Revelations from mitogenome studies of northwestern Gulf of Mexico octocorals

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with additional data obtained in collaboration with
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National Oceanic and Atmospheric Administration
Northern GOM mesophotic reefs

Boundary of Flower Garden Banks National Marine Sanctuary, which has bottom-contact restrictions, is indicated in red.
Who’s who and where?

Which yellow coral am I?

In what conditions do I thrive?

Can you predict where I live?

Am I resilient to change?

If I die, will the ecosystem be healthy without me?
Without the who and where, we cannot

- Discover
- Observe
- Monitor
- Understand

3 species in 3 genera:
1 each distinguishable by color

5 species in four genera:
sp. 1 (yellow) and sp. 2 (red) in genus 1,
sp. 3 (yellow or purple) in genus 2,
sp. 4 (yellow) in genus 3, and sp. 5 (red or purple) in genus 4
Without the who and where, we cannot

- Understand
- Apply
- Model

## Conclusion vs Reality

<table>
<thead>
<tr>
<th>Conclusion</th>
<th>Reality</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 species in 2 genera:</td>
<td>2 species in 2 genera:</td>
</tr>
<tr>
<td>1 each distinguishable by color</td>
<td>sp. 1 (yellow)</td>
</tr>
<tr>
<td>Purple species &lt; 80 m</td>
<td>sp. 2 (yellow or purple)</td>
</tr>
<tr>
<td>Yellow species &gt; 80 m</td>
<td>sp. 1 &gt; 100 m</td>
</tr>
</tbody>
</table>

50 m

100 m

150 m
Without the who and where, we cannot

• Understand
• Conserve
• Respond

<table>
<thead>
<tr>
<th>Conclusion</th>
<th>Reality</th>
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<tbody>
<tr>
<td>2 species in 2 genera:</td>
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</tr>
<tr>
<td>1 each distinguishable by color</td>
<td>sp. 1 (yellow)</td>
</tr>
<tr>
<td>Purple species impacted</td>
<td>sp. 2 (yellow or purple)</td>
</tr>
<tr>
<td>Yellow species impacted</td>
<td>sp. 1 not impacted</td>
</tr>
<tr>
<td></td>
<td>sp. 2 impacted</td>
</tr>
</tbody>
</table>
Tools to find who’s who and where

**Morphology**
- In situ images
- Macroscopic evaluation
- Microscopic evaluation

**Genetics**
- Complete mitogenomes
- Mitochondrial barcodes
- Nuclear barcodes
- Environmental DNA (eDNA)
Genetic-based biodiversity assessments are complicated by

- traditional barcodes fail for many octocoral species
  - primers do not work when mitochondrial gene rearrangements are present
  - mitochondrial barcode regions are insufficiently variable
- limited reference data for eDNA assessments
  - census of corals is not comprehensive
  - GenBank sequence data is not peer reviewed
  - paired taxonomic and genetic efforts are lacking
Project goals to resolve problems in determining who’s who and who’s where

• Sequence and map complete mitogenomes of select octocoral species to provide reference data

• Identify extended or new barcode regions that may resolve relationships among species and populations

• Develop a field guide with reference data for consistent and accurate identifications within the limits of the assessment tool (e.g., in situ images, collected specimens, eDNA)
Mapping octocoral mitogenomes

- 14 protein-coding genes
- 2 ribosomal RNA (rRNA) genes
- 1 transfer RNA (tRNA) gene
- Circular, double-stranded
- Genes coded on either strand as indicated by gene direction (clockwise, counterclockwise)
- Gene order rearrangement among taxa at black arrows

Callogorgia cf. gracilis
18,937 bp

USNM1507967
GenBank, MH719202
https://doi.org/10.1080/23802359.2018.1544042

Linear representation with traditional primer pairs illustrated by
Results

GenBank data (1 April 2020):
• 85 complete Octocorallia mitogenomes
  • 73 putative species
  • 38 genera

This project:
• Sequenced 26 complete mitogenomes (30% increase)
  • 15 species
• Sequenced 8 partial mitogenomes (9% increase)
  • 7 species
• Mapped mitogenomes of 20-30% of ~75 known northern GOM mesophotic octocorals
  • 1 published (excluded from GenBank total)
  • 1 in press
  • 14-21 species not in GenBank (19-28% increase)
  • 6-9 genera not in GenBank (16-24% increase)
• Identified taxa not reported for GOM and taxa for taxonomic revision
Results

Scleracis divergence values:
~2.25% between species
<0.07% within species

~400 vs. < 20 differences across 18,729 bases

Scleracis divergence values:
~0.1-7.8% (0.2-3%) between species (same genus)
<0.15% (<0.08%) within species
>2% (3-7%) among genera (same family)
>4% (10-20%) among families
1.5% divergence across mitogenome
0.2% across *mutS* barcode region
Target region insufficiently variable?

**Consensus**

**Known location of gene rearrangement**

**SNPs (single nucleotide polymorphisms)**

*Muricea purpurea*

*Muricea crassa*
Identified new potential target regions

Designed dozens of new primers that work across a range of octocoral taxa
Best alternative to traditional cox2-cox1 barcode region

Traditional region, extended by pairing an alternative primer with an existing primer

Best three alternatives for extended + traditional region

Best alternative when cox2 not adjacent to cox1

Best alternative to traditional region

Best three alternatives for extended region

and appear to work well on recent museum specimens (collected <10 y ago) when traditional primers fail
Were differences observed in the barcode regions?

<table>
<thead>
<tr>
<th></th>
<th>44% Traditional barcode</th>
<th>Extended barcode 56%</th>
<th>Extended region more divergent? 44%</th>
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</thead>
<tbody>
<tr>
<td>Muricea pendula</td>
<td>no</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Paracis cf. enopla sp. 1</td>
<td>no</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Paracis cf. enopla sp. 2</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Thesea nivea</td>
<td>no</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Paramuricea sp. 1</td>
<td>YES</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Scleracis sp. 1</td>
<td>YES</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Scleracis sp. 2</td>
<td>YES</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Swiftia exserta</td>
<td>no</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Thesea cf. rubra</td>
<td>YES</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>20 other taxa (interspecific)</td>
<td>YES = 18 (90%)</td>
<td>YES = 15 (75%)</td>
<td>YES = 7 (35%)</td>
</tr>
</tbody>
</table>

Extended barcode region

- **cox2 to cox1**
- **Muricea pendula**
  - SNP
  - 2 SNPs
- **Swiftia exserta**

Traditional barcode region

- **cox2 to cox1**
- **Muricea pendula**
  - SNP
  - 2 SNPs
- **Swiftia exserta**
Were differences observed in the barcode regions?

<table>
<thead>
<tr>
<th></th>
<th>50% Traditional barcode</th>
<th>Extended barcode</th>
<th>88%</th>
<th>Extended region more divergent? 63%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muricea pendula</strong></td>
<td>no</td>
<td>YES</td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td><strong>Paracis cf. enopla sp.1</strong></td>
<td>no</td>
<td>no</td>
<td></td>
<td>no</td>
</tr>
<tr>
<td><strong>Paracis cf. enopla sp.2</strong></td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thesea nivea</strong></td>
<td>no</td>
<td>YES</td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td><strong>Paramuricea sp. 1</strong></td>
<td>YES</td>
<td>YES</td>
<td></td>
<td>no</td>
</tr>
<tr>
<td><strong>Scleracis sp. 1</strong></td>
<td>no</td>
<td>YES</td>
<td></td>
<td>YES</td>
</tr>
<tr>
<td><strong>Scleracis sp. 2</strong></td>
<td>YES</td>
<td>YES</td>
<td></td>
<td>YES</td>
</tr>
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<td><strong>Swiftia exserta</strong></td>
<td>YES</td>
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<td></td>
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<tr>
<td><strong>Thesea cf. rubra</strong></td>
<td>YES</td>
<td>YES</td>
<td></td>
<td>no</td>
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</table>

21 other taxa (interspecific)  
YES = 19 (90%)  
YES = 19 (90%)  
YES = 12 (57%)

**ND4L to mutS**

<table>
<thead>
<tr>
<th></th>
<th>Traditional barcode region</th>
<th>Extended barcode region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muricea pendula</strong></td>
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SNP = Single Nucleotide Polymorphism  
2 SNPs = Two Single Nucleotide Polymorphisms
Summary

• Substantially increased available octocoral reference mitogenome data, some new gene orders
• Identified taxa not reported for GOM and taxa needing taxonomic revision
• Designed dozens of new primers that work across a range of octocoral taxa
  • May improve amplification success rate across octocoral taxa
  • May improve amplification success rate for museum specimens
• Identified extended barcode regions that may reveal overlooked genetic diversity
Thank you

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