

Marine Mammal Society 2023

Small Grants in Aid of Research

To support marine mammal research in places where funding is needed most.ⁱ

Application template and prompts – please paste responses into the SMM web portal to submit your proposal. Use the tab field to move between fields. Check your word count for each section. Thank you for completing this form in English.

Project Information

Title Conservation of the African manatee in Cameroon: assessing a country-range genetic diversity and connectivity through haplotypes' identification

Location Various manatee habitats (rivers, lakes, ocean) in Cameroon

Applicant Information

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Student Early Career Experienced Investigator (> 5 y post degree or equivalent)

Biography

I am Clinton Factheu, a Cameroonian and PhD student at the University of Yaounde 1. I am a passionate of marine mammal biology and conservation, with a particular interest on manatees. My PhD thesis focuses on African manatee genetics, acoustic detection and habitat use in Lake Ossa. I also work with the African Marine Mammal Conservation Organization (AMMCO), a Cameroonian NGO that is dedicated to the conservation of aquatic megafauna and their habitat. At AMMCO, I am in charge of designing and conducting marine mammal research projects.

Overview

What do you want to do? Explain the background, rationale, and purpose of the study. Summarize current knowledge of the topic, identify knowledge gaps, and state the specific research question or hypothesis you will address. You may include essential numbered references [1].

The African manatee (*Trichechus senegalensis*) is an aquatic omnivorous mammal, classified as Vulnerable in the IUCN Red-List [1] and in Appendix I of both the Convention on the International Trade of Endangered Species of Wild fauna and flora (CITES) and the Convention on Migratory Species (CMS). It is the least studied sirenian and the least studied large mammal in Africa [2]. The major threats to this species are poaching, bycatch in fishing nets, and habitat degradation [1] with consequences on its population numbers, fewer than 10,000 individuals, of which 30% or more are highly likely to vanish within ninety years due to threats [1]. One of the challenges of the African manatee conservation is the limited available data, due in part to the species' cryptic, elusive and mildly social behavior. In

Cameroon, manatees are widely distributed. They occur throughout the country's coasts, in the Ndian, Wouri, Sanaga, Nyong, Cross, Benoue, Nkam, and Ntem Rivers, and in Lake Ossa and Lake Tssongu [1, 3, 4, 5]. The Downstream of the Sanaga River Watershed (DSRW) is one of the most important and the most monitored manatee habitat in Cameroon and approximately 250 manatees inhabit the area [5]. Very little is known about manatees of the rest of their distribution range in Cameroon. In Cameroon, manatees are widely poached throughout their range and manatee meat can be found in some local markets including the Youpwe market in Douala, the economic capital of Cameroon. Through questionnaires and discrete investigations, manatee poaching has been confirmed to be severe in the Nkam, Wouri and Sanaga Rivers and in Lake Tssongu and the estuary. Thus, at many areas, manatees could be locally and silently poached to extinction without any scientific information known about their population. Meanwhile it is known that manatees within the DSRW constitute a single population [5] and exhibit a low genetic diversity [1, 5], nothing is known about the genetics of the populations from the rest of their distribution range. The molecular understanding of genetics now plays a crucial role in the effective management and conservation of species as it allows one to deduce connectivity, population structure, and origins of populations and taxonomic groups [6, 7]. The level of genetic diversity in a species reflects its resilience to environmental challenge [5]. Haplotypes are a combination of alleles on adjacent loci that are inherited together, which are indicators of genetic diversity, and can inform researchers about relatedness between populations [8]. With 22 haplotypes, the African manatee exhibits the greatest control region diversity among sirenians [5, 8, 9]. However, Keith-Diagne [8] found a low mtDNA nucleotide diversity which indicates a possible reduction in effective population size. In addition, most of haplotypes (5/6) found in Cameroon are endemic to the country, suggesting the isolation of the Cameroon coastal population from the other coastal populations potentially due to the rough waves and the scarcity of food along the coast, which might prevent migration between coastal habitats or populations in that region [8]. Most samples used in previous manatee genetic studies were collected within the DSRW. Collecting and processing samples from other manatee habitats in Cameroon will help in understanding manatee genetic diversity and population connectivity which is important to the establishment of a country-range conservation program.

The research questions of this project are: 1) what is the control region haplotype diversity of manatees in Cameroon? 2) Are manatees from different habitat genetically connected? 3) Are they distinct manatee populations in Cameroon?

The overall objective of this project is to contribute to the establishment of a country-range manatee conservation program in Cameroon. More specifically we aim to 1) determine the number of manatee control region haplotypes in Cameroon; 2) determine if manatees are genetically connected across the country and 3) determine if manatees constitute a single population in Cameroon.

Approach

How will you do it? Briefly explain the methods you will use to collect, analyze, and share data. You may include essential numbered references [1].

Manatee tissue and fecal samples collection: African manatee tissues and fecal samples have been and are still being collected. Fecal samples have recently been proven to be an excellent source of DNA for African manatees [5]. Tissue and fecal samples are collected across manatee distribution range in Cameroon. Through a collaboration with the fishermen network of the African Marine Mammal Conservation Organization (AMMCO, www.ammco.org), manatee fecal samples are collected opportunistically by the fishermen of SIREN network. This is a citizen science network established in 2016 by AMMCO and mainly constituted of local fishermen from different water systems in Cameroon. Fishermen collect fecal samples during their routine fishing activity. Manatee tissue samples are also collected opportunistically on washed-up manatee carcasses when available. In addition, manatee tissue

samples are collected on poached manatees sold at already identified manatee meat trafficking markets in Douala, the economic capital of Cameroon. Also, through a collaboration with the African Aquatic Conservation Fund (AACF) which conducts African manatee threat assessment projects in Cameroon, manatee tissue and fecal samples collected during these projects' fieldtrips are included in our study samples. Collected manatee fecal and tissue samples are preserved in 50 mL sterile tubes half filled with 95% ethanol and stored in the laboratory.

To date, 24 manatee tissue samples have already been collected in the Ntem, Sanaga, Nkam, Wouri and Ndiang (Moko) Rivers and the country's northern coast (a washed-up manatee carcass in Limbe). Samples are still being opportunistically collected by both the fishermen network (AMMCO) and the manatee threat assessment project (AACF).

Lab processing: the Qiagen Blood and Tissue Samples Kits (Qiagen Inc., Valencia, CA) will be used to extract DNA from tissue samples whereas methodology developed by Takoukam [5] will be used to extract DNA from fecal samples. For both sample types, primers CR-4 and CR-5 [10] will be used to amplify a portion of 410bp the mtDNA control region following protocols developed by Keith-Diagne [8] and Takoukam [5] accordingly.

DNA amplicons will be sequenced through a collaboration already initiated with the World Wildlife Fund (WWF) whereby their cyclor of the Campo Ma'an National Park program (south Cameroon) will be used for DNA sequencing.

Analytical approach: the obtained mtDNA control region sequences will be aligned with a reference sequence (AY963894.1, [9]) using the MUSCLE algorithm in the GENEIOUS Software. Then the sequences will be trimmed to 410bp and examined for error using the same software. The summary statistics including haplotype, nucleotide and sequence diversities, the number of polymorphic sites, and the number of haplotypes, will be calculated using the DNAsp software. The analysis of molecular variance (AMOVA) will be computed using ARLEQUIN software, which will also serve for the tests of differentiation between manatees from different locations in Cameroon. All tests will be set at P-value <0.05. Thus, the number of haplotypes in each location and in general and the haplotype distinctiveness across the species range will be determined.

Significance

Why is it important? Explain the anticipated outcomes of your proposed study and their significance to our understanding of the biology and/or conservation of marine mammals. You may include essential numbered references [1].

This will be the first country-range genetic study of manatees in Cameroon. While site-specific surveys are important, country range genetic studies are crucial as they provide substantial information on the species ecology, plasticity and their likelihood to survive in the mid-term. Thus, this survey will orient manatee conservation actions and determine key habitat/populations that necessitate urgent intervention.

Timeline

What is your timeline? Give a brief timeline of expected research milestones. Funds should be used within 12 months of the award. A short summary report will be requested at the end of the year. An extension may be requested and granted at that time if needed.

Manatee tissue and fecal samples have been collected for the past five years. We will begin sample processing (including DNA extraction, amplification and sequencing) in December 2022. Sequence analyses will be done in January and February 2022. We plan to submit a paper in March 2023 and a project report in April 2023.

Expenses

What support is needed and how will it be used? Provide a brief project budget and justify the expenses (breakdown by category and explain requested costs within each category). A maximum of 25% of the budget can be requested as a stipend. As these awards are modest, please describe other available funding or explain how the project can be completed with available resources. Grants are awarded to individuals, therefore no grant fees or overhead should be included.

The total budget of this project is \$5,000. The African Aquatic Conservation Fund has already supported this project with \$2,000. An application to obtain \$1,000 through the Society for Conservation Biology's Graduate Student Research Grant has been submitted. We request \$2,000 to the Society for Marine Mammalogy to complete the project total budget. SMM grant will be used as follows:

- 2 kits of QIAMP FAST DNA Stool Kit: $\$300 \times 2 = \600
- 2 kits of QIAGEN Dneasy Blood and Tissue: $\$175 \times 2 = \350
- 1 package of Sterile PCR water (25 mL): \$200
- 1 Master mix for the mitochondrial amplification: \$330
- GENEIOUS software license: \$300
- Publishing: \$220
- Total funds requested: \$2,000

Preparation

Tell us about your preparation or qualification for this study. If working with a mentor and/or team, briefly describe. **200 words max.**

I hold a Bachelor and a Master's degree in Animal Biology and Physiology and currently following PhD program in Applied Zoology. Thus, I have the theoretical background necessary to carry on genetics analyses. Moreover, I have been following an internship in a molecular biology laboratory where I have been practicing DNA extraction and purification for the past few months. In addition, I am supervised by two African manatee experts, Dr Lucy Keith Diagne and Dr Aristide Takoukam, who have solid backgrounds in manatee genetics.

Animal Welfare Considerations

What are the legislative requirements for this study in this region and have the necessary permits been applied for or received?

The legislative requirement for this study is a research permit. This requirement is fulfilled, as this research is carried out under the control of AMMCO, which has a MoU with the Cameroonian Ministry of Forestry and Wildlife. This MoU allows AMMCO to carry out research activities throughout the study sites.

Are there additional animal welfare requirements and does this study have the necessary approvals applied for or received?

No other animal welfare requirement is needed in this study.

If applicable, does this study comply with the [Guidelines of Treatment of Marine Mammals in Field Research](#)? (yes/no)

Not applicable.

Societal and Research Ethics Considerations

The Society for Marine Mammalogy recognizes the importance of advancing diversity, equity, and inclusion (DEI), and its essential role in generating novel research and conservation practices that truly reflect local needs.

Does this study comply with the [Ethical Standards of the Society for Marine Mammalogy](#)? (yes/no) **Yes**

Please affirm that you have reviewed these important standards and best practices by stating so here.

I have read and understood the standards and best practices.

Please provide a short statement that highlights how throughout your project you will be contributing to the Society's DEI goals. We recognize that this section might not be applicable to everyone. However, we would appreciate it if you could envision ways in which you can advance diversity, equity, and inclusion (DEI) in the country where your research will take place. **100 words max.**

This project has been and will continue to be carried out in respect, where applicable, of the society's DEI goals.

Citations

- [1] Keith Diagne, L. (2015). "Trichechus senegalensis," IUCN Red List Threatened Species, <https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T22104A81904980.en> (Last viewed 06/16/2022).
- [2] Trimble, M. J., and Van Aarde, R. J. (2010). "Species inequality in scientific study: Species inequality in science," *Conservation Biol.* 24, 886–890.
- [3] Nishiwaki, M., Yamaguchi, M., Shokita, S., Uchida, S., and Kataoka, T. (1982). "Recent Survey on the Distribution of the African Manatee," *Scient. Rep. Whale Res. Inst.* 34, 137-147.
- [4] Powell, J. A. (1996). *The Distribution and Biology of the West African Manatee (Trichechus senegalensis Link, 1795)*. United Nations Environmental Program, Regional Seas Program, Oceans and Coastal Areas, Nairobi, Kenya, p. 68.
- [5] Takoukam, A. (2019). "Genetic diversity, diet, and habitat quality of the African manatee (*Trichechus senegalensis*) in the downstream of the Sanaga river watershed, Cameroon," Ph.D. dissertation, University of Florida, Gainesville, FL.
- [6] Frankham, R, Briscoe, D., & Ballou, J. (2002). *Introduction to conservation genetics*. Cambridge university press.
- [7] Schwartz, M. K., Luikart, G., & Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, 22(1), 25–33. <https://doi.org/10.1016/J.TREE.2006.08.009>
- [8] Keith-Diagne, L. (2014). *Phylogenetics and feeding ecology of the African manatee, Trichechus senegalensis*. University of Florida.

[9] Vianna, J. A., Bonde, R. K., Caballero, S., Giraldo, J. P., Lima, R. P., Clark, A., ...294 Santos, F. R. (2006). Phylogeography, phylogeny and hybridization in trichechid sirenians: Implications for manatee conservation. *Molecular Ecology*, 15(2), 433–447. <https://doi.org/10.1111/j.1365-294X.2005.02771.x>

[10] Southern SO, Southern PJ, Dizon AE (1988). Molecular characterization of a cloned dolphin mitochondrial genome. *Journal of Molecular Evolution*, 28, 32-42.

ⁱ The Society's International Relations Committee and Committee of Scientific Advisors have defined all countries except the following as eligible countries: Applicants cannot be from Australia, Austria, Belgium, Canada, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Luxembourg, Netherlands, New Zealand, Norway, Poland, Portugal, Singapore, Slovenia, Spain, Sweden, Switzerland, Taiwan, United Arab Emirates, United Kingdom, and United States. See <https://marinemammalscience.org/awards-funding/awards-and-scholarships/grants-in-aid-of-research-information/>