Genetic analysis of "*R. bonasus*"

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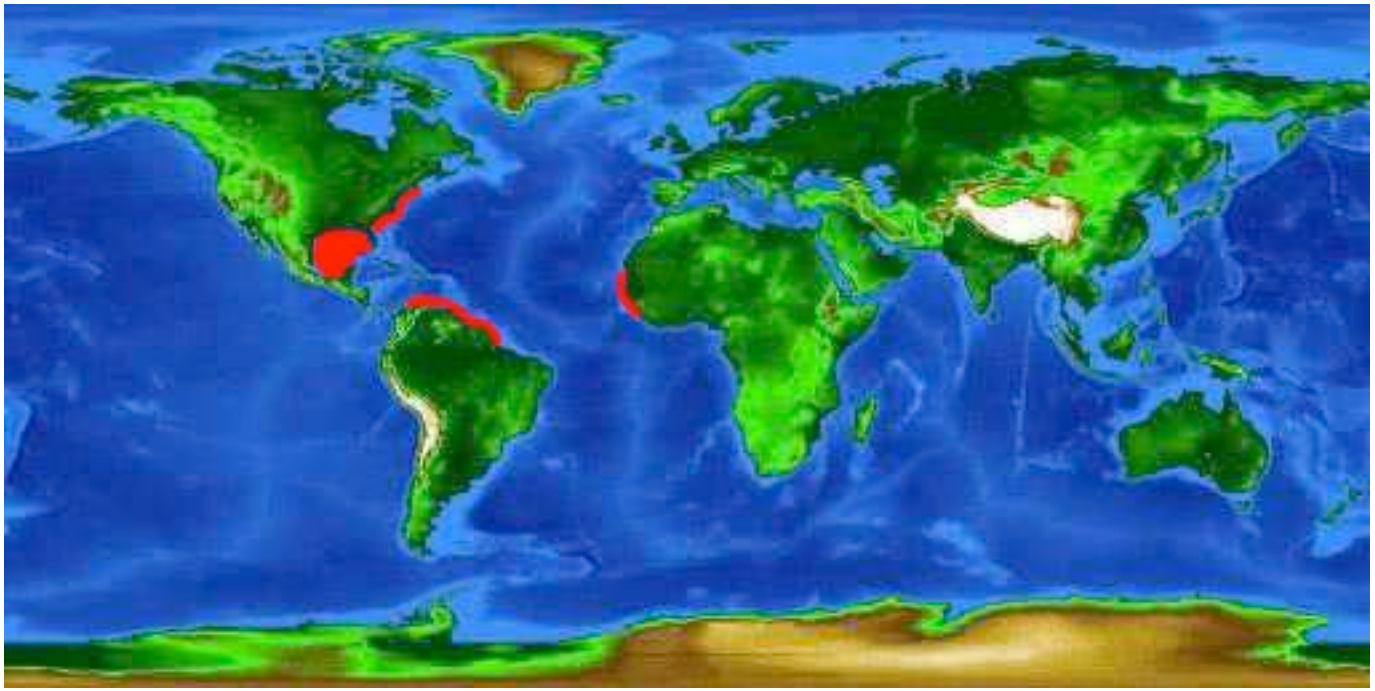
Background

Rhinopteridae include at least 7 known species of coastal pelagic rays (Compagno 2005).

R. bonasus is the only species that occurs along the east coast of the United States (including GOM).

Range



Distributed from southern New England to Brazil and throughout the Gulf of Mexico.

http://www.flmnh.ufl.edu/fish/Gallery/Descript/cownoseray/cownoseray.html

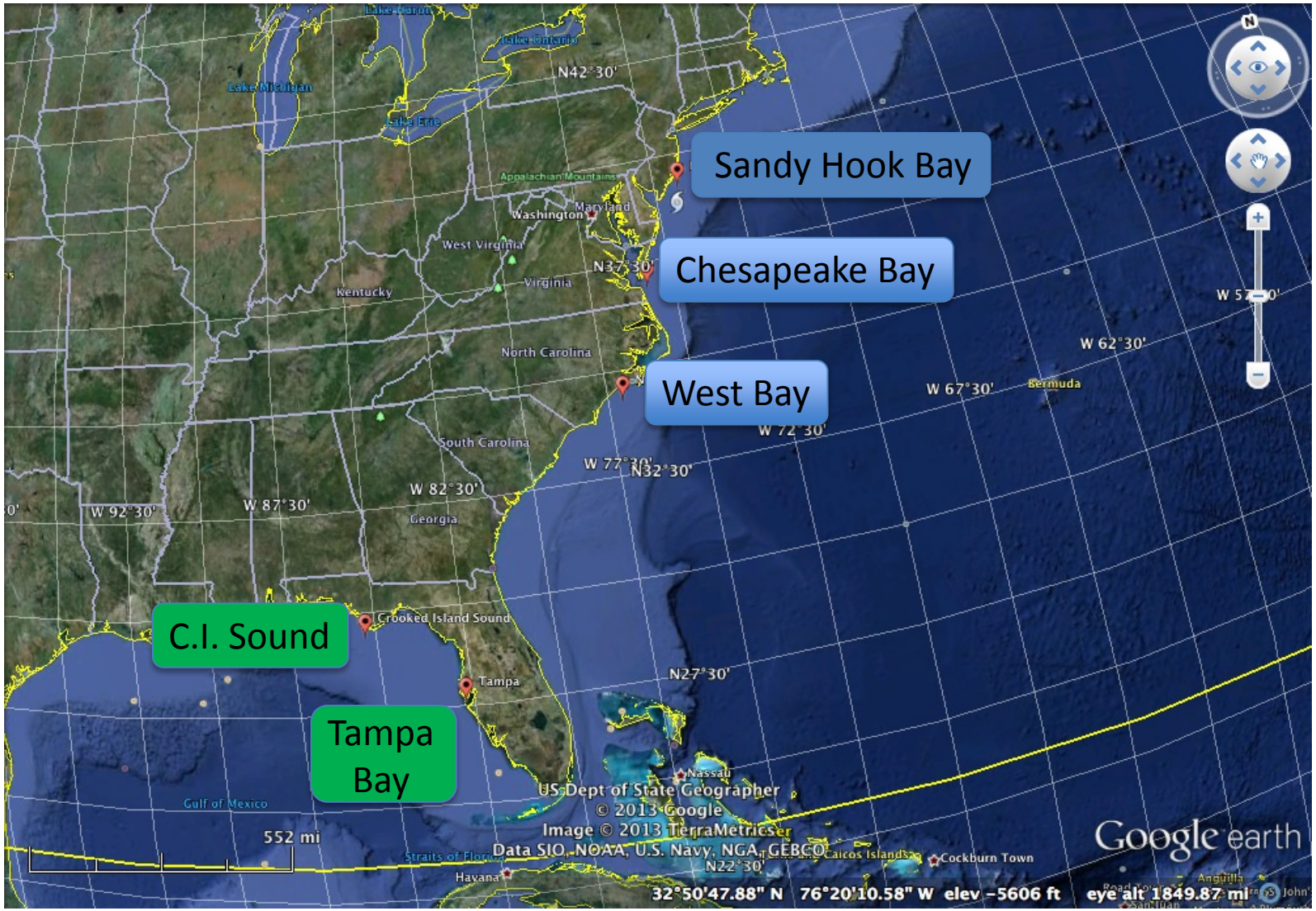
Nursery Areas

Two known cownose ray nursery and breeding areas in the western Atlantic.

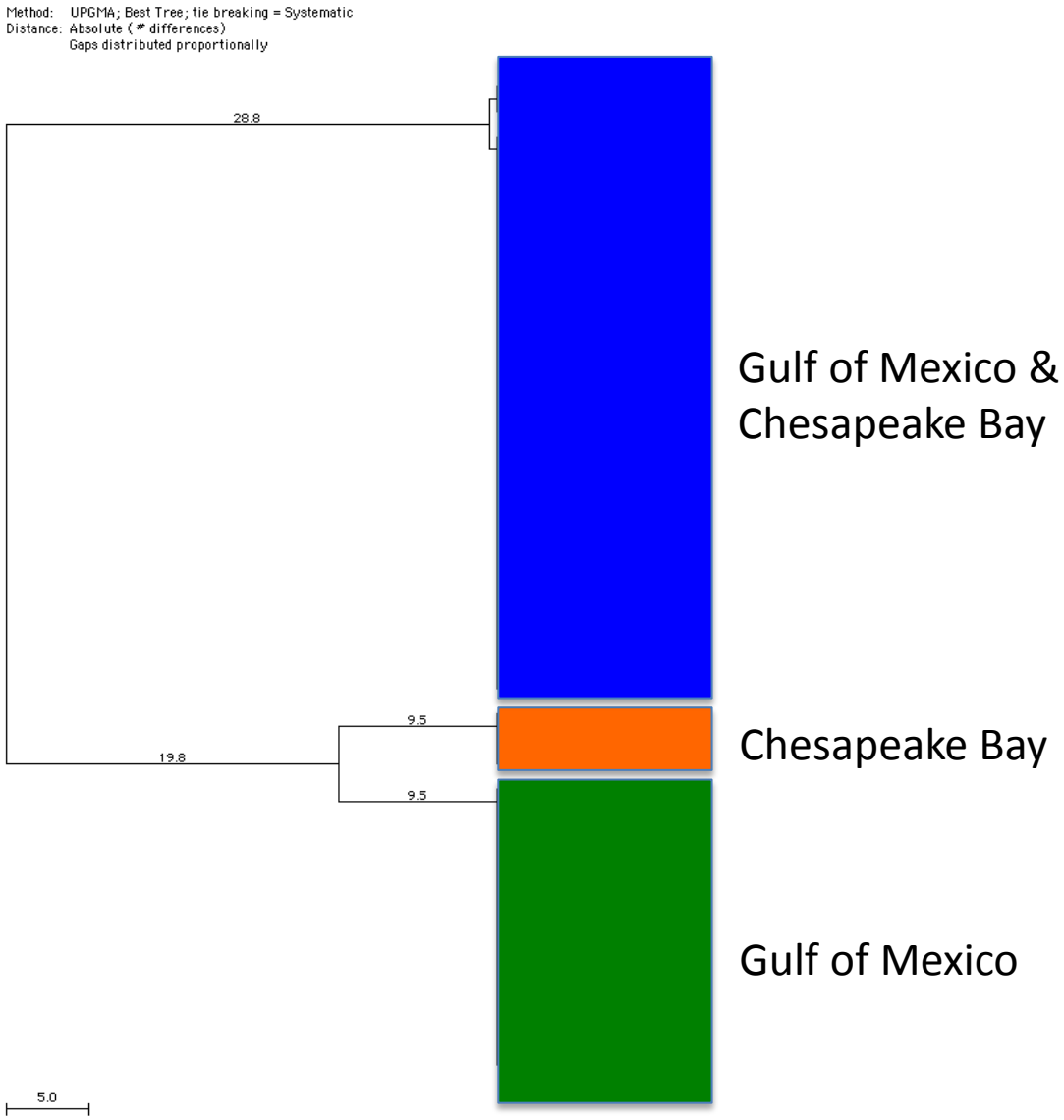
Chesapeake Bay nursery serves cownose rays that reside off the Eastern Seaboard.

Gulf of Mexico nursery used by rays that live in the Gulf and the Caribbean Sea.

Project: Discrimination of cownose ray, *Rhinoptera bonasus*, stock structure using microsatellite markers and mtDNA sequencing



Preliminary ND2 analysis



Meaning?

3 groups of haplotypes with little variation within groups, but high divergence among groups.

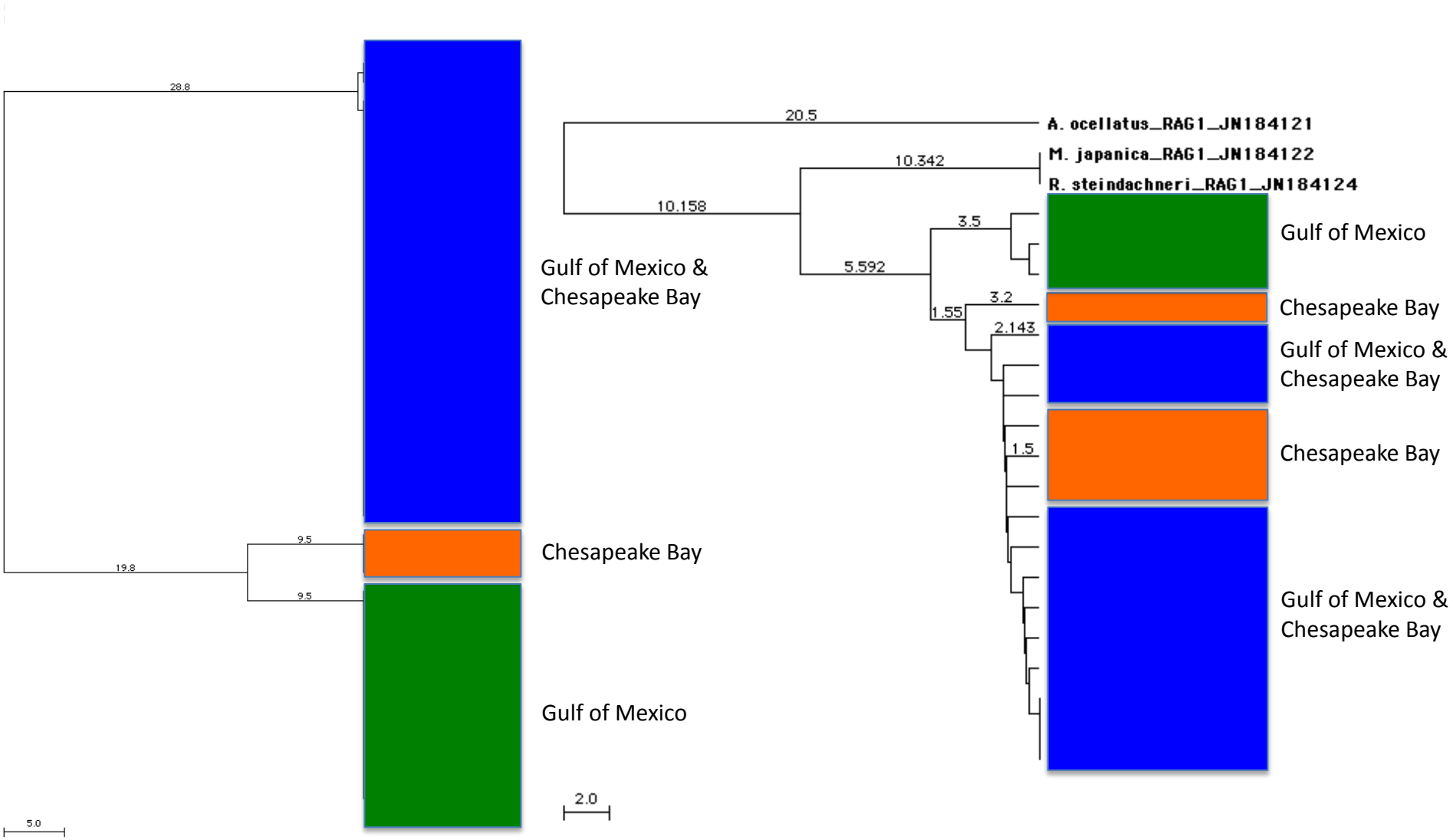
Possibilities:

Could be indicative of historical signature.

Could be more than one species.

Need nuclear a locus: RAG1

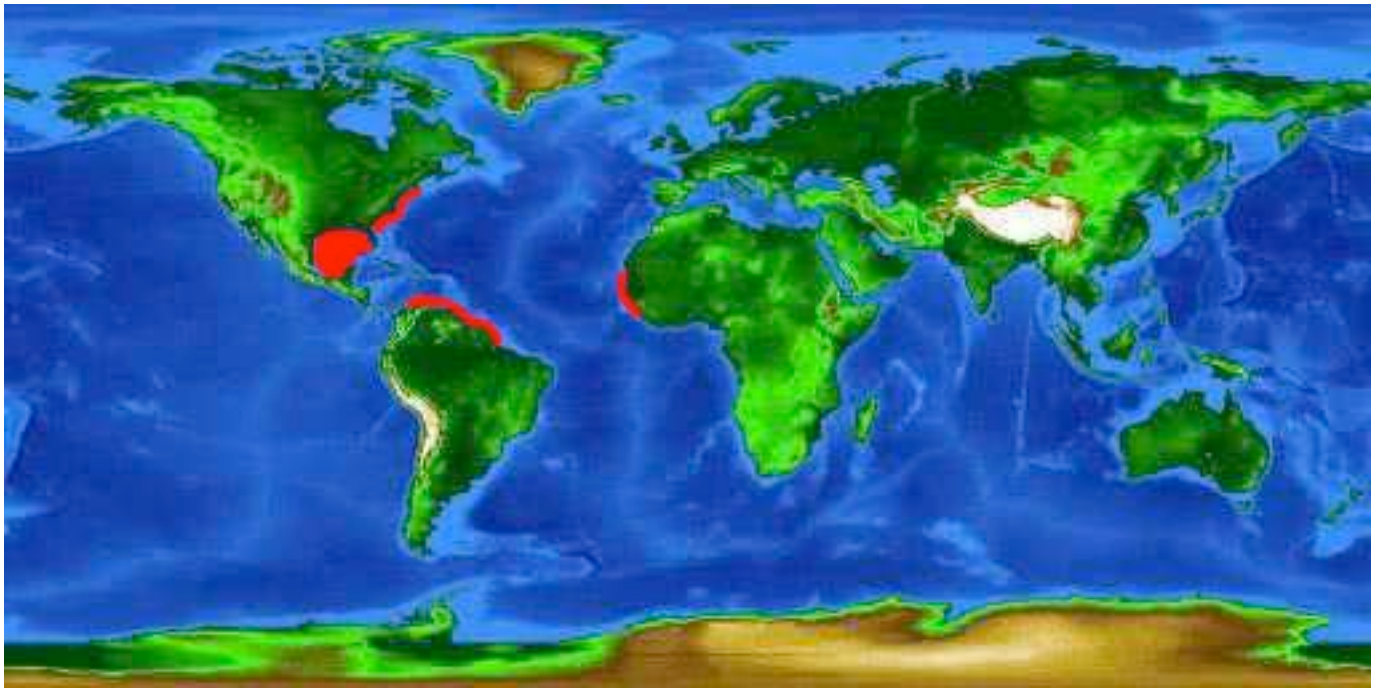
Mitochondrial ND2 vs. Nuclear RAG1



2 species, one found only in the Gulf of Mexico, and a historical signature of separation?

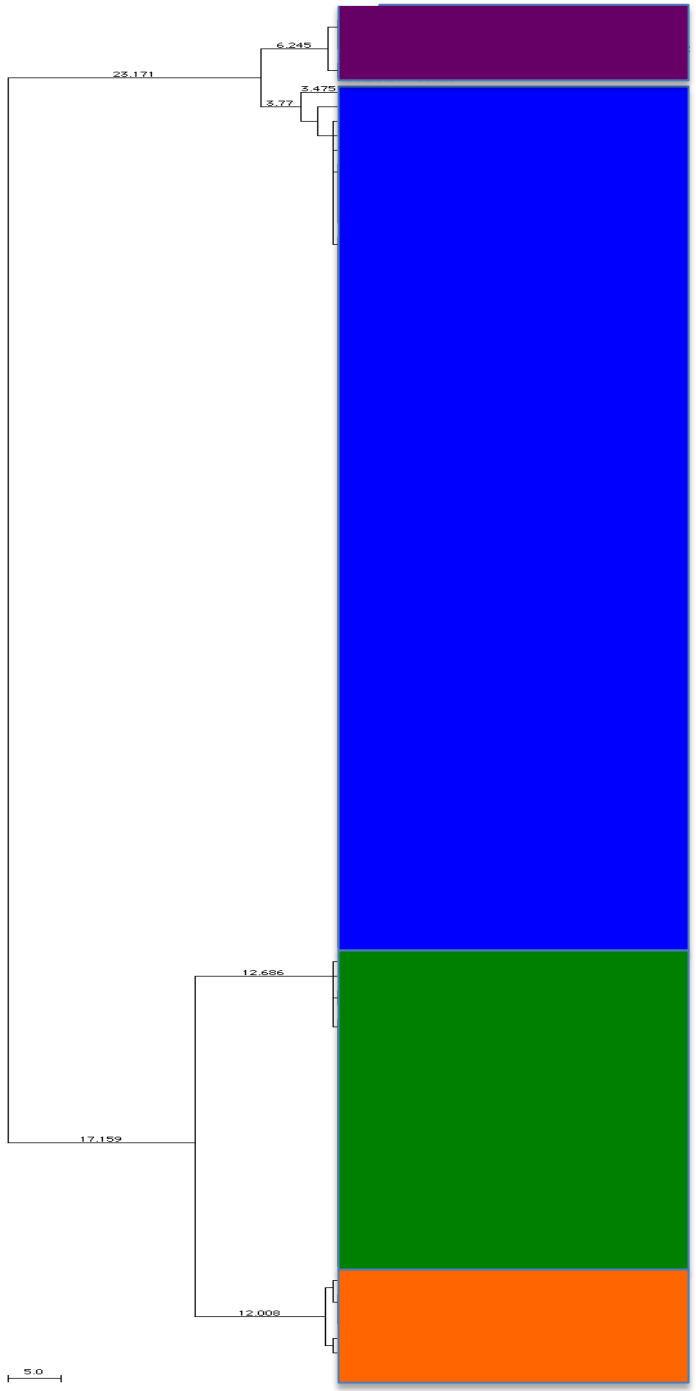
More samples for comparison

Samples collected from Brazil (n=6*) and Columbia (n=5)



*Two samples form Brazil were identified as *R. bonasus* and four were identified as *R. brasiliensis* by Brazilian researchers.

ND2



R. bonasus from Brazil and Columbia

R. bonasus from Gulf of Mexico & Chesapeake Bay

R. brasiliensis from Brazil + samples with haplotype only found in the Gulf of Mexico

R. bonasus from Chesapeake Bay

Preliminary investigation of mitochondrial genetic variation in the cownose ray *Rhinoptera bonasus* from the Chesapeake Bay and Gulf of Mexico

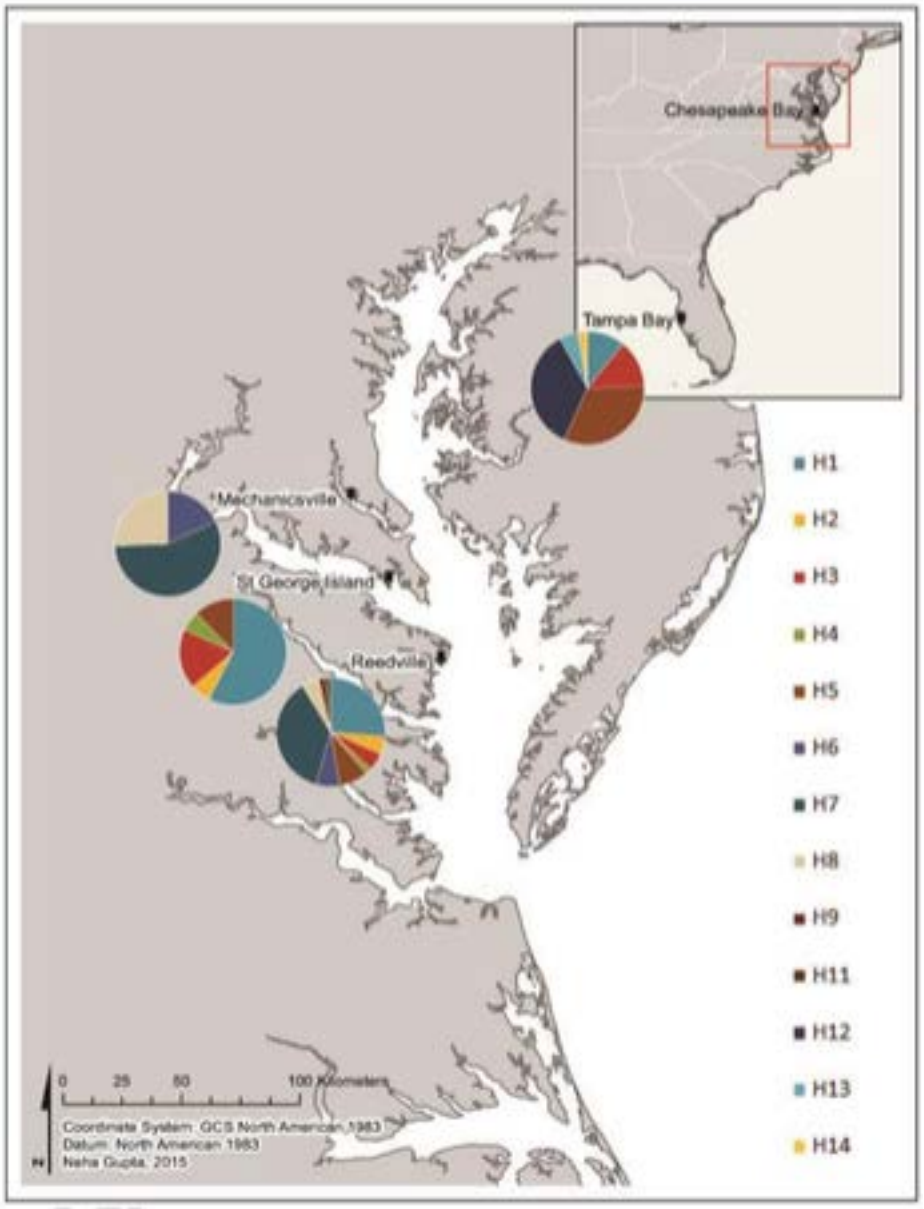
S. L. Carney, D. M. McVeigh, J. B. Moss, M. D. Ferrier, J. F. Morrissey

Cytochrome oxidase I, cytochrome *b* (967 bp total)

A total of 175 samples collected

- Patuxent River, Mechanicsville, MD June 2012 (n=39, all female)
- St. George Island, MD, Summer of 2010 (n=33, ~11 male)
- Reedville, VA June 2011 (n=66, 11% male i.e. ~7)
- Tampa, FL during fall 2010 and winter 2011 (n =37, sex not recorded)

Results



6 polymorphic sites

14 haplotypes

Table 4. Pairwise ϕ_{ST} values with corresponding *P* values below the diagonal and Jost's D values, including 95% confidence intervals, above the diagonal for combined COI and cytb haplotypes in *Rhinoptera bonasus*. From left to right and top to bottom, Mechanicsville is the northernmost site sampled while Tampa is the southernmost site.

Population	Mechanicsville	St. George Island	Reedville	Tampa
Mechanicsville	---	1	0.294 (0.119-0.469)	1
St. George Island	0.385*	---	0.436 (0.235-0.637)	0.586 (0.340-0.832)
Reedville	0.100*	0.131*	---	0.526 (0.324-0.729)
Tampa	0.322*	0.203*	0.119*	---

* Significant at $P < 0.01$ after sequential Bonferroni correction

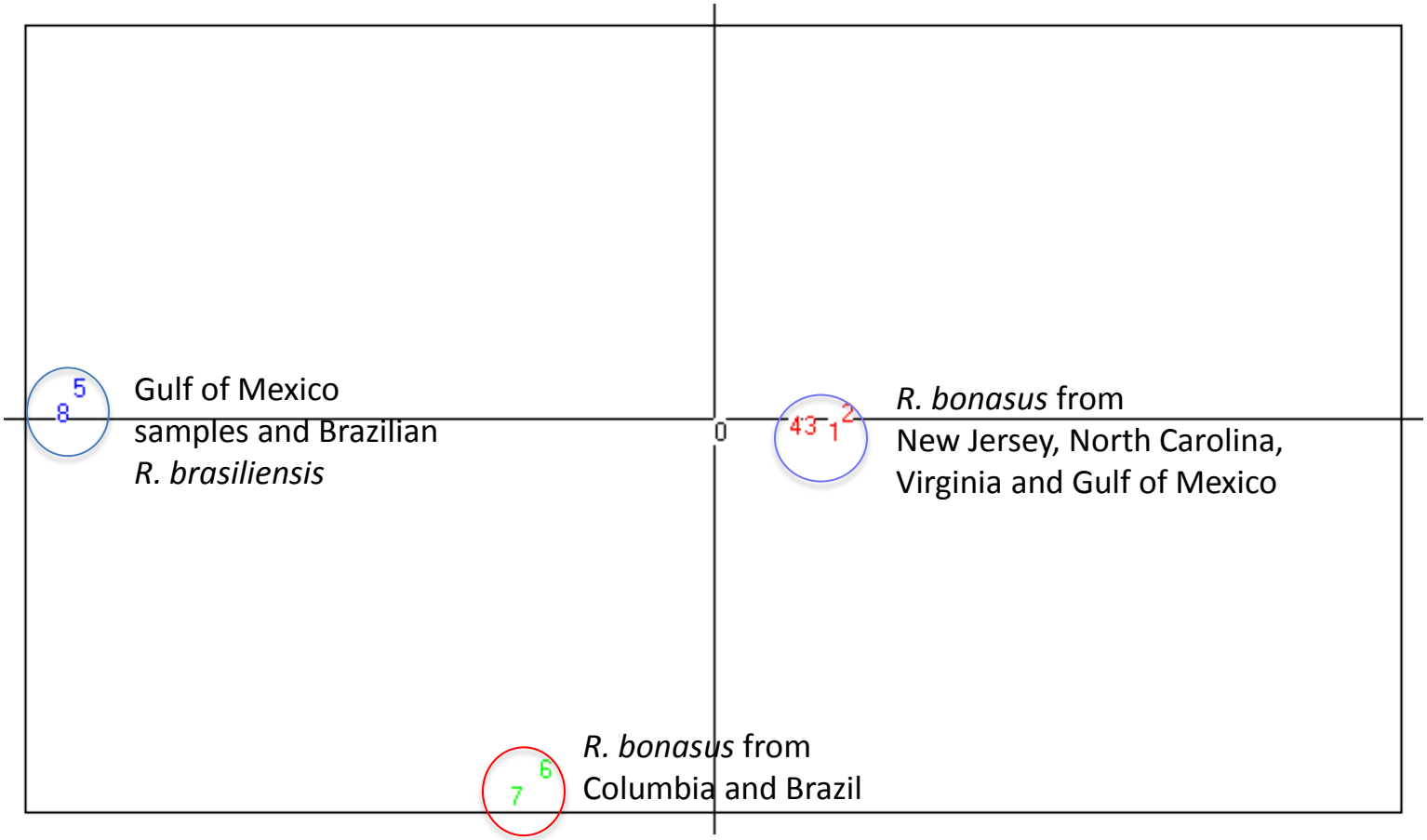
Conclusions

- Genetic variation not uniformly distributed among sampling locations.
- Different groups may use different areas of the Bay upon their return to forage, pup and mate.
- Female philopatry?
- Effect of year of collection? More samples needed.
- Need more variable markers.

Microsatellites
(highly variable nuclear markers)

- Microsatellite markers from high throughput sequencing data.
- 13 loci developed (having a cryptic mix of species complicated marker development).
- 11 loci worked consistently and were in HWE within *bonasus* at each collection location. 7 worked well in *brasiliensis-like.*

PCA (212 samples, 11 loci)



Stock Structure in *R. bonasus*?

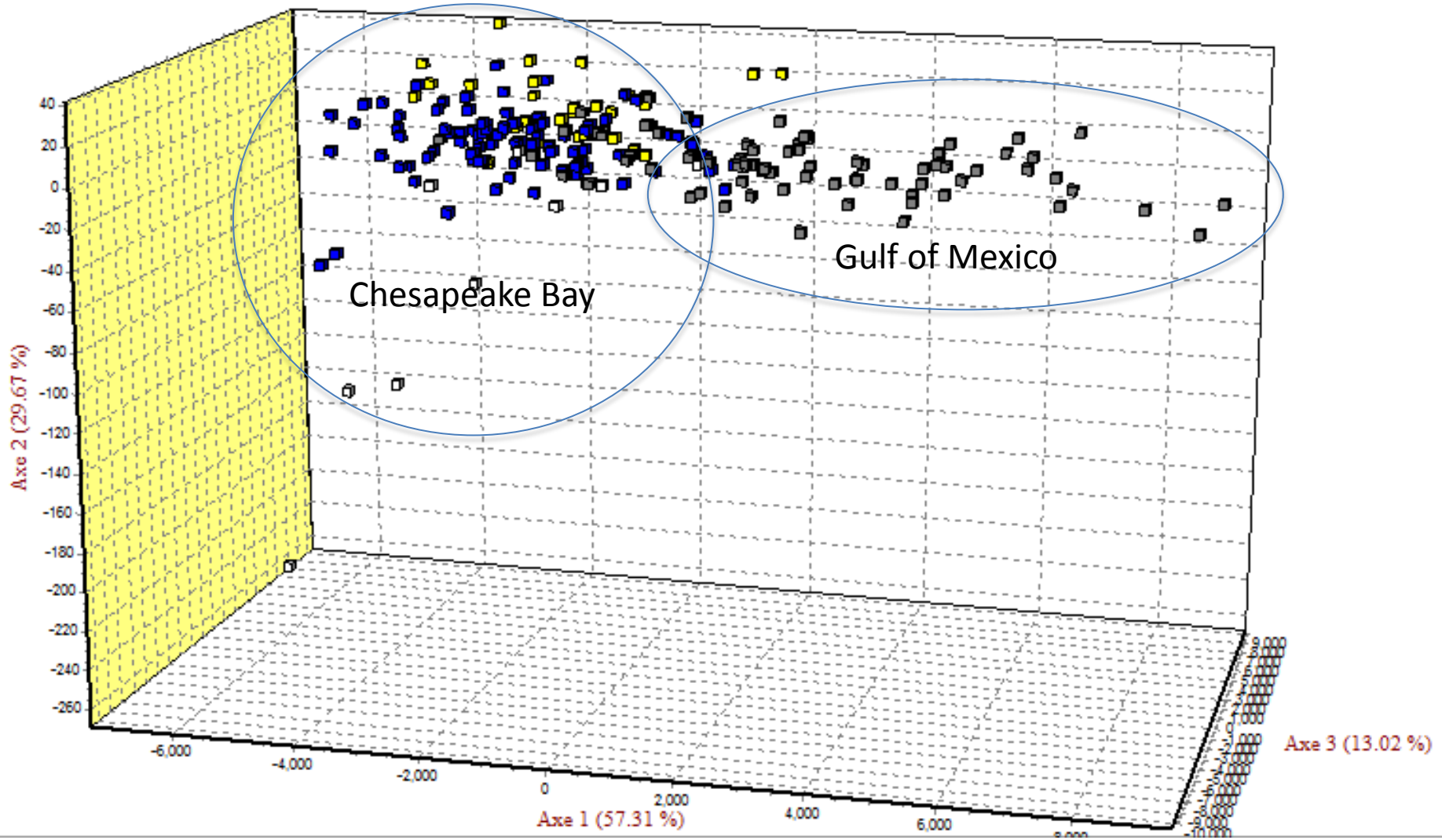
No significant differences among U.S. east coast samples

	NJ	VA	NC	GOM	BRA	COL
NJ	0.00000					
VA	0.00096	0.00000				
NC	0.01407	0.01301	0.00000			
GOM	**0.01290**	**0.02754**	**0.02916**	0.00000		
BRA	**0.41214**	**0.44352**	**0.46793**	**0.40493**	0.00000	
COL	**0.43383**	**0.46875**	**0.49283**	**0.41554**	0.17842	0.00000

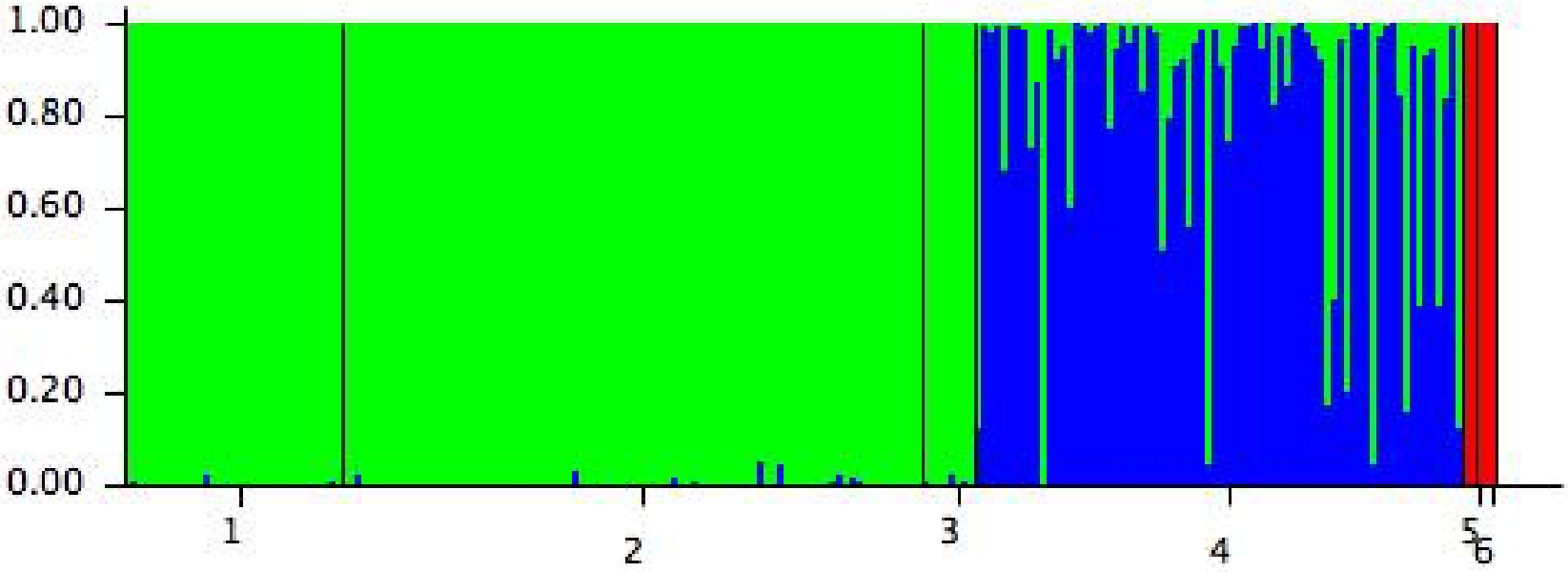
AMOVA FST = 0.02 $p < 0.001$

Key	
NJ	New Jersey
VA	Virginia
NC	North Carolina
GOM	Gulf of Mexico
COL	Columbia
BRA	Brazil

Factorial Correspondence Analysis of *R. bonasus* microsatellite data



STRUCTURE analysis
R. bonasus only



Chesapeake Bay (1-3)

Gulf of Mexico (4)

Brazil and Columbia (5, 6)

Overall Conclusions

- Evidence suggests more than one species is present in the Gulf of Mexico and they overlap spatially and temporally.
- The Gulf of Mexico and Chesapeake Bay represent genetically distinct stocks of *R. bonasus.*
- *R. bonasus* from Columbia and Brazil appear to be a third stock.
- *R. bonasus* may exhibit female philopatry/natal homing.
- *R. brasiliensis* may not be endemic to Brazil.

Now what?

- What progress has been made?

  Gulf of Mexico and western Atlantic are separate genetic stocks.

  There is heterogeneity among Chesapeake Bay sampling locations based on mtDNA, which may indicate philopatry.

  No significant differences among Chesapeake Bay estuaries using nuclear microsatellites, but sample sizes were small .

- What do we still need to know?

  Is there a single western Atlantic stock?

  Female philopatry/natal homing?

- Need more intensive sampling

  Especially from nursery grounds

Acknowledgements

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