

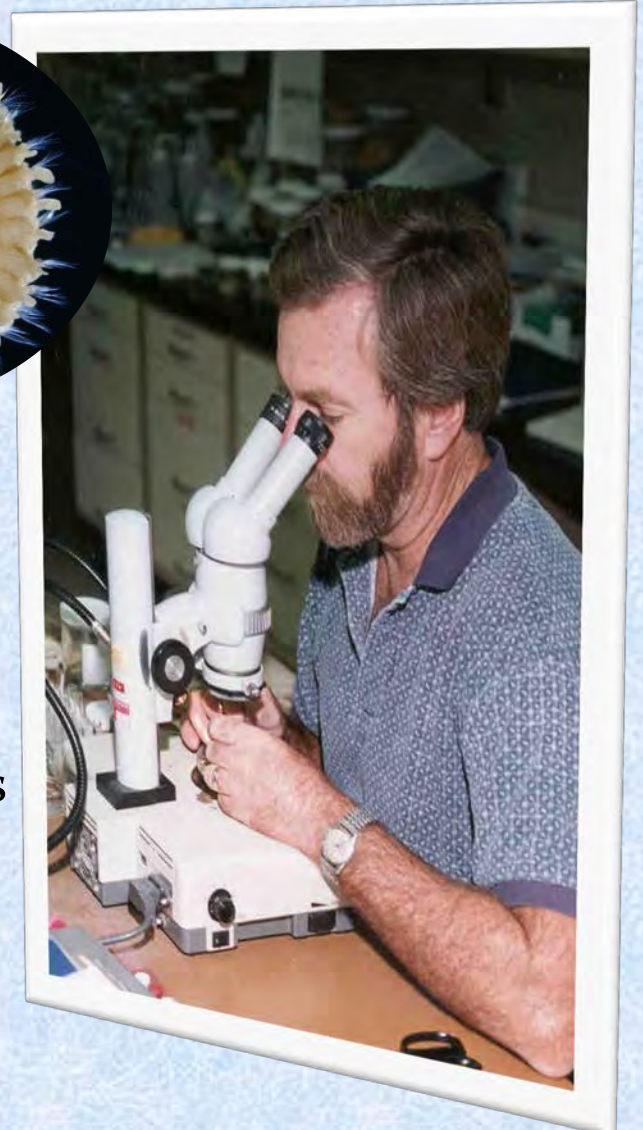
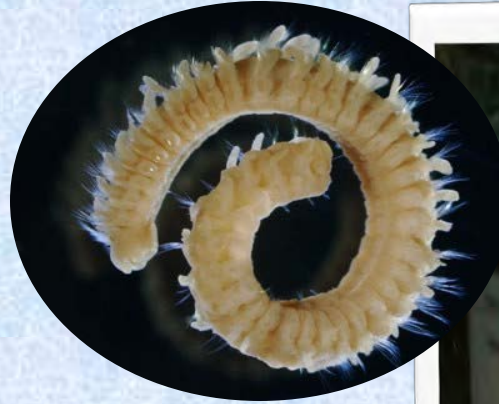
# DNA Barcoding as Tool for Marine and Freshwater Bioassessment

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# The Promise of Molecular Methods

- Faster answers
  - Weeks vs. months
- Less expensive
- Better data
  - Recognizing misidentifications
  - Improving taxonomic keys
  - Helping with difficult to ID taxa
  - Supports QA programs
  - Less dependent on availability of taxonomists
  - Gateway to new biological indicators



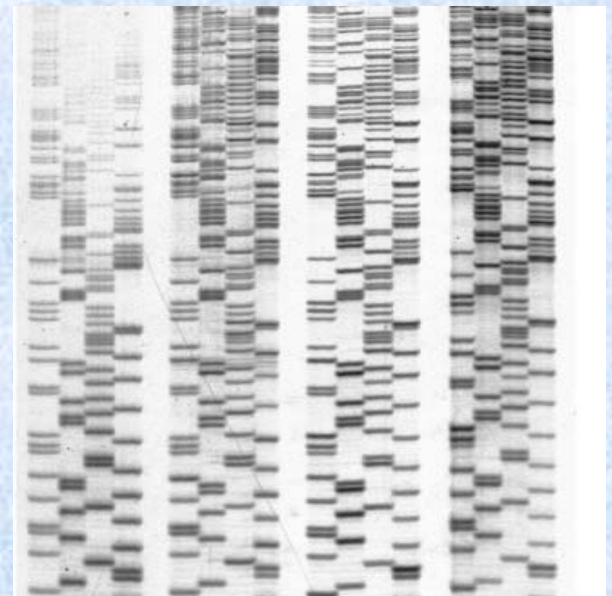


# Barcoding as a New Tool

A DNA barcode is a short gene sequence taken from standardized portions of the genome, used to identify species.



Similar to the UPC, DNA barcodes provide a universal system of unique tags for each species.



# How Does Barcoding Work?



Specimen



Collection Data



Tissue Sample



Photograph



Extract DNA

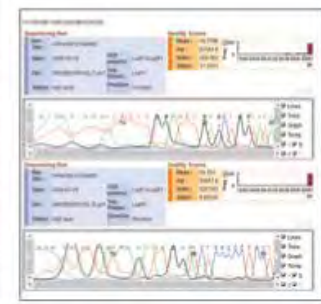


PCR Amplify



Sequence

## Barcode of Life Database (BOLD)



Web-Accessible Data and DNA Barcodes



# Transition to Routine Bioassessment

## *Issues that need to be addressed*

Routine Implementation → Standardized Methods

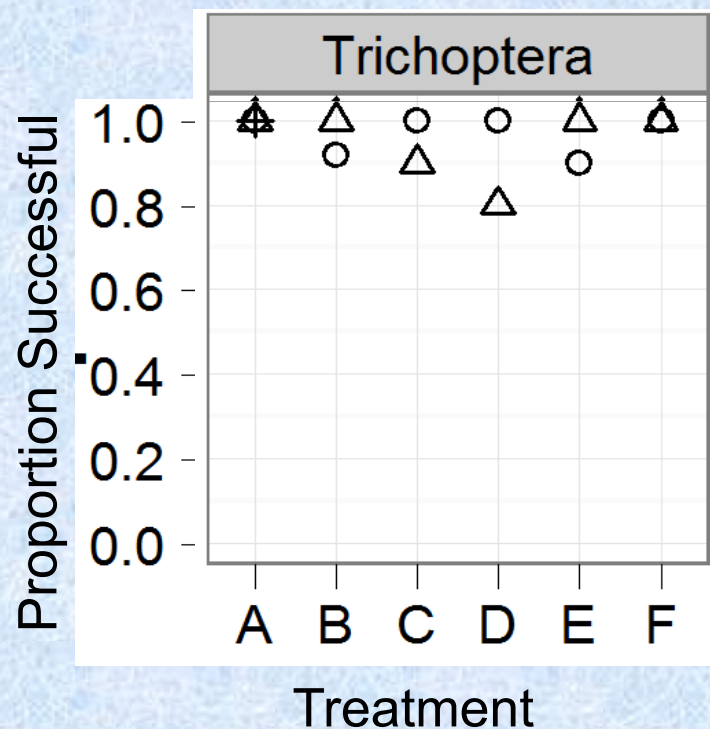
- *Develop sampling and preservation methods*
- *Develop a Reference Library*
- *Evaluate efficacy of molecular approaches*
- *Standardize species delimitation methods*
- *Test performance of indices*
- *Integrate into environmental monitoring programs*
  - *Quality control, data management, etc.*

# Preservation Methods: Study Approach

- Test preservatives
  - Volume of ethanol
  - Number of ethanol replacements
  - Addition of glycerin
- Test holding times of 1 week – 6 months
- Barcode all samples to determine effect of preservation method

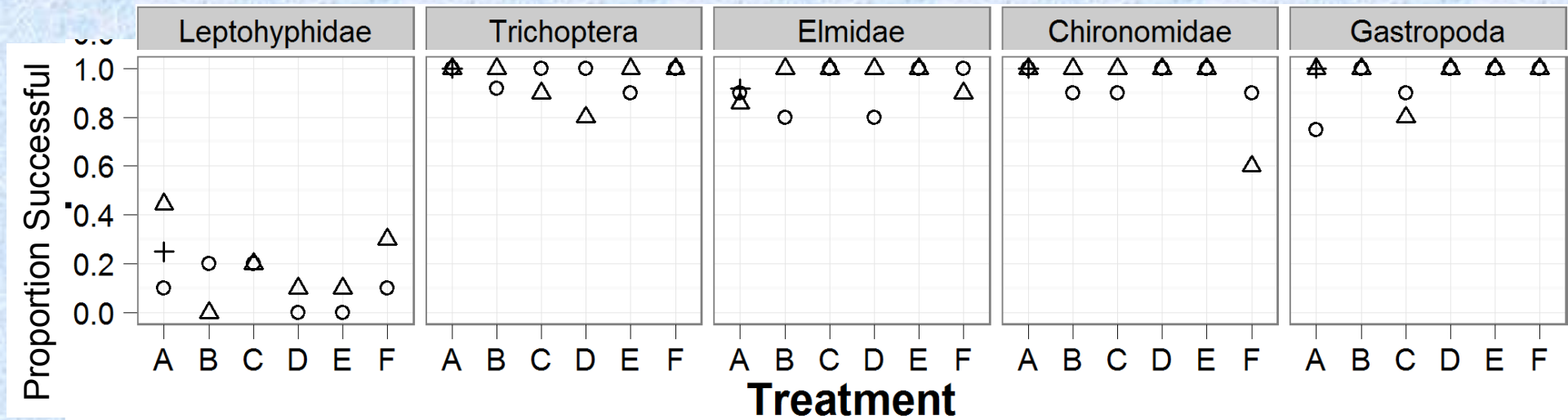


# Ethanol Preservation is OK



Treatment	Ratio	Solution	Holding Time	
			In matrix	DNA Extraction
A	5:1	95% ethanol	7 d	50 d
B	2:1	95% ethanol	7 d	50 d
C	2:1	95% ethanol + 5% glycerin	7 d	50 d
D	2:1	95% ethanol	30 d	50 d
E	2:1	70% ethanol	30 d	50 d
F	2:1	70% ethanol	6 m	174 d

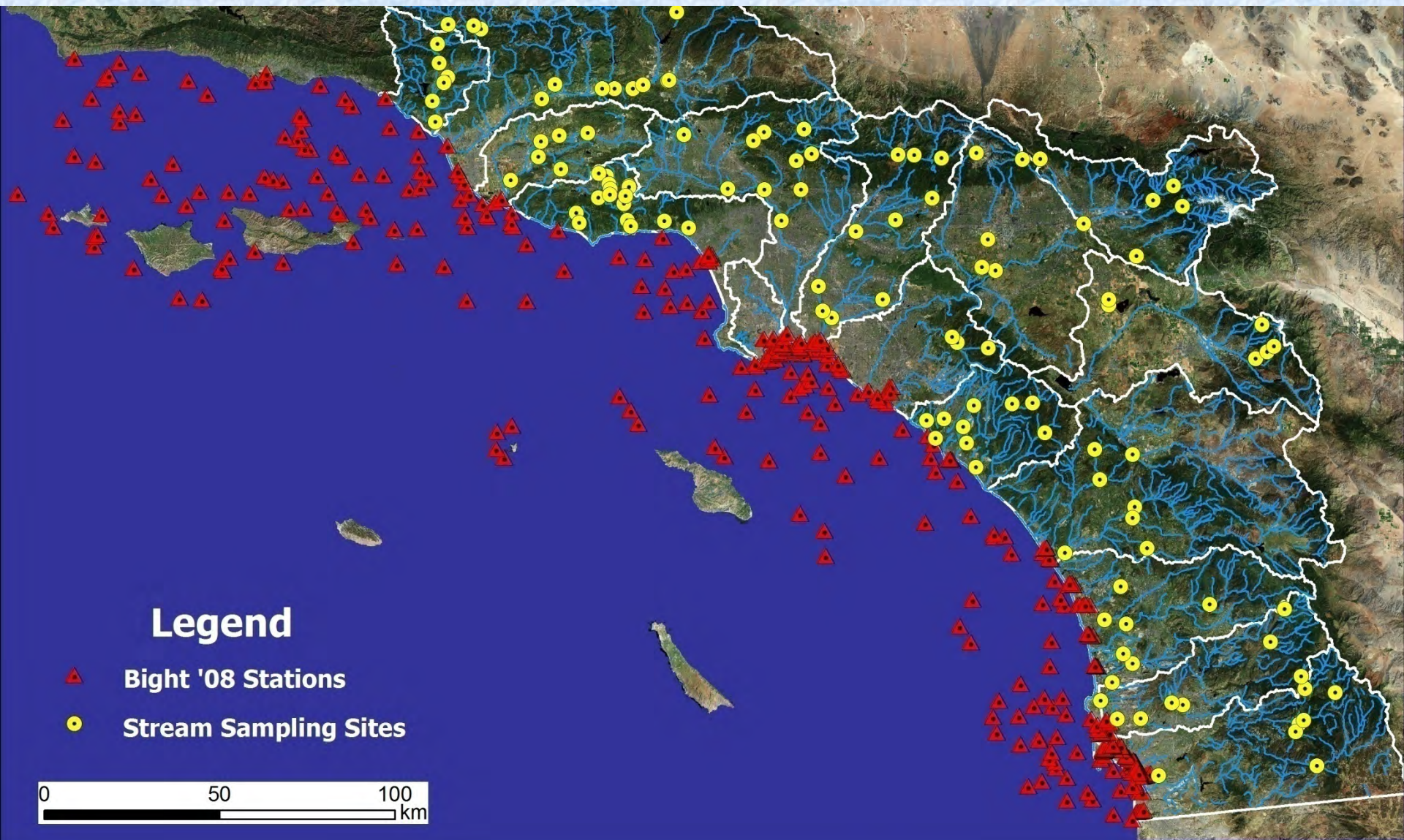
# Ethanol Preservation is OK



Plans to repeat study with marine organisms using formalin

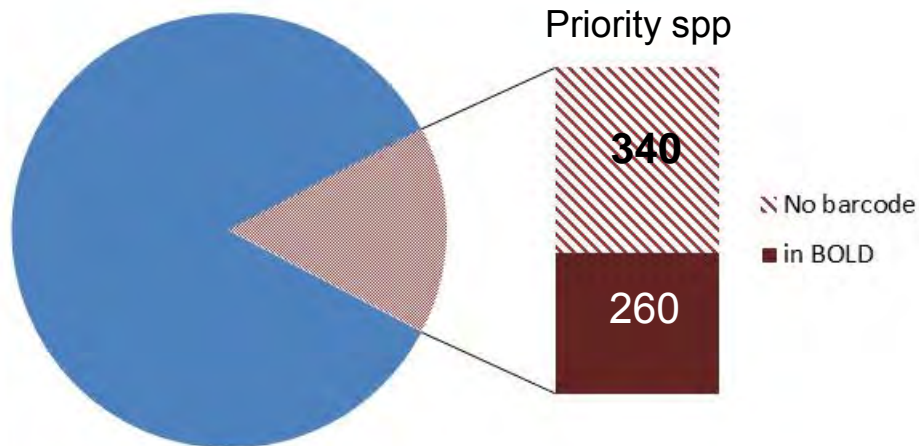


# Building Reference Through Regional Monitoring

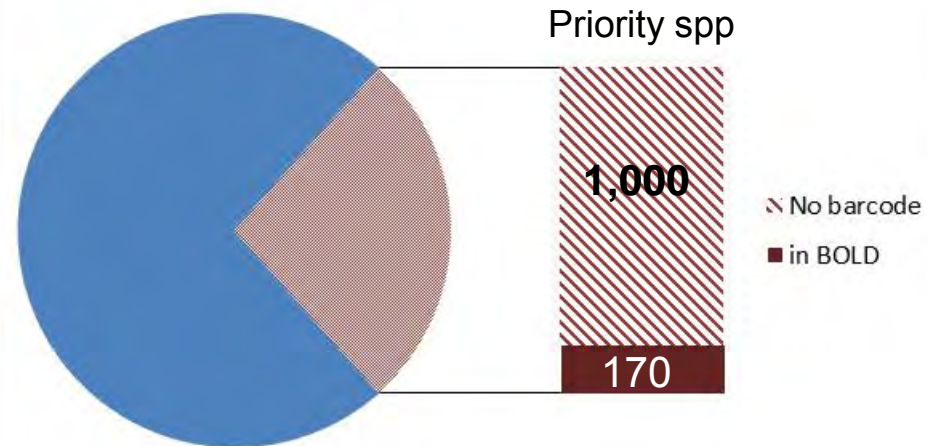




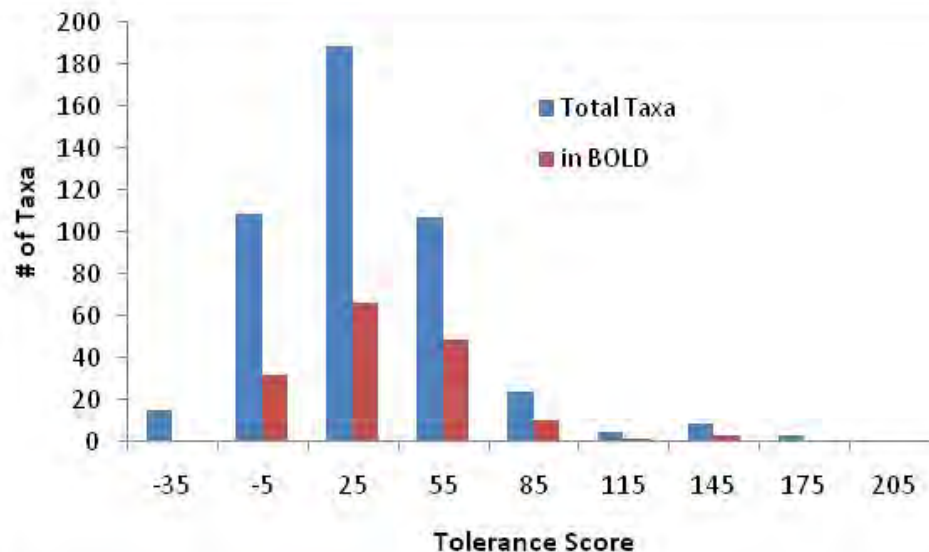
# Building the Reference Library



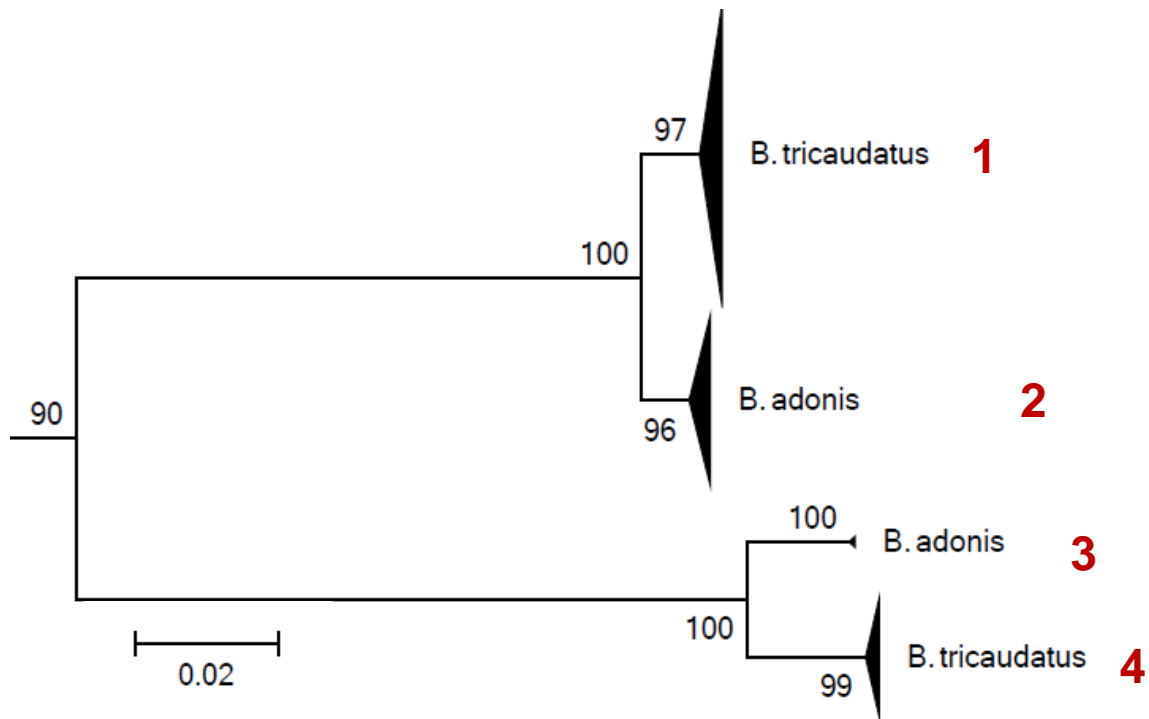
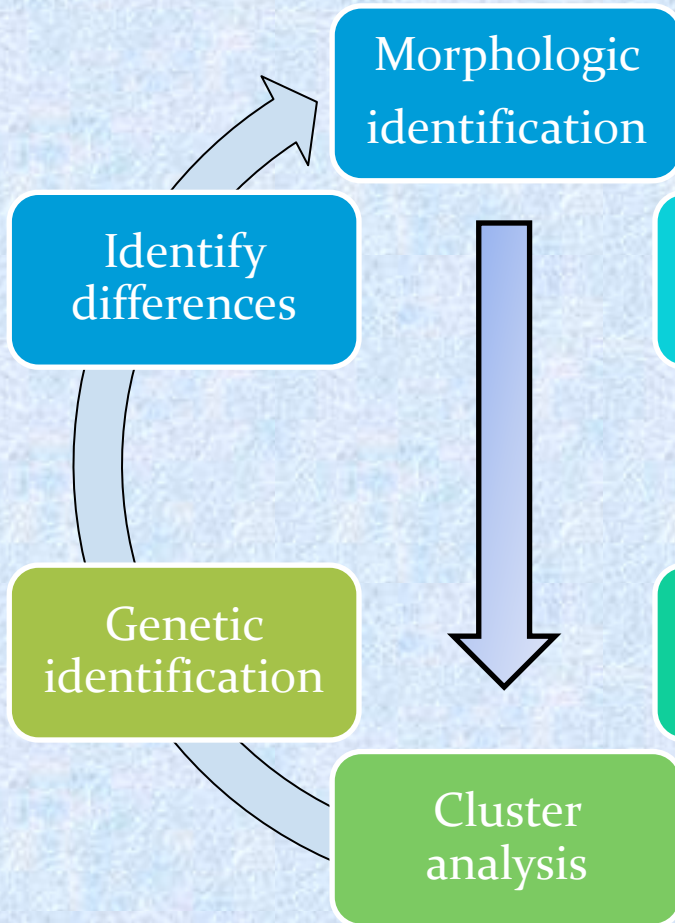
Freshwater sponges



Marine sponges



# Improved Taxonomic Identification





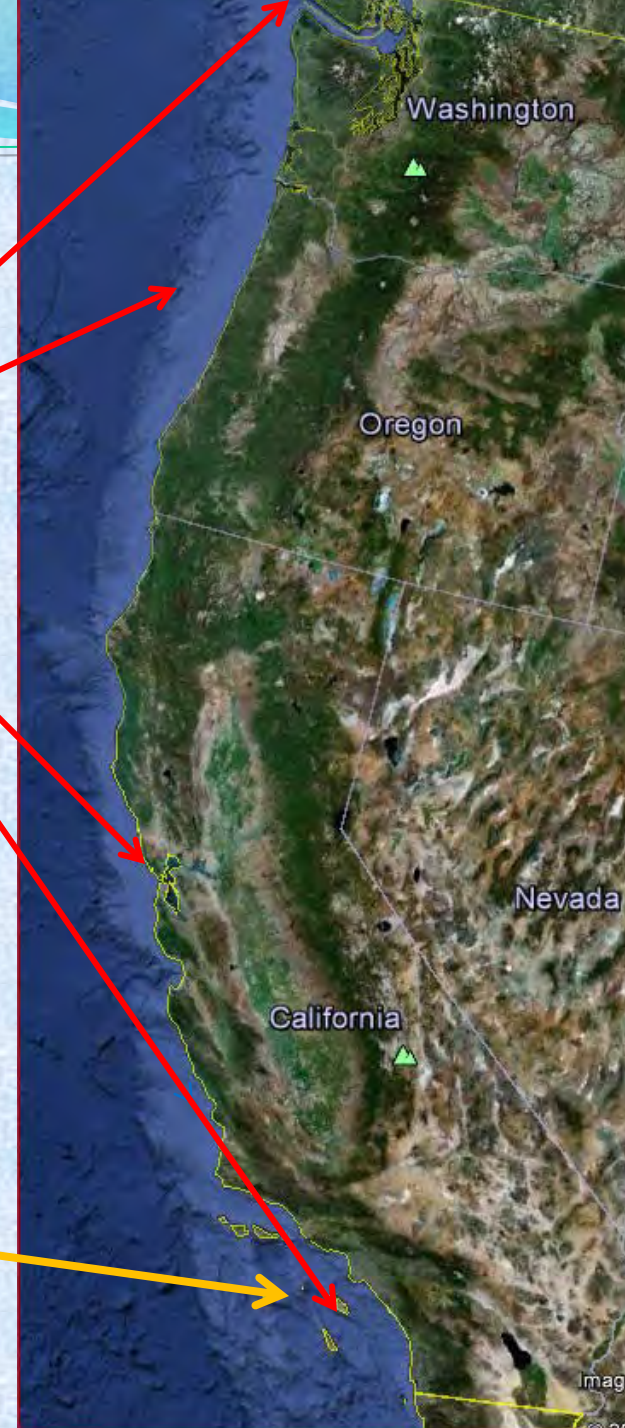
# Can Barcoding Aid in Marine Benthos Identification?

## Cosmopolitan spp

Species	coarser taxonomic level
<i>Ampelisca agassizi</i>	gammarid
<i>Ampelisca careyi</i>	gammarid
<i>Euphilomedes carcharodonta</i>	ostracod
<i>Nephtys caecoides</i>	phyllodocid
<i>Nephtys ferruginea</i>	phyllodocid
<i>Spiophanes berkleyorum</i>	spionid
<i>Spiophanes norrisi</i> (bombyx)	spionid
<i>Tellina modesta</i>	tellinid

## Cryptic spp

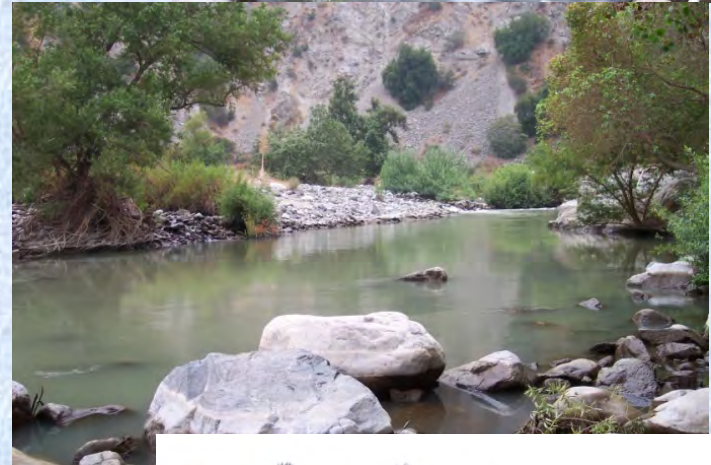
Taxon
<i>Aphelocheata glandaria</i> complex
<i>Capitella capitata</i> complex
<i>Leptochelia dubia</i>
<i>Pholoe</i> spp.
<i>Protomedeia</i> spp.
<i>Scolopolus arminger</i> complex
<i>Spio filicornus</i>
<i>Tellina</i> spp.





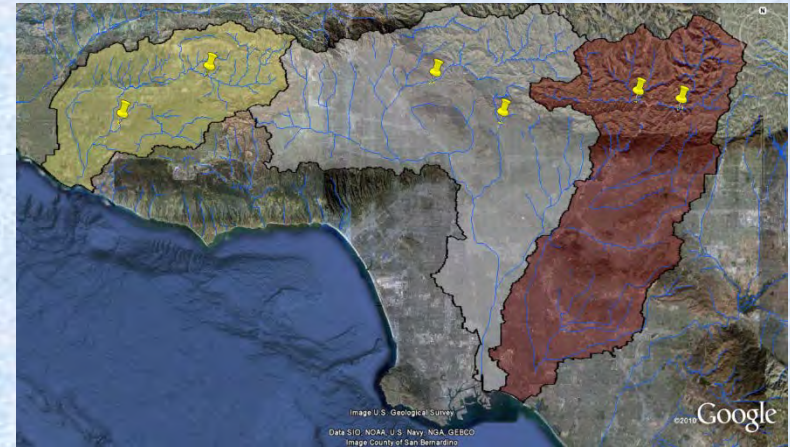
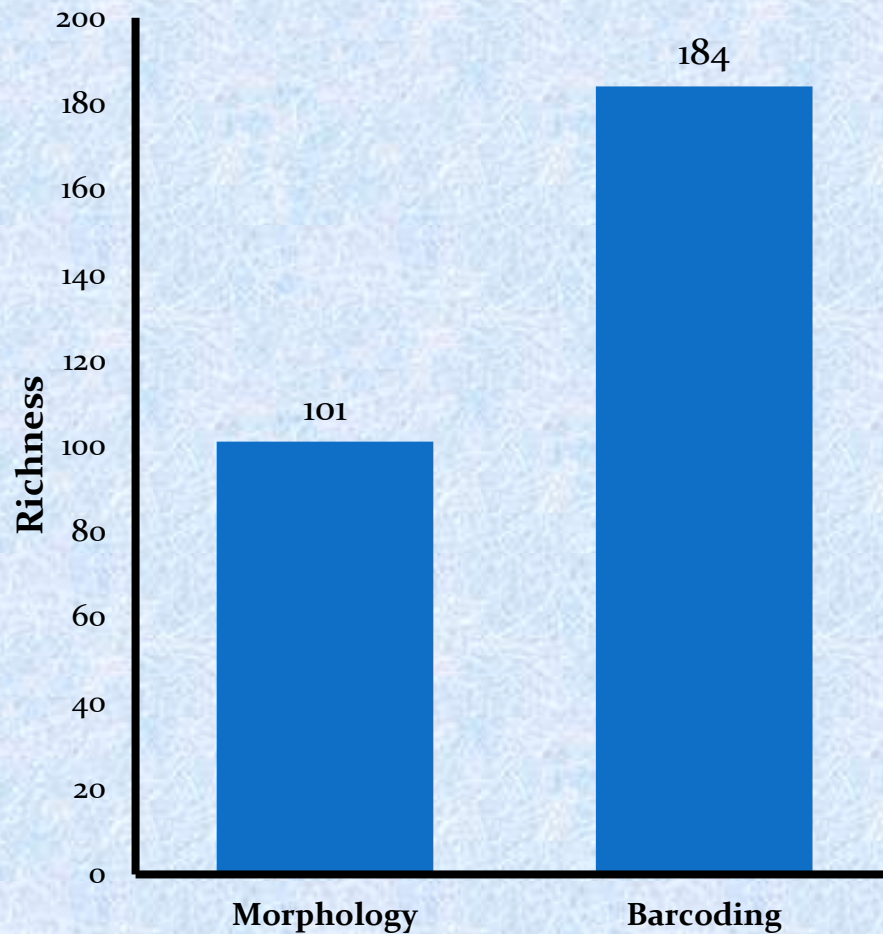
# Does Barcoding Improve Indices?

- How does information on species and community composition vary between barcoding and traditional taxonomy?
- What effect might this have on “Biological Indices?”



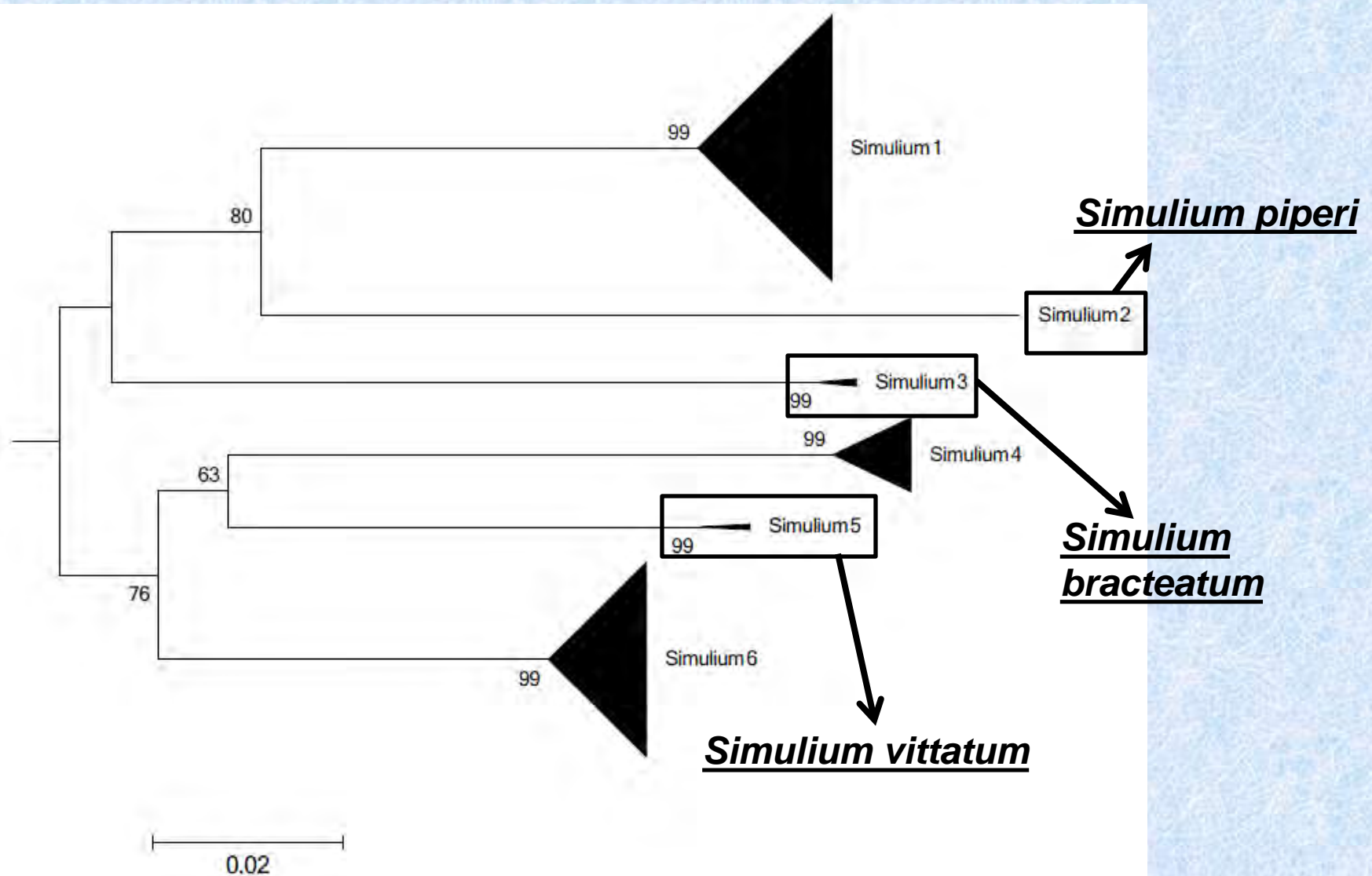


# Richness Measures





# Match Unidentified Specimens to Existing Libraries



# Improved Taxonomic Resolution



## *Simulium piperi*

- High quality sites
- Cool water
- Good vegetated margins



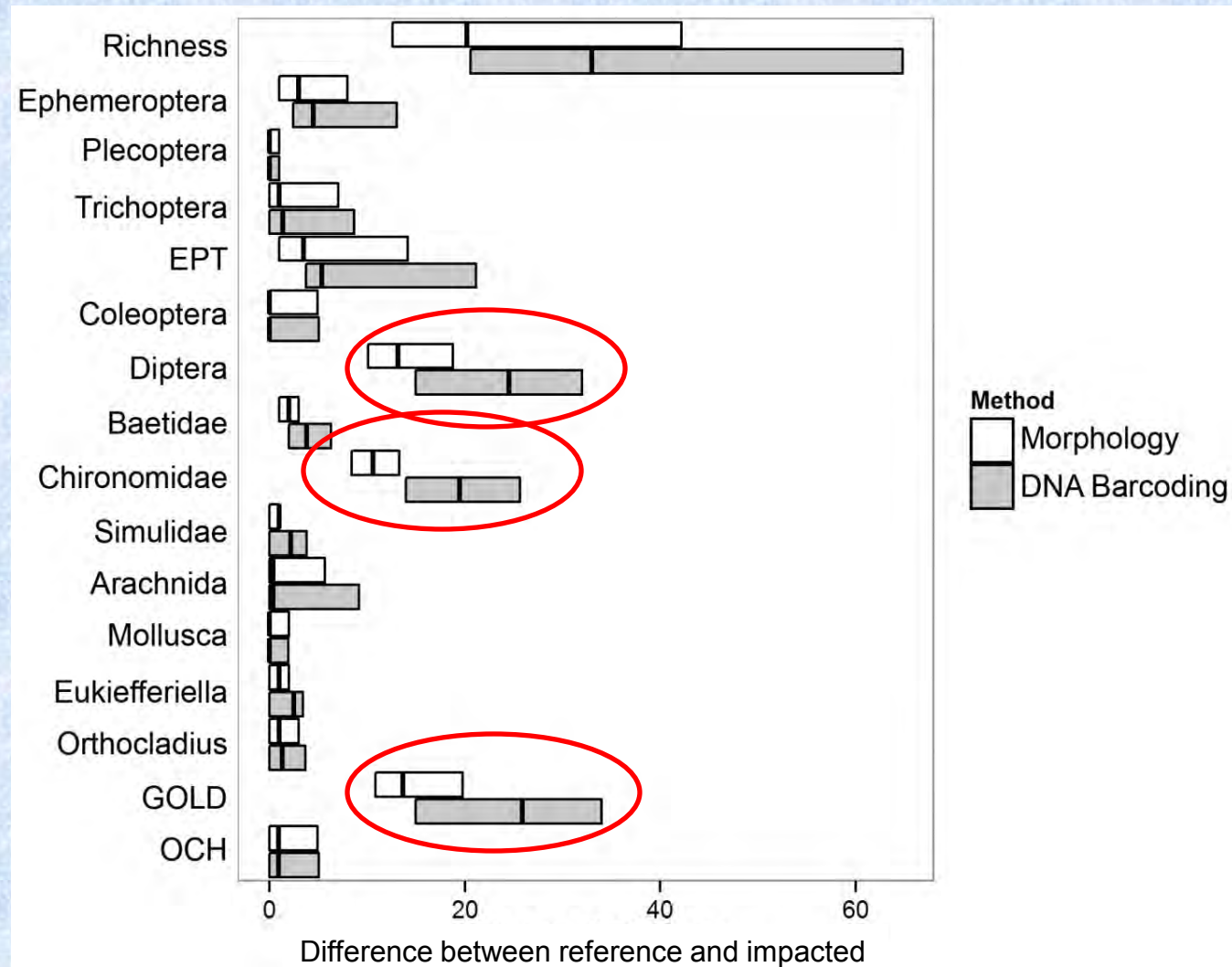
## *Simulium vittatum*

- Tolerant species
- Extreme temperature
- Low oxygen
- Often associated with agriculture





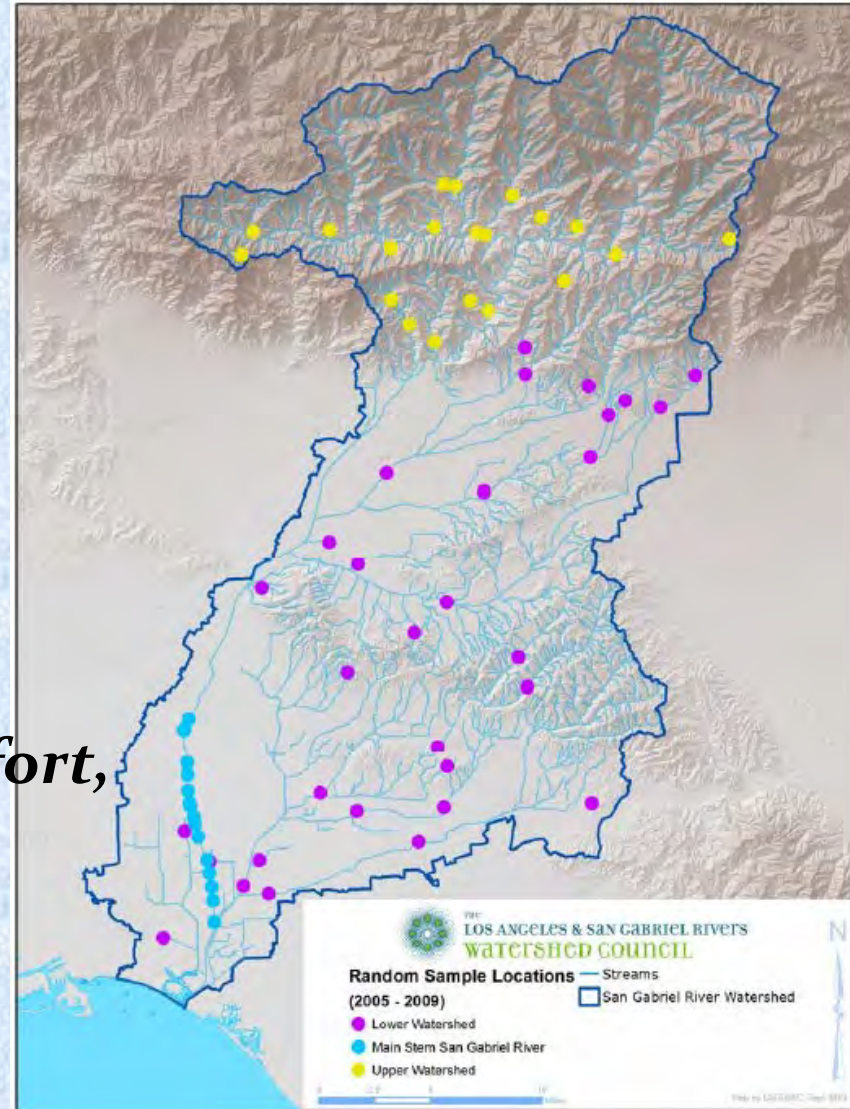
# Some Barcode Derived Metrics are More Sensitive



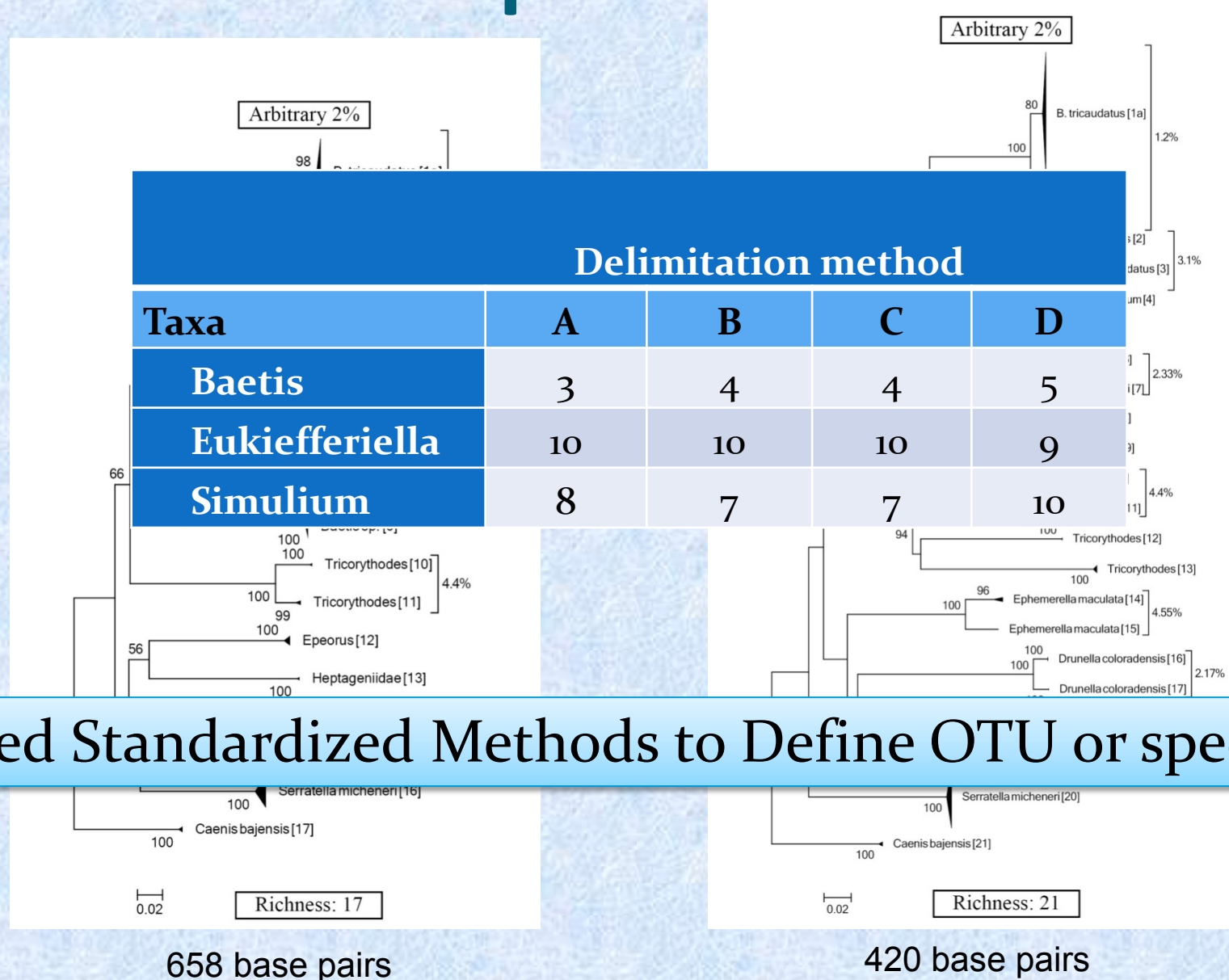


# San Gabriel Watershed Demo

- Implement barcoding in context of a routine monitoring program
- High, medium, low quality sites
  - Test metric performance
- 7,200 total specimens
  - Barcoding in process
- *Rigorously document relative effort, time, challenges, and costs*
  - Field collection
  - Sample processing
  - Barcoding



# Standardized Species Delimitation



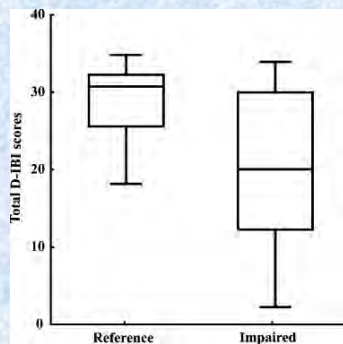
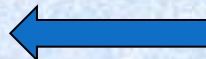
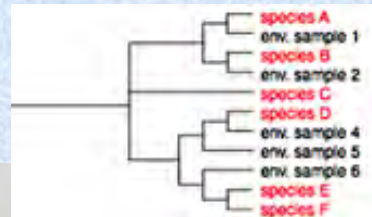
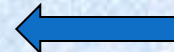
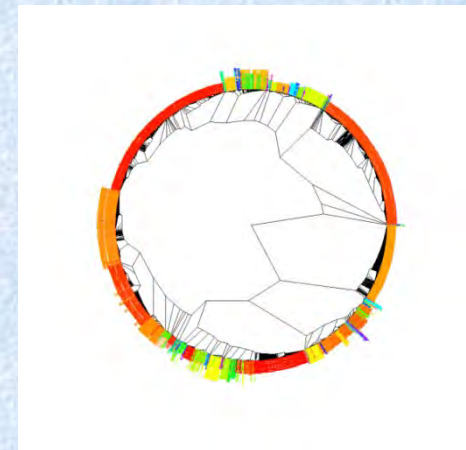
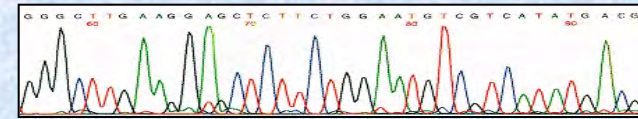
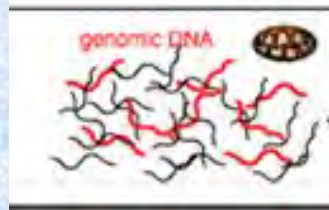
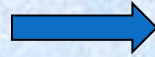


# Where are We Going Next?





# Bulk Sampling Using Next-generation Methods



# Evaluating Ability to Use Bulk Sampling

- Created virtual composites from archived specimens
- 3 “treatments” representing H, M, L conditions based on taxonomic composition
  - Approximately 50 individuals from 10 taxa in each treatment
- Bulk DNA extraction from each → PCR
- Blind DNA extract samples to labs to test next-generation sequencers
- *Results in process*



# Bulk Sampling with Environmental DNA (eDNA)

- Nuclear or mitochondrial DNA released from an organism into the water column
- Persists for 7-21 days depending on conditions
- Initial applications for detecting invasive species
- Potential future application of broader community analysis



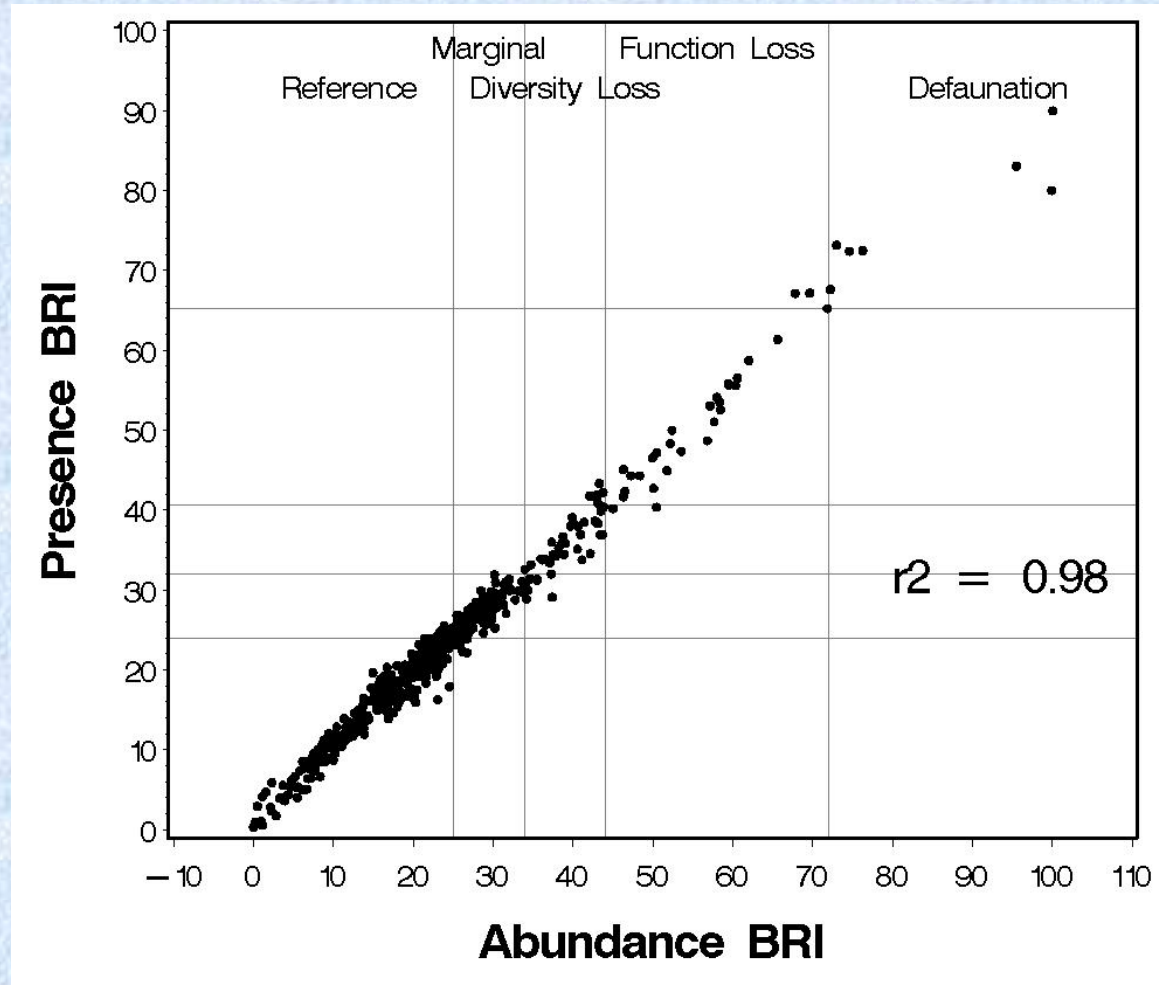
Updated March 2011

For the most updated maps, please visit: <http://nas.er.usgs.gov/taxgroup/mollusks/newzealandmudsnaildistribution.aspx>



# Can Existing Indices Accommodate Next-generation Methods?

- Lack of abundance data should not be a limitation
- Presence BRI and AMBI perform well
- Able to discern both spatial and temporal gradients



# Are We There Yet?

## Molecular

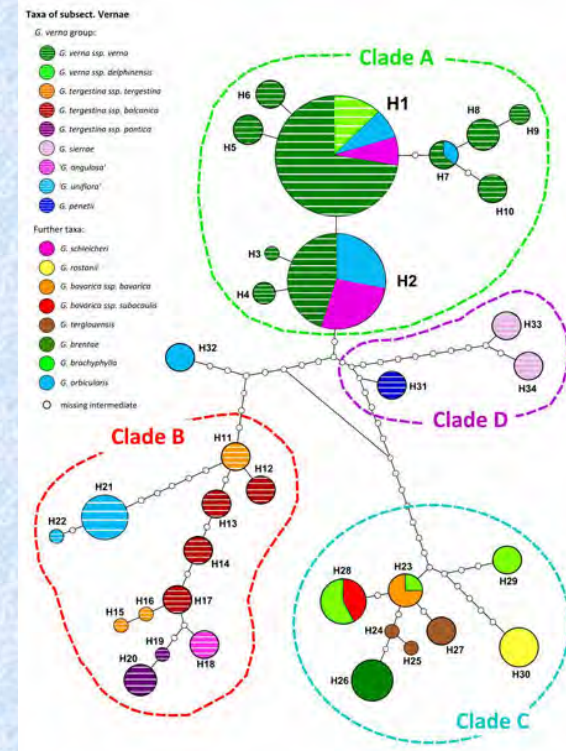
- Additional markers besides COI
- Improved primers
- Next generation sequencing
- Ability to process bulk samples

## Bioassessment

- Species delimitation
  - Character based analysis
- Revised bioassessment scoring tools
- Additional taxonomic groups
  - algae, prokaryotes, meiofauna

## Bioinformatics

- Adequate vouchering (specimens & DNA)
- Data management and analysis tools







# THANK YOU

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