DNA Barcoding as Tool for Freshwater Bioassessment

Eric D. Stein

Southern California Coastal Water Research Project

Biology Department

erics@sccwrp.org

www.sccwrp.org

Today's Talk

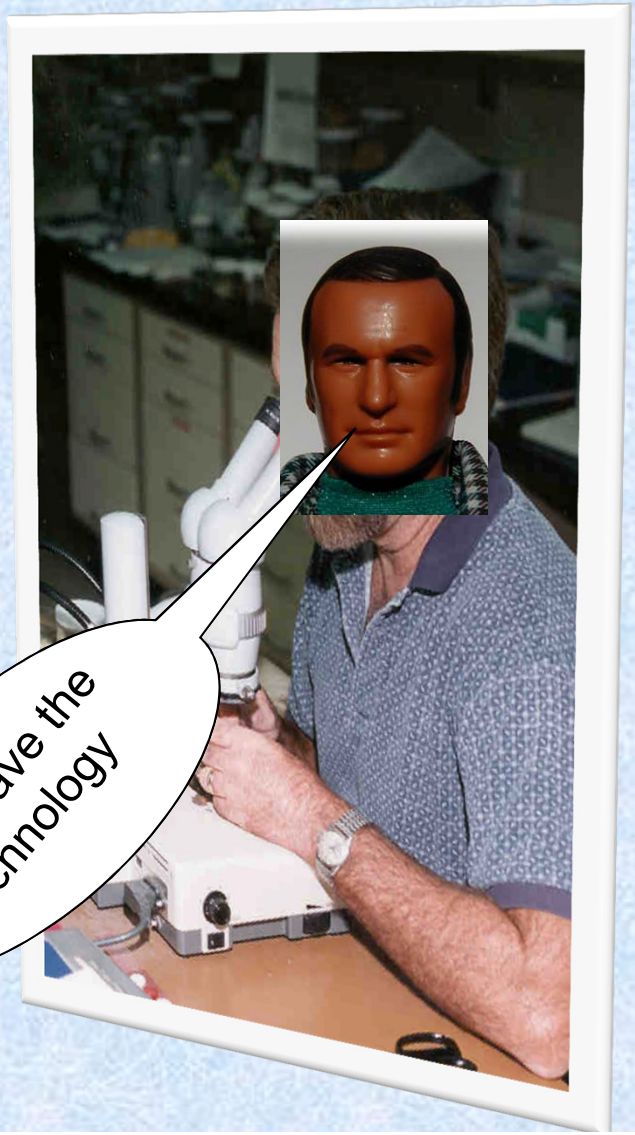
- What is barcoding?
- Why use barcoding for bioassessment?
- What are some of the technical challenges?
- What is the status of the application of barcoding for routine bioassessment?
- What does the future hold?

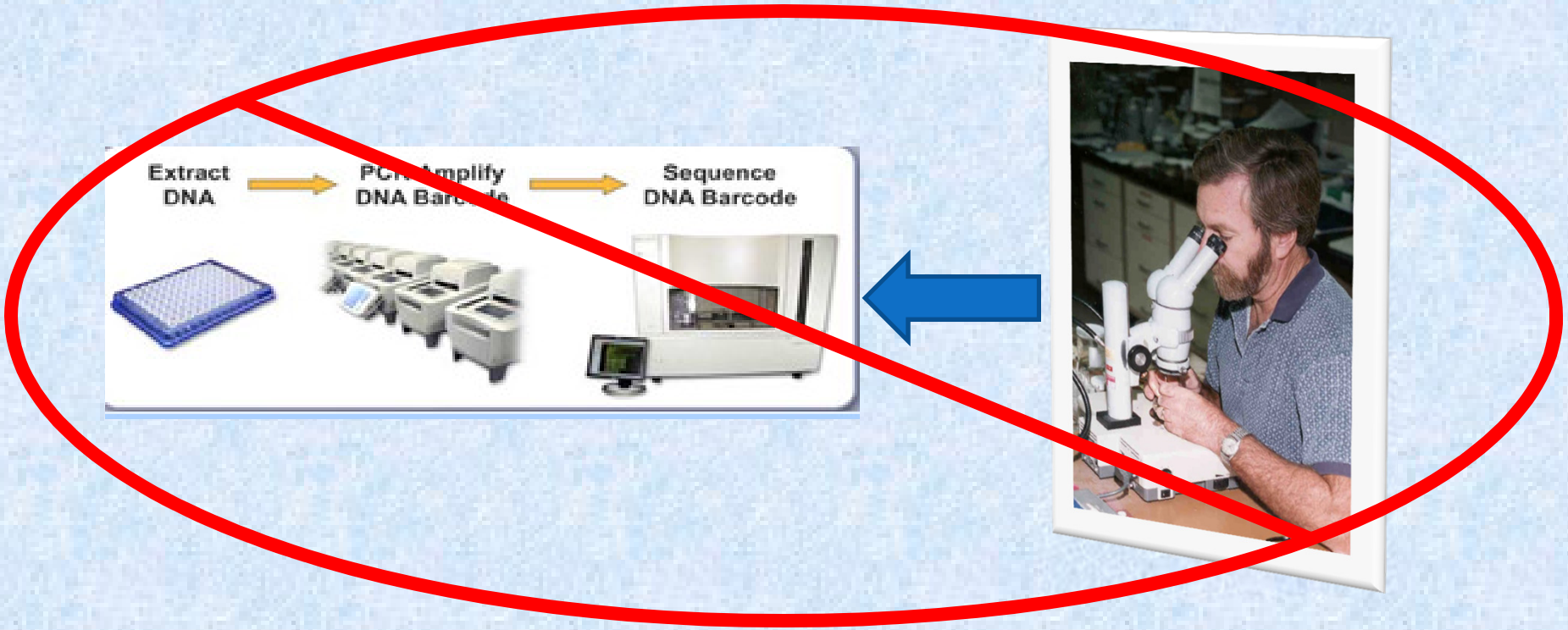
Challenges of Traditional Taxonomy

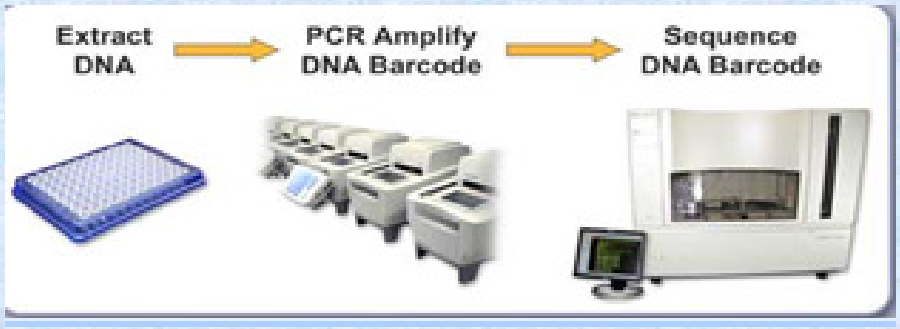
- Time required to get results
- Expense of detailed taxonomy
- Taxonomic capacity
- Unpredictable quality of specimens
- Challenges of traditional taxonomy
  - Rare and cryptic species
  - Morphology of various life stages
  - Sexual dimorphism

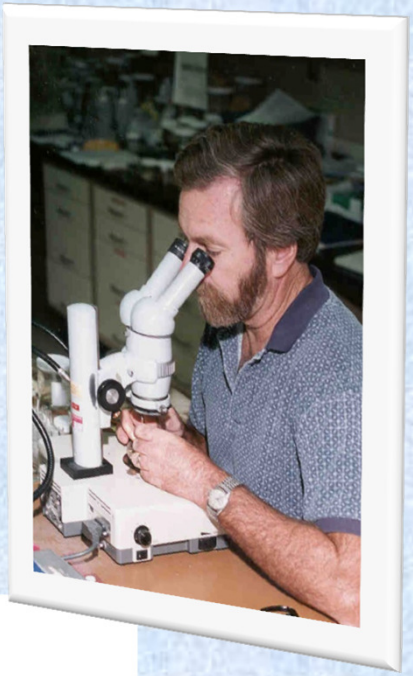
Barcoding is Part of the Solution

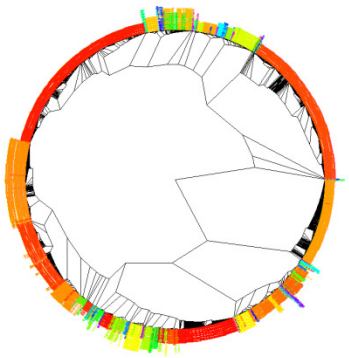
- Faster answers
  - Weeks vs. months
- Less expensive
  - Current barcoding costs comparable with traditional taxonomy
  - Next generation sequencing costs could be 60% cheaper
- Improved taxonomy (better data)
  - Recognizing misidentifications
  - Improving taxonomic keys
  - Helping with difficult to ID taxa
  - Supports QA programs




Extract
DNA
Sequence
DNA Barcode





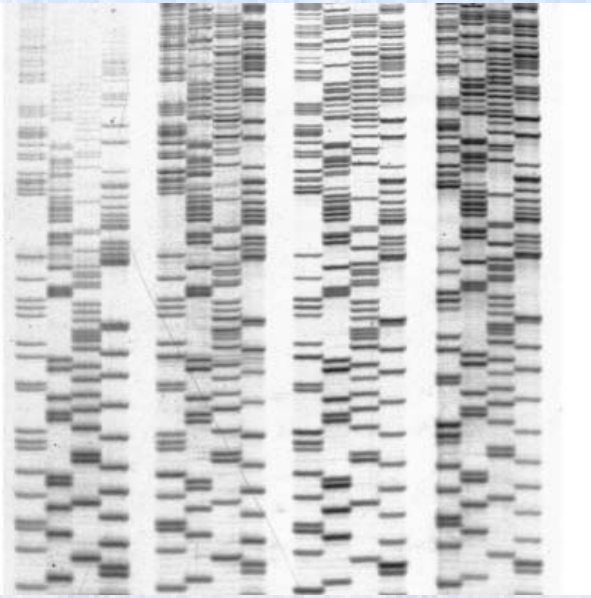


More robust understanding of community composition

What is barcoding?

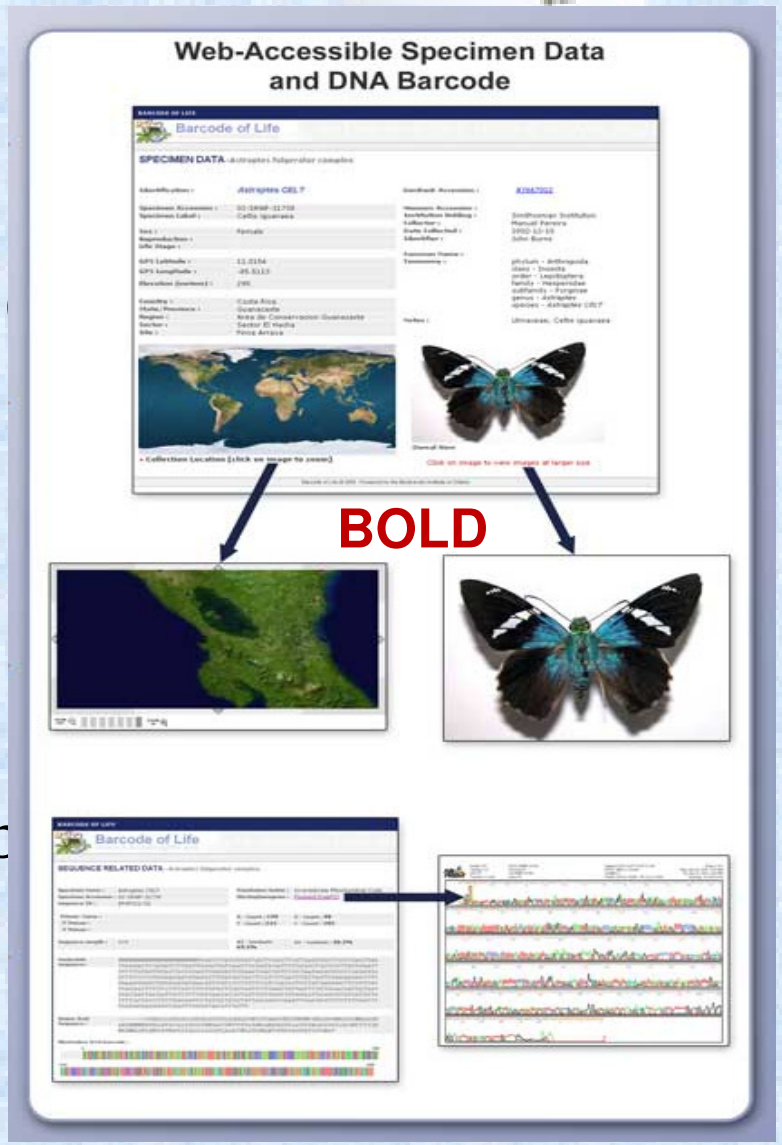
A DNA barcode is a short gene sequence taken from standardized portions of the genome, used to identify species.

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



How Does Barcoding Work?

- Collect samples
- Remove small amount of tissue
- Extract and amplify DNA
- Isolate DNA “marker”
- Compare marker to reference lib
- Identify species



Technical Challenges

Sounds Simple ….. But There are Technical Challenges

- ***Sampling***
- ***Development of a Reference Library***
- ***Efficacy of DNA Markers***
- ***Application to Environmental Monitoring***

Sampling Challenges

- Normal sample preservation methods don't work
- Formalin used in routine bioassessment
  - DNA degradation
- High volume ethanol used in molecular biology
  - Increases brittleness of samples
  - Logistically more difficult to take in the field
  - More hazardous
- Need alternative sample preservation methods

Preservation Methods: Study Approach

- Test preservatives
  - 7 different treatments
  - Volume of ethanol
  - Number of ethanol replacements
  - Addition of glycerin
- Test holding times of 1 week – 12 months
- Barcode all samples to determine effect of preservation method
- ***Initiated Sept. 2011***

Building the Reference Library

- Routine barcode application depends on a robust library
  - Do barcodes match correct taxa?
  - Are barcode results consistent?
  - Do barcode sequence have correct amount of genetic variation?

- Progress to date
  - Collected approximately 5,500 freshwater invertebrates from CA
  - *Vouchers for all specimens barcoded to date*
  - Approximately 2,500 species on SAFIT list
  - 258 species in BOLD ≈ 75% from S. CA.

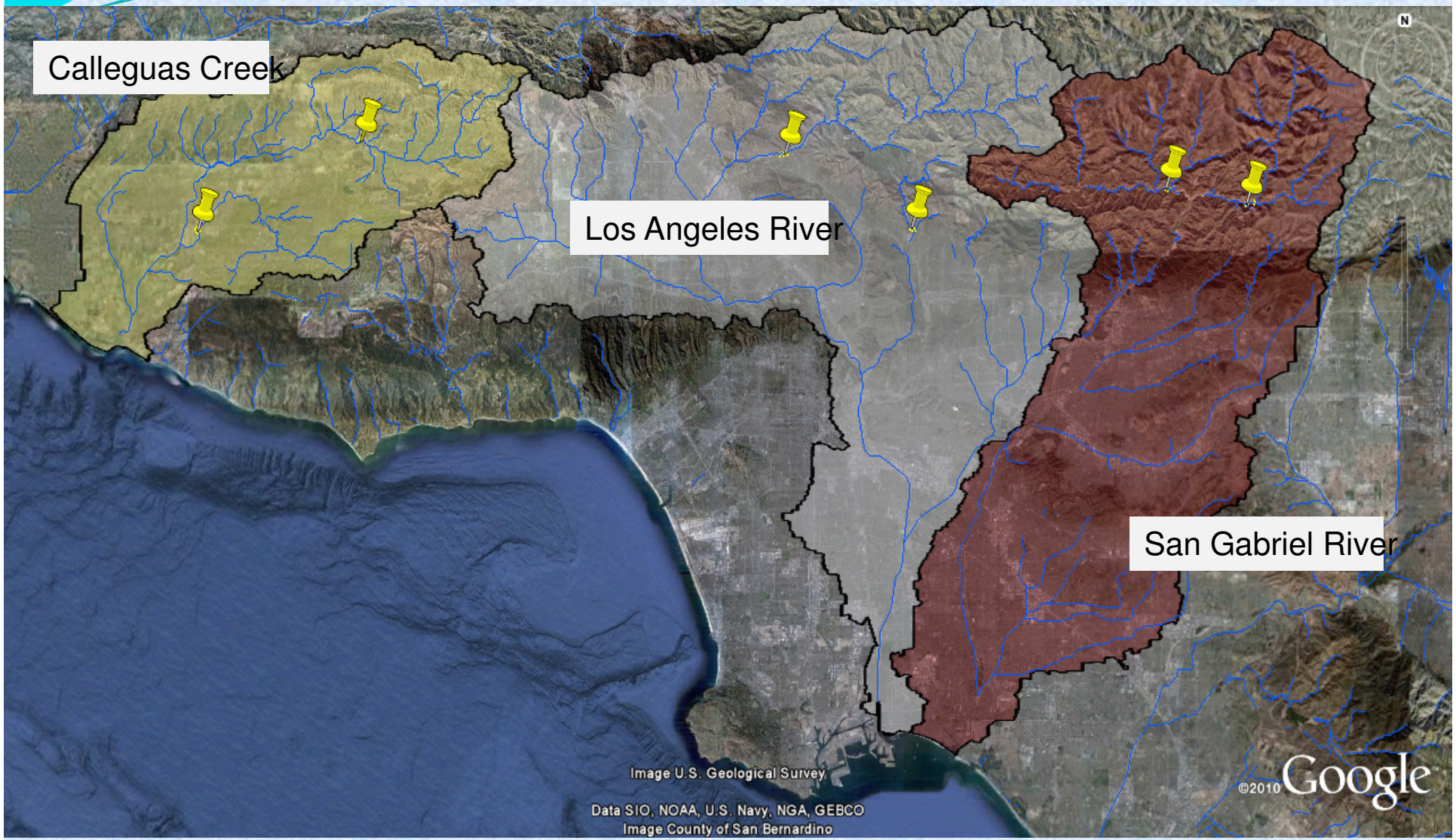
Relationships to Traditional Taxonomy
Efficacy of DNA Markers

- How does information on species and community composition vary between barcoding and traditional taxonomy?
  - What effect might this have on Indices of Biotic Integrity?

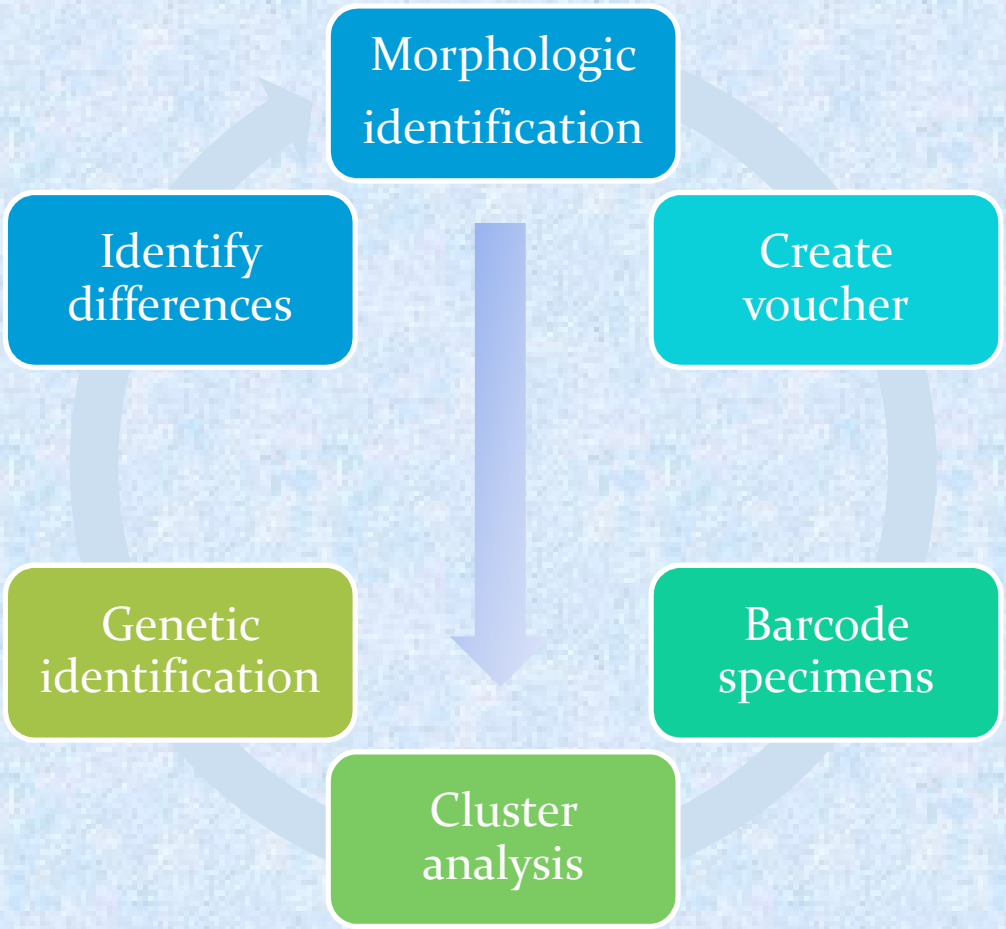
- Side-by-side comparisons of two approaches for freshwater benthic macroinvertebrates
  - Barcoding and morphology based identification
  - ***Vouchers for every specimen***

- Incorporated into existing study on the effects of channel armoring on benthic communities.

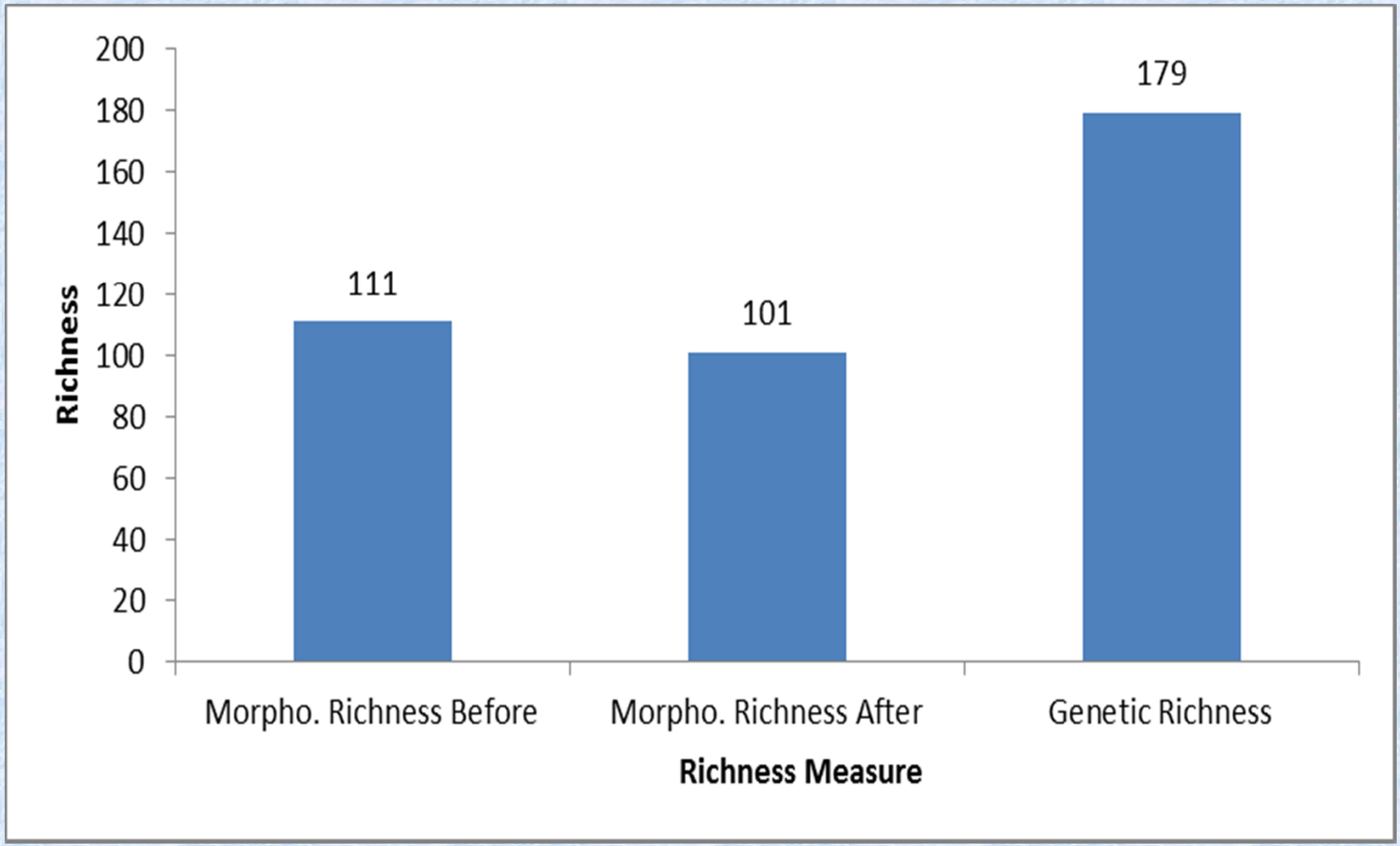
Study Sites


Calleguas Creek
Los Angeles River
San Gabriel River
Image U.S. Geological Survey
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image County of San Bernardino
©2010 Google

Taxonomic Identification



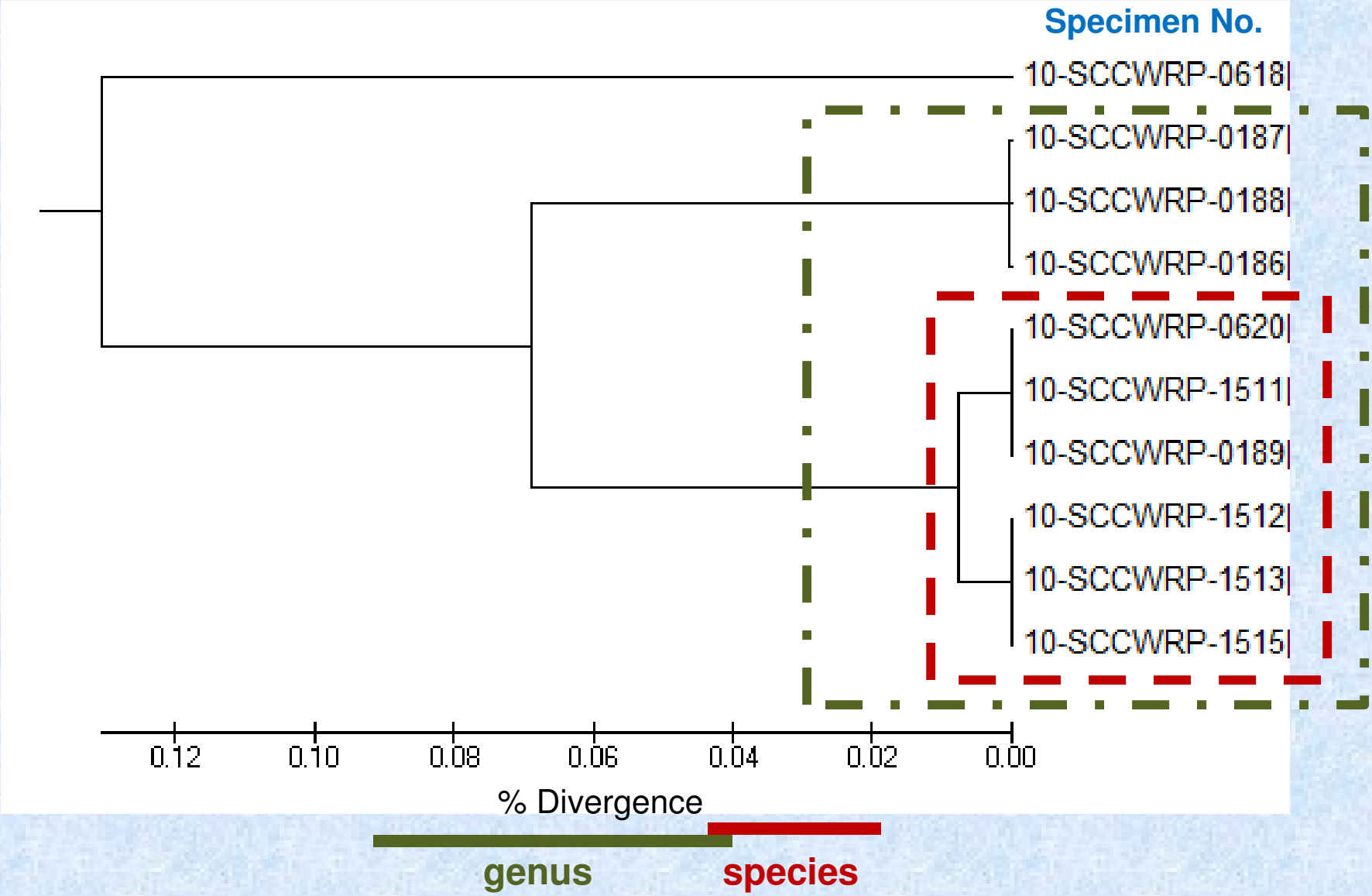
Richness Measures

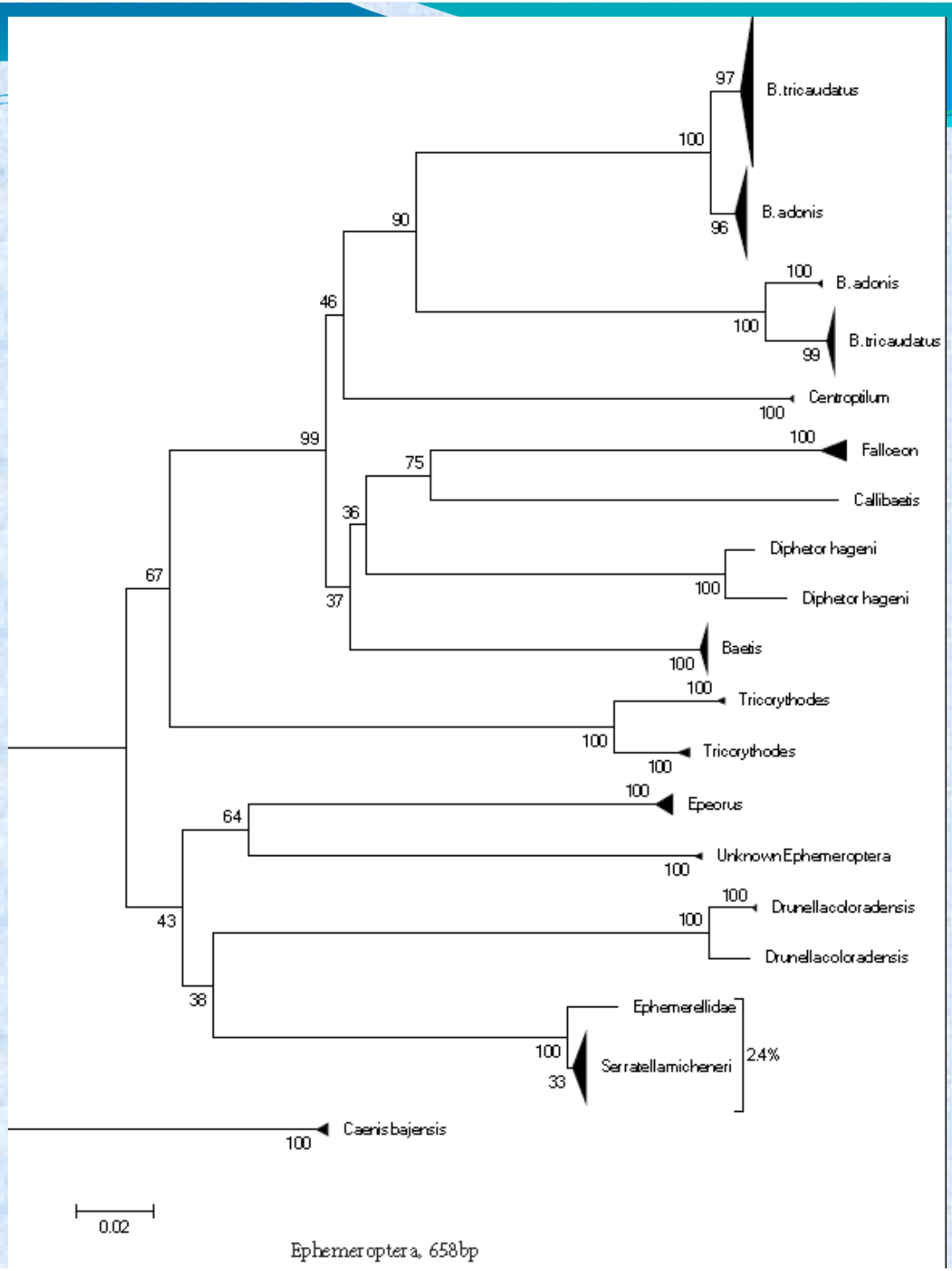


Results Summary

Taxa	Seqs. > 420bp	Specimens	% Success	Morpho. Richness	Genetic Richness	% Change
Ephemeroptera	1203	1378	87.3%	15	18	20%
Diptera (Non-Chiro)	935	937	99.8%	17	20	18%
Diptera (Chironomidae)	1277	1428	89.4%	34	92	171%
Trichoptera	1004	1059	94.8%	13	18	38%
Plecoptera	13	13	100.0%	2	2	0%
Coleoptera	40	40	100.0%	7	6	-14%
Arachnida	142	145	97.9%	6	16	167%
Corbicula	451	465	97.0%	1	1	0%
Ostracods, Odonates, Snails	12	13	92.3%	6	6	0%
Total	**5107**	**5509**	**92.7%**	**101**	**179**	77%

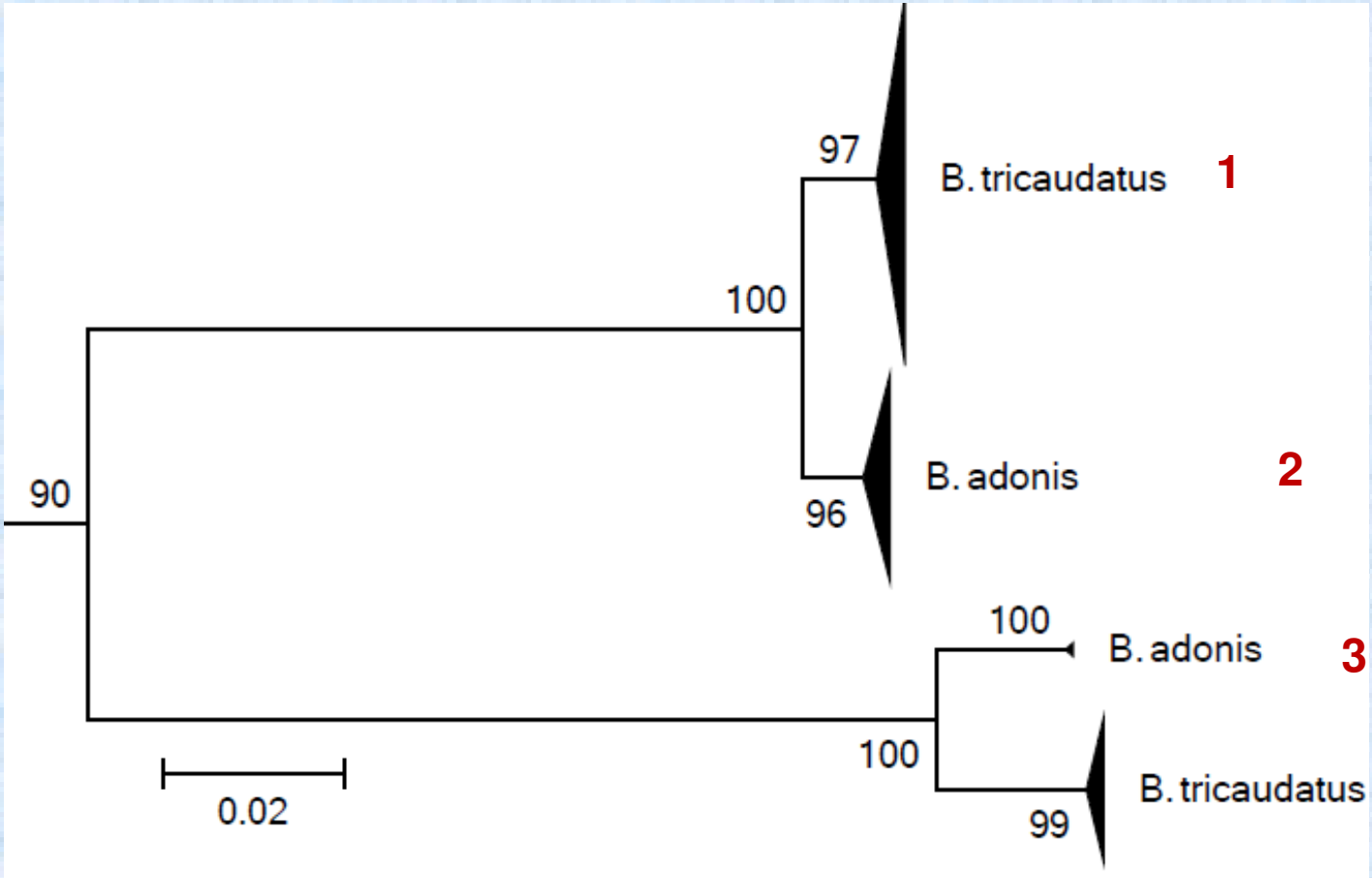
Neighbor-joining Grouping



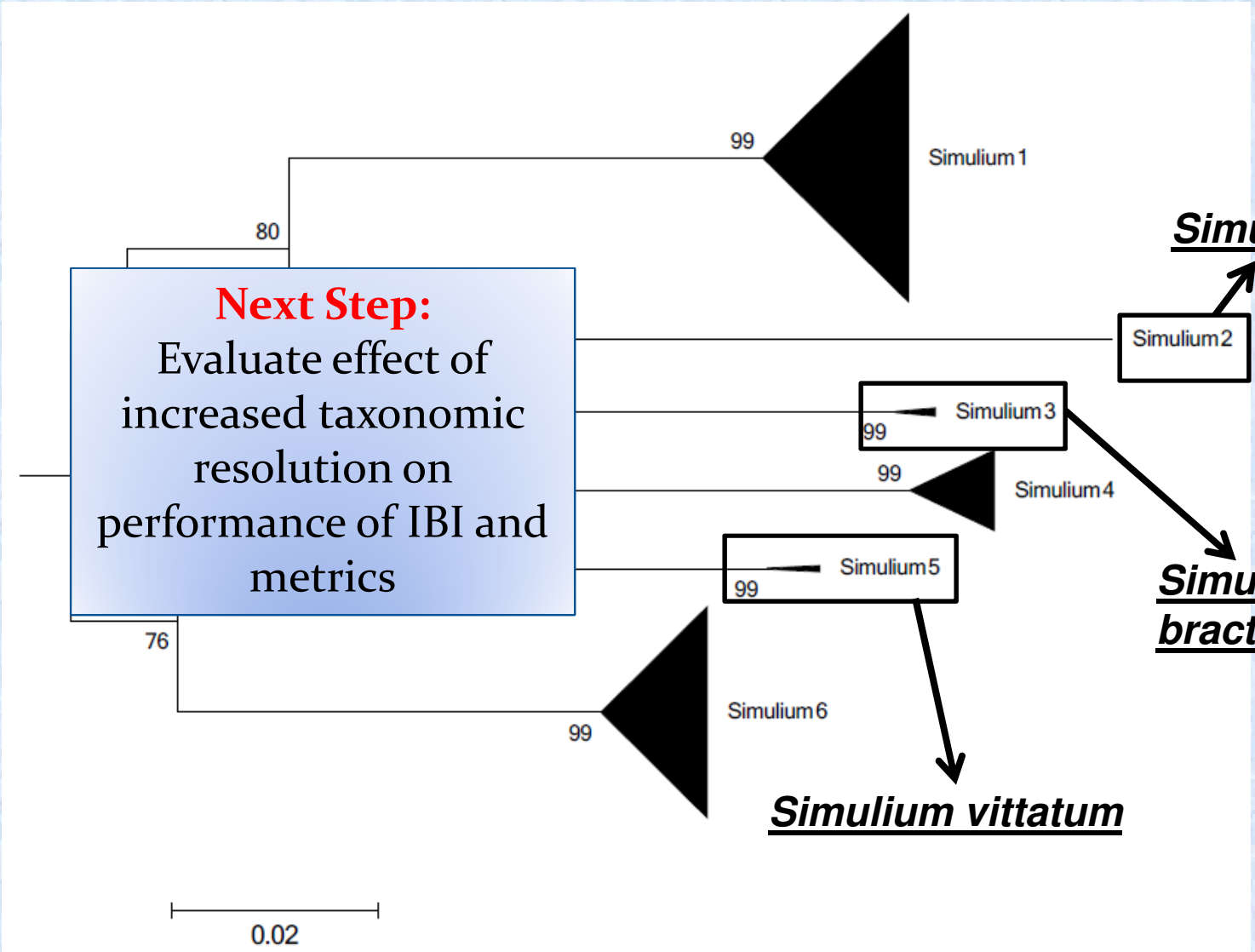

B.tricaudatus
B.adonis
B.adonis
B.tricaudatus
Centroptilum
Falloeon
Callibaetis
Diphetor hageni
Diphetor hageni
Baetis
Tricorythodes
Tricorythodes
Epeorus
Unknown Ephemeroptera
Drunella coloradensis
Drunella coloradensis
Ephemerellidae
Serratella micheneri
2.4%
Caenis bajensis
0.02
Ephemeroptera, 658bp

Species Complex Resolution

Potentially "new" or "rare" species



Match Unidentified Specimens to Existing Libraries



Next Step:
Evaluate effect of increased taxonomic resolution on performance of IBI and metrics

Near-term Applications of Barcoding

- Improved taxonomic resolution
- Improved taxonomic keys
- Identification of cryptic species
- Resolving errors in original identification / QA
- Identification of additional species
  - Immature life stages
  - Previously undescribed taxa
  - Focused morphodiagnosis to answer specific questions
- Phylogenetic analysis
- More finely tuned biotic indices

SAFIT

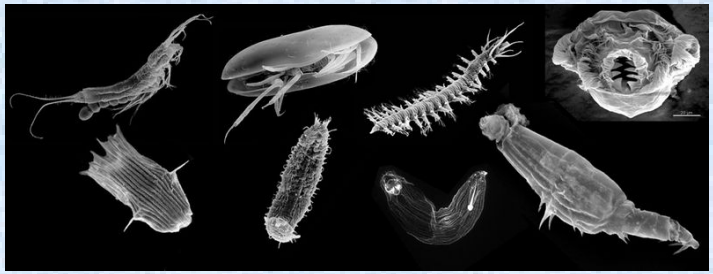
The Long View

10-15 Year Vision

- Incorporate barcoding into routine bioassessment
  - Faster & cheaper results

- Develop and test next-generation sequencing
  - Analysis of bulk environmental samples

- ***Develop new applications***
  - Additional taxa (algae, prokaryotes, meiofauna)
  - Early screening applications for invasive species, etc.
  - E-DNA stressor evaluation
  - Mechanistic investigations
  - Phylogeny and systematics
  - Yet unimagined applications



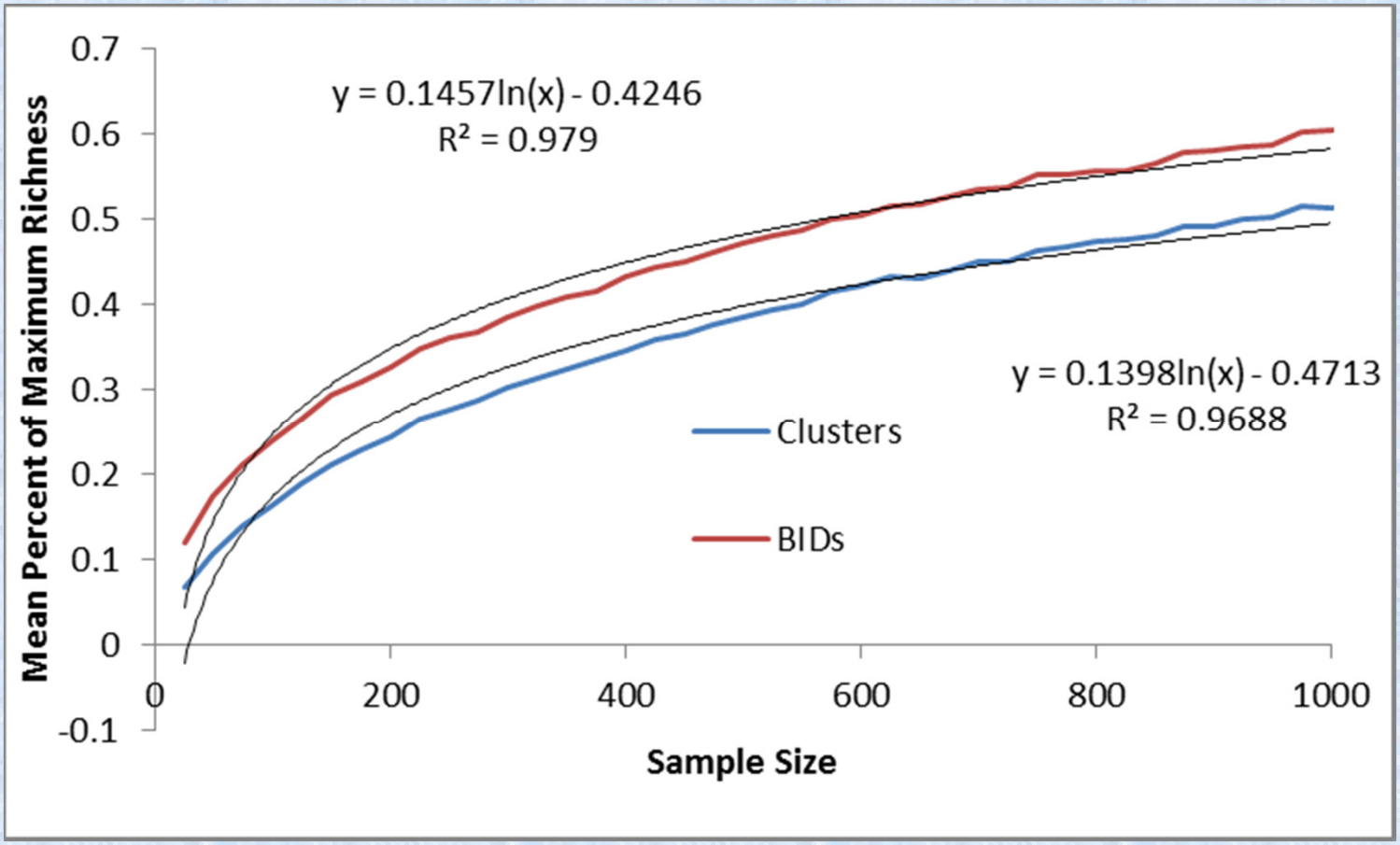
THANK YOU

Eric Stein

714-755-3233
erics@sccwrp.org

EXTRA SLIDES

Richness Accumulation Curve



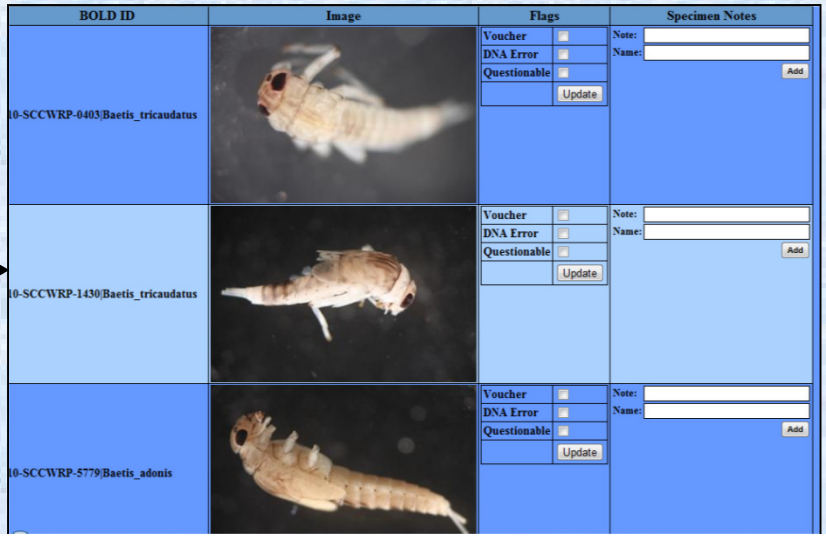
Bioinformatics Tools

SCCWRP-BOL Cluster Browser

Projects | **Add_Project** | **Search** | **Help**

Organization: SCCWRP | **Clusters:** 179 | **Group:** Everything | **Tree File:** | **Temporary Master Tree:** SCCWRP - Everything Set

Reference ID	Cluster Number	Number ID's	Abundance	% Of Cluster	Max K2p %	Detail	Flags	ID List
10-SCCWRP-2831.1|Hydroptila	5	1	807	100.00% =>	0.64%		-	^^^
10-SCCWRP-0766|Baetis_tricaudatus	69	2	798	100.00% =>	3.64%		-	^^^
10-SCCWRP-4834|Simulium	68	1	555	100.00% =>	3.08%		-	^^^
10-SCCWRP-6797|Corbicula	179	2	444	100.00% =>	0.36%		1	^^^
10-SCCWRP-5356|Eukiefferiella	132	2	301	100.00% =>	3.63%		-	^^^
10-SCCWRP-1305|Simulium	63	1	273	100.00% =>	3.65%		-	^^^
10-SCCWRP-0500.1|Serratella_micheneri	49	1	133	100.00% =>	6.14%		-	^^^
10-SCCWRP-2913|Baetis_adonis	70	2	122	100.00% =>	3.93%		-	^^^
10-SCCWRP-0849|Helicopsyche	14	1	94	100.00% =>	0.93%		-	^^^



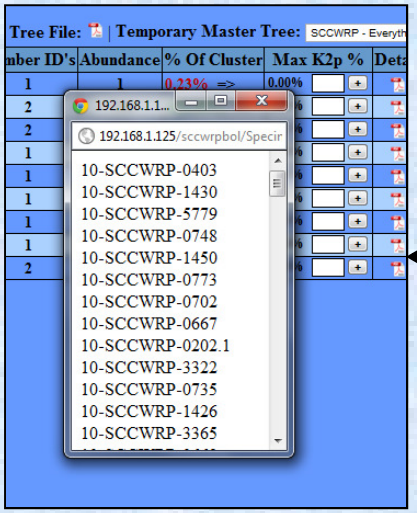
BOLD

↓

Cluster Analysis

↓

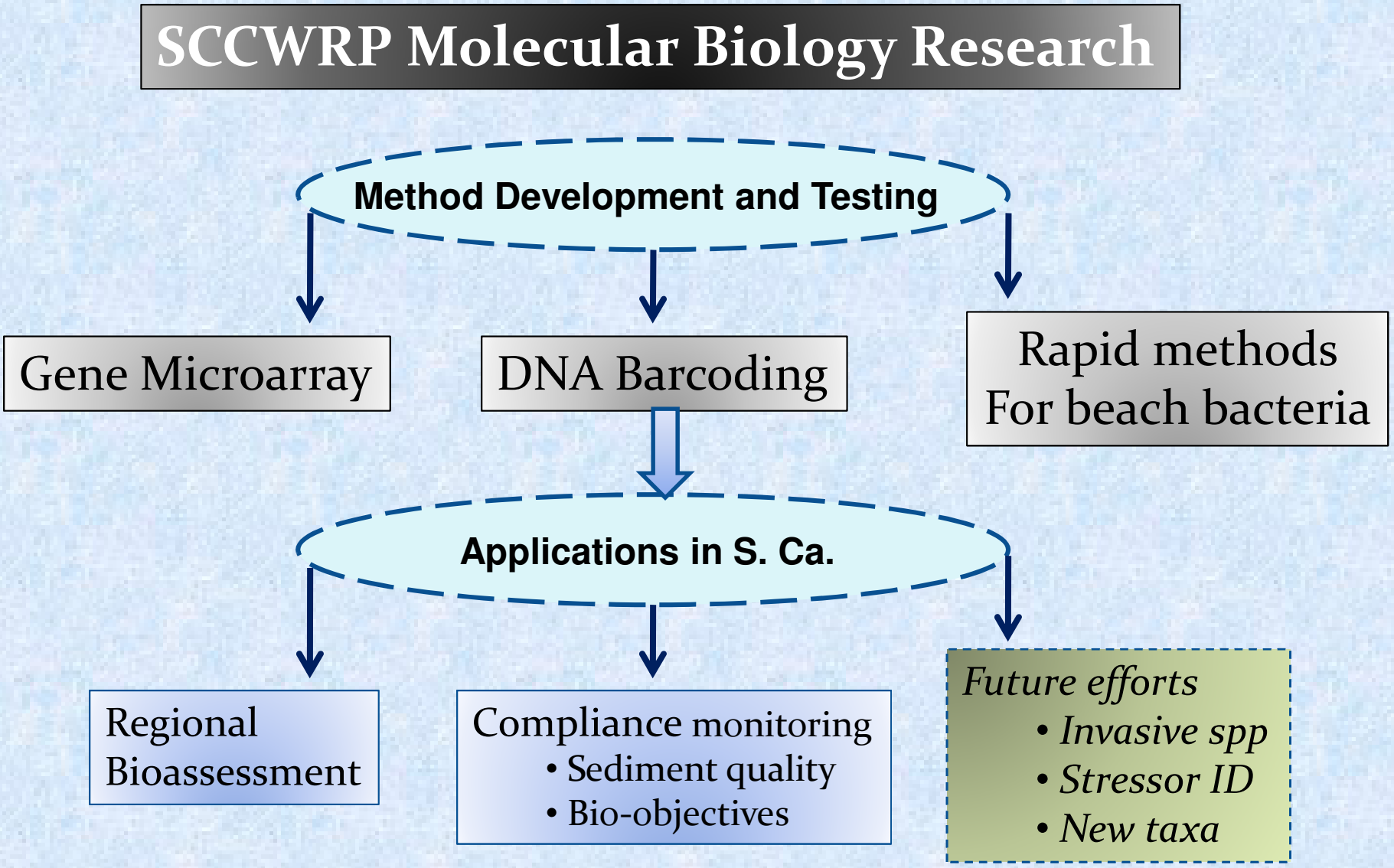
Biotic Indices



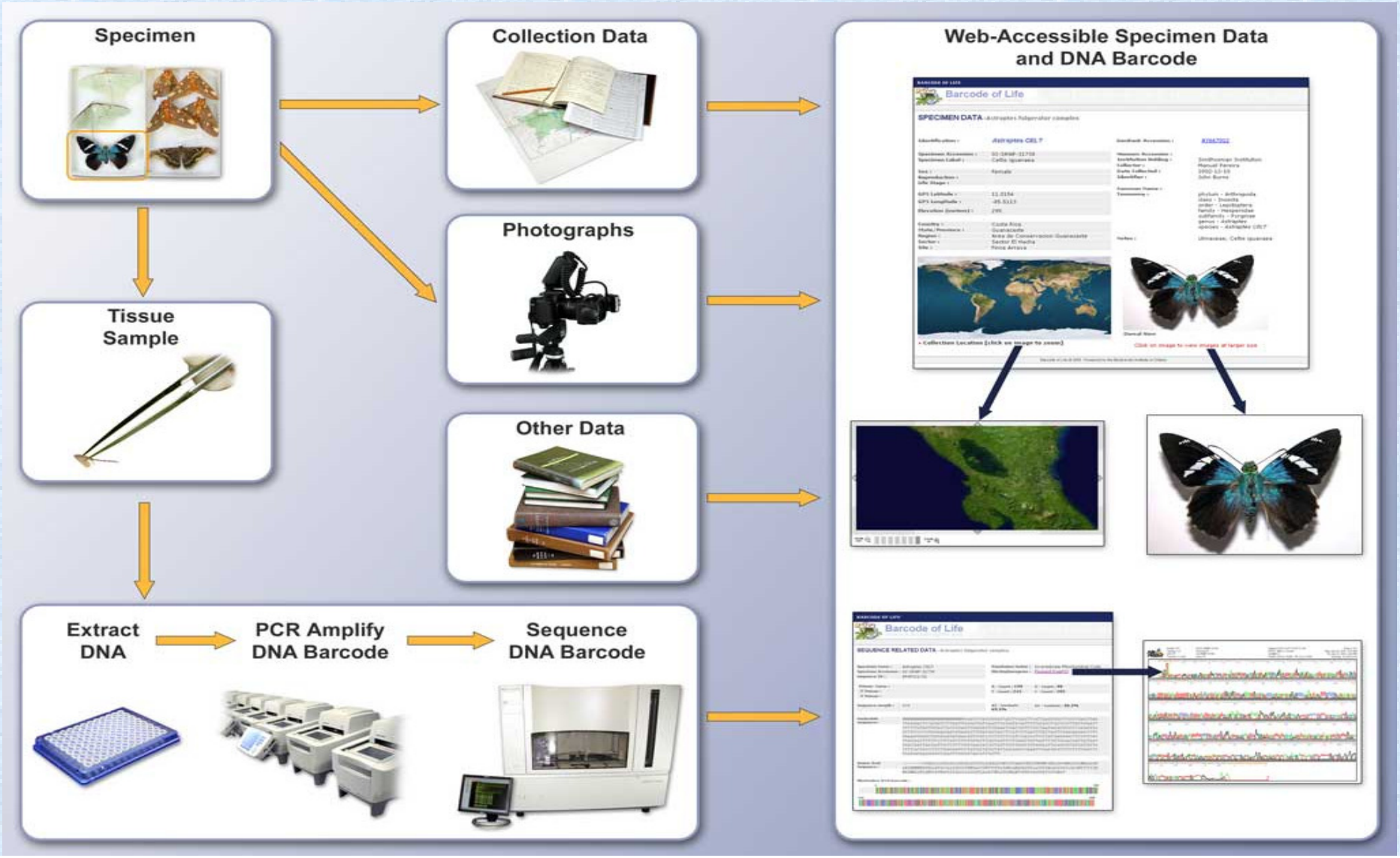
Topics for Discussion

- Why is SCCWRP pursuing this research line?
- What is barcoding?
- How does barcoding fit into the larger molecular biology research agenda?
- What are our ultimate/long-term goals?
- What are some of the key technical challenges?
- What is our plan/roadmap for achieving our goals?
- Who are our partners?
- What progress have we made thus far?
- What are the next steps?

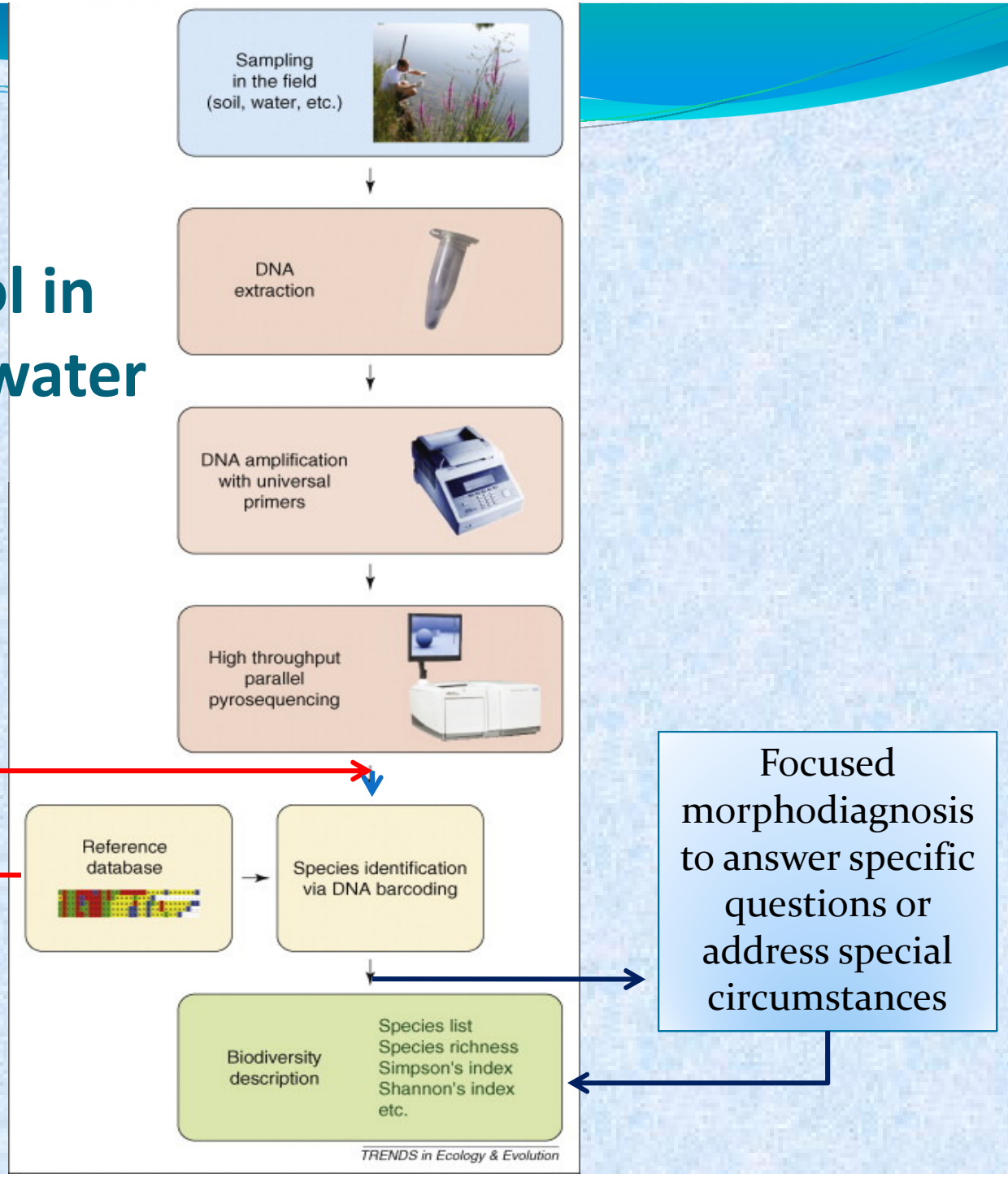
Barcoding in Context



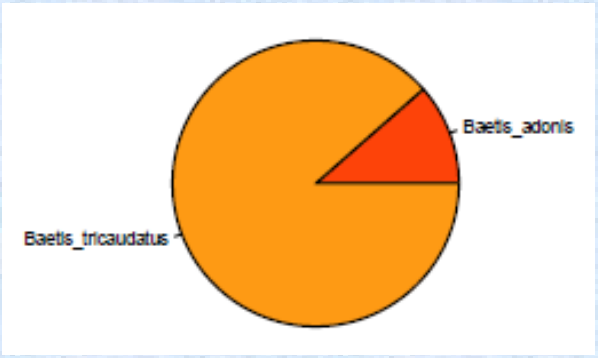
How Does Barcoding Work?

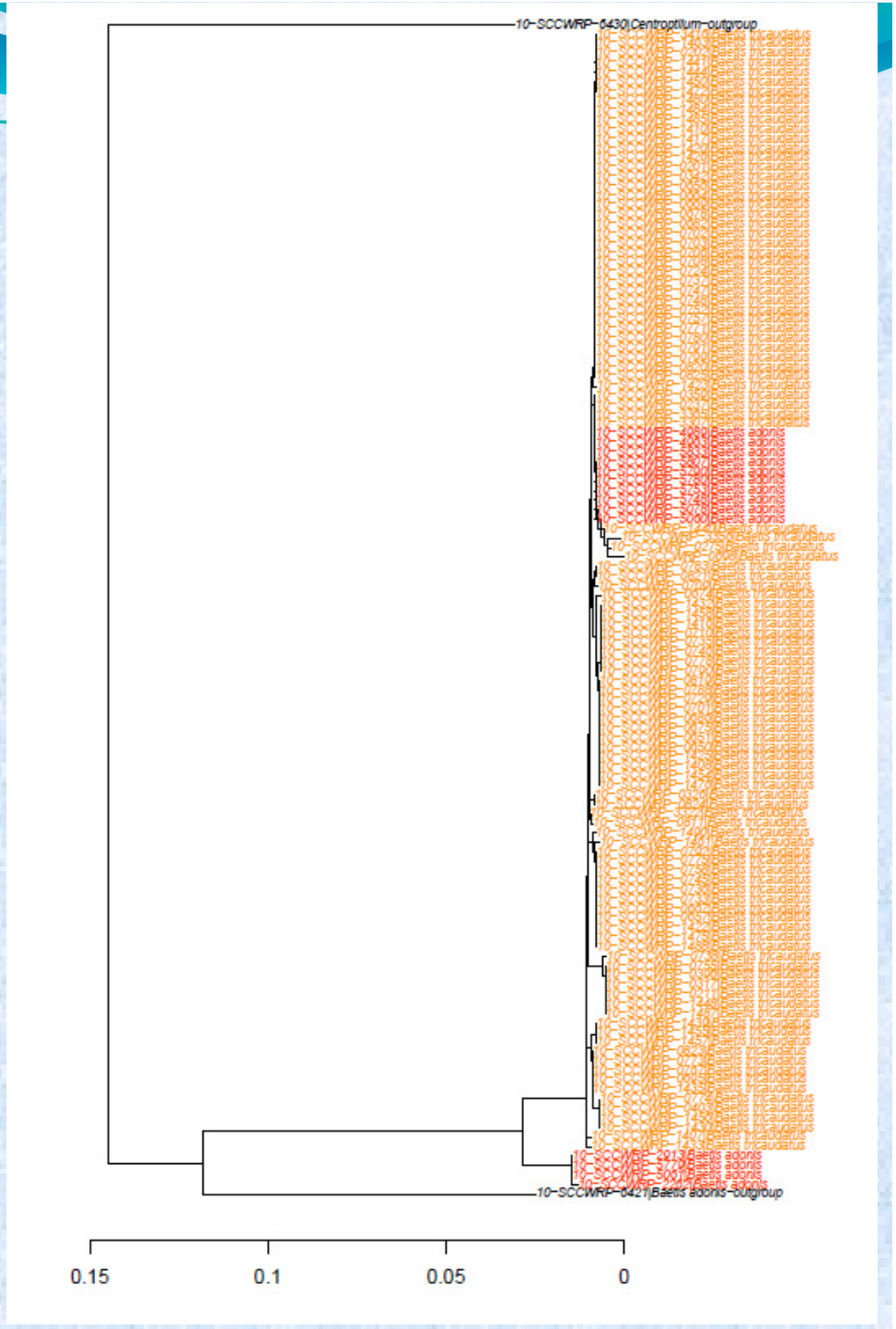
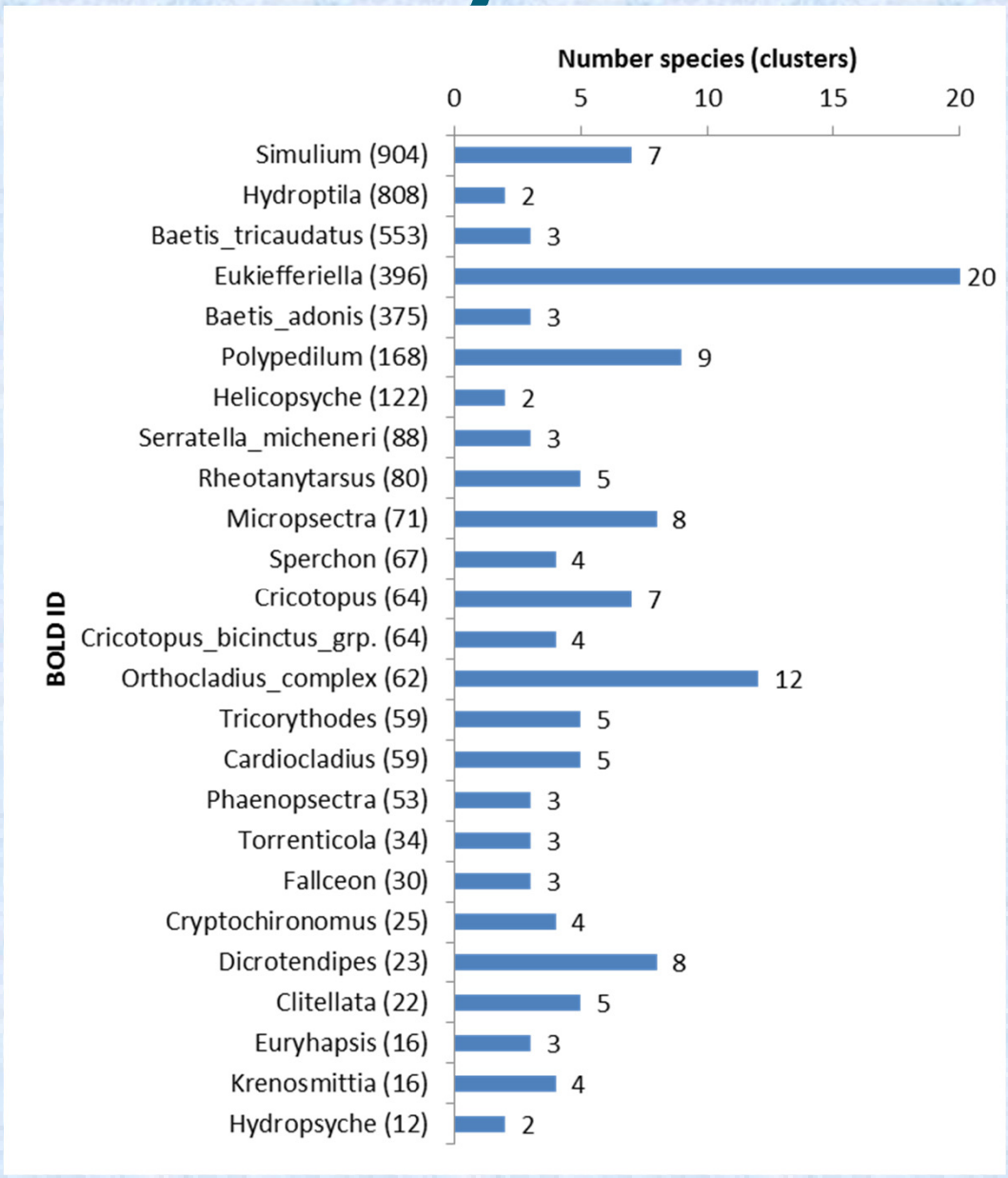


Ultimate Role of Barcoding as a Tool in Marine and Freshwater Bioassessment



Improved Taxonomic Understanding





Species Diversity