Barcoding Palmyra Atoll: Comparing Genetic Methods for Marine Biodiversity Assessment

Authors: Jessica Freedman[1], Zhazha Mahootian[2]
Mentors: Eugenia Naro-Maciel,[3,4] Patrick Shea[4,5]
[1]*Columbia Grammar & Preparatory School*, [2]*Bronx High School of Science*, [3]*Liberal Studies, New York University*,
[4]*Center for Biodiversity and Conservation, American Museum of Natural History*, [5]*The Graduate Center, City University of New York*

ABSTRACT

Palmyra Atoll is a National Wildlife Refuge in the Central Pacific with threatened and understudied coral reefs. In this project, we identified selected invertebrates with which to investigate Palmyra's marine biodiversity as revealed by DNA barcoding, in comparison to assessments made through environmental DNA (eDNA) metabarcoding. Whereas the initial DNA barcoding primers amplified 10 of 53 invertebrate samples, newer COI primers amplified 40 of 43 samples. In addition, we used turtle-specific primers on available eDNA extracts for sea turtle detection, but results were largely inconclusive. While eDNA results provide resolution at higher taxonomic levels, such as Phylum, the barcoding results of this project provide new species-level information about the biodiversity of crustaceans at Palmyra Atoll and suggest that individual specimen barcoding can seem more straightforward than eDNA metabarcoding.

INTRODUCTION

DNA barcoding is an important global initiative for documenting life on earth and identifying species (Hebert et al. 2003), but new sequencing methods such as environmental DNA analysis of water or soil, known as metabarcoding, have the potential to vastly increase the number of organisms studied, more rapidly and relatively inexpensively, through next-generation technology (Bohmann et al., 2014; Thomsen & Willerslev, 2015).

Coral reefs are among the most endangered and biologically diverse ecosystems in the world. Palmyra Atoll (Figs. 1,2) - located in the Central Pacific Ocean - is considered a natural laboratory for biodiversity study, as it is currently uninhabited except for limited research (The Nature Conservancy, n.d.). As such, we investigated diversity of anomuran squat lobsters and caridean shrimp through barcoding specimens from Autonomous Reef Monitoring Structure (ARMS) units (Fig. 3). Furthermore, we attempted amplification of marine turtle DNA from eDNA extracts of coral reef water, as past research has shown difficulty in doing so (Servis et al., in preparation). By examining its biodiversity, conservationists will be better able to protect the integral coral reef ecosystems of Palmyra Atoll.

Figure 1. Aerial view of Palmyra Atoll. Source: abovetopsecret.com

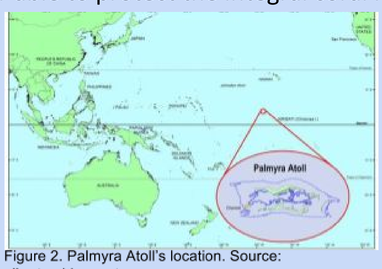

Figure 2. Palmyra Atoll's location. Source: albertan.blogspot.com

Figure 3. Autonomous Reef Monitoring Structure (ARMS) unit, composed of nine 23cm x 23cm PVC plates. Source: Smithsonian National Museum of Natural History

MATERIALS & METHODS

Invertebrate Barcoding

- Ethanol-preserved shrimp & lobster specimens obtained from ARMS around Palmyra Atoll, Central Pacific
- DNA Extraction: Qiagen DNeasy Blood & Tissue Kit
- DNA Amplification: PuReTaq Ready-To-Go PCR beads and COI primers LCO1490 and HC02198; Folmer et al., 1994)
- Gel Electrophoresis: PCR-amplified products & invertebrate positive control
- Second round of PCR with updated COI primers mlCOIintF and jgHCO2198 (Leray et al., 2013)
- Analysis of gels and sequencing by GENEWIZ, Inc. (South Plainfield, NJ)
- DNA Subway bioinformatics pipeline

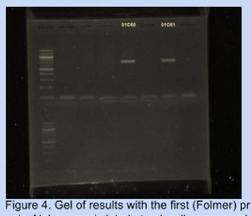

Figure 4. Gel of results with the first (Folmer) primer pair. Alphanumeric label at gel well corresponds to ARMS unit # and specimen ID.

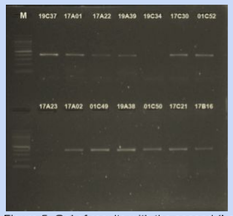

Figure 5. Gel of results with the second (Leray et al.) primer pair.

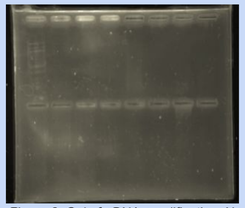

Figure 6. Gel of eDNA amplification. No lanes showed distinct bands.

Environmental DNA Amplification (metabarcoding)

- PCR: Turtle-specific mitochondrial control region primers TCR5 and TCR6 (Norman et al., 1994) and L-turtCOI and H-turtCOIb primers (Stuart & Parham, 2004). Thermocycler conditions described in sources for respective primer pairs.
- Gel electrophoresis and analysis of gels.

RESULTS

The initial Folmer primers yielded only 10 visible gel bands from 53 samples (Fig. 4). Updated COI primers had a higher percentage of successful amplification, with 40 of 43 samples providing visible bands (Fig. 5). Most shrimp (Fig. 7) were classified to the genus *Synalpheus*, while most lobsters (Fig. 8) were classified as genus *Sadayoshia* or species within *Galathea* (Table 1). The eDNA gels were initially unclear and, following restaining, exhibited no signs of successful amplification (Fig. 6). Smearing was visible in several cases, but did not correspond to anticipated fragment sizes. Accordingly, these PCR products were not sequenced.

Table 1. Barcoding results for (A) Caridean shrimp, (B) Anomuran squat lobsters, and (C) Brachyuran crabs.

Infraorder	**(A) Caridea (45.8%)**								
Family	Lymatidea (1.3%)	Hippolytidae (4%)		Alpheidae (30.4%)			Palaemonidea (7.1%)		Unidentified (2.9%)
Genus	Lysmata (1.3%)	Saron (1.3%)	Unidentified (2.7%)	Alpheus (4.9%)		Synalpheus (25.2%)	Brachycarpus (1.3%)	Periclimenes (5.8%)	Unidentified (2.9%)
Species	*argentopunctata* (1.3%)	*neglectus* (1.3%)	Unidentified (2.7%)	*estuariensis* (1.3%)	*cylindricus* (3.6%)	Unidentified (25.2%)	*biunguiculatus* (1.3%)	Unidentified (5.8%)	Unidentified (2.9%)

Infraorder	**(B) Anomura (50%)**			
Family	Galatheidea (28%)		Munidiae (19.9%)	Unidentified (2.1%)
Genus	Galathea (28%)		Sadayoshia (19.9%)	Unidentified (2.1%)
Species	*tongi* (12.8%)	*imitata* (15.2%)	Unidentified (19.9%)	Unidentified (2.1%)

Infraorder	**(C) Brachyura (2.1%)**	
Family	Epialtidae (1%)	Xanthidae (1%)
Genus	Acanthonyx (1%)	Xantho (1%)
Species	*peteverii* (1%)	*hydrophilus* (1%)

Figure 7. (Above) Caridean Shrimp
Figure 8. (Near Left) Anomuran Squat Lobster
Figure 9. (Far Left) Brachyuran Crab. All images from Wikimedia.

DISCUSSION

This project helps create a more detailed picture of life at Palmyra Atoll. The results suggest that standard methods of individual specimen barcoding can provide more resolution, such as species identification, than large scale environmental metabarcoding. Because eDNA is a new technique, comparing metabarcoding to standard barcoding methods can help future researchers understand the differences between the methods and decide which one is suitable for their research. Future work extending from this project could further examine marine turtle DNA amplification from eDNA. Experimenting with different primer pairs and increased PCR amplification cycles could improve their detection. Barcoding efforts could also be expanded through use of additional reference databases.

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