

Proposal: Exploring the Genetic Connectivity Between Madagascar and the Indo-Pacific Through the Distribution of Mollusk Species

1. OBJECTIVES

The **general goal** of this project is to observe the wide range of endemic species of mollusks in South Madagascar and the Indo-Pacific region to assess their phylogeny for cryptic biodiversity due to their similar morphologies.

Specific Aims: We will analyze the DNA sequences of mollusk species found in Madagascar as well as the Indo-Pacific region and build a phylogenetic tree. We can then investigate whether the dispersal type of the larvae (long vs. short disperser) caused certain species to survive in different habitats and be related, or if there is cryptic biodiversity. Therefore, we will compare and contrast molecular (DNA sequences) and morphological variation of these mussel species.

2. BACKGROUND

Madagascar is recognized as a biodiversity hotspot and for the endemism of its marine life, but there has been limited research on bivalves. Even though freshwater mussels are known as poor dispersers across oceanic and terrestrial barriers, and their presence in Madagascar was assumed to be due to continental drift, further studies have shown that they have also arrived by transoceanic dispersal (Graf & Cummings, 2009). The majority of Madagascar's plant and animal species are endemic and have evolved from their closest relatives millions of years ago. New genetic approaches have helped with the differentiation of species that had previously been thought to be single species (Ganzhorn et al., 2014). Therefore, Madagascar can be used as a model for exploring numerous evolutionary processes, such as convergent evolution.

The behavioral range of many marine invertebrates such as dispersal range and larval development period has an influence on the diversity of these species. Genetic structure and connectivity across the range of mollusk species can therefore be caused by the interaction of the ocean currents, which cause dispersal during the larval stage (Lal et al., 2017).

Previous studies have observed high levels of phenotypic variation, differences in shell morphology, and variation in physiology and behavior in Australian freshwater mussel species. It was then concluded that these species may be cryptic species, and molecular processes can improve our understanding of diversity and evolution in mussels in Madagascar as well (Baker et al., 2003). We predict that if the species is a long-distance disperser then it will be a broad range species, but if a species in Madagascar is a short time/distance disperser (not feeding) and found in multiple habitats, then we would expect to observe at least 2 species.

3. RESEARCH QUESTIONS

Q1. Does the dispersal type (long vs. short distance) of the larvae affect the geographic distribution of mollusk species in the Madagascar and Indo-Pacific region?

Q2. Do mollusk species found throughout the Indo-Pacific region and Madagascar represent a broad range species or cryptic biodiversity?

Q3. By observing the phylogenetic trees, can we predict how many species there are by dispersal?

4. EXPERIMENTAL PLAN

Study Species: The species we are looking into were collected at an expedition to Madagascar by The Muséum National d'Histoire Naturelle (MNHN, Paris), the Institut

d'Halieutique et des Sciences Marines, University of Toliara (IH.SM), and the Wildlife Conservation Society (WCS) Madagascar Programme in April-June 2010. The species were then matched with their MNHN numbers and their locations were noted using the World Register of Marine Species (WoRMS).

Tissue Sampling and DNA extraction have already been completed and the data of the DNA sequences of the species below will be provided by the Serb lab at Iowa State University.

Data Summary:

Gene sequences: 12S, 16S, 18S, 28S, H3

Taxa: 80 species + outgroup (Spondylidae)

Treatments:

1. Different alignment options: look at the possible differences between MAFFT options with different algorithms (L-INS and E-INS) to align the sequences.
2. Different partition schemes: Test whether a partition for nuclear (18S, 28S, H3) and mitochondrial genes (12S, 16S) would be better than single partition with all sequences concatenated.
3. Gaps: Use sequences with no gaps removed and sequences with gaps removed.

General Procedure:

1. Organize all the sequences by gene and taxa.
2. Align sequences using MAFFT and check for abnormalities/differences.
3. Trim overlapping regions.
4. Inspect the dataset after removing gaps.
5. Concatenate gene alignments (single partition and two partition schemes).
6. Test for model of nucleotide substitution with RAXML (choose best ML tree -> find model with highest L for that tree) or MrBayes (same but with posterior probabilities instead of ML).
7. Run analyses for each partition and respective model in IQTree (or RAXML).
8. Also infer a Bayesian phylogeny and decide on a method of choosing if they're different. After getting our trees and choosing the best one to use, we can observe if the "same" species found in different habitats related by observing what part of the tree they are at (how similar their sequences are), or if there is a possibility of cryptic diversity. After that, we can compare the species morphologies and larval states to see if they match with our tree.

5. BROADER IMPACTS

Previous research in the Pacific Ocean shows that ocean currents and geographic distances are major influences on population connectivity. The inland waters of Madagascar and the Indian Ocean islands have a high diversity of aquatic species and high levels of endemism. This study will help emphasize the biodiversity within Madagascar and provide detailed information of behavioral information of mollusk larvae and its effects on convergent evolution and genetic connectivity. By assessing these mollusk species' distributions among Madagascar and the Indo-Pacific region, we can identify biodiversity to assess conservation priorities.

References

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