Genetic Variability in *Acropora palmata and A. cervicornis*

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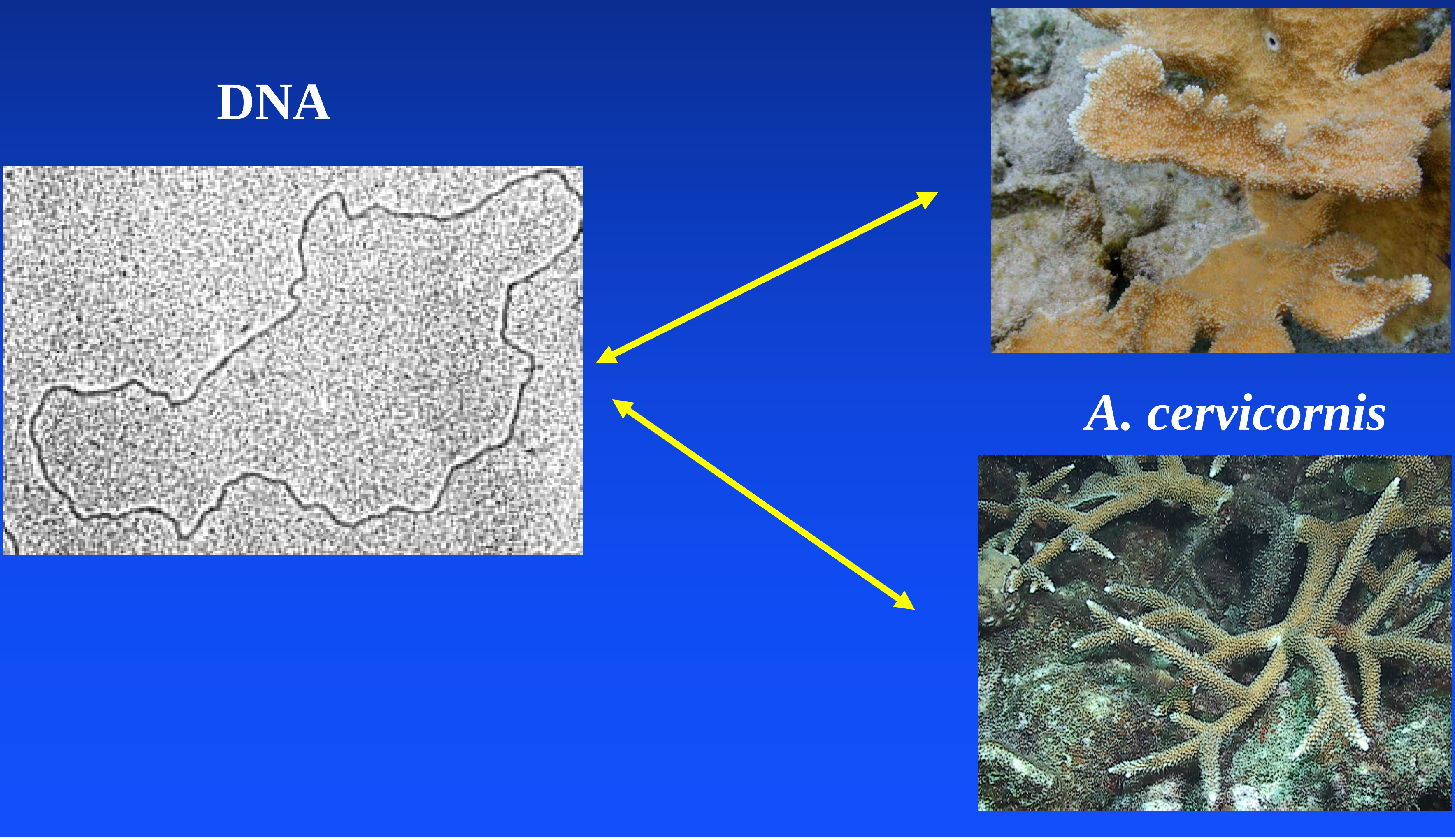
Goal of this proposal

- **Evaluate the genetic variability of *A. cervicornis* and *A. palmata* at different levels of tissue organization**



DNA

A. palmata

A. cervicornis

Motivation

1) Levels of genetic variation may be used as a proxy for the long term survival of the species

2) Identification of distinct populations may aid decisions in management and conservation issues

HOW?

Hierarchical Design

We are evaluating levels of genetic diversity:

1) **Within discrete patches of *Acropora***
2) **Among discrete patches of *Acropora* within sampling locations**
3) **Among sampling locations within islands**
4) **Among different islands**

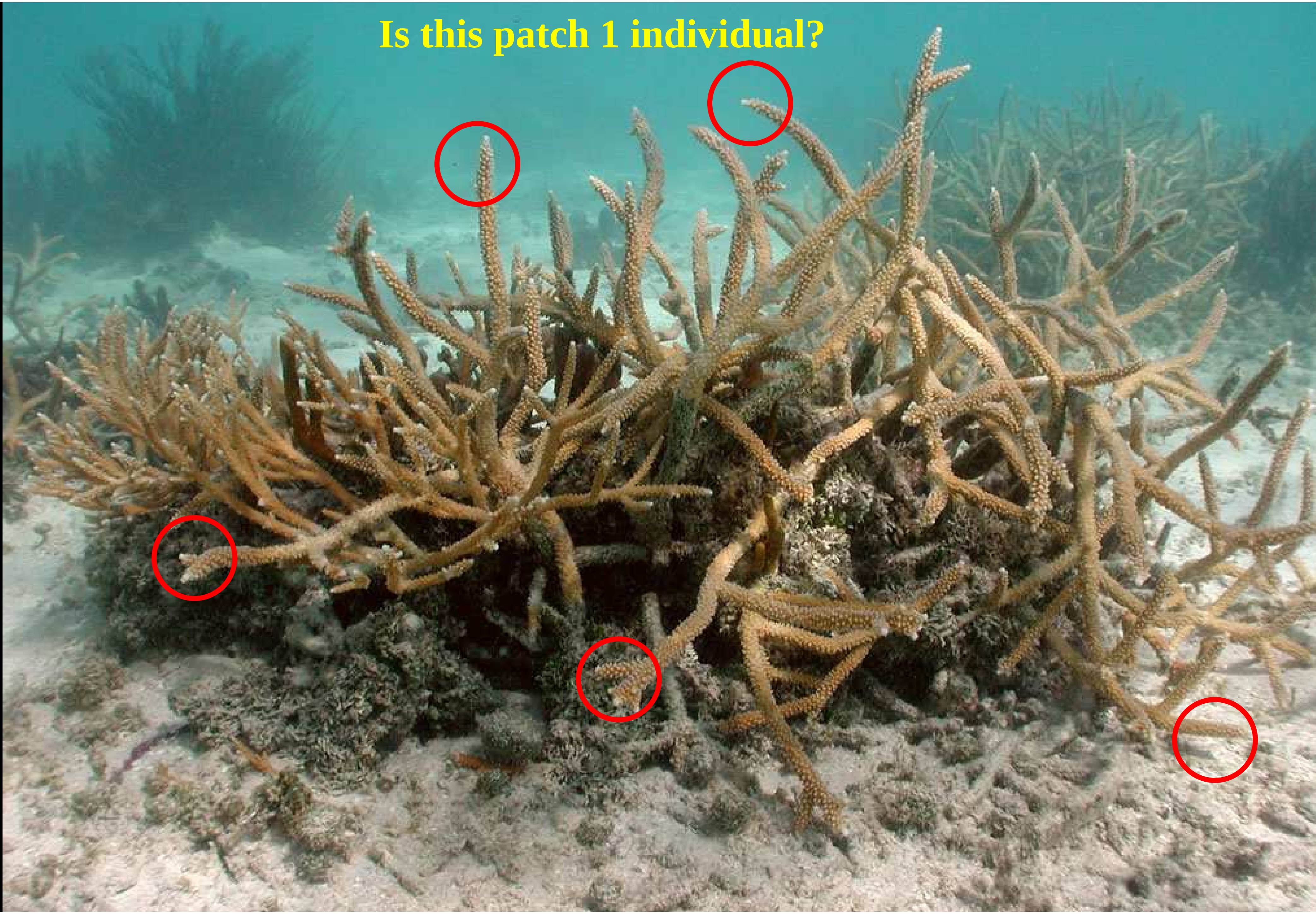
Proposed Sampling Locations of *Acropora*

Collection Sites

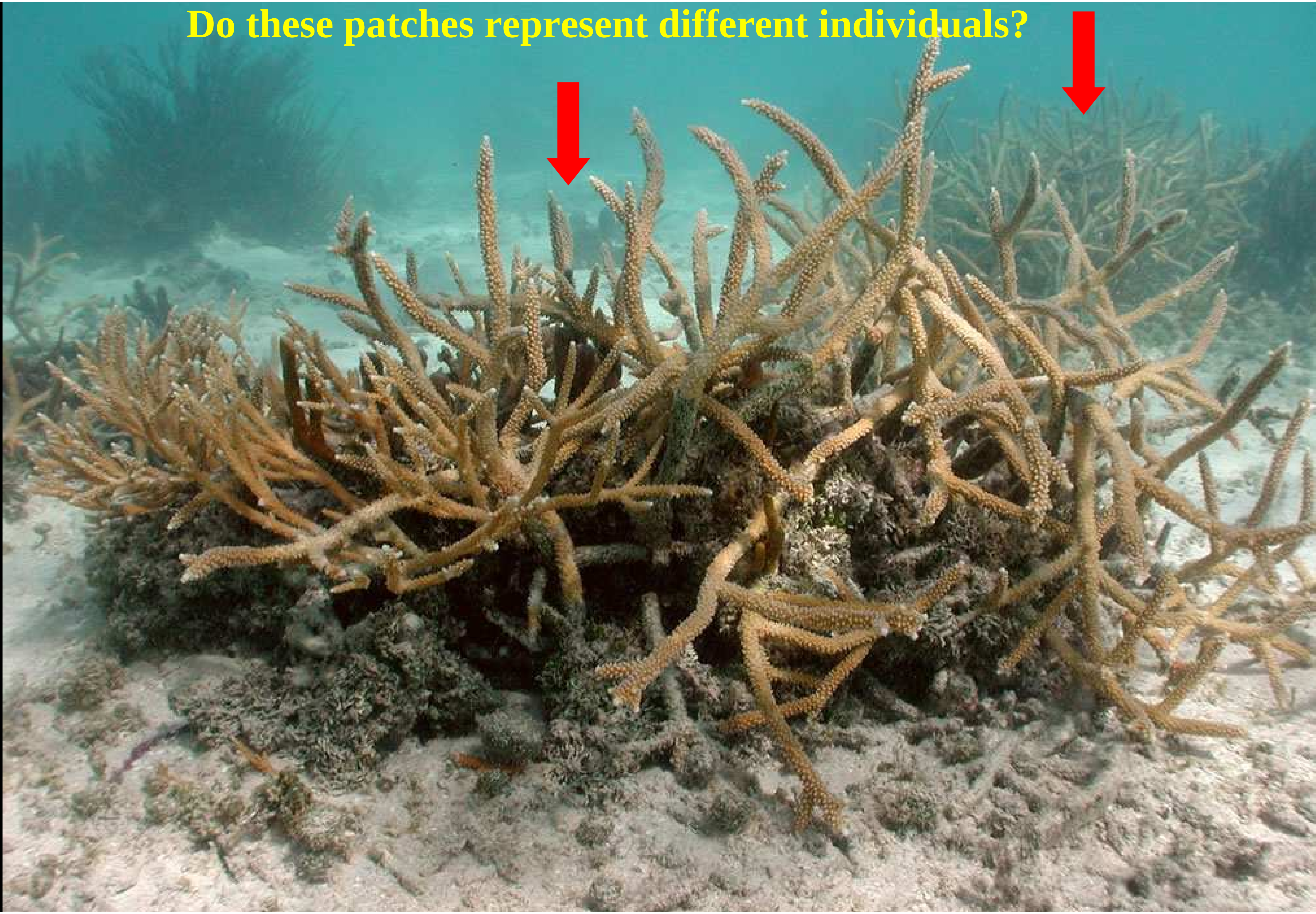
- La Parguera
  - Cayo Enrique
  - Cayo Laurel
  - Cayo Media Luna
  - Cayo San Cristobal
  - Cayo Wineberg*
  - Cayo Romero
- Culebra
- Lee Stocking Island, Bahamas (outgroup)

* Samples from the shelf edge

Is this patch 1 individual?



Do these patches represent different individuals?



Joselyd Garcia (MS student) collecting polyps of *Acropora cervicornis*

Materials and Methods

Candidate Genes:

MtDNA: putative control region.

Nuclear DNA: ITS-1, and introns from *Pax-C* and calmodulin

Course of Action

- **Develop a reliable DNA extraction technique in *Acropora*** ✓
- **Optimize PCR amplification conditions for 4-6 genes per specimen** ✓
- **Expand collection of *Acropora* to other locations** ✓
- **Analyze data**

PaxC Intron (507 bp)

Polymorphism in *Acropora cervicornis*

Sample location	# of sequences	# colonies:
Culebra	4	2
Cayo Enrique	4	2
Cayo Laurel	3	2

Results of *A. cervicornis* from Pax-C

1. No genetic variation within colonies
2. No single point mutations detected between colonies
3. 1 colony in Laurel and 1 colony in Culebra were heterozygous at 2 nucleotide positions

Calmodulin Intron (357 bp)

A. cervicornis

Sample location	# of sequences	# colonies:
Cayo Enrique	3	2
Cayo Laurel	3	2

Results of *A. cervicornis* from Calmodulin

1. No genetic variation within colonies
2. No single point mutations detected between colonies
3. 1 colony in Laurel was heterozygous at 4 nucleotide positions

PaxC Intron (507bp)

A. palmata

Sample location	# of sequences	# colonies:
Cayo Enrique	2	1
Cayo Laurel	7	5
Culebra	8	4

Results of *A. palmata* from PaxC

1. No genetic variation within colonies
2. No single point mutations detected between colonies

Calmodulin Intron (357bp)

A. palmata

Sample location	# of sequences	# colonies:
Cayo Enrique	1	1
Cayo Laurel	4	3

Results of *A. palmata* from Calmodulin Intron

1. **No genetic variation within colonies**
2. **No single point mutations detected between colonies**

Preliminary Conclusions

- Introns from two nuclear genes indicate that there is no or little genetic variation among our samples
- Identified allelic variants within individuals (i.e. heterozygotes)
- Current data suggest high levels of gene flow
- Sampling is limited, more geographic samples are needed to understand the levels of variability (e.g. Mona, Desecheo)
- Current genes may not be providing well enough resolution

Proposed Schedule

- **Fall 2005-Summer 2006**
    - **Collection of *Acropora* from all locations**
    - **Culebra, Mona, Desecheo, North Coast of PR**
    - **Collection of data**
    - **Data Analysis**

- **Fall 2006**
    - **Manuscript Preparation**

Sequenced Regions from both *Acropora* species

Gene	**Sequence Length**
***Control Region**	**673 bp**
ITS region	**239 bp (*A. palmata*); 188 bp (*A. cervicornis*)**
***Calmodulin* intron**	**357bp**
***PaxC* intron**	**507 bp**
In Total	**1776 bp**

*** Mitochondrial DNA**

Control Region (673 bp)

Polymorphism (*A. palmata*, n=3)	**Divergence**
0	**15.76% (106 mutations)**

ITS region (239 bp)

Polymorphism (*A. palmata*, n=2)	Divergence
1	5.9% (11 mutations out of 188)

4 putative sequence gaps in the ITS aligment

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A_palmata1ITS      TGATCACACATCTTTGTTACTTAGTCAGTCGGACCTCGGCT
A_palmata2ITS      TGATCACACATCTTTGTTACTTAGTCAGTCGGACCTCGGCT
A_cerv1ITS         T------------TTG--AATCAGTCAGTCGGACCTCGGTT
                   *            ***  * * ***************** *
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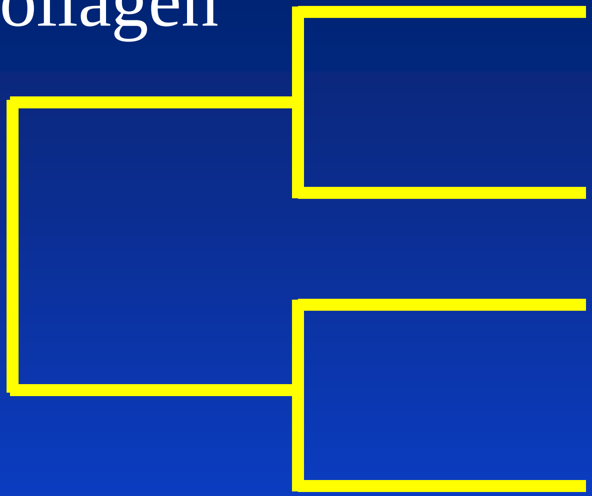
Sequence divergence in minicollagen, calmodulin, and PaxC (0.6%-2.1%). In ITS-1 is up to 13.2%.



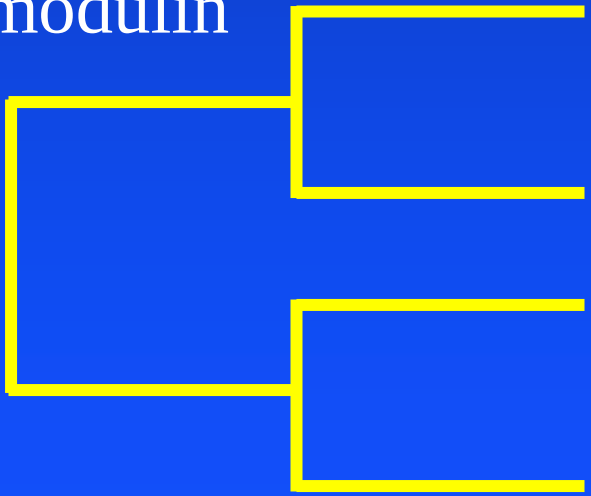
Minicollagen

A. cervicornis

A. cervicornis

A. palmata

A. palmata



Calmodulin

A. cervicornis

A. cervicornis

A. cervicornis

A. palmata