2019 SMALL GRANT IN AID OF RESEARCH – FINAL SUMMARY REPORT

Assessment of the effectiveness of noninvasive free-floating fecal samples of the African manatee as a source of DNA for genetic analysis using mitochondrial, microsatellite, and sex identification markers.

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Abstract

Noninvasive genetic sampling approaches have been widely used to study terrestrial species but applied scantly to their aquatic counterparts. The African manatee is the least known of sirenian species and their cryptic nature hampers direct observational studies. We investigated the reliability of DNA isolated from free-floating manatee feces for genetic analysis using mitochondrial, microsatellite and sex-specific DNA markers. We also assessed the effect of habitat on the quality and quantity of fecal DNA yields. We optimized the QIAmp Fast DNA Stool Kit protocol to isolate DNA from 235 free-floating African manatee feces collected in Lakes Ossa (n=93), Tissongo (n=60) and Sanaga River (n=82), Cameroon, between 2016- 2017. From 235 isolates, we selected 110 with a total DNA concentration (t[DNA]) >10ng/µl, DNA was purified from possible PCR inhibitors, and we amplified a 410-bp segment of the mitochondrial Control Region (CR). We used the pre-amplification PCR approach to amplify 13 microsatellites and three sex-specific loci in four to eight PCR replicates per individual and constructed a consensus genotype. Overall, t[DNA] averaged 15.3ng/µl, and about 92% of the samples yielded adequate CR sequence lengths with an average HQ% of 94.6%. PCR success rate was high (80%) and allelic dropout rate moderate (ADO=24%). We successfully assigned sex to 86% of isolates. Total DNA concentration and PCR success were significantly (P=0.0002) higher for samples from rivers (21.9ng/µl, 87.5%) than lakes (11.8ng/µl, 79.4%). ADO was significantly (P=0.006) lower in rivers compared to lakes (18.5% versus 29.1%, respectively). This suggests that fecal samples from rivers are fresher than those found in lakes, where water can be stagnant and feces can remain in the system for longer, allowing for a greater chance of collecting older fecal samples. For the first time, noninvasive manatee fecal DNA was used to generate reliable microsatellite and sex genotypic profiles. This approach is

cost-effective and would make genetic sampling of this imperiled manatee more accessible.

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