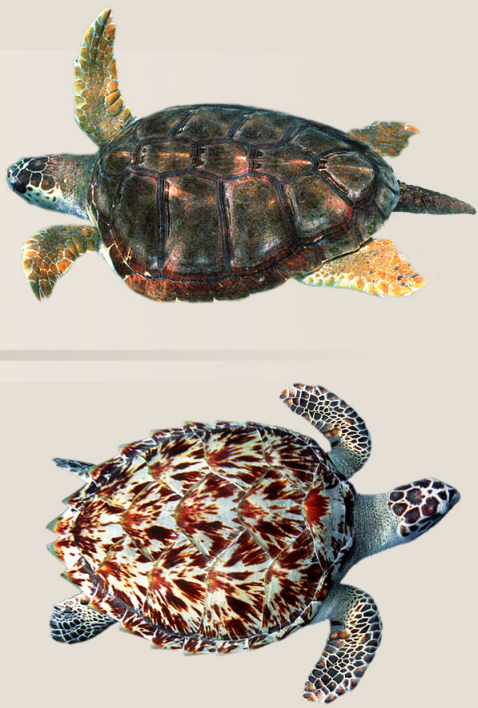




Unidirectional Introgression Between *Eretmochelys imbricata* and *Caretta caretta* analyzed by PCR-RFLP



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Abstract

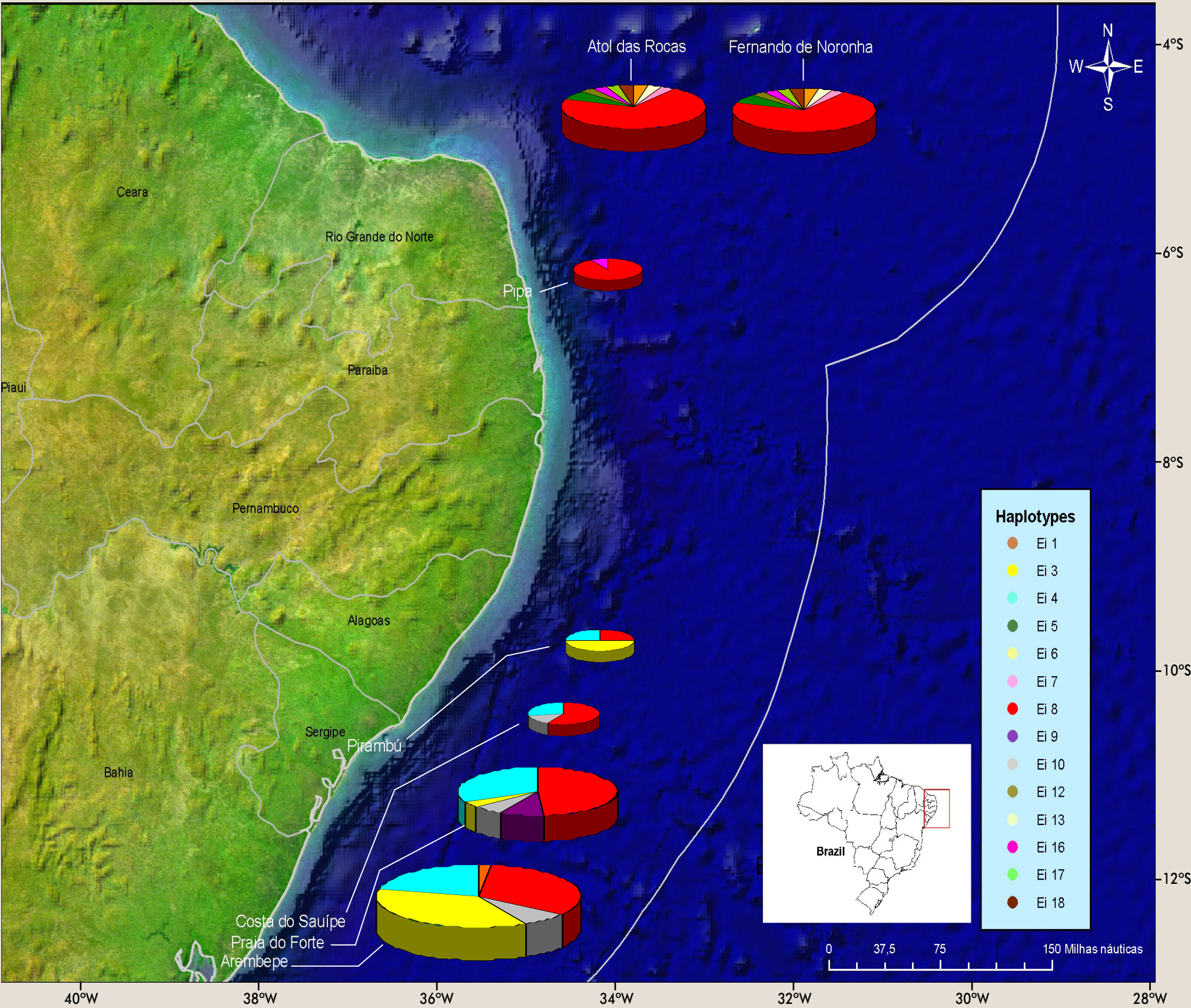
The hawksbill turtle, *Eretmochelys imbricata*, occurs in tropical seas of the Atlantic, Indian and Pacific oceans. It is considered critically endangered by the IUCN and in Brazil, due to fisheries, killing and egg collecting, there are few hatchling sites left. The loggerhead turtle, *Caretta caretta*, is distributed in the Atlantic, Indian and Pacific oceans and tropical waters of Mediterranean. It is considered endangered by the IUCN and by the Brazilian environmental agency Ibama. Hybridization between these two species has been known for many decades from morphology studies. Recently we used mitochondrial DNA (mtDNA) analysis to detect hybrids in Brazilian nesting beaches and feeding grounds. In this study, we aimed to investigate the hybridization process between *E. imbricata* and *C. caretta* though analyses of three autosomal markers. Here we present research data produced in cooperation between LBEM, Projeto Tamar-Ibama, and PETROBRAS/CENPES as part of the project "Mamíferos e Quelônios Marinhos". A total of 42 individuals from Bahia State in Brazil, identified as *E. imbricata* and bearing a *C. caretta* mtDNA, and 133 individuals identified as *E. imbricata* by morphology and mtDNA were analyzed with these autosomal markers to detect likely *C. caretta* genomic ancestry in this population. In all individuals tested, three anonymous regions of nuclear DNA were amplified by PCR with primers previously described to Chelonia mydas, and the inter-specific variation was identified using one or two restriction enzymes. Among 42 previously detected hybrids, four (15.1%) showed both specific alleles of *E. imbricata* in at least one locus, but no hybrid presented both *C. caretta* alleles in any locus. Furthermore, another eleven hybrids, with incomplete genotyping, have also occurred in 4 individuals with at least one *E. imbricata* homozygous locus. Additionally, in the 42 hybrids 52.4% of the alleles belonging to *E. imbricata* and only 47.6% to *C. caretta* were observed. These data indicate some introgression between parental species and hybrids due to the existence of hybrids of generations >F1. It would also indicate a likely bias to preferential mating with *E. imbricata*. This unidirectional introgression can be an evidence of the incapacity or failure in meeting of F1 female hybrids to mate with males of the parental taxa *C. caretta*. This apparently unsuccessful introgression with *C. caretta* could be due either to the low mating success of this species with hybrids or low survival/fecundity of >F1 hybrids with *C. caretta*, since all hybrids analyzed were females in oviposition. It is interesting to note that F1 female hybrids with *C. caretta* mtDNA are result of the mating of a female *C. caretta* with a male *E. imbricata* and we do not observe any evidence of opposite gender pairing. Likely, this hybridization process can be biased by the gender of the parental species, and can be an important factor in introgression. The hybridization observed in sea turtles can be due to the inexistence of pre and post-zygotic barriers allowing interbreeding. The detection of this intensive hybridization process and the sex biased introgression can direct differential management strategies for conservation of this species in Brazil.

Methods

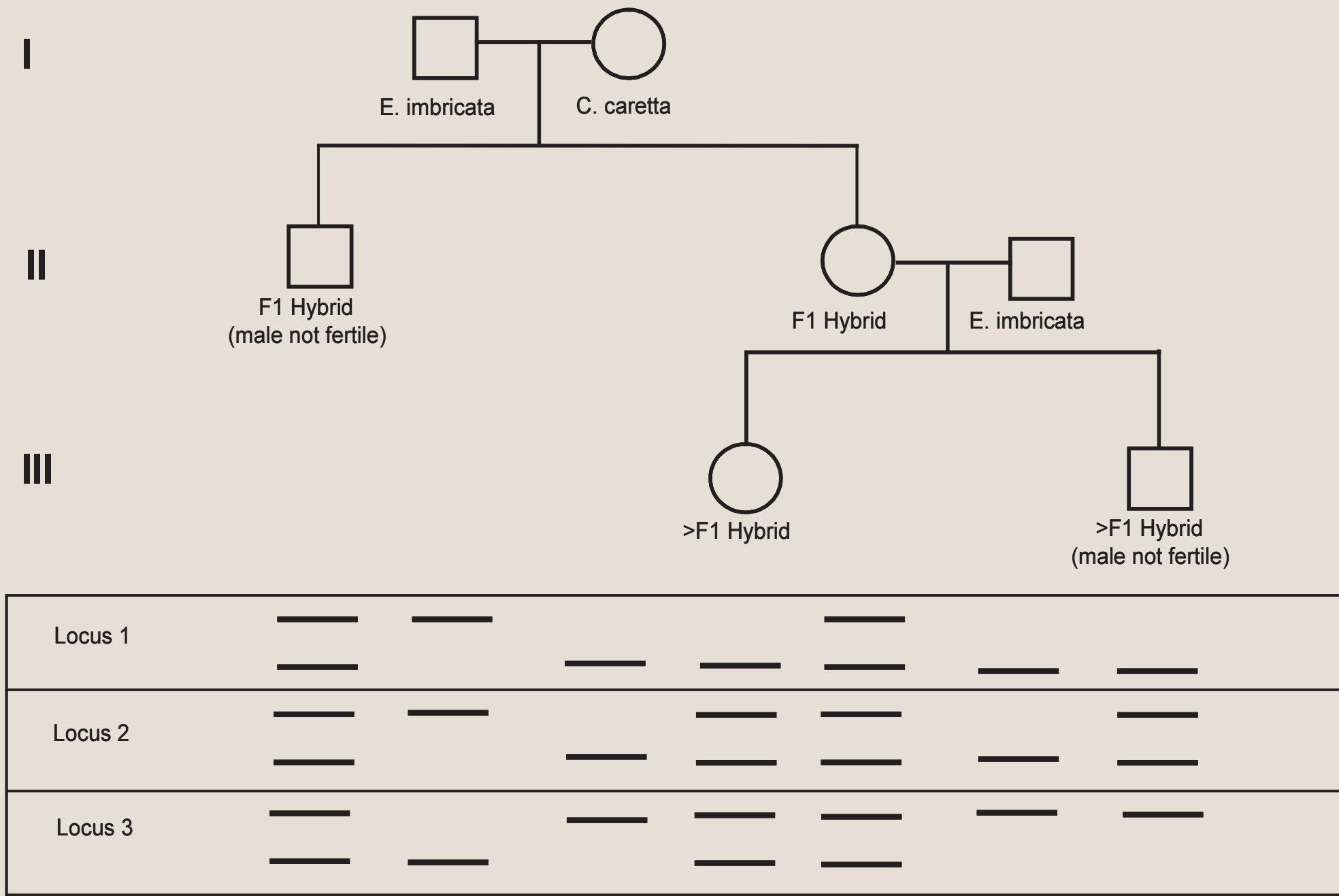
We analyzed 42 individuals identified morphologically as *E. imbricata* and bearing a *C. caretta* mtDNA haplotype, and 133 individuals identified as *E. imbricata* by morphology and mtDNA (Lara-Ruiz et al., 2006). Figure shows the location of the population studied:

Primer	Sequence	Restriction enzyme
CM 12	F - 5' TGCCTCTCAGAACCA GTT 3' R - 5' AGCTGAAGCCAATGAAGAAGAA 3'	DraI RsaI
CM 14	F - 5' AGTATTTGGGCAGAACAGAA 3' R - 5' TCCAGCTGCAGGTGCAACAT 3'	HaeIII
CM 28	F - 5' GATTGCTGGTCTCTGGAAGGCT 3' R - 5' TAAATGCCAGGTATGTAATCT 3'	BstI

Primers and restriction enzymes used to identify each locus inter-specific variation, according to Karl et al., 1995:



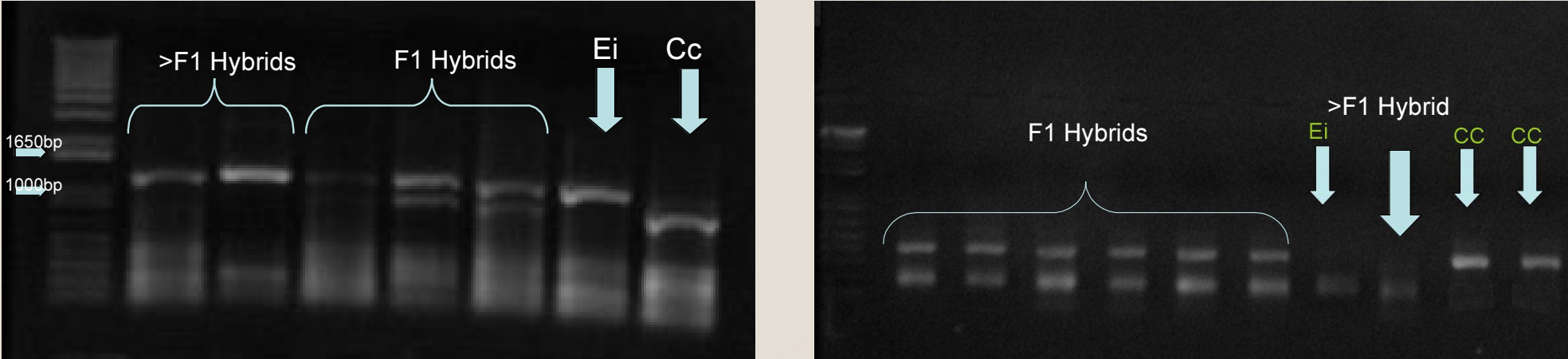
Results



Schematic representation of the hybridization between *E. imbricata* e *C. caretta*. Individuals were genotyped for three nuclear markers. The hybridization initiates with the breeding within a *E. imbricata* male and a female *C. caretta*. The F1 hybrid male (II.1) is probably infertile because no alleles of *C. caretta* were found in individuals with *E. imbricata* morphology and mtDNA haplotypes. The F1 hybrid females (II.2) are fertile and there is where the introgression occurs. This females breed with the parental taxa *E. imbricata* (II.2 x II.3) and the progeny (III.1 and III.2; further generation hybrids, >F1) has the *E. imbricata* marker in at least one locus.



Hybrids in Praia do Forte, Bahia, Brazil. (Tamar Image Bank).



Agarose gel 0.8% stained with ethidium bromide of locus CM14 (left) and CM28 (right), showing the observed band pattern of individual F1 hybrids, >F1 hybrids and control individuals. Cc=*Caretta caretta* e Ei=*Eretmochelys imbricata*.

Conclusions

- The 42 individuals with *E. imbricata* morphology and *C. caretta* mtDNA were genotyped: 11.9% of the individuals typed for the three nuclear markers had at least one locus homozygote for the *E. imbricata* specific marker. The others 88.1% had the two alleles of both species in the three loci.
- The introgression was only detected with the parental taxon *E. imbricata*.
- Among the 133 control individuals that displayed *E. imbricata* morphology and mtDNA haplotypes, no alleles of *C. caretta* were found. These result suggest that the introgression only occurs between *C. caretta* females or F1 hybrid females with *E. imbricata* males
- This intense hybridization is not found in other locations, and can be due to a specific process affecting these two species in Bahia, Brazil.

References

- Karl, SA, Bowen BW, Avise JC. 1995. Hybridization among the ancient mariners: identification and characterization of marine turtle hybrids with molecular genetic assays. *Journal of Heredity* 86: 262-268
- Lara-Ruiz P, Lopez GG, Santos FR, Soares LS. 2006. Extensive hybridization in hawksbill turtles (*Eretmochelys imbricata*) nesting in Brazil revealed by mtDNA analyses. *Conservation Genetics*, 7: 773–78