RIO GRANDE FEDERAL UNIVERSITY
OCEANOGRAPHY INSTITUTE
MARINE MOLECULAR ECOLOGY LABORATORY

PARTIAL REPORT

Juvenile hybrid turtles along the Brazilian coast

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PROJECT TEAM

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M.Sc. Luciano Soares (Ph.D. candidate in Animal Ecology, university of Florida, U.S.A.)
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Cíntia Brito (M.Sc. student in Biological Oceanography, FURG, Brazil)

BACKGROUND

Hawksbill turtles (*Eretmochelys imbricata*) are globally distributed in coastal waters at tropical latitudes, and can also occur at subtropical/temperate latitudes. This species generally inhabits coral or rocky reefs, using shallow coastal waters for feeding mainly on incrusting benthic organisms such as sponges, zoanthids and anemones. It is believed that hawksbills contribute to reef health and diversity by altering the composition of benthic organisms through feeding, by exposing colonization substrate and releasing nutrients and food in the water.

The hawksbill turtle is classified as critically endangered by the International Union for Conservation of Nature (IUCN), having suffered massive population declines. In Brazil, reproductive populations currently display an increasing trend due to intense conservation efforts at nesting beaches, with approximately 700 females nesting along the northeast coast. However, these populations are still threatened by poaching, habitat degradation, bycatch in fisheries, and marine pollution. Additionally, hawksbills in Brazil are faced with an extensive hybridisation phenomenon, which could pose a serious threat to the recovery of their populations. Interspecific hybridisation occurs naturally or as a result of anthropogenic actions, and may lead to lower fitness and fertility, and even genetic extinction of species. In Brazil, hawksbill and loggerhead breeding groups present exceptionally high hybridisation rates. The largest rookeries of both species overlap along the coast of Bahia state, and studies have shown that 42% of nesting females with hawksbill morphology were actually hybridised with loggerheads, presenting typical loggerhead mitochondrial DNA (mtDNA) haplotypes. Despite the extremely high hybridisation rates between these species in Bahia, how hybrid offspring

disperse and where they recruit to is still poorly studied. Until recently, no immature hybrids resulting from the hawksbill x loggerhead crosses had ever been reported. In the beginning of 2014, we recorded four of these immature hybrids in 157 immature hawksbill samples in Brazil, which was a major step towards a better understanding of this phenomenon. This project aims to better understand the occurrence, distribution, parental species and generations of hawksbill x loggerhead hybrid offspring, through a combination of mitochondrial and nuclear DNA marker analyses of immature animals sampled along the Brazilian coast.

PARTIAL RESULTS

We obtained 66 new samples from immature turtles morphologically identified as hawksbills from the Northeast, at Ceará state (Almofala), Bahia state (Arembepe, Praia do Forte and Sauípe) and the Abrolhos National Marine Park, and South Brazil, at Rio Grande do Sul state (Cassino). The previous RSGF project conducted by this team detected four hybrids among the 157 samples collected along the coast. DNA was extracted and the mtDNA D-Loop amplified through PCR using primers LCM15382/H950, using an Applied Biosystems Veriti 96-well Thermocycler acquired with the current financial support of the RSGF (see photo at end of report). The obtained PCR products were purified and sequenced in both directions, and sequences were aligned and classified according to GenBank®. In the 66 new samples, we found high frequency of haplotype Ei-A01 (n=52), and lower occurrence of haplotypes Ei-A32 (n=4), Ei-A61 (n=1) and Ei-A62 (n=4) (Figure 1). Haplotype Ei A01 is the most common haplotype in the Atlantic Ocean, and the remaining haplotypes occur frequently in Brazilian nesting and feeding populations. We also encountered four additional hawksbill x loggerhead hybrids (haplotype Cc-A4.2), and one hawksbill x olive ridley (*Lepidochelys olivacea*) hybrid (haplotype Lo-78920). This brings us to a total of nine hybrids in 224 immature sea turtles sampled in Brazil.

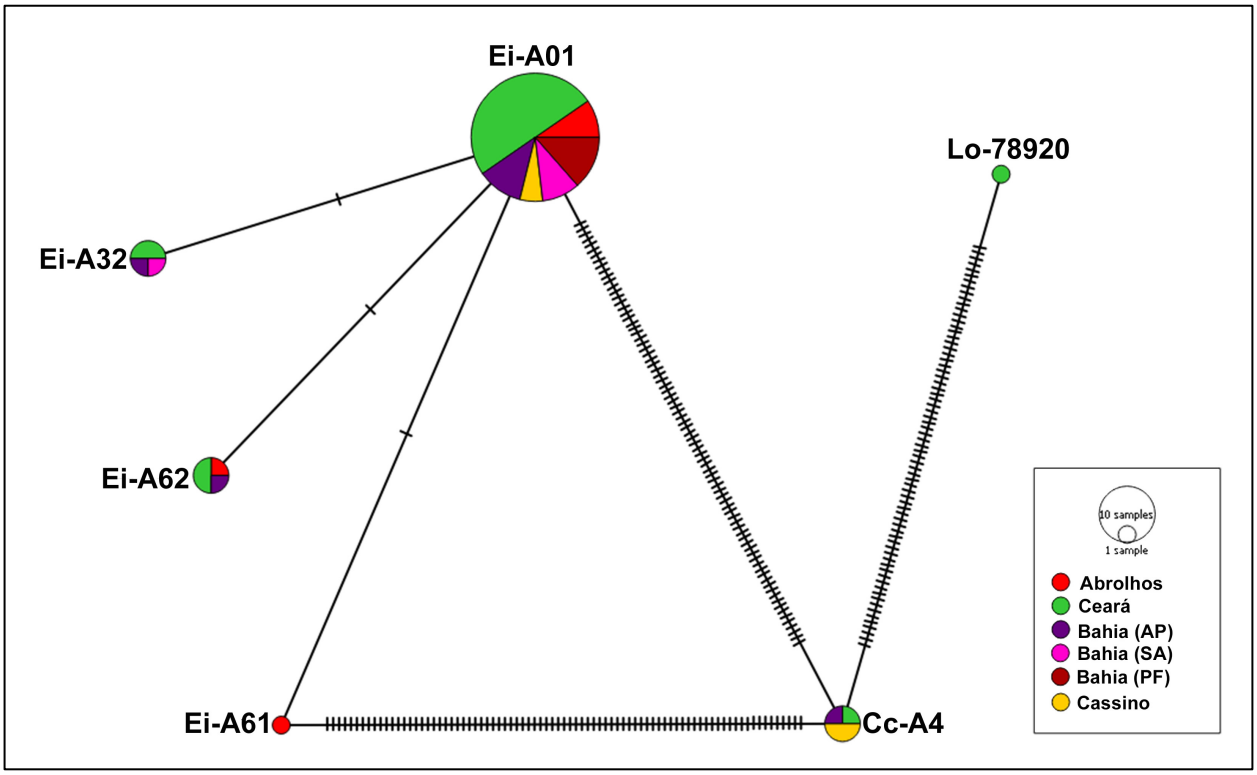

Figure 1. Network between haplotypes found at our study areas. Dashes along lines indicate differences between haploytpes (1 dash = 1 difference). Cc-A4 and Lo-78920 are haplotypes belonging to hybrid turtles.

Three of the four hawksbill x loggerhead hybrids were once again observed at an area uncommon for hawksbills, the cold waters along the extreme South of the coast (Figure 2). The hawksbill x olive ridley hybrid was found at Ceará and is one of the first records of this type of hybridization worldwide. Our genetic analyses continue to reveal that immature hybrids are relatively common in Brazilian populations when compared to other areas in the world. Classified as a priority in the National Plan for Marine Turtle Conservation, this subject requires continued investigation in order to better understand the distribution and management strategies of hybrid animals.

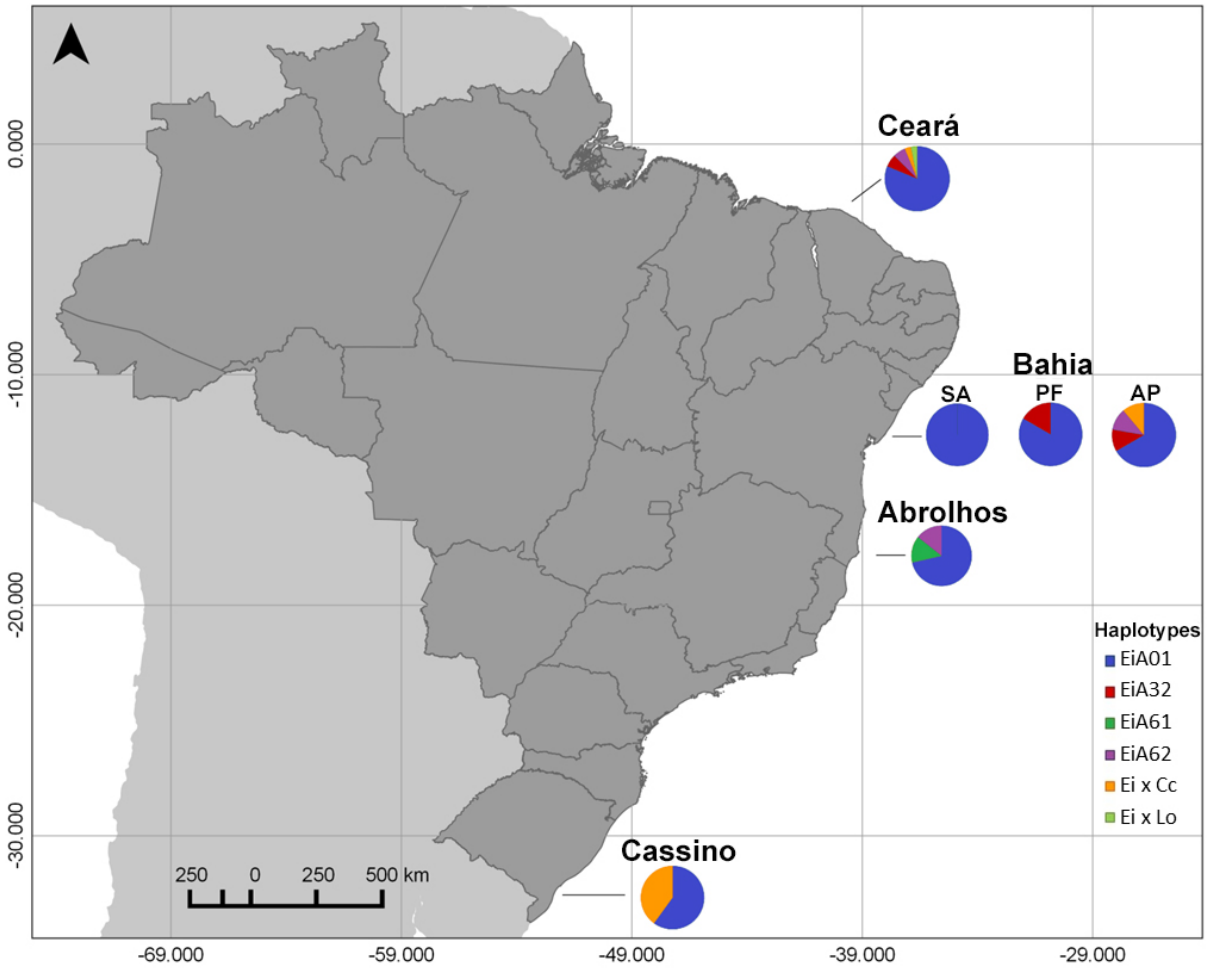

Figure 2. Haplotype frequencies of new immature hawksbill samples along Brazil (Ceará state, Bahia state, Abrolhos Park and Cassino Beach). SA, PF and AP = beaches at Bahia state. Orange (seen at Bahia and Cassino) and light green (seen at Ceará) indicate hawksbill x loggerhead and hawksbill x olive ridley hybrids, respectively.

NEXT STEPS

The next step in this project is to evaluate nuclear DNA markers of all samples (n = 224). We are currently waiting for the primers necessary for the amplification and genotyping of ten nuclear markers, which will be compared to pure species (hawksbills and loggerheads) in order to determine parental species. The extent of hybridisation (generations) will be evaluated through grouping methods using STRUCTURE, NewHybrids and HybridLab software. Results will be evaluated and discussed in view of available literature, as well as according to results currently being produced on the reproductive parameters, survival and stable isotopes of hybrid sea turtles from Bahia state (conducted by Luciano Soares, also a participant of this proposal). By analyzing biparentally inherited nuclear DNA, we believe an even higher frequency of

immature hybrids along the coast of Brazil will be found, and therefore we will be able to better infer on the distribution and possible ecological alterations of these hybrid sea turtles.

MATERIAL PRODUCED

The grant from the Rufford Foundation was used to acquire a state-of-the-art Applied Biosystems Veriti Thermocycler, which is an essential part of the success of the project. Apart from being used to study sea turtle hybrids, it is being used for other conservation genetics studies being conducted in the laboratory. This includes population genetics of the five sea turtle species that occur in Brazil, kinship and population analyses of bottlenose dolphins, and diversity and structure of exploited fishery stocks such as silversides, engraulids, blue crabs, and red and pink shrimp.

So far, we have submitted two abstracts on the subject of the sea turtle hybrids in Brazil. The undergraduate student Cíntia Brito presented one of these abstracts orally on October 27, 2015, at the Rio Grande Federal University's Scientific Showcase. I presented the second abstract orally at the 36th International Sea Turtle Symposium in Lima, Peru, in March 2016. Final results will be presented at the 37th International Sea Turtle Symposium (Las Vegas, U.S.A.) and communicated in the media, as well as in a scientific paper submitted to a high-impact journal.

Additionally, over the course of the project I produced a short video to communicate in an accessible manner our findings on the hybridization process in Brazil. This video was submitted to the EURAXESS Science Slam, a science communication contest, and was selected as one of the five finalists (https://scienceslambrasil.splashthat.com/). I presented the video live in Rio de Janeiro at the event's final, and although it was not the winning presentation, it received very positive comments from the review panel. The video can be watched (English subtitles are available) at the following link: https://www.youtube.com/watch?v=FGjuEDej_BU. It has been watched over 1700 times and shared extensively on Facebook and blogs.

Image: PCR tubes being placed into the Veriti Thermocycler acquired with the grant.