

## Evidence of olive ridley mitochondrial genome introgression into loggerhead turtle rookeries of Sergipe, Brazil

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**Abstract** The coastline of Sergipe state hosts the main Brazilian nesting sites of *Lepidochelys olivacea* (Eschscholtz, 1829). The second most abundant species of turtles in Sergipe is *Caretta caretta* (Linnaeus, 1758). Both sea turtle species, respectively known as olive ridley and loggerhead, are currently listed as endangered by the International Union for the Conservation of Nature and Natural Resources. The genetic diversity of the Sergipe loggerhead population ( $N = 51$ ) was assayed by analyzing 627 bp from the control region of mitochondrial DNA in nesting females. Three haplotypes were identified: CC-A4, CC-A24 and CC × LO. The last one was recorded for specimens considered hybrids because they represent *L. olivacea*'s mtDNA, but had the external morphology of *C. caretta* or of a mixture of both species. Based on the two types of hybrids, it was hypothesized that at least two hybridization events had occurred: a more ancient hybridization event, accompanied by introgression (F2 or later backcrosses), and a recent one (F1), both of which involving the same *L. olivacea* haplotype. The incidence of *L. olivacea* mitochondrial genome introgression into the *C. caretta* rookeries was only observed in Sergipe, which could be related to the large numbers of *L. olivacea* in this

region and an overlap of reproduction periods and distribution areas of both species. This may also be associated to global warming since it might alter the sex ratio of sea turtles, thus facilitating interspecific mating. Awareness of gene flow between these species will significantly influence the development and implementation of adequate management strategies.

**Keywords** Sea turtles · Hybridization · mtDNA control region · Genetic diversity · Conservation strategies

### Introduction

Until the nineteenth century, marine turtles were abundant in tropical and subtropical seas. However, environmental pressure caused by human interference, as in egg and female harvesting, commercial fisheries, bycatch and the destruction of natural habitats, has forced conservation agencies to declare several marine turtle populations as being currently threatened (Lutcavage et al. 1997; IUCN 2008).

The coastline of Sergipe (10°30'34"S 36°24'26"W–11°07'41"S 37°09'04"W) is the main nesting area of *Lepidochelys olivacea* (Eschscholtz, 1829) in Brazil. The second most abundant species in Sergipe is *Caretta caretta* (Linnaeus, 1758) (Marcovaldi and Marcovaldi 1999; Silva et al. 2007). During the nesting season of 2005/2006, from a total of 2,369 nests, 85% were made up of olive ridley turtles, while 12% were loggerhead nests (TAMAR-IBAMA 2006).

Frequent examples of hybridization in nature are often attributed to environmental degradation (Mallet 2005). Therefore, the occurrence of hybrids among sea turtle

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species may point to the existence of anthropogenic pressure that needs to be investigated. Equally, if we consider that the process of hybridization has important evolutionary consequences, it becomes essential to evaluate its impact on the genetic diversity and identity of the species involved.

Although hybridization events have previously been reported for marine turtles (Conceição et al. 1990; Bowen et al. 1992, 1994; Karl et al. 1995; Bass et al. 1996; Bowen and Karl 1996; Lara-Ruiz et al. 2006), this work is the first notification of hybridization between *C. caretta* and *L. olivacea* on the Brazilian coast.

Fossil evidence suggests that time of separation among *L. olivacea* and *C. caretta* is around 10–20 million years ago (Zangerl 1980; Dodd and Morgan 1992). Sea turtles are likely the most ancient vertebrates hybridizing under natural conditions, since hybrids exist between *Caretta* and *Chelonia* genera, which were separated about 50 million years ago (Bowen et al. 1992, 1994; Karl et al. 1995).

The capacity of sea turtles to generate fertile hybrids seems to be related to the slow chromosomal and anatomic evolution. The maintenance of chromosomal number and structure may allow genomic compatibility among species (Bickham 1981). The fitness success of the carapace and accompanying morphological adaptations should favor interspecific mating. Moreover, a dearth of behavioral barriers to hybridization may be an issue, as well as the fact that male turtles are notably indiscriminate in mating preferences (Bowen 2003).

Herein, we report on the distribution and frequency of interspecific hybrids within loggerhead nests in Sergipe, Brazil, evaluated using mtDNA sequences. We also discuss the relevance of these findings for the conservation management of these species in the region.

## Methods

During the nesting seasons (from September to March) of 2004/2005 and 2005/2006, 51 tissue samples from female *C. caretta* individuals were collected in nesting beaches in Sergipe state (Abaís, Pirambu and Ponta dos Mangues) by the use of 6 mm disposable biopsy punches. Furthermore, 10 *L. olivacea* female samples were also collected from the Sergipe rookery (Pirambu) for comparative analysis. The samples were collected by biologists from Projeto TAMAR-ICMBio (The Brazilian Sea Turtle Conservation Program).

Genomic DNA extraction, mtDNA control region amplification and sequencing, sequence assembly and alignment and phylogenetic relationship analyses were performed as described in Reis et al. (2009). Mitochondrial haplotypes were classified according to haplotypes

deposited in the DNA database from the Archie Carr Center for Sea Turtle Research (ACCSTR 2009).

## Results

Out of 627 bp of mtDNA control region we identified three haplotypes in the *C. caretta* nesting population in Sergipe: CC-A4 (61%), CC-A24 (12%) and CC × LO (27%). The latter was recorded for specimens considered hybrids because they represent *L. olivacea* mtDNA, except having external morphology of *C. caretta* (64%) or a mixture between *C. caretta* and *L. olivacea* (36%). In the latter, specimens show a combination of morphological characteristics from both species, i.e., number of lateral scutes on the carapace and on the head, the format of the carapace and the animal's biometry. The CC-A4 haplotype has already been reported in Rio de Janeiro, Espírito Santo and Bahia rookeries, as well as in the Elevação do Rio Grande feeding area, and the CC-A24 haplotype in Bahia rookeries (Reis 2008).

If we consider the traditional 380 bp fragment deposited at the ACCSTR DNA database, the CC × LO haplotype differs from the CC-A4 by 37 polymorphic sites (24 transitions, six transversions and seven indels), and from the CC-A24 by 38 (including one more transition) (Table 1).

As seen in Table 1, the CC × LO haplotype is identical to the *L. olivacea* (LO) haplotype obtained from the Sergipe rookery ( $N = 10$ ), and therefore it is not a true *C. caretta* haplotype. However, the *C. caretta* population from Sergipe presented a high proportion of CC × LO hybrids (~30%), which lead to a considerable increase in the total population nucleotide and genetic diversity values (Sergipe overall; Table 2).

As observed in the Neighbor-Joining tree (Fig. 1), the CC × LO haplotype groups into a branch with a bootstrap support of 100 with the *L. olivacea* sequence obtained from GenBank (accession number AF051773), as well as with the LO sequences obtained from Sergipe rookeries. A monophyletic group with a bootstrap support of 85 is observed for the *C. caretta* (CC) from Brazilian nesting and feeding areas. The other three species from the Cheloniidae Family are clearly separated.

## Discussion

Here we report on a relatively high frequency of hybrids between loggerhead and olive ridley turtles from Sergipe (northeast Brazil), identified based on mtDNA sequences. This contrasts to a previous mtDNA study (Soares 2004) that used 81 loggerheads from nesting grounds from Bahia and Espírito Santo (northeast Brazil) and 43 bycatch

**Table 1** Polymorphic sites among three mtDNA control region haplotypes found for nesting ( $N = 51$ ) and bycatch ( $N = 01$ ) *Caretta caretta* (CC) samples and the unique *Lepidochelys olivacea* (LO) ( $N = 10$ ) haplotype sampled in Sergipe, Brazil

Haplotypes	Polymorphic site number																													
	3	5	5	6	6	7	7	8	8	9	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
CC-A4	C	A	A	A	A	G	T	A	T	C	T	G	A	C	T	A	A	C	G	T	G	T	T	C	C	A	C	G	A	G
CC-A24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
CCxLO	T	G	-	-	G	A	C	T	A	A	G	A	C	T	.	T	G	T	A	C	A	C	C	A	A	.	A	A	G	T
LO	T	G	-	-	G	A	C	T	A	A	G	A	C	T	.	T	G	T	A	C	A	C	C	A	A	.	A	A	G	T

Haplotypes	Polymorphic site number (continued)																														
	2	2	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5
CC-A4	A	A	A	A	G	C	G	T	A	G	T	C	T	A	T	A	A	C	C	-	-	-	-	-	-	-	A	T	G	T	
CC-A24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CCxLO	G	G	.	T	A	T	A	C	G	A	C	T	C	G	A	G	G	T	T	G	C	A	C	G	A	T	G	C	A	C	
LO	G	G	.	T	A	T	A	C	G	A	C	T	C	G	A	G	G	T	T	G	C	A	C	G	A	T	G	C	A	C	

The additional polymorphic sites (obtained from the analysis of a 627 bp fragment) are highlighted in gray. CC-A4 and CC-A24, *C. caretta* (CC) haplotypes; CC × LO, hybrids between *C. caretta* and *L. olivacea*

**Table 2** Standard diversity indexes calculated for each nesting beach (Abaís, Pirambu and Ponta dos Mangues) in Sergipe, Brazil and for the total *Caretta caretta* population having considered (overall) or not (CC only) the hybrid samples

Populations	Diversity indices						
	<i>N</i>	<i>bp</i>	<i>S</i>	<i>H</i>	<i>h</i>	$\pi$	<i>k</i>
Abaís	8	627	57	2	0.43 ± 0.17	0.0390 ± 0.0219	24.43 ± 12.04
Pirambu	38	627	58	3	0.61 ± 0.05	0.0408 ± 0.0203	25.57 ± 11.47
Ponta dos Mangues	5	620	0	1	0	0	0
Sergipe overall	51	627	58	3	0.55 ± 0.05	0.0373 ± 0.0185	23.37 ± 10.45
Sergipe CC only	37	620	1	2	0.28 ± 0.08	0.0005 ± 0.0006	0.28 ± 0.31

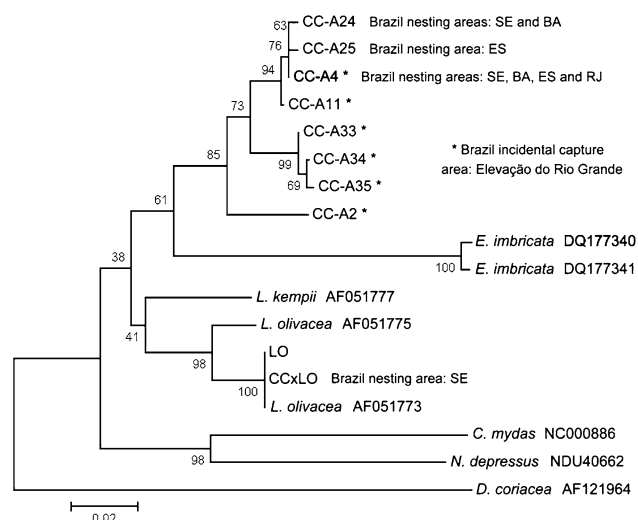
*N* sample size, *bp* usable fragment length in base pairs, *S* number of variable sites, *H* number of haplotypes, *h* haplotype or genetic diversity,  $\pi$  nucleotide diversity, *k* mean number of pairwise differences

loggerheads from the southern Brazilian coast and did not register any olive ridley mtDNA haplotype.

The incidence of *L. olivacea* mitochondrial genome introgression into *C. caretta* nesting areas in Sergipe, which is the largest Brazilian population of olive ridley turtles, may be related to an overlap in the reproduction period and area of both species. Recalling that the *L. olivacea* nesting population is larger than the *C. caretta* (TAMAR-IBAMA 2006), we assumed that the same proportion is maintained at the reproduction colony. Naturally, the availability of *L. olivacea* females was greater than *C. caretta*, favoring the interspecific mating of loggerhead males with olive ridley females. The scenario is even more severe since the sex ratio is female biased (Marcovaldi et al. 1997).

From the 14 individuals genotyped as CC × LO hybrids, nine (64%) presented *C. caretta* external morphology and five (36%), mixed morphological characteristics from both *C. caretta* and *L. olivacea*. These two types of hybrids led us to suppose that at least two hybridization

events may have occurred between these two species. An ancient event resulted in individuals with *L. olivacea* mtDNA haplotypes without morphological vestiges of this species. In this case, hybrids will most likely be F2 or >F2 and introgression probably occurred by backcrossing of female hybrids with *C. caretta* males. A second more recent event resulted in individuals with mixed morphological characteristics from both *C. caretta* and *L. olivacea*, possibly from direct interspecific coupling, therefore, F1. This scenario, however, cannot be distinguished from an ongoing process of hybridization over the last few millennia without nuclear DNA data. An additional hypothesis considers the CC × LO hybrids as a result of a long and antique process of introgressive hybridization, although it predicts a morphological gradient of characteristics which was not observed in the present scenario. Comparing sequences from the mtDNA control region in CC × LO hybrids with that of ten *L. olivacea* (LO) samples from the same area (Table 1; Fig. 1), it can be noted that only one *L. olivacea* haplotype underwent introgression into the *C.*



**Fig. 1** Neighbor-Joining tree produced from the alignment of *Caretta caretta* (CC), *Lepidochelys olivacea* (LO) and hybrids between *C. caretta* and *L. olivacea* (CC × LO) 380 bp mtDNA control region sequences found for nesting and bycatch areas on the Brazilian coast (Soares 2004; Reis 2008). GenBank sequences of *Eretmochelys imbricata*, *Lepidochelys olivacea*, *Lepidochelys kempii*, *Chelonia mydas*, *Natator depressus* and *Dermochelys coriacea* (with respective accession numbers) were also considered in the analysis. Location: SE Sergipe, BA Bahia, ES Espírito Santo and RJ Rio de Janeiro states

*caretta* rookeries of Sergipe. Thus, even if more than one hybridization event had taken place, they involved the same *L. olivacea* mtDNA haplotype.

We only observed hybrids between *L. olivacea* females and *C. caretta* males, and never between *C. caretta* females and *L. olivacea* males (see also Hahn et al. 2007). This suggests that only unidirectional hybridization and subsequent backcrossing occurred between these two species. Karl et al. (1995) suggested that a numerical predominance of hybrids with mothers from the more common species is due to a constant “error” rate in the choice of mate per female.

Considering that sexual determination in marine turtles is defined by egg incubation temperatures, where higher temperatures determine females and lower determine males (Mrosovsky 1994), the inappropriate management of clutches and the global warming process are some of the factors which may have contributed to the altered proportion of females, consequently facilitating interspecific breeding. At present, clutch relocation to safer sites is recommended to protect them against rapid shore habitat changes caused by real estate investments, native vegetation devastation, photopollution and increased vehicle traffic. Any relocation, however, may lead to substantial changes in the environmental incubation and in the characteristics of hatchlings (Foley et al. 2000; Carthy et al. 2003). In Sergipe, a higher proportion (around 50%) of nests from the 2004/2005 and 2005/2006 nesting seasons were

relocated from the original site to other more secure sites on the same beach or to artificially protected open-air hatcheries elsewhere (TAMAR-IBAMA 2005, 2006). Nevertheless, females currently nesting along the Sergipe coast were hatchlings about 25–30 years ago, which return to the same region in which they hatched due to philopatric behavior. Therefore, the distorted sex ratio cannot be attributed to the management of rookeries since this strategy was not used back then. There are not enough studies correlating this strategy and its consequences on population sex proportion, but the significant presence of hybrids in these areas alerts to the need of constant evaluation of management plans.

If this occurrence is favored by global warming, some researchers have suggested that sea turtles could adapt to the new conditions created by this process. This may be possible through adjustments in pivotal temperature, spatial alteration of nesting areas (moving to cooler beaches) or temporal alteration of nesting seasons (Hays et al. 2003; Pike et al. 2006).

The unusually high proportion of hybrids between *C. caretta* and *L. olivacea* in Sergipe sea turtle populations (present study), as well as in related hybridization events between *E. imbricata* and *C. caretta* or *E. imbricata* and *L. olivacea* in Bahia (Lara-Ruiz et al. 2006), and between *C. caretta* and *C. mydas* also in Bahia (Bowen et al. 1992, 1994; Karl et al. 1995), represents a serious concern for the conservation of these species in Brazil. This raises a controversial issue about conservation efforts focusing on hybrid populations (Allendorf et al. 2001). Although hybrids are normally rare in populations, a few hybrids may form a bridge which allows a trickle of alleles to be transferred between species; thus, if species that hybridize are common, even low rates of hybridization per individual can have important evolutionary consequences (Mallet 2005).

Considering that there is extensive hybridization among sea turtle species of the Cheloniidae Family and that hybrids can mate with parental species or other hybrids, it is important to evaluate natural sex proportion in those species. Moreover, the monitoring of natural temperature changes is crucial to better understand this ongoing hybridization process. The limited information provided by maternally inherited markers makes it difficult to establish how many hybridization events took place and how long ago these events occurred. Further studies with biparentally inherited nuclear markers are needed to better understand the implications and causes of such events, and their impact on the genetic diversity and identity of these species. This information, combined with other ecological and environmental data, will provide important clues about the effect of anthropogenic pressures acting on sea turtle populations.

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