

## Molecular evidence supporting a recurrent stranding event in West Indian manatees

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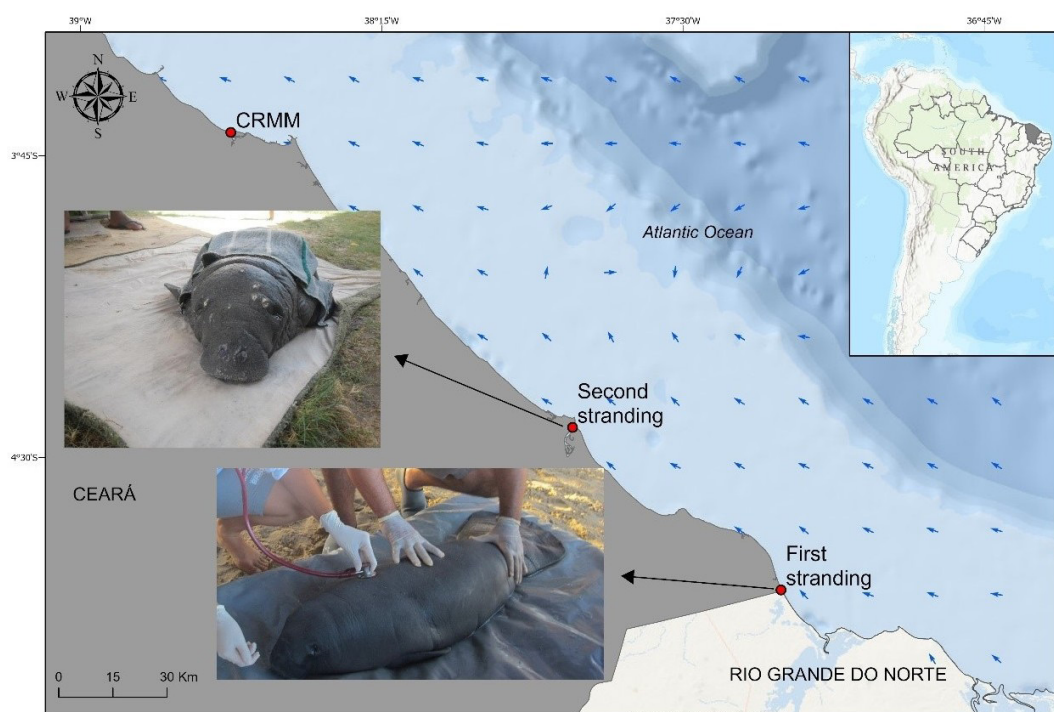
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The West Indian manatee (*Trichechus manatus*, Linnaeus 1958), classified as vulnerable by the IUCN (Deutsch and Morales-Vela, 2024) and as endangered by the Brazilian Ministry of the Environment (Brasil, 2022), faces significant threats in northeastern Brazil due to environmental degradation, motorized coastal vessels, and the destruction of mangroves and estuaries by development (Lima et al., 2011; Meirelles et al., 2024). Stranding of neonates in this region is a major conservation concern, as human impacts appear to force some manatees to give birth in open waters, exposing neonates to increased risks of

mother-calf separation or abandonment (Meirelles et al., 2024; Lima et al., 2011).

The highest rates of live stranding of calves on the Brazilian coast occur in a stretch of less than 400 km along the eastern coast of the state of Ceará and northwestern of the state of Rio Grande do Norte (Balensiefer et al., 2017). In this area, manatees no longer enter estuaries and are found in marine waters (Choi et al., 2017). On December 1st, 2014, a newborn male manatee, 114 cm long with an open umbilical scar, stranded on Ceará Beach, Icapuí, in the state of Ceará, Brazil (Figure 1). The Aquasis' rescue team identified the calf (Aquasis record #02S0111/67) as only a



**Figure 1.** Stranding locations on the coast of the Brazilian state of Ceará and the site of the rehabilitation center (CRMM). The photographs show the manatee (*T. manatus*) rescued after each event. The blue arrows indicate the current's direction.

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few days old (based on Marmontel, 1995). Morphometric data and biological samples (blood and skin) were collected. Since the newborn appeared healthy and adult manatees were nearby, the rescue team followed Brazilian protocols and released the calf.

Later, on December 28th, a manatee calf, male, with a body length of 103 cm, extremely debilitated was found stranded on the Jaguaribe River's mouth (Figure 1), in Fortim, Ceará (Aquasis record #02S0111/68). The individual's age was estimated to be 20–30 days, considering the condition of the umbilical scar and the weight. Showing signs of malnutrition, weighing only 20 kg, when a newborn weighs an average of 34 kg (Borges et al., 2012), it was taken to the Aquasis' Marine Mammal Rehabilitation Centre (CRMM), in Iparana, Caucaia, Ceará, due to its poor health. The morphological characteristics of the new stranded manatee, including body size and tail shape suggested it was the same individual found stranded earlier, on Ceará Beach, given the short interval between events. The observed difference in the total length can be explained by inter or intra-researcher variation (Waite and Mellish, 2009). Additionally, during periods of fasting, marine mammals can experience noticeable reductions in body mass, including muscle and fat stores, which may affect their overall body measurements.

Blood and tissue samples were collected and preserved in alcohol for genetic analysis. To investigate the suspicion, molecular analyses were conducted using samples collected from both individuals. The DNA was extracted using the Wizard Genomic Purification kit, and ten microsatellite loci (SC5, J02, Kb60, SC13, E14, E07, E1, E08, E11, A09) were amplified from each sample following Moreira et al. (2022). The products were applied in an ABI 3500XL, the alleles were identified with GeneMapper 4.1 and added to the IECOS/UFPA genotype database of 53 *T. manatus* specimens from Ceará and Rio Grande do Norte. Genetic similarity between the samples collected from the two stranded individuals was investigated using the relatedness index ( $r_{xy}$ ) from TrioML and WANG estimators in Coancestry (Wang, 2011). Finally, we analyzed the matching genotypes within the whole database of the GeneCap program (Wilberg and Dreher, 2004).

Comparison of genetic profiles revealed identical genotypes for both samples across all loci, with  $r_{xy}$  values of 1, indicating they were genetically identical. The GeneCap analysis confirmed that samples #02S0111/67 and #02S0111/68 shared the same genotype. The identical genetic profile, along with similar morphometrics and the proximity of events, suggest that both samples came from the same individual.

Although the genetic profile is also compatible with monozygotic twins, this hypothesis is not the most likely, since twinning is an extremely rare event in manatees, especially with identical twins, which have never been documented in wild manatees. In this species, a single proven case of multiparity in nature was found to be dizygotic twins (Moreira et al., 2022).

Although the region between the states of Ceará and Rio Grande do Norte has the highest rates of strandings of live calves in Brazil (Balensiefer et al., 2017), cases of recurrent stranding of these animals had never been

reported. This is the first study in which it was possible to prove this event, which contributes to new reflections on release protocols.

This study highlights the effectiveness of genetic tools with biparental inheritance, combined with rescue data (e.g., weight and morphology), in identifying potential cases of double strandings in manatees. It emphasizes the critical importance of collecting biological samples and detailed morphological data during all stranding events to enhance research, improve conservation strategies, and support the long-term protection of this endangered species.

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