



Census of Marine Zooplankton



DNA barcoding of marine zooplankton: a 'Rosetta Stone' for taxonomic and biodiversity studies

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Zooplankton images by Russell R. Hopcroft (Univ. of Alaska)
and Laurence P. Madin (Woods Hole Oceanographic Inst.)

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Outline of Talk



Introduction to DNA barcoding

The ZooGene approach

Census of Marine Zooplankton (CMarZ)

Barcoding at sea: NW Atlantic (April 2006)

Applications of barcoding

Conclusions and future prospects



What is DNA Barcoding?

- ***Definition:*** Derivation of short DNA sequence(s) that enables species identification or recognition in a particular domain of life (e.g., eucaryotes).
- ***Focus to date:*** For animals, a 658 base-pair fragment of the mitochondrial gene, cytochrome oxidase subunit I (mtCOI).

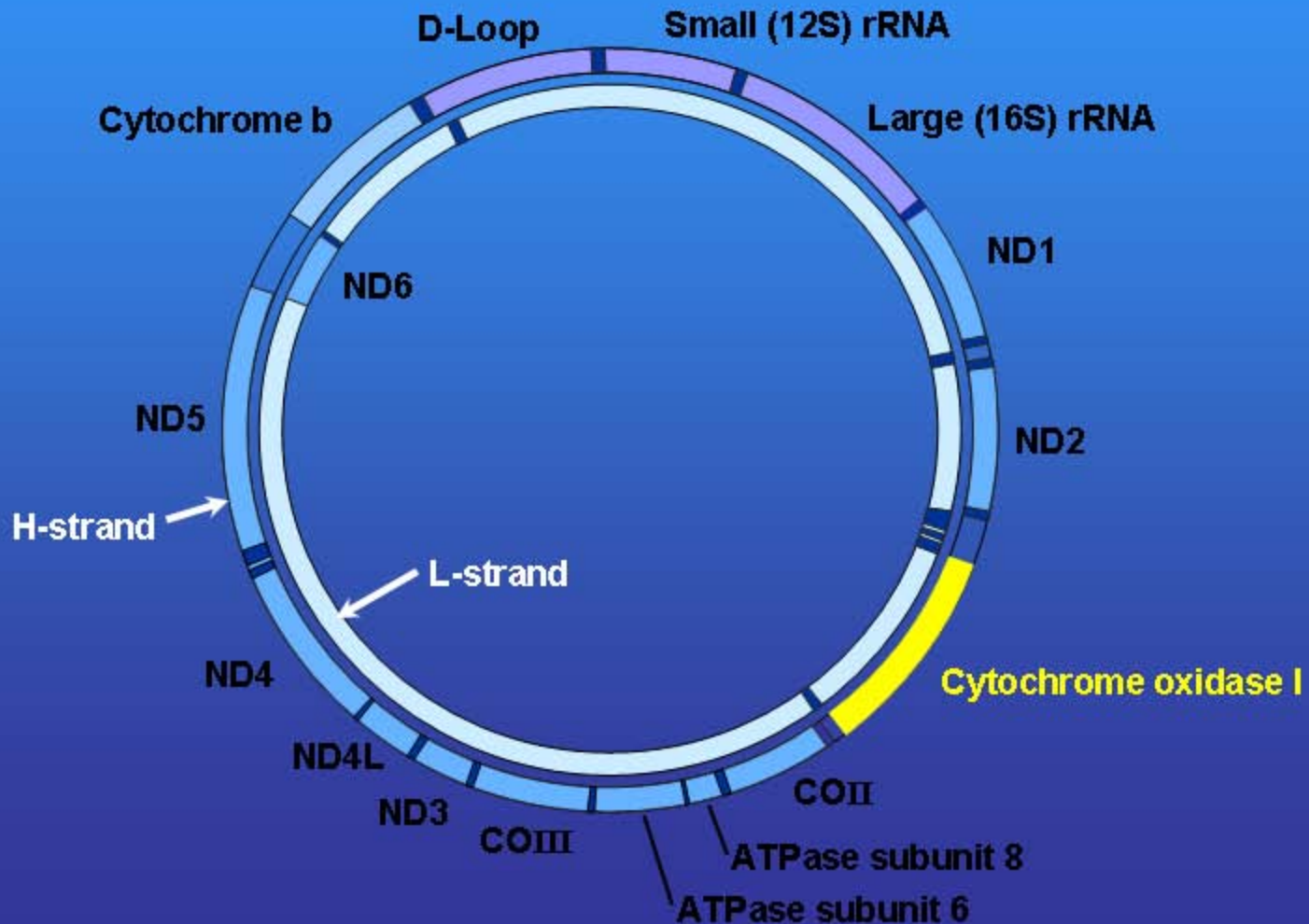
What isn't DNA Barcoding?

- Existing taxonomic practices will not be supplanted or invalidated;
- Barcoding is not DNA taxonomy; it does not equate species identity, formally or informally, with a particular DNA sequence.
- Barcoding will not duplicate or compete with efforts to resolve deep phylogeny (e.g., *Assembling the Tree of Life*, ATOL).

Why Mitochondrial DNA?

Why mtDNA: many copies of the genome; rapid evolution; maternal clonal inheritance

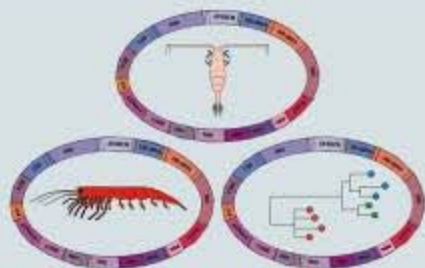
Why mtCOI: pattern of variation within and between animal species



Why Barcode Zooplankton?

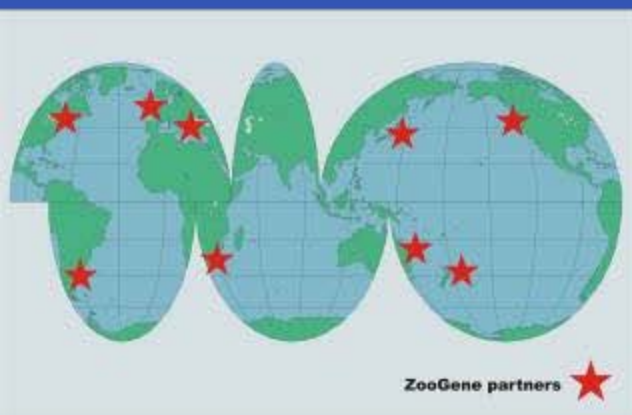
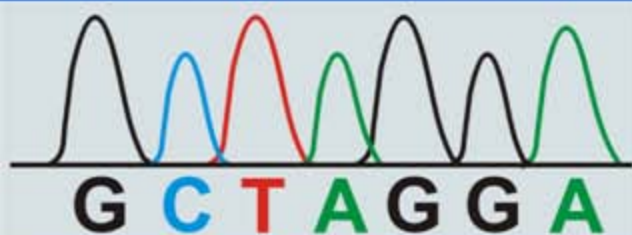


- Improve species identification of rare, fragile, and/or small organisms
- Add new character to discriminate morphologically cryptic species
- Reveal taxonomically important geographic variation within circumglobal species
- Discover new species for description by morphological taxonomists
- Rapidly identify species by querying DNA database
- Future: develop automatable DNA based approaches taxonomic analysis of samples



Zooplankton Genomics

ZooGene™



➤ Project funded 2000 – 2004

➤ Lead investigators – **Ann Bucklin (UNH/UConn)**, **Bruce Frost (UW)**, **Peter Wiebe (WHOI)** and **Mike Fogarty (NOAA/NMFS/NEFSC)** – with 13 expert taxonomists for crustacean zooplankton from 7 countries.

➤ MtCOI “reference DNA sequences” were determined for copepods and euphausiids

➤ Published studies: taxonomy, molecular systematics, phylogeny, phylogeography

www.ZooGene.org



CMarZ Overarching Question:

“what are the patterns of zooplankton biodiversity throughout the world ocean, and how are they generated and maintained”?

CMarZ Goals 2004 – 2010:

- Describe species diversity, biomass, biogeography, and genetic diversity of ~7,000 described species of holozooplankton by 2010
- Determine DNA barcodes for identified specimens
- Develop new sampling technologies, analytical tools
- Create distributed database with for all species data and information, with ‘Species Pages’
- Enhance education, professional training, and capacity-building in zooplankton taxonomy

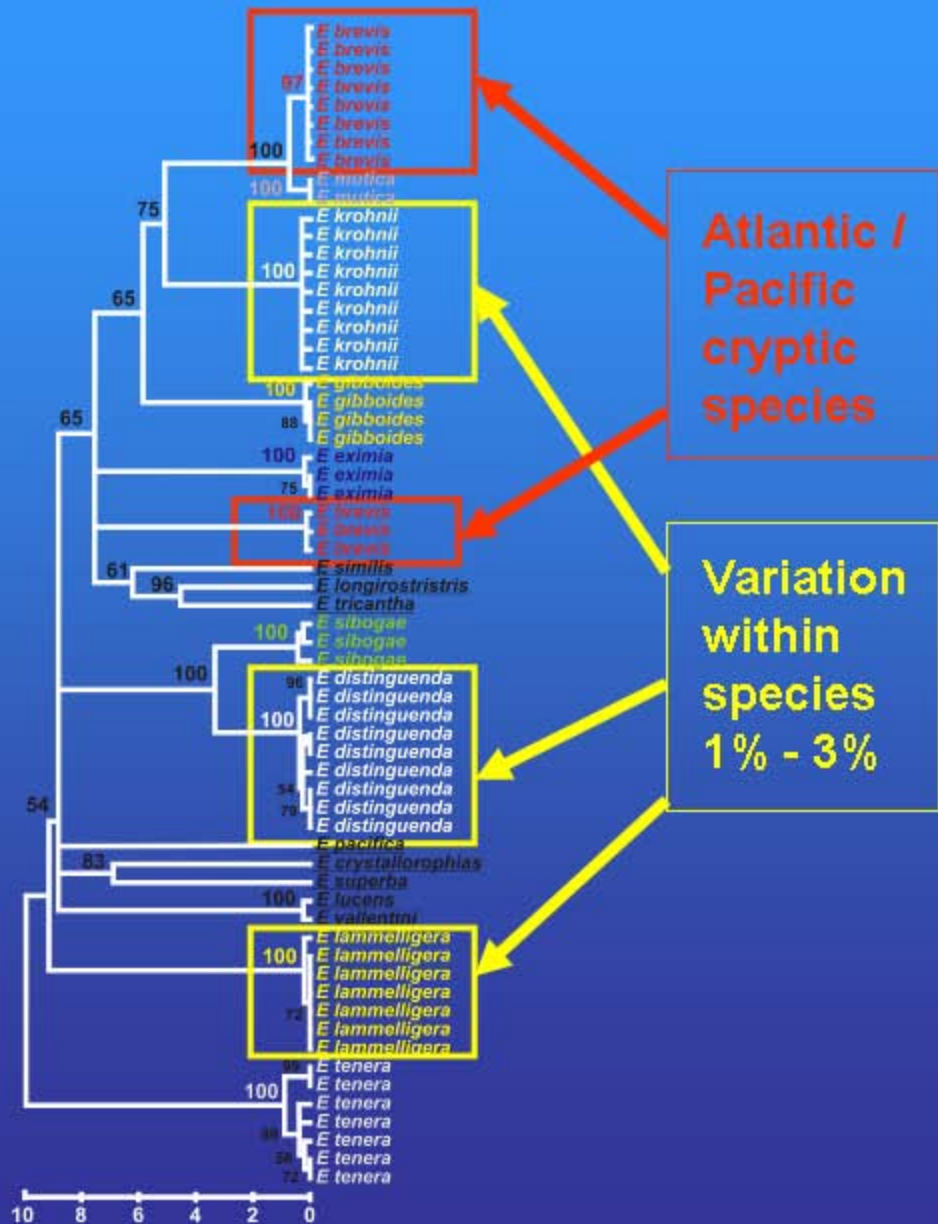
See www.CMarZ.org

Forty of 86 species barcoded by Bucklin, Wiebe et al. (2007), 13 more species by others.

Twenty of 31 species of *Euphausia* barcoded.

Barcodes accurately and uniquely identify and discriminate species

Barcodes can reveal cryptic species within widespread taxa.



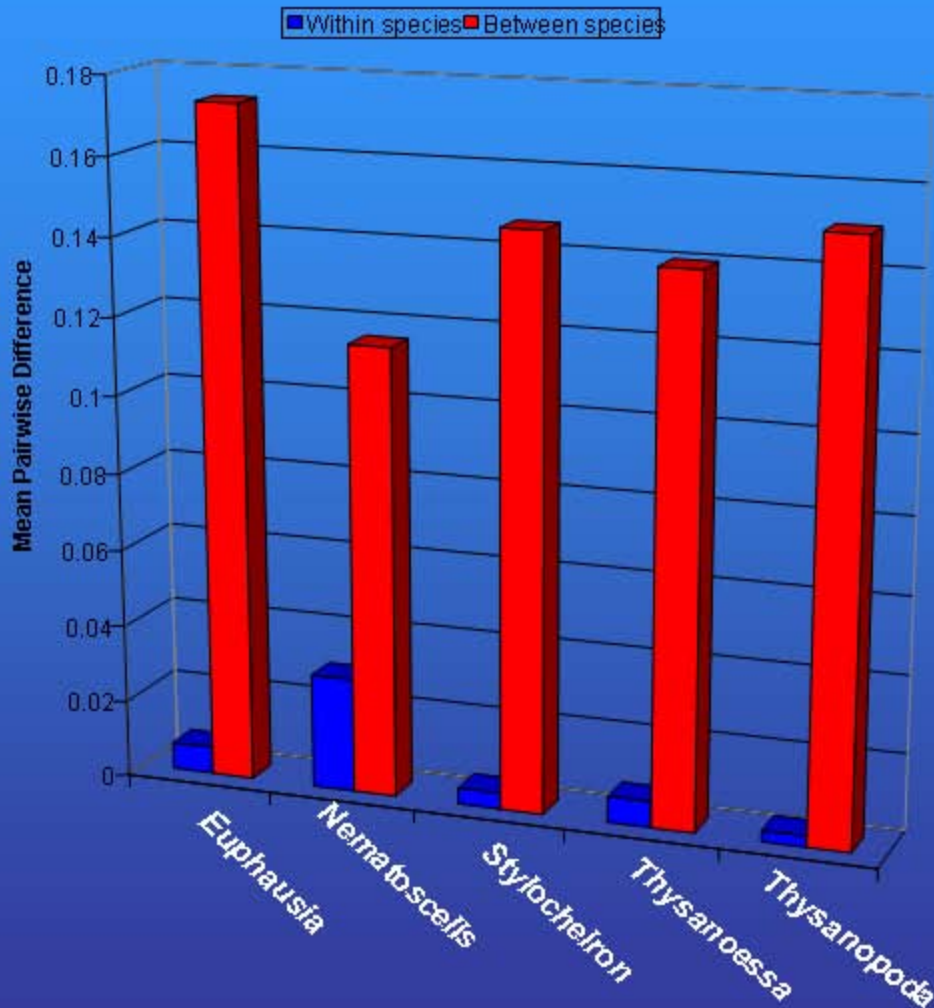
Atlantic /
Pacific
cryptic
species

Variation
within
species
1% - 3%

Variation
within
species
1% - 3%

MtCOI Variation of Euphausiids

Bucklin, Wiebe, et al. (2007) J. Plankton Res.



Pairwise differences in mtCOI sequences ranged from 1% to 3% within a given species.

Pairwise differences between species – of the same or different genera – ranged from 7% to 24%.

This pattern of sequence divergence is desirable and necessary for DNA barcoding.

MtCOI suggested one 'species group' of *Euphausia* is an evolutionary lineage.

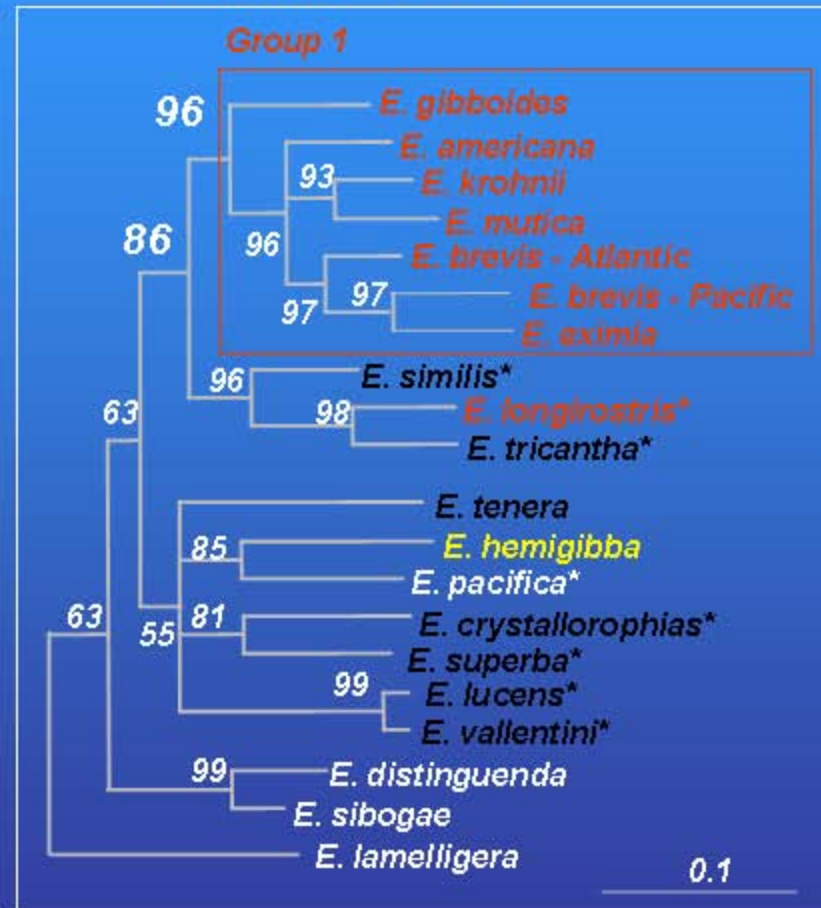
MtCOI resolves evolutionary relationships among closely related species of same genus.

MtCOI does not resolve deeper evolutionary relationships.

Species Groups



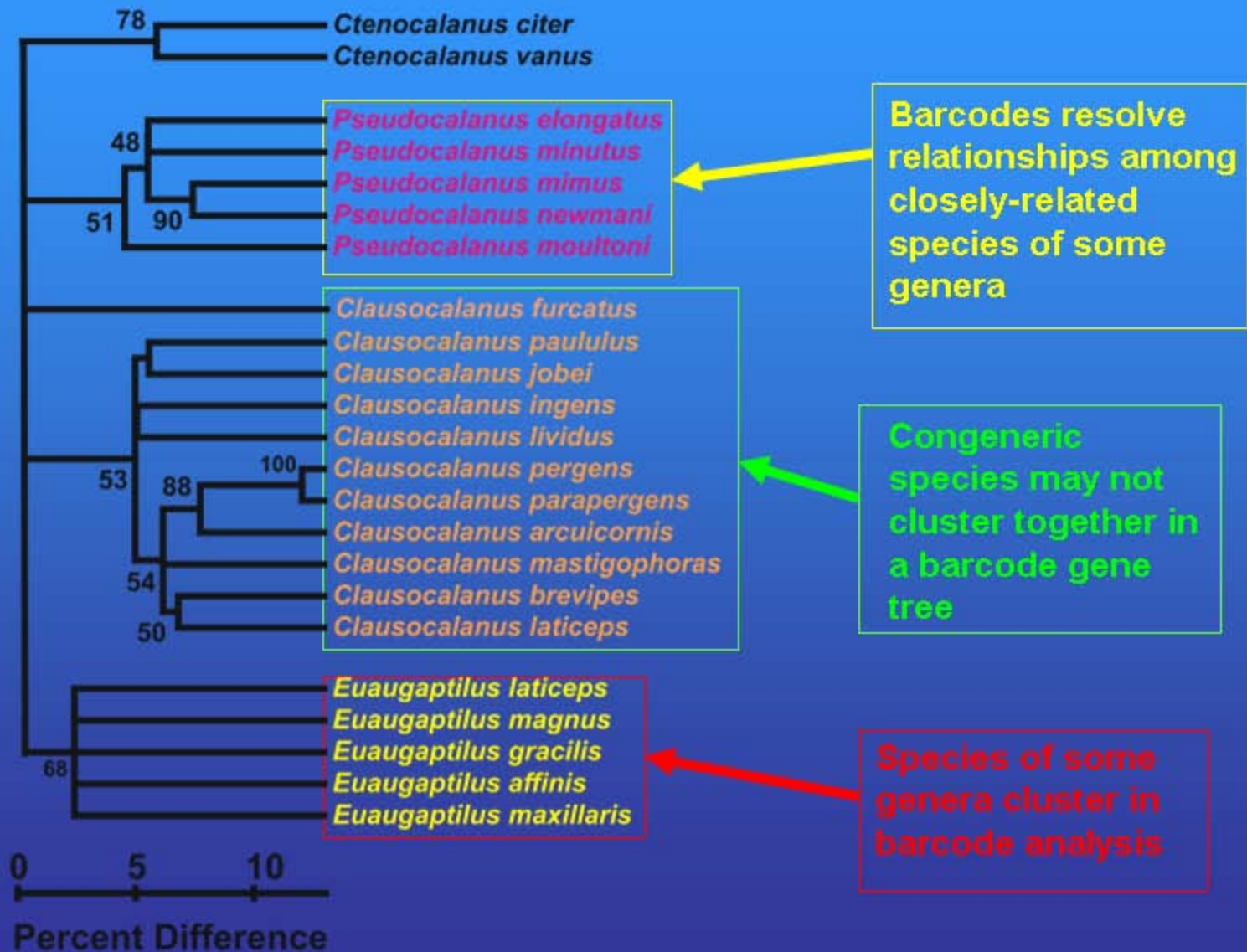
Molecular Phylogeny*



*Bayesian analysis, MrModeltest GTR+G+I model selection

Barcoding Copepods

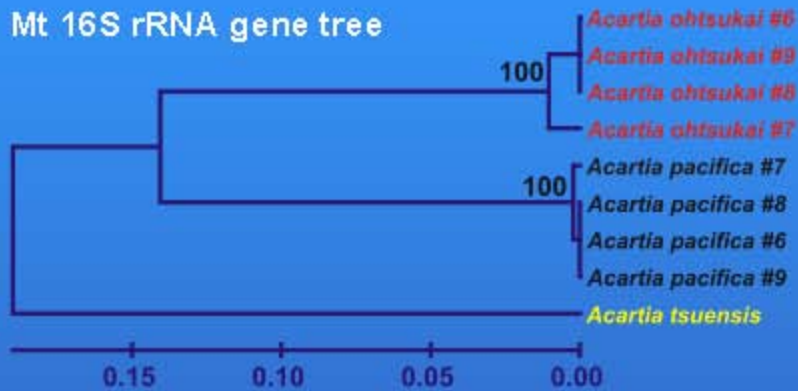
A. Bucklin, N.J. Copley, L. Nigro, CJ Sweetman, others



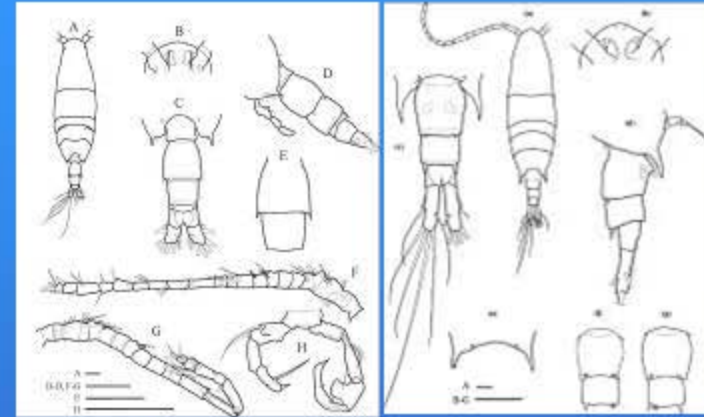
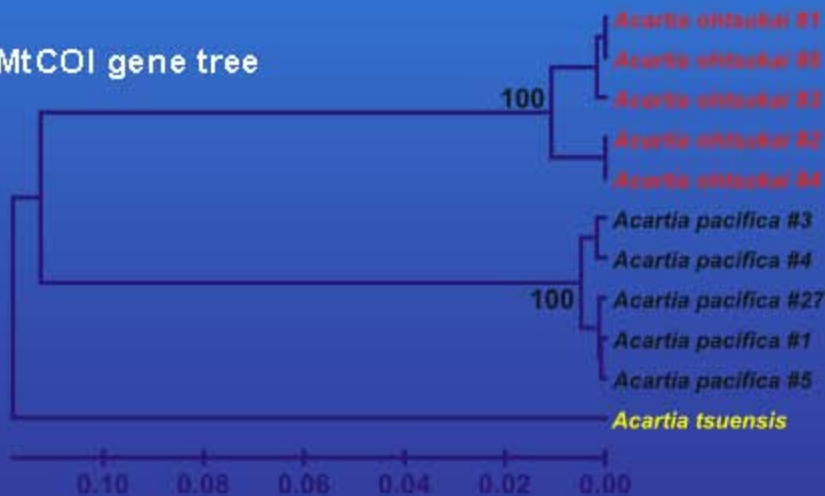
Barcoding Copepods: *Acartia*

Ueda and Bucklin (2005) *Hydrobiologia*

Mt 16S rRNA gene tree



MtCOI gene tree



Acartia ohtsukai (Ueda & Bucklin, 2005)

MtCOI and mt16S rRNA sequence divergence supported morphological taxonomic analysis.

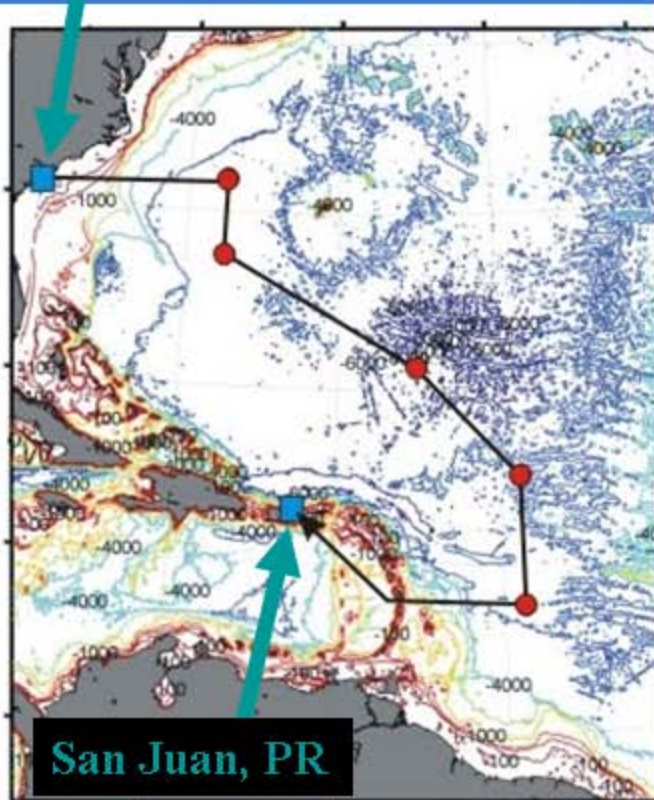
Species description of *Acartia ohtsukai* included both morphology and DNA.

Species cannot be described using DNA sequence data alone; DNA barcoding is not DNA taxonomy.

In April 2006, Peter Wiebe (WHOI) led a CMarZ biodiversity survey to sample zooplankton and fish from the deepest waters (to 5,000 m) of the Sargasso Sea in the NW Atlantic. Participants included CMarZ Steering Group members, students, staff from 14 countries. Funded by NOAA Ocean Exploration Program.



Charleston, SC



San Juan, PR





DNA Sequencing at Sea

UConn Team DNA set up a DNA sequencing laboratory on board the *RV Ron Brown* with PCR machines and ABI 3130 4-capillary DNA sequencer.



Brian Ortman, Rob Jennings, Paola Batta Lona, Ebru Unal, Leo Blanco Bercial, Jason Beaudet



UConn Team DNA

Barcoding Ostracods (Crustacea)

M. Angel, L. Nigro, others



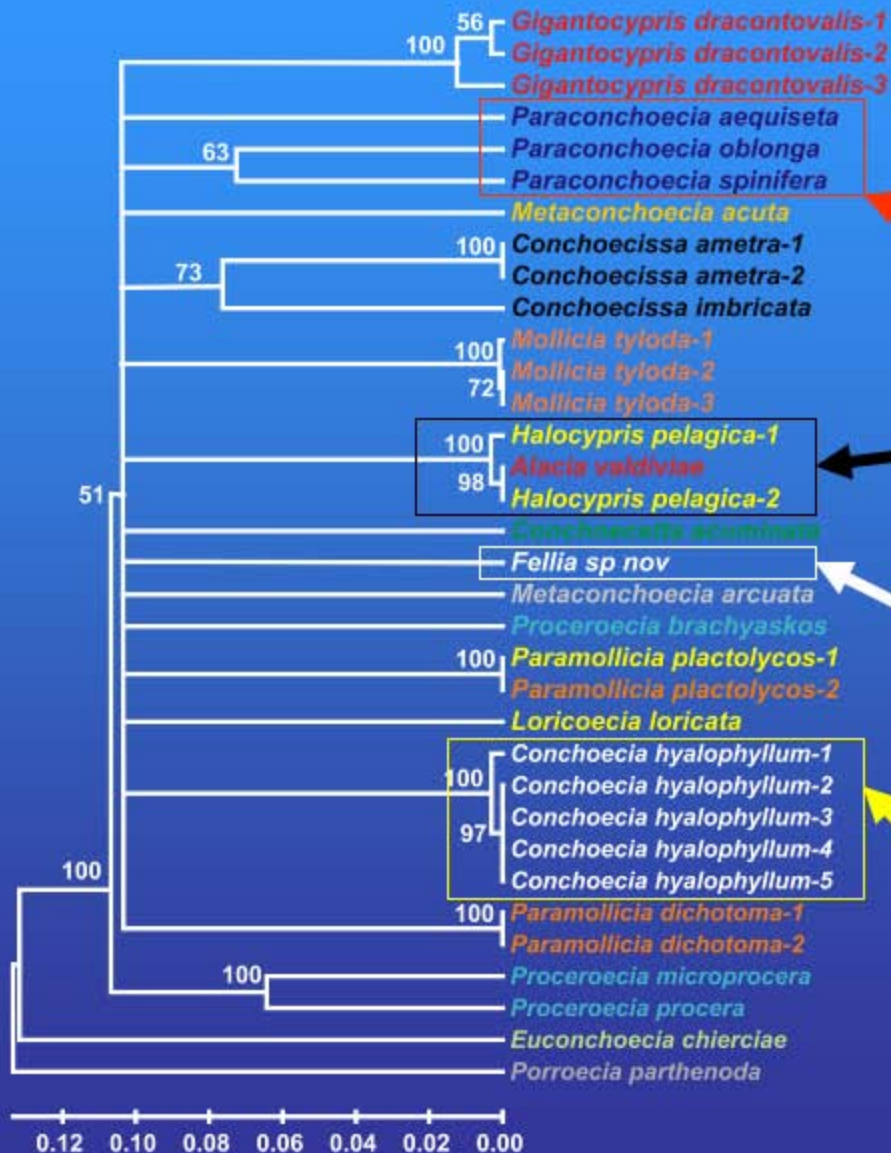
Martin Angel (center)



MtCOI barcodes clearly discriminate species.

Cluster diagrams are not optimized to resolve relationships among species; mtCOI may contain phylogenetic information.

Data: Lisa Nigro / UConn



Variation between species
15% - 26%

Species ID error?

New species discovery

Variation within species
1% - 3%

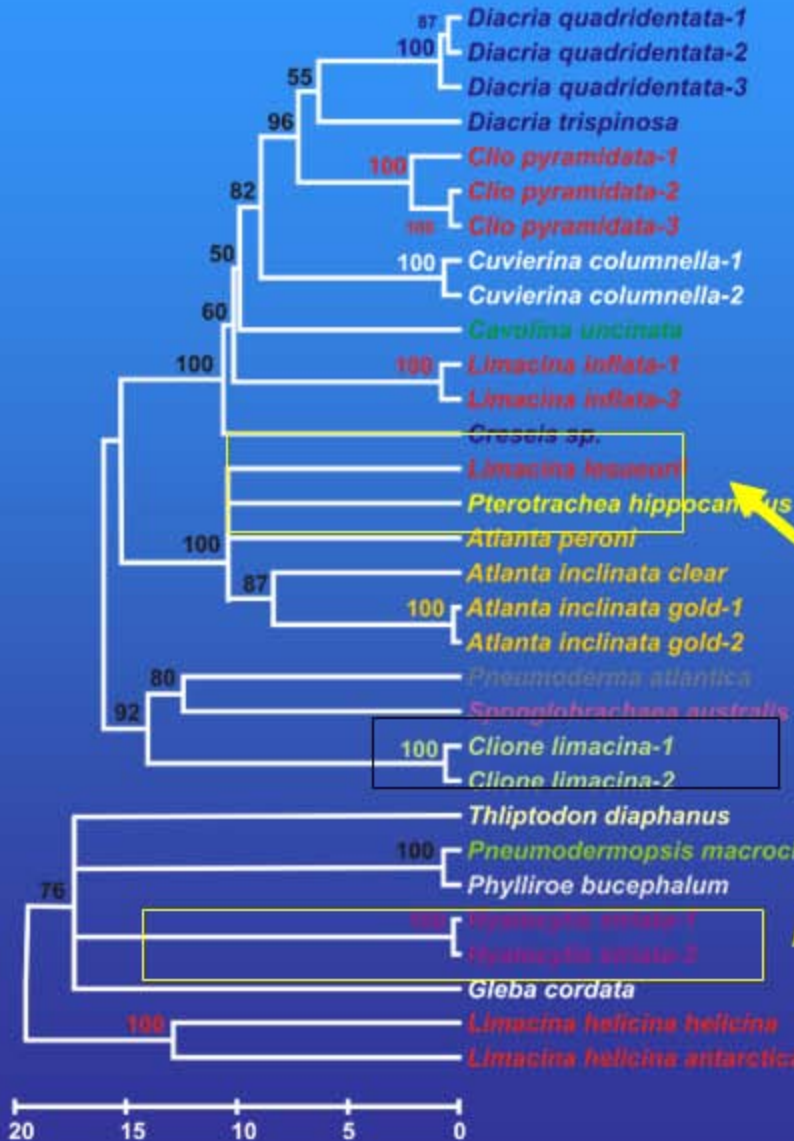
Barcoding Planktonic Gastropods

R.M. Jennings, R.R. Hopcroft, et al.



Russ Hopcroft and
Dhugal Lindsay at sea

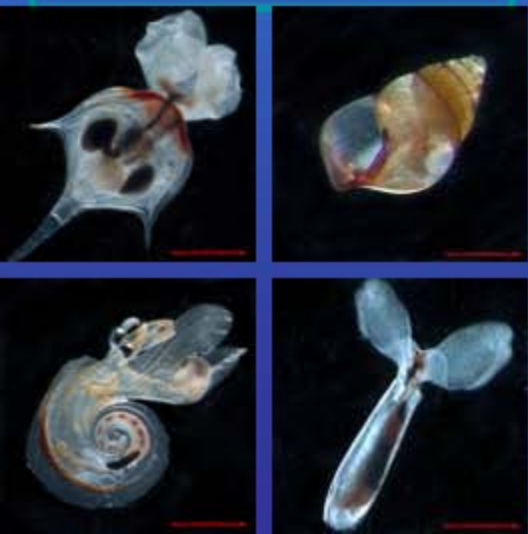
Group	Species Barcodes
Pteropods	36
Nudibranch	1
Octopods	5
Squids	6

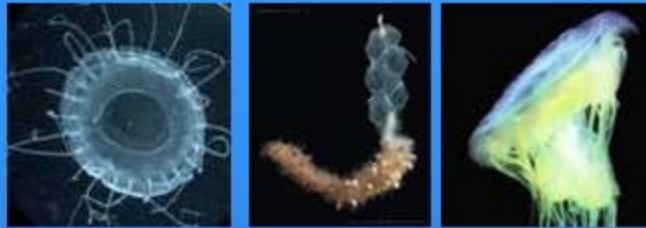


MtCOI barcodes differ by 14% - 40% between species of pteropods

Species ID and barcode validity need re-checking

Barcodes indicate morphological forms may be species





Francesc Pagès at sea

Francesc Pagès (ICM, Spain)
and Larry Madin (WHOI, USA);
barcoding by Brian Ortman
(UConn/USA)

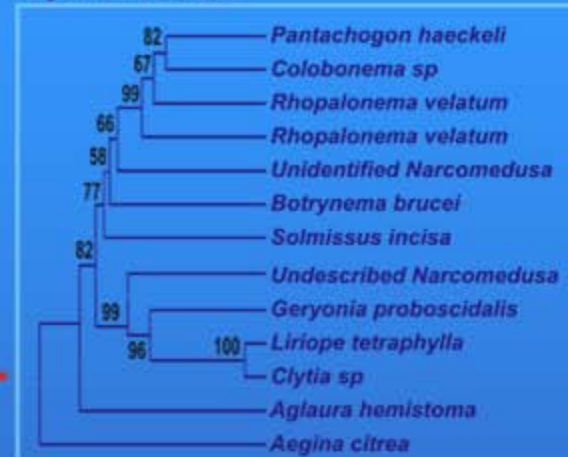
Species barcoded / collected

Hydromedusae: 13 / 22

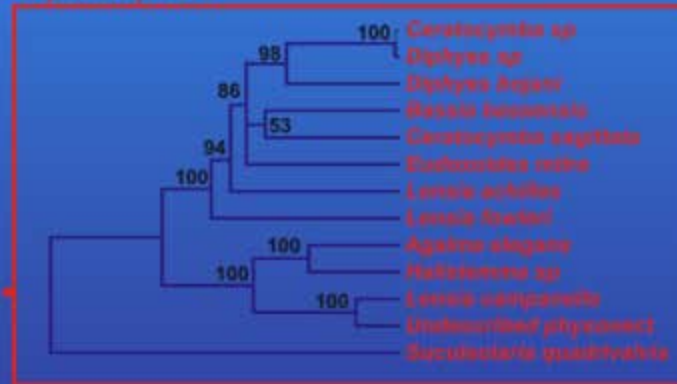
Siphonophores: 55 / 74

Scyphomedusae: 4 / 8

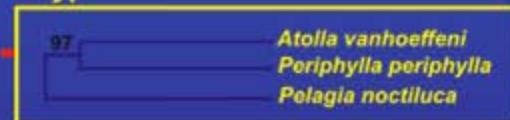
Hydromedusae



Siphonophora



Scyphozoa



30 25 20 15 10 5 0
Percentage difference

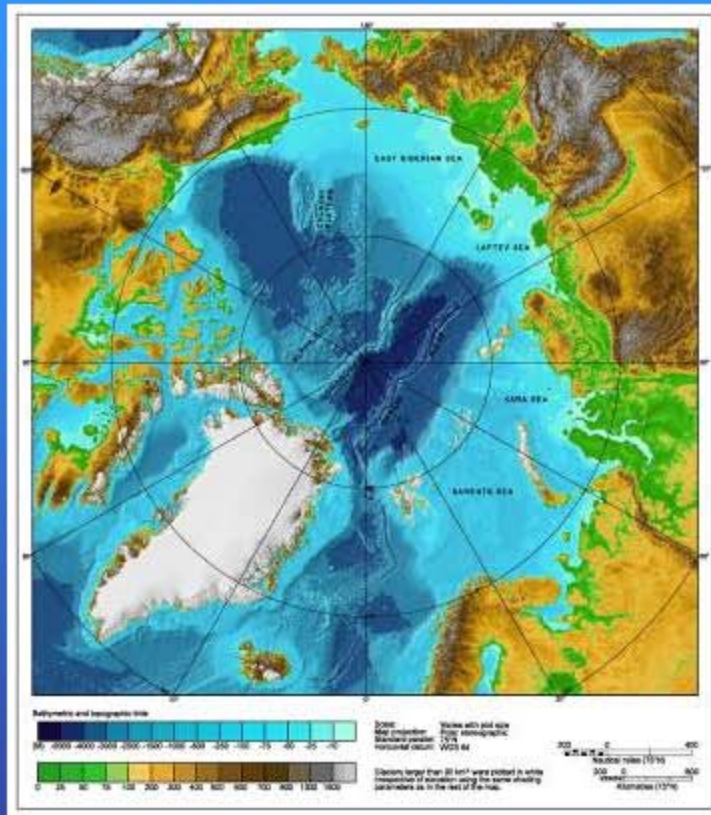


Group	-----Species-----			
	Collect	Barcode	Known	
Ctenophora	22	13	90	
Cnidaria	Hydromedusae	33	12	842
	Siphonophora	70	55	160
	Scyphozoa	7	4	161
Crustacea	Amphipoda	31	12	400
	Copepoda	138	38	2,000
	Euphausiidae	14	14	86
	Ostracoda	58	36	169
	Other Crustacea	18	15	23
Mollusca	Gastropoda	44	36	144
	Other Mollusca	27	12	--
Others	Larvacea	12	5	64
	Nemertea	1	1	99
	Polychaeta	3	3	110
	Thaliacea	14	2	45
Totals	534	258	4869	



Barcoding by Region: Arctic Ocean

R.R. Hopcroft (Univ. of Alaska), A. Bucklin (Univ. Conn), et al.



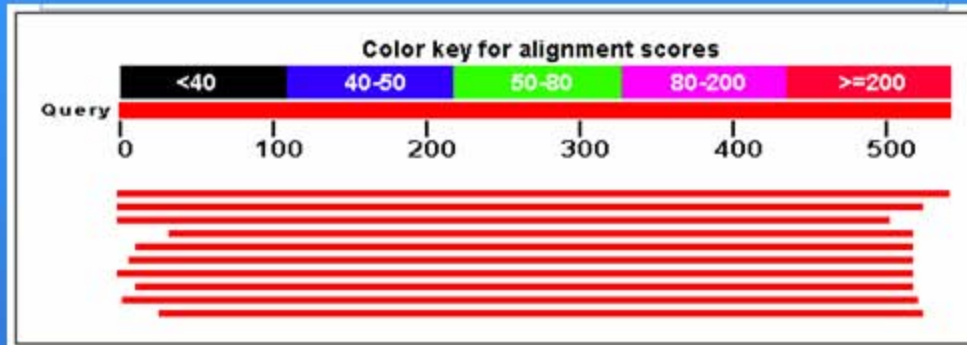
- ArcOD (Arctic Ocean Diversity) sends identified specimens of Arctic zooplankton for barcoding by CMarZ.
- DNA database now includes ~100 species of the Central Arctic assemblage.
- Goal is DNA barcoding of ~400 Arctic zooplankton species, including ~150 hydromedusae and ~150 copepods.



DNA Barcodes for Zooplankton

Phylum	Taxon	Species	GenBank	Unpubl	Source
Foraminifera	Foraminifera	49	35	200	C. deVargas
Actinopoda	Acantharea	150	14		GenBank
	Polycystinea (Rad)	350	27		GenBank
Cercozoa	Phaeodarea (Rad)	350	3		GenBank
Ciliophora	Aloricate Ciliata	150			
	Tintinnida	300	52		B. Costas
Cnidaria	Hydromedusae	842	5	25	GenBank
	Siphonophora	160	16	80	GenBank, S. Haddock, B. Ortman
	Cubomedusae	18	0		GenBank
	Scyphomedusae	161	10	15	GenBank, B. Ortman, others
Ctenophora	Ctenophora	90	20	12	GenBank, B. Ortman
Rotifera	Rotifera	50?			
Platyhelminthes	Platyhelminthes	3?	1	20	GenBank, M. Liviatos
Nematomorpha	Nectonema	5			
Nemertea	Nemertinea	99	1	3	GenBank, R. Jennings
Annelida	Polychaeta	110	5	4	GenBank, R. Jennings
Mollusca	Gastropoda	144	100	30	GenBank, R. Jennings
	Cephalopoda	370	86	5	GenBank, R. Jennings
Arthropoda	Cladocera	8			
	Ostracoda	169	0	35	A. Bucklin
	Isopoda	20	0	14	S. Brix
	Copepoda	2000	41	275	A. Bucklin, R. Machida, others
	Mysidacea	700	4		GenBank
	Amphipoda	400	15	50	GenBank
	Euphausiacea	86	45	10	A. Bucklin, S. Jarman
	Decapoda	50	75	100	GenBank, K. Crandall
	Insecta	5		5	R. Jennings
Chaetognatha	Chaetognatha	93	10	30	A. Pierrot-Bults, R. Machida, R. Jennings
Chordata	Appendicularia	64	1	10	L. Madin, A. Bucklin, GenBank
	Pyrosoma	8	1		GenBank, Wada
	Doliolida	17	1		GenBank, Wada
	Salpidae	45	0	5	L. Madin, A. Bucklin
TOTALS		7,013	568	928	
TOTALS (no Protista)		5,664	437	728	

BLAST results showing sequence overlap and ranked alignment scores



Minimum Evolution Tree Generated by GenBank BLAST

Maximum
Similarity



UNKNOWN clusters with mtCOI sequences from two different studies: AF513649, Bucklin, Frost, et al. (2003) and AY145431, Eyun, Soh, et al. (2002)

MtCOI sequence must be in database to identify unknown specimen.

GenBank BLAST search algorithm returns maximum similarity percent and scores.

Many species differ by ~ 15% - 25% regardless of taxonomic separation.

No trustworthy information if unknown is not in GenBank

Barcodes from different laboratories will match, assuming specimens collected from same location or little or no geographic variation.

Barcode Applications: A Rosetta Stone



Hippopodius hippopus



Sapphirina metallina



Limacina helicina



Salpa cylindrica

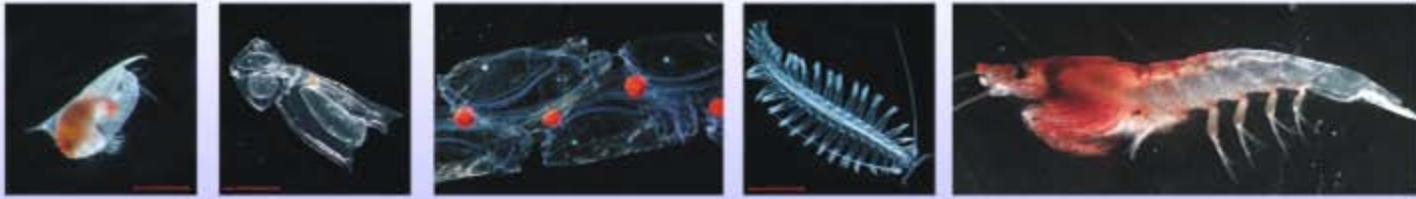


- **Rosetta stone:** a key for decryption or translation; places terms from different languages side-by-side to allow rapid conversion from one to another.
- **DNA barcoding** translates between species name, morphology, and DNA sequence.
- Neither morphology nor genetics should be over-simplified; both can assess variation within and between populations and species, evolutionary relationships among species and higher taxa, and processes associated with speciation.
- Much may be lost in translation, but goals of morphologists and geneticists are the same: to gain genuine knowledge of species diversity of life in the ocean.

Barcode Applications: DNA-based Taxonomic Analysis



- DNA barcode database will provide useful means to identify known species; reveal cryptic, unknown, or undescribed species.
- Known species for which barcodes are present in database can be accurately and reliably identified using barcodes.
- Rapid species detection may eventually be done using region- and taxon-specific DNA microarrays.
- Lab-on-a-chip miniaturization and automation may allow remote species detection from oceanographic platforms.

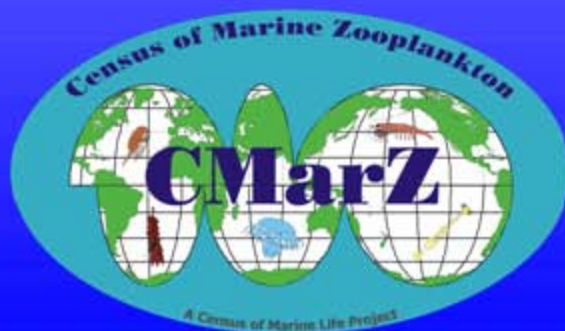


Barcoding Marine Zooplankton

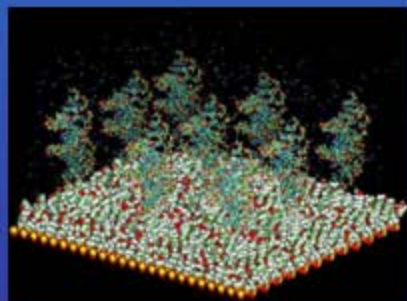
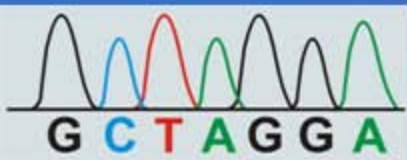
Got specimens? CMarZ encourages and challenges the CoML community to provide identified specimens of holozooplankton groups for barcoding. A DNA barcode (700 base-pair region of mtCOI with replicates / metadata) will be determined for appropriately preserved (i.e., frozen or in alcohol) and identified holozooplankton specimens. Contact Rob Jennings (robert.jennings@uconn.edu).

Got data? DNA barcodes for zooplankton should be submitted to:

1. BARCODE section of GenBank (www.ncbi.nlm.nih.gov/BankIt/barcode)
2. Barcode of Life Database (BOLD; see www.barcodinglife.org).

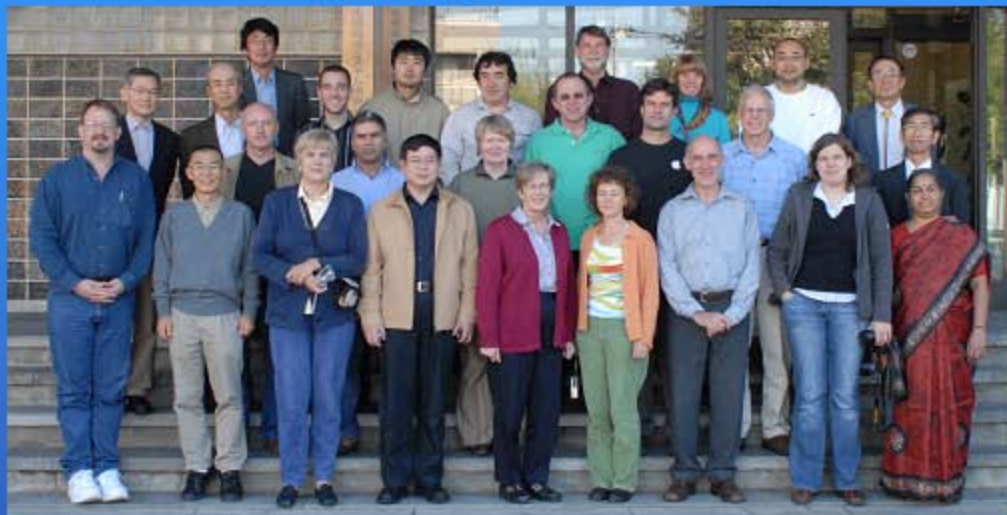


UConn address for specimen shipment:
Ann Bucklin
Department of Marine Sciences
University of Connecticut - Avery Point
1080 Shennecossett Road, Groton, CT 06340 USA
Email: ann.bucklin@uconn.edu

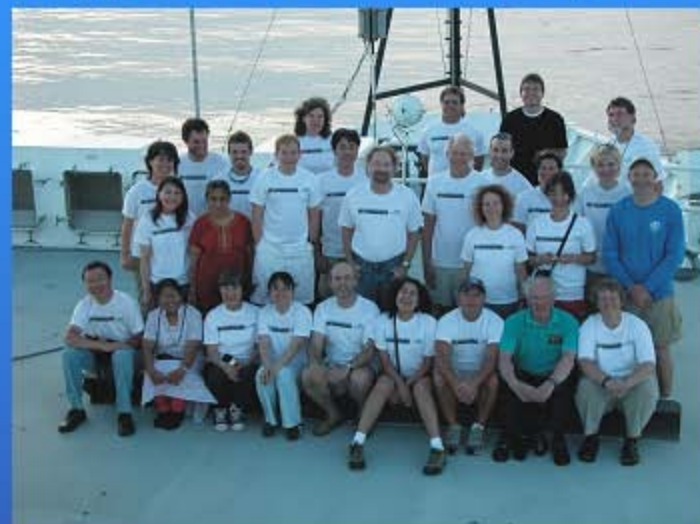


- DNA barcoding of described species of marine holozooplankton is about 20% complete, with plans for completion by 2010.
- CMarZ is working toward a global biodiversity assessment, using ships of opportunity, dedicated cruises.
- Integrated morphological and molecular analysis of zooplankton species is needed for accurate biodiversity assessments.
- At-sea taxonomic analysis of samples and DNA sequencing is an effective and efficient approach to barcoding zooplankton.
- New species of zooplankton will be found in biodiversity hotspots, under-sampled regions (deep sea), in rare and fragile groups, and within geographically-widespread species.

CMarZ Steering Group members



CMarZ/NOAA Ocean Exploration
Sargasso Sea Cruise Participants



UConn Team DNA

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- The Alfred P. Sloan Foundation
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- US NSF Biological Oceanography
- US NSF Office of Polar Programs