

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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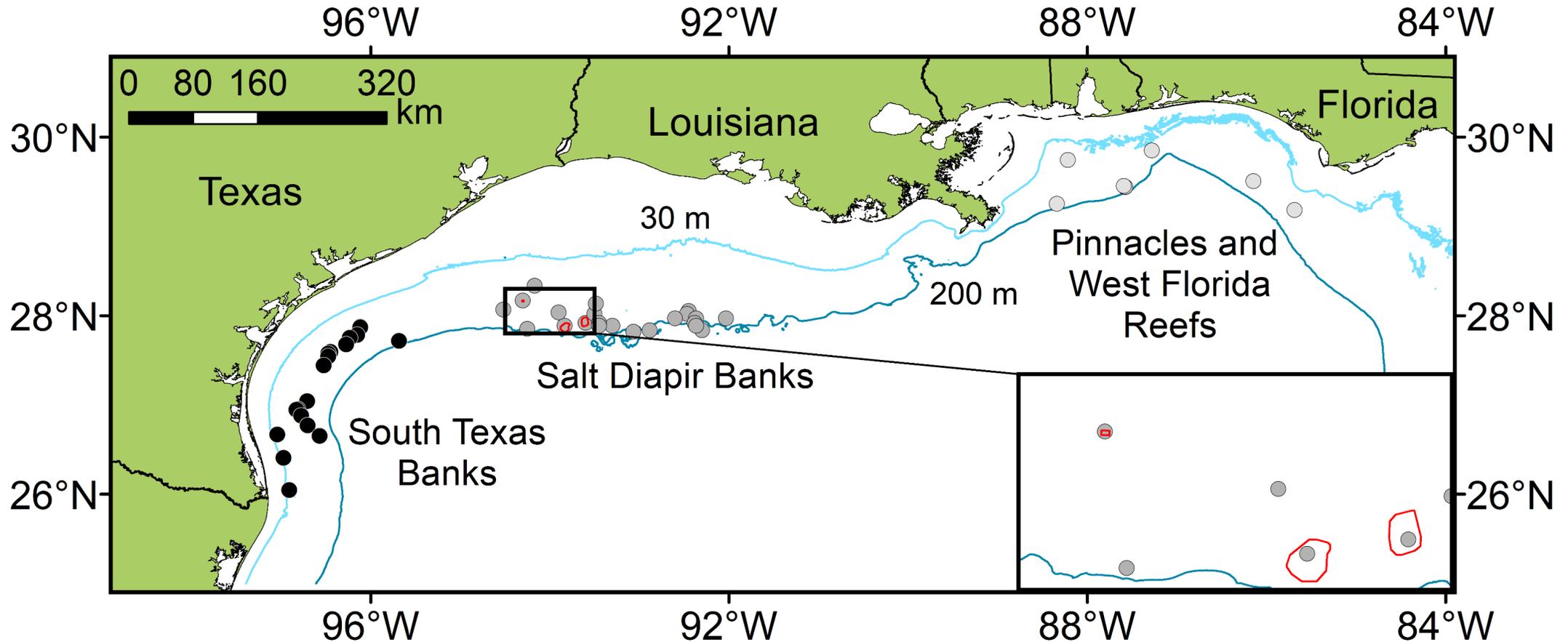
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NOAA CCME
NOAA Center for Coastal & Marine Ecosystems



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

50 m

- Observe

- Monitor

100 m

- Understand

150 m

Observation	Reality
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

50 m

100 m

150 m

Conclusion	Reality
2 species in 2 genera: 1 each distinguishable by color	2 species in 2 genera: sp. 1 (yellow) sp. 2 (yellow or purple)
Purple species < 80 m Yellow species > 80 m	sp. 1 > 100 m sp. 2 50-150 m

Without the who and where, we cannot

- Understand
- Conserve
- Respond

Pre-disturbance

Post-disturbance

Conclusion	Reality
2 species in 2 genera: 1 each distinguishable by color	2 species in 2 genera: sp. 1 (yellow) sp. 2 (yellow or purple)
Purple species impacted Yellow species impacted	sp. 1 not impacted sp. 2 impacted

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)

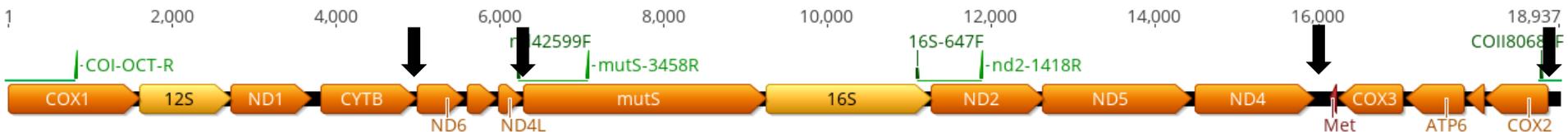
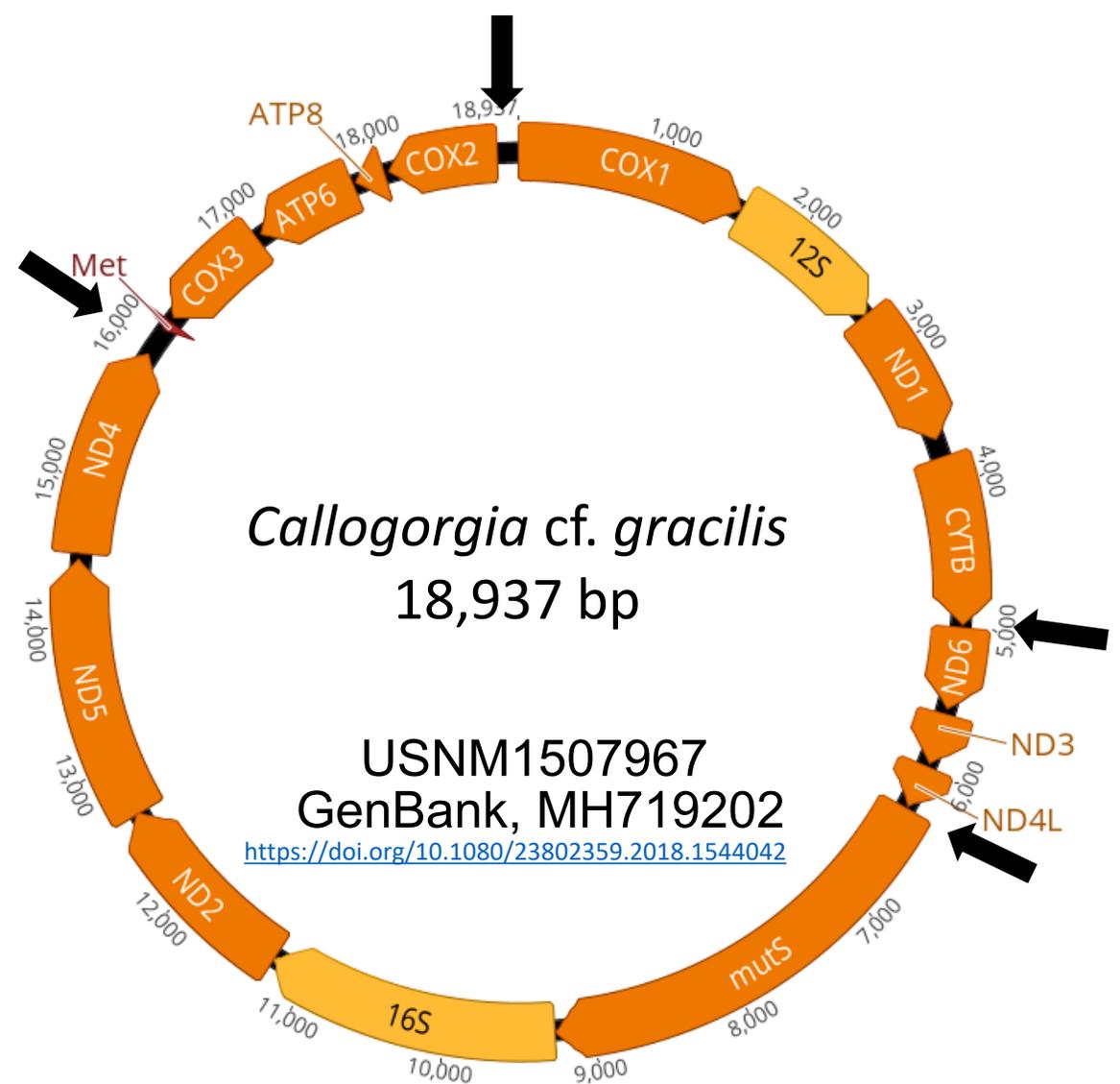
Mapping octocoral mitogenomes

- ✓ 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 



Linear representation with traditional primer pairs illustrated by 

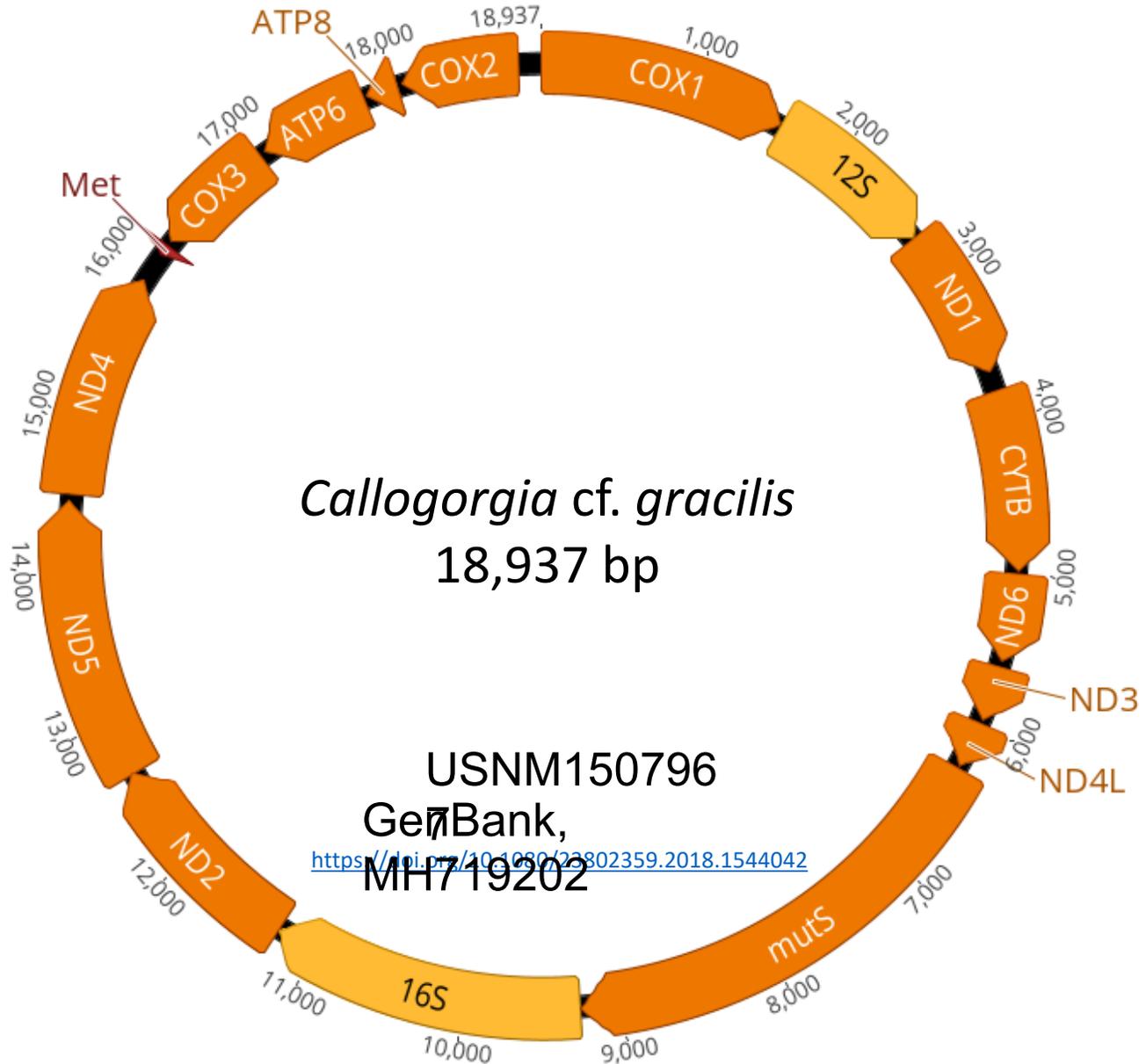
Results

GenBank data (1 April 2020):

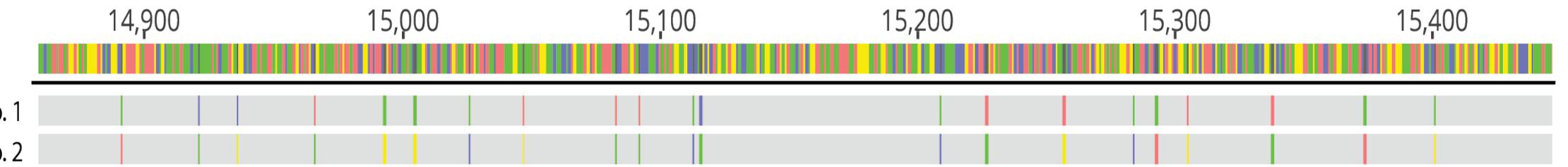
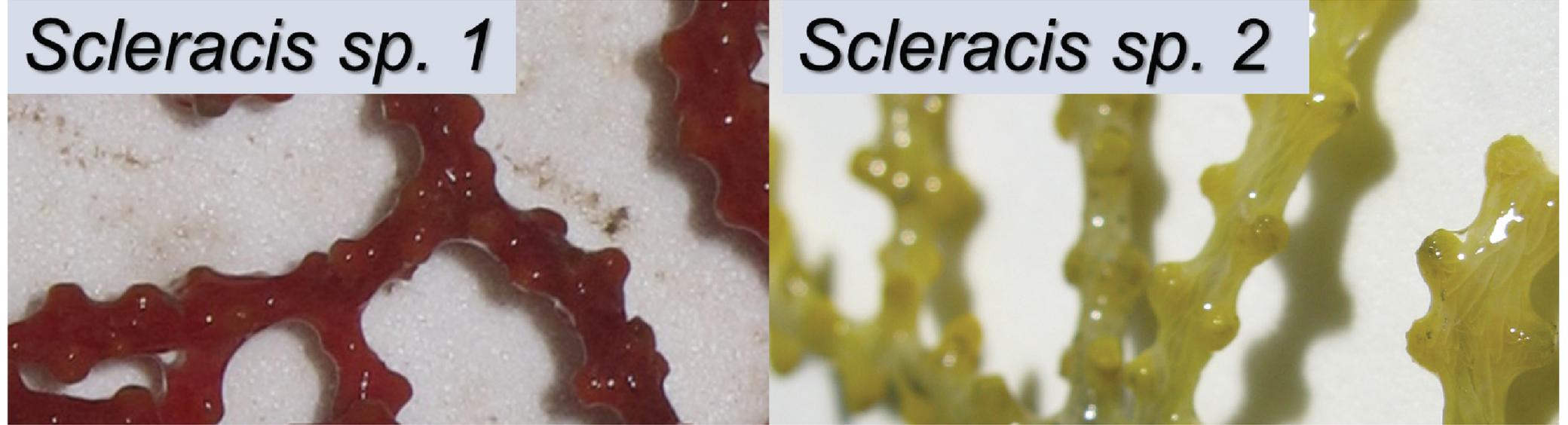
- 85 complete Octocorallia mitogenomes
 - 73 putative species
 - 38 genera

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



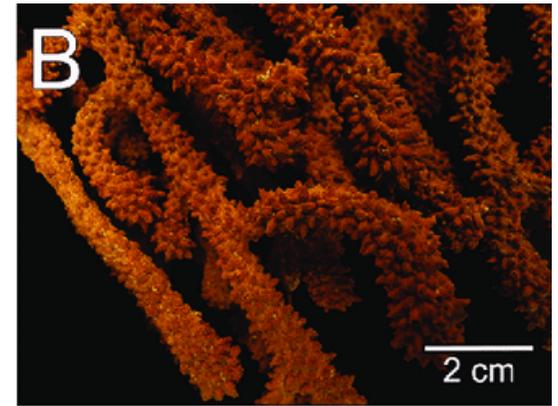
Scleracis divergence values:
~2.25% between species
<0.07% within species
~400 vs. < 20 differences across 18,729 bases

Octocorallia 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

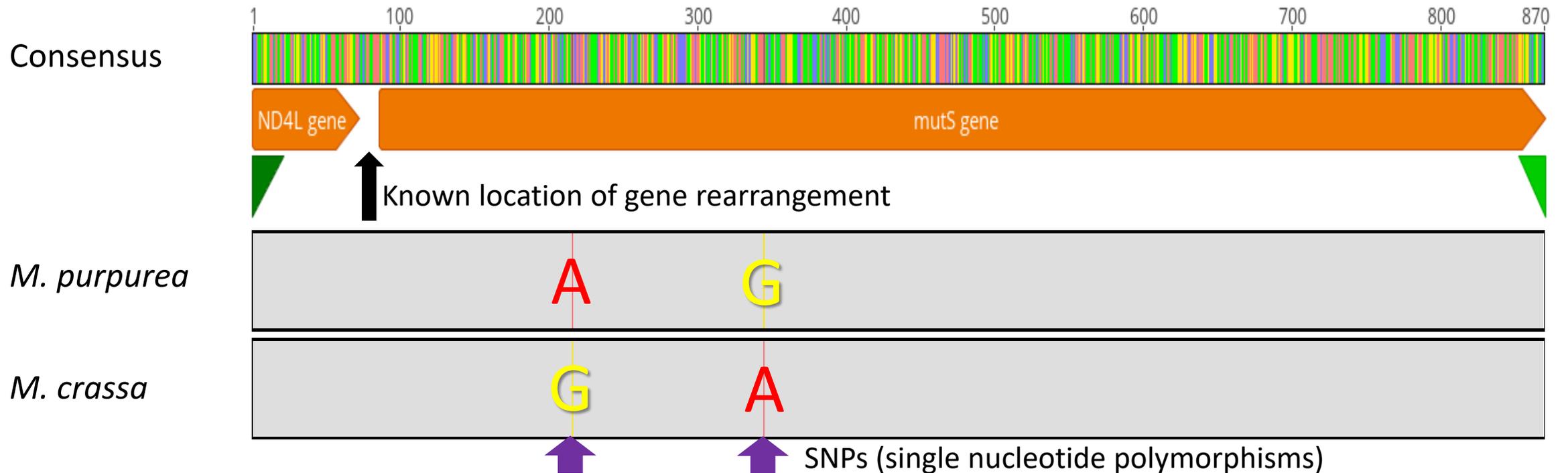


Muricea purpurea

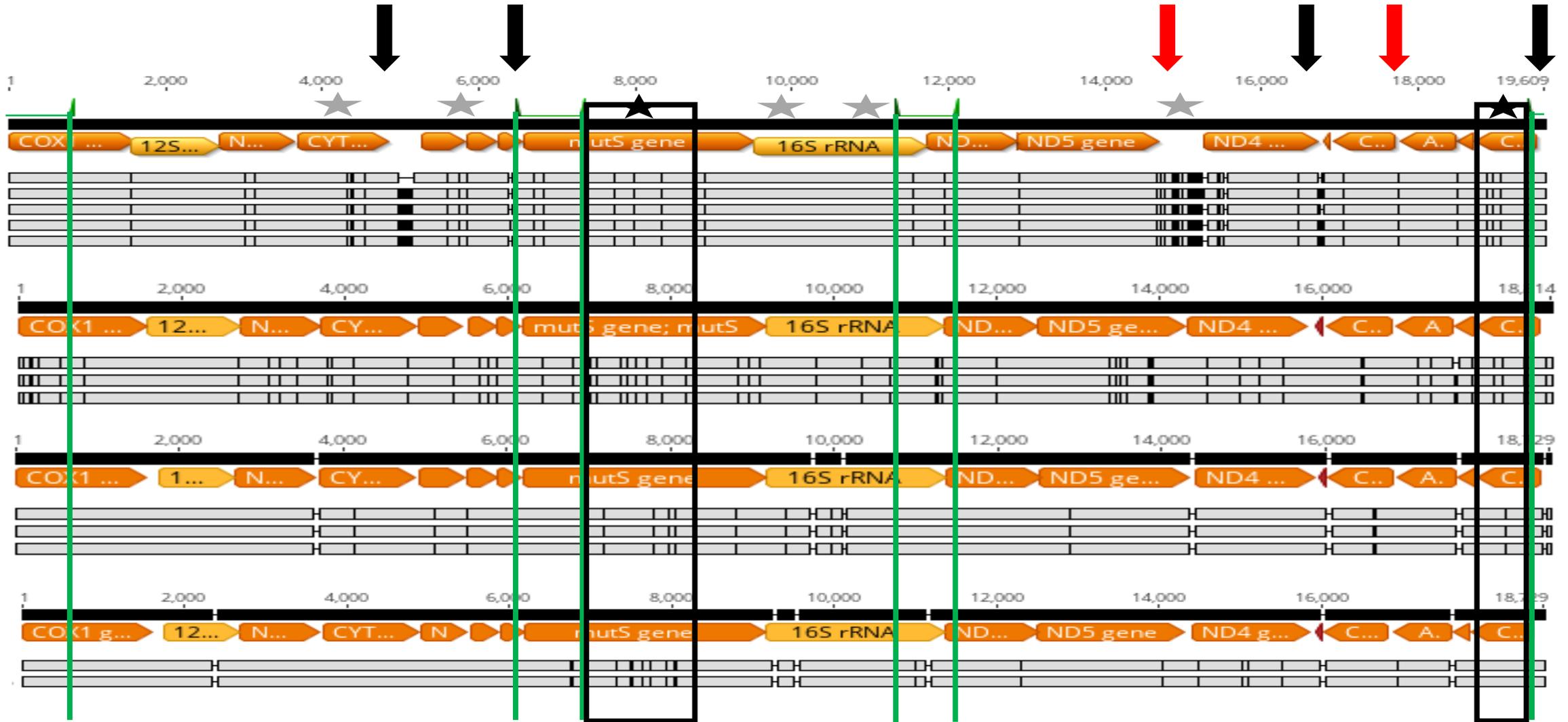
1.5% divergence across mitogenome
 0.2% across *mutS* barcode region
 Target region insufficiently variable?



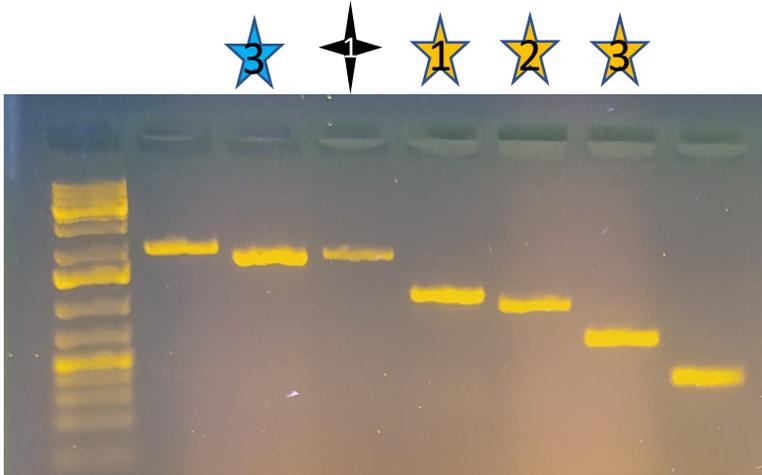
Muricea crassa



Designed dozens of new primers that work across a range of octocoral taxa
 Identified new potential target regions



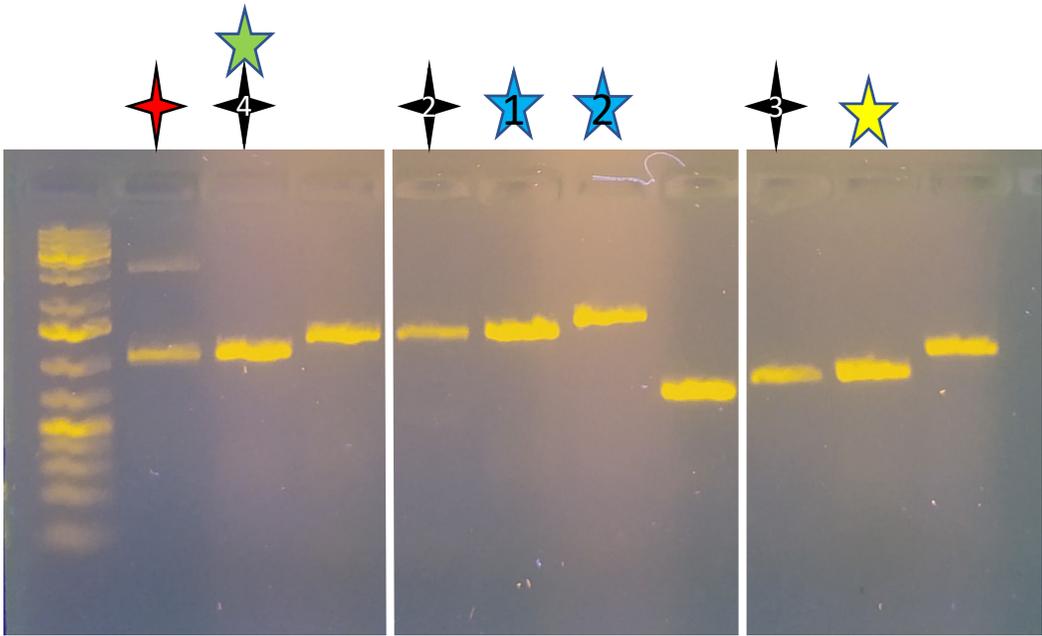
★ Primary candidate ★ Secondary candidate black arrows = known rearrangement zone , red arrows = new zone of gene rearrangement



- Existing primer pair for 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



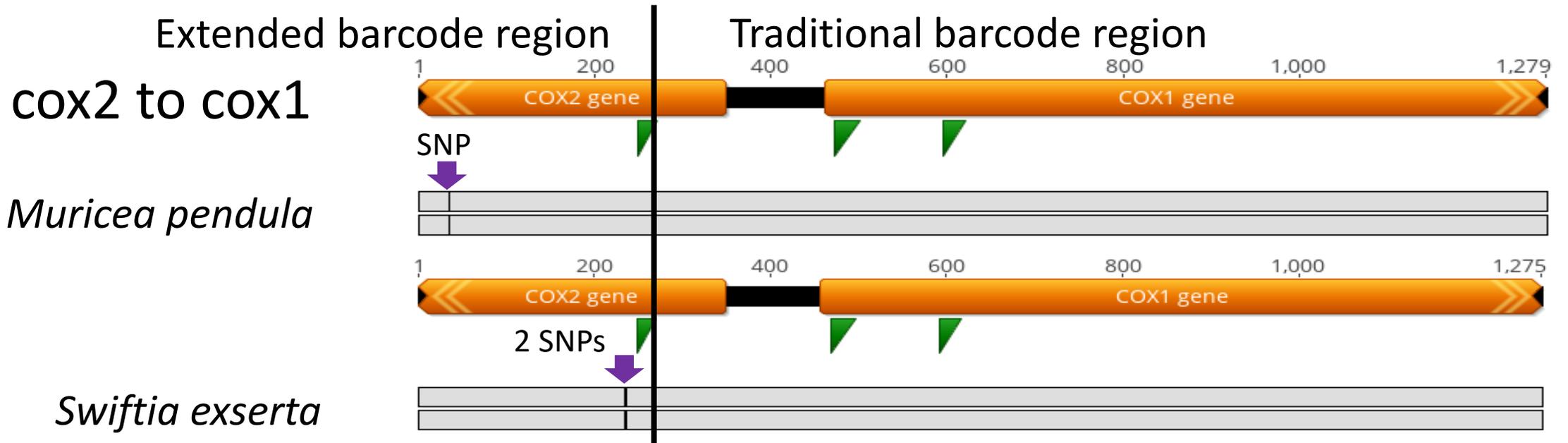
Best alternative to traditional region

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?

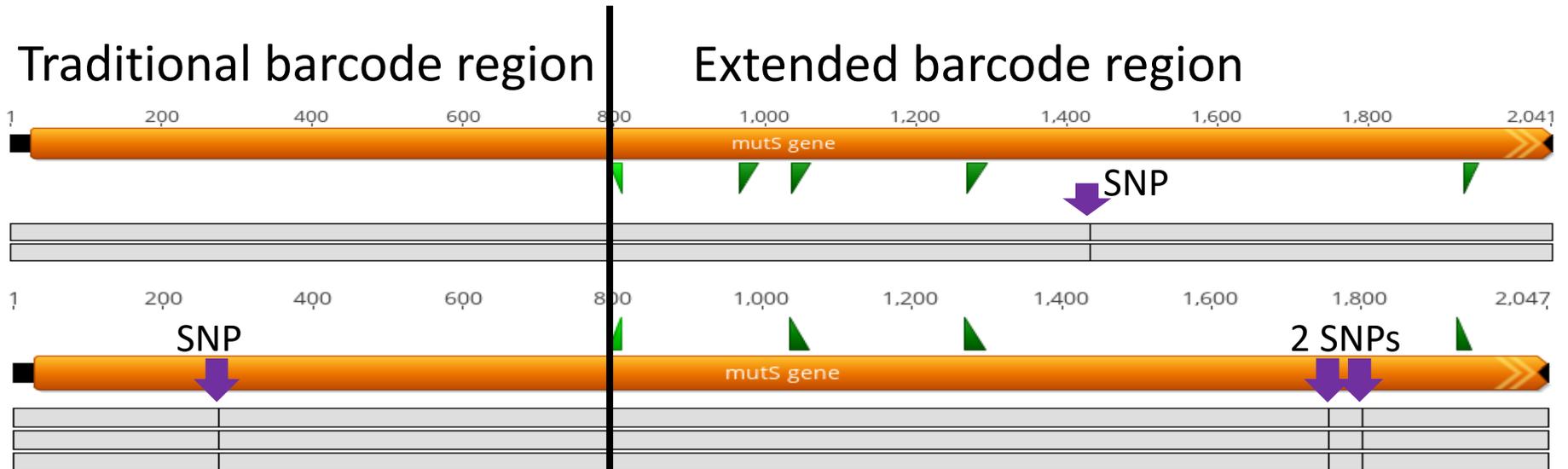
	44% Traditional barcode	Extended barcode 56%	Extended region more divergent? 44%
➔ <i>Muricea pendula</i>	no	YES	YES
<i>Paracis cf. enopla</i> sp .1	no	YES	YES
<i>Paracis cf. enopla</i> sp .2	no	no	no
<i>Thesea nivea</i>	no	YES	YES
<i>Paramuricea</i> sp. 1	YES	no	no
<i>Scleracis</i> sp. 1	YES	no	no
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



Were differences observed in the barcode regions?

	50% Traditional barcode	Extended barcode 88%	Extended region more divergent? 63%
➔ <i>Muricea pendula</i>	no	YES	YES
<i>Paracis cf. enopla</i> sp .1	no	no	no
<i>Paracis cf. enopla</i> sp .2	---	---	
<i>Thesea nivea</i>	no	YES	YES
<i>Paramuricea</i> sp. 1	YES	YES	no
<i>Scleracis</i> sp. 1	no	YES	YES
<i>Scleracis</i> sp. 2	YES	YES	YES
➔ <i>Swiftia exserta</i>	YES	YES	YES
<i>Thesea cf. rubra</i>	YES	YES	no
21 other taxa (interspecific)	YES = 19 (90%)	YES = 19 (90%)	YES = 12 (57%)

ND4L to mutS



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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