance between the Nigerian samples and another from the Mediterranean. This supports the hypothesis of Naro-Maciel (2010) [3] which suggests a recent dispersal from near equatorial glacial refugia because the waterbodies are not contiguous. Table 2 below shows the evolutionary distances.

Table 2: Intraspecific evolutionary distances between the Nigerian samples and others

	GQ152878.1	GQ152880.1	GQ152881.1	GQ152877.1	MW996713.1	ST2	ST1/ST4	ST3/ST5/ST6/ST7
GQ152878.1								
GQ152880.1	0.008							
GQ152881.1	0.010	0.012						
GQ152877.1	0.002	0.007	0.008					
MW996713.1	0.010	0.012	0.000	0.008				
ST2	0.046	0.052	0.047	0.048	0.047			
ST1/ST4	0.048	0.054	0.048	0.050	0.048	0.002		
ST3/ST5/ST6/ST7	0.048	0.054	0.048	0.050	0.048	0.002	0.000	0.00

The best evolutionary model using the maximum likelihood fits for the twenty-four nucleotide substitution models is TN93+G. The model is the determinant of the phylogenetic tree below. Six Hundred and sixteen (616) sites were conserved and 32 were polymorphic. Sequences conservation is 0.948. There were a total of thirty four (34) mutations in the sequences. And a nucleotide diversity of 0.02758 and a Tajima's D index of 1.563. The average codon usage also followed the pattern A (29.3) > C (28.7) > T (27.3) > and a G (14.8). The phylogenetic tree had two major clades, one represents the Nigerian samples from our research and the other further split other species into two clades. The bootstrap support for the grouping of the Nigerian species into one clade is high (100%). Figure 2 below shows the phylogenetic tree. The CO1 gene sequences significantly discriminated other species cum location from the Nigerian species despite a very small genetic diversity within and between the species despite irrespective of location.

The discovery of species-specific CO1 sequences which enhances the possibilities of identifying marine Turtle species by the use of DNA barcode methods is an important aid in systematics and taxonomy of any group, especially those that occur widely.

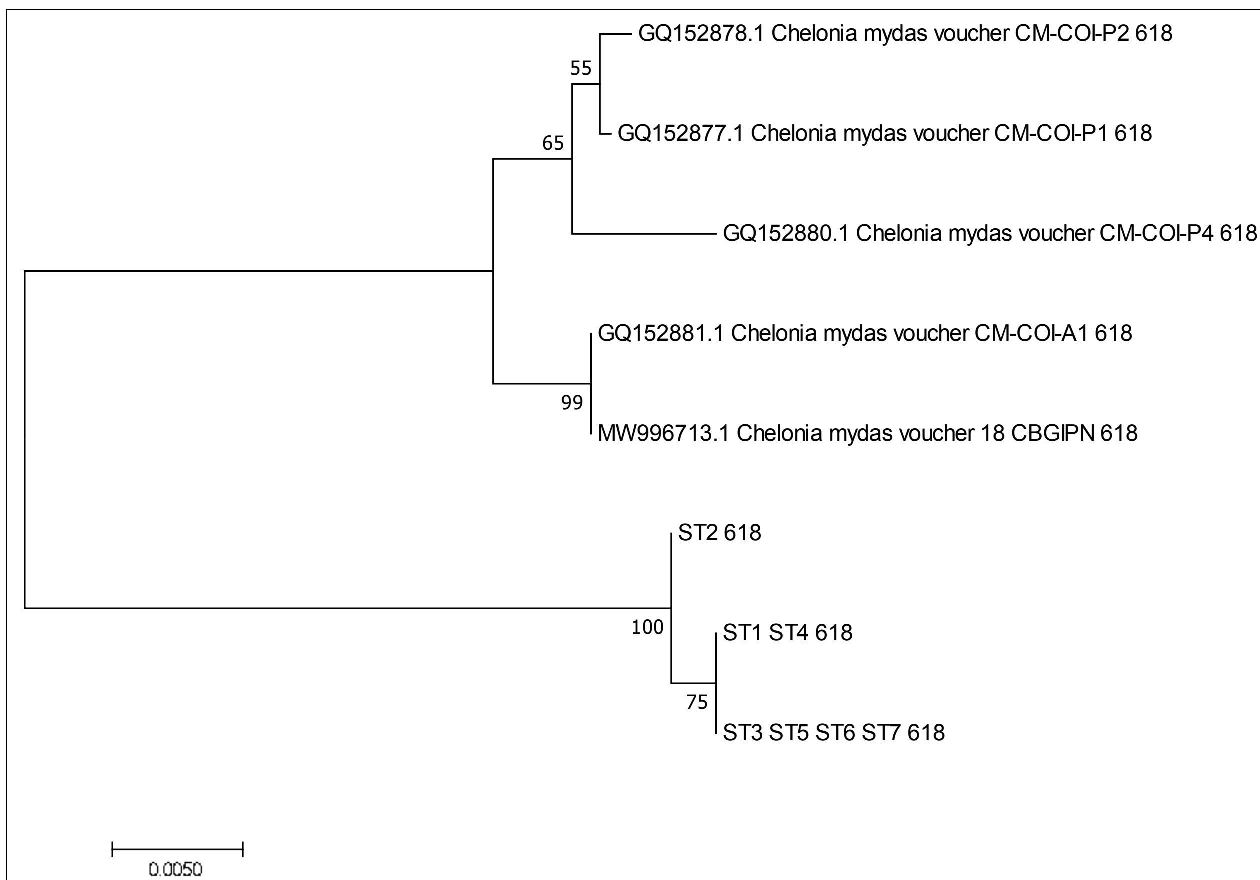

Fig 2: The phylogenetic tree using Neighbor-joining for 8 sequences from Nigeria and another 5 from GenBank with a 500 bootstrap and the value of the tree indicated at the nodes of the branches.

CO1 DNA barcoding is a very promising and powerful tool also for species identification, population structure, phylogenetic relationship and conservation status especially because of its high variability and the availability of several off-the shelf metazoan primers that have been useful in previous studies. Remarkably to is the mapping of a sample from Mexico (MW996713.1) mapping next to Nigerian species despite the distance separating both locations.

Conclusions

There is currently no reference publication using the CO1 gene to identify the *Chelonia mydas* population in Nigeria despite the concern expressed The International Union for the Conservation of Nature (IUCN) indicating the critical nature of the species in most of the developing world where they are a constant part of their meals. Compounding the threat to the group in the rapid destruction of their habitats

and the use of sea shores as nesting sites making them very vulnerable to being picked up by Man and a few of the carnivorous birds. It is documented here that truly these species are *Chelonia mydas* and that their genetic diversity is very low. Despite their occurrence and spread, the very low genetic diversity is a serious cause for concern although Avise *et al*., 1992 [9] and Fitzsimmons *et al*., 1995 [10] had noted that the group is an ancient taxa with relatively slow molecular evolution. The usefulness of the CO1 gene marker as an effective identification tool which can and has been used for species identification is also established. The classifications were reliable and this research has substantially added and expanded the sequence database for *Chelonia mydas*, especially the Nigerian population which before now had none.

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References

1. Kurniarum M, Prihanta W, Wahyuni S. Pengetahuan dan sikap masyarakat terhadap konservasi penyu dan ekowisata di Desa Hadiwarno Kabupaten Pacitan sebagai sumber belajar biologi J Pendidikan Biologi Indonesia. 2015;1(2):24-137.
2. Thorbjarnarson J, Lagueux C, Bolze D, Klemens M, Meylan A. Human use of turtles: A worldwide perspective; c2000.
3. Naro-Maciel E, Reid B, Fitzsimmons NN, LEM, Desalle R, Amato G. DNA barcodes for globally threatened marine turtles: a registry approach to documenting biodiversity. 2010 Mar;10(2):252-63.
4. Lara-Ruiz P, Lopez GG, Santos FR, Soares LS. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mt DNA analyses. Conserv Genet. 2006;7:773-781.
5. Folmer OM, Black W, Hoeh R, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3:294-299.
6. Alaa H Al-Darraji. The actual truth about different procedures of DNA and its extractions processes. Int. J Adv Biochem Res. 2020;4(2):33-36. DOI: 10.33545/26174693.2020.v4.i2a.54
7. Naro-Maciel E, Reid B, Fitz Simmons N, Le M, Desalle R, Amato G. DNA barcodes for globally threatened marine turtles: A registry approach to documenting biodiversity. Molecular ecology resources. 2010;10:252-263.
DOI: 10.1111/j.1755-0998.2009.02747.x.
8. Camacho-Sanchez FY, Aguirre A, Acosta-Sánchez HH, Rodriguez-González H, López-Hernández M, Reyes-Lopez MA. DNA Barcoding of Kemp's Ridley (*Lepidochelys Kempii*) in México +. Presented at the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution. 2021;2(1):37.
DOI: https://doi.org/10.3390/BDEE2021-09392
9. Avise JC. Molecular population structure and the biogeographic history of a regional fauna: A case history with lessons for conservation biology. Oikos 1992 Feb 1;63:62-76.
10. Fitz Simmons NN, Moritz C, Moore SS. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Molecular biology and evolution. 1995 May 1;12(3):432-40.
11. Steel MA, Lockhart PJ, Penny D. Confidence in evolutionary trees from biological sequence data. Nature. 1993 Jul;364(6436):440-2.