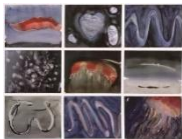


Name that Species

The Need for Reference DNA Barcode Databases for Analysis of Zooplankton Diversity using Barcoding and Metabarcoding



Ann Bucklin
Department of Marine Sciences
University of Connecticut



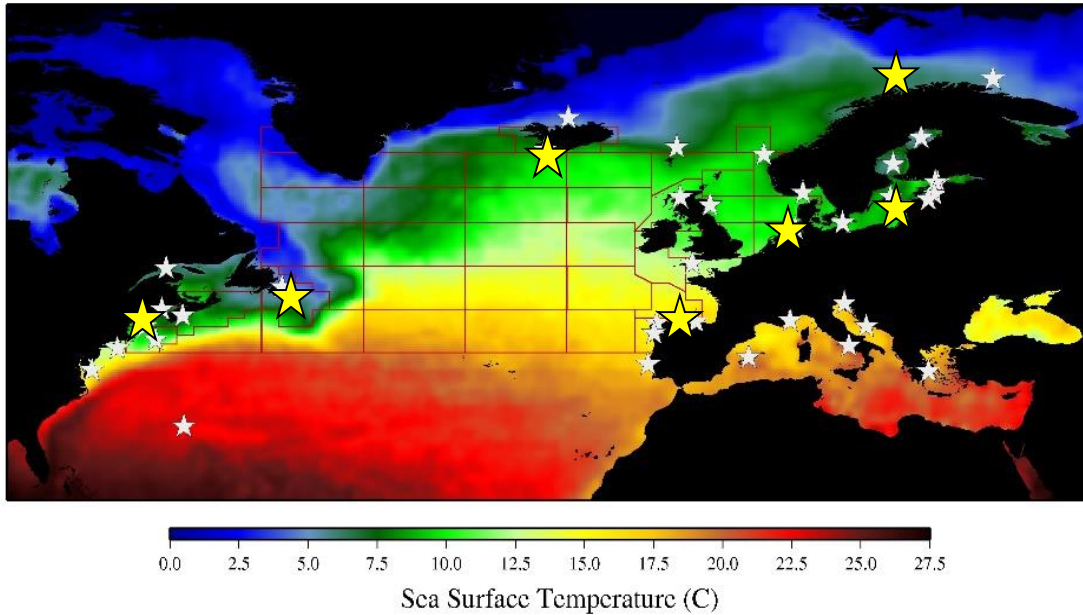
ICES/PICES 6th Zooplankton Production Symposium
*Workshop 7. Toward a taxonomically-comprehensive global
reference database for DNA barcodes of marine zooplankton.*
May 11, 2016 – Bergen, Norway

Outline of Presentation

- Importance of analyzing species-level diversity
- Integrative taxonomy
- DNA barcoding: COI and other barcode genes
- Metabarcoding: promise and pitfalls
- Call to action: Need for reference sequence databases



Why Analyze Species-level Diversity: Species are Better Indicators

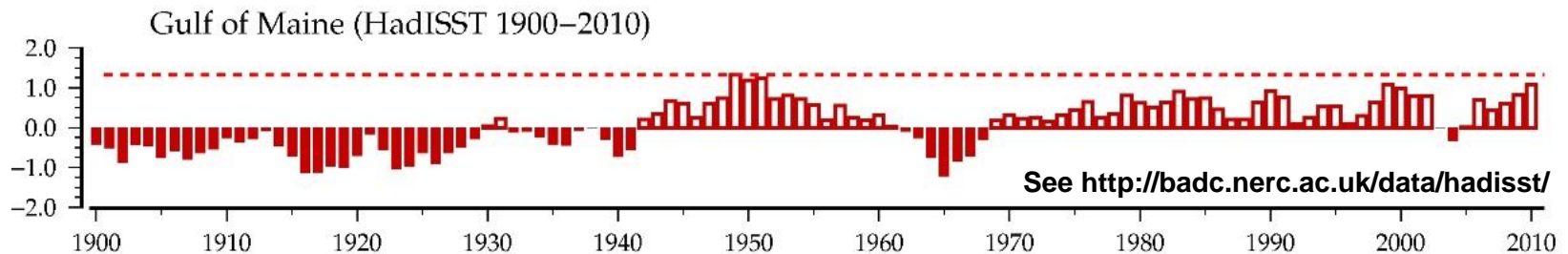


40 zooplankton monitoring sites (white stars) and
7 Continuous Plankton Recorder standard areas.

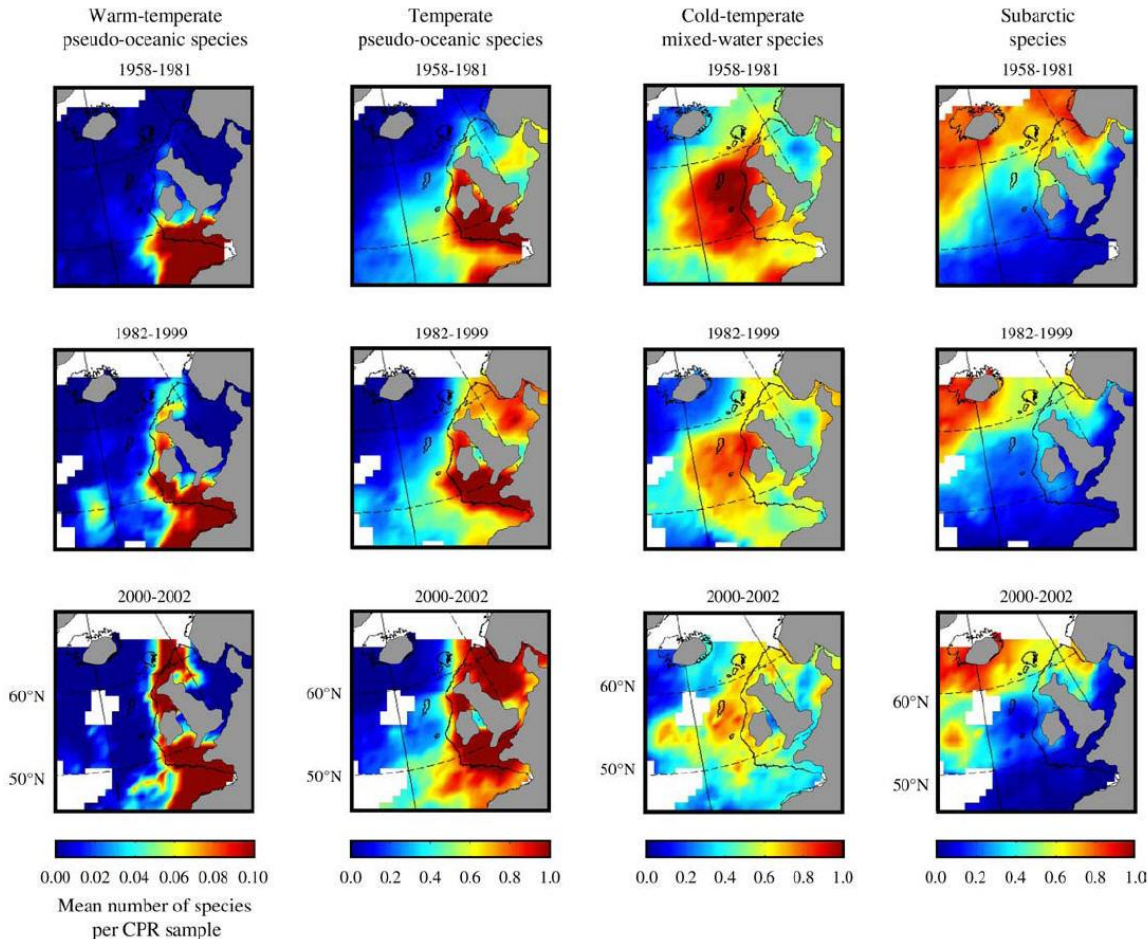
*From: O'Brien, Wiebe, and Falkenhaus (2013)
ICES Zooplankton Status Report*

- Species provide more information to evaluate nature of changes and biological / environmental mechanisms responsible.
- Changes in species abundance / biomass, phenology show larger changes than biomass.

Mackas and Beaugrand (2010)
J. Marine Systems 79: 286–304



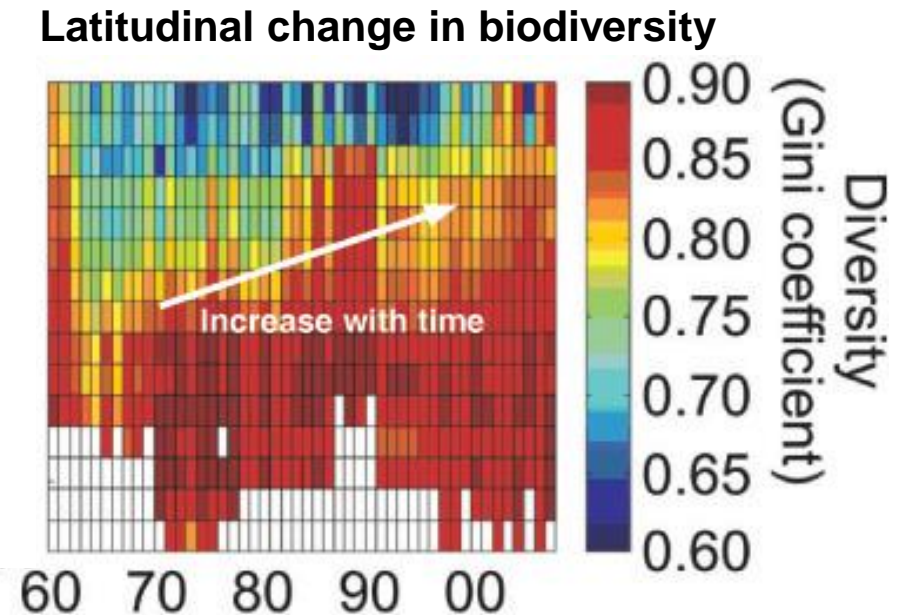
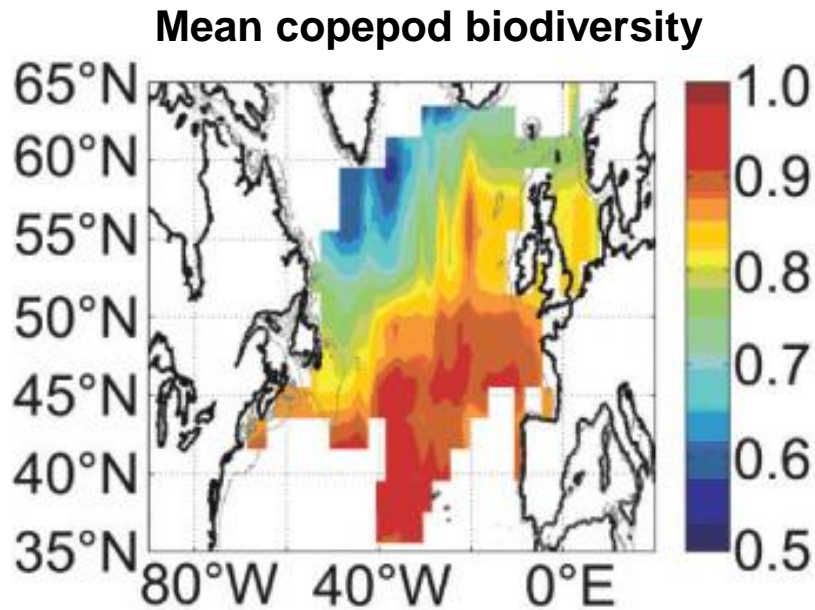
Why Analyze Species-level Diversity: Species-Specific Impacts of Warming



- Long-term changes in species abundances are coincident with NE Atlantic warming.
- Abundances based on Continuous Plankton Recorder (CPR) surveys.
- Warm-water species distributions extended north by $>10^\circ$ latitude.
- Cold-water species decreased in abundance and range.

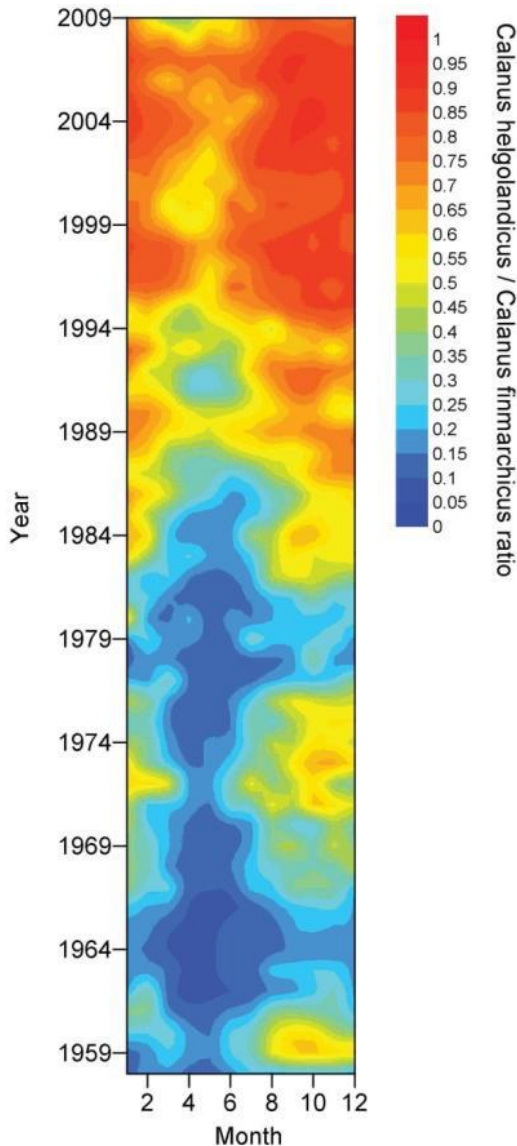
Why Analyze Species-level Diversity: Species-Specific Impacts of Warming

Multi-decadal northward shift in copepod biodiversity coincide with increased SST at high latitudes.

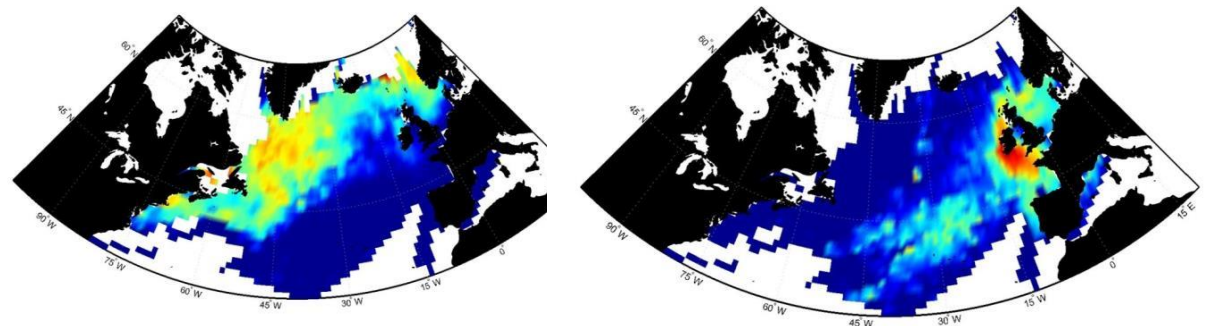


Beaugrand et al. (2010) PNAS 107: 10120–10124

Why Analyze Species-level Diversity: Species-Specific Impacts of Warming



- Dramatic shift in ratio of two *Calanus* species in North Sea: *C. helgolandicus* increases, *C. finmarchicus* decreases.
- Differences in species life history timing and role in food web impact whole ecosystem.

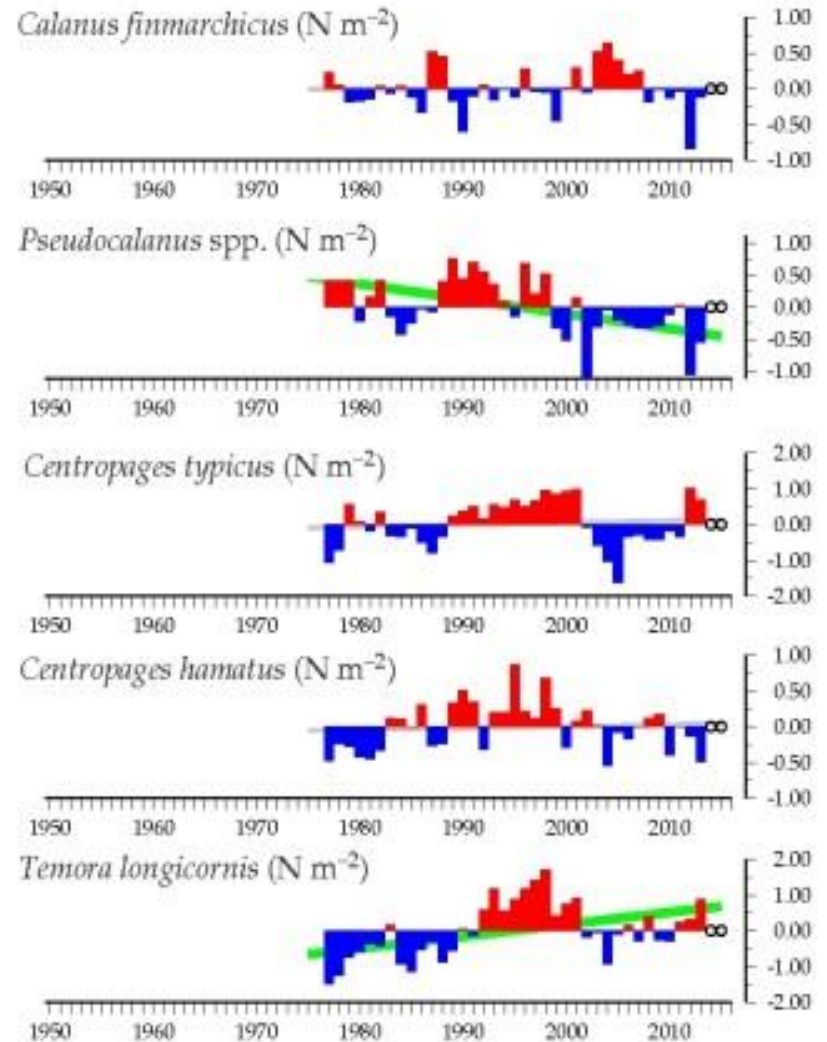
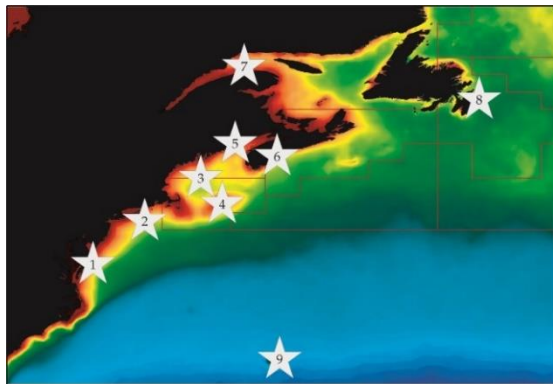
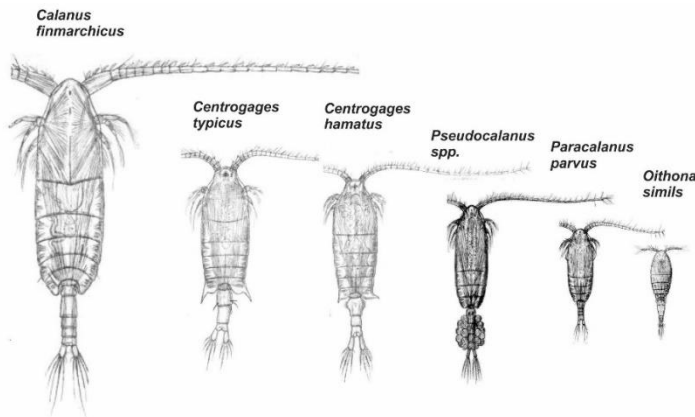


Mean spatial average for the last 50 years: *Calanus finmarchicus* (left) and *C. helgolandicus* (right)

From: SAHFOS Marine Ecological Status Report 2010

Why Analyze Species-level Diversity: Species-Specific Responses to Large-Scale Forcing

Dominant copepods on Georges Bank show species-specific patterns of decadal variability associated with Arctic Ocean Oscillation (AOO).



O'Brien, Wiebe, and Falkenhaus (2013)

Contributions of Integrative Taxonomy to Biodiversity Science

Integrative taxonomy: science that aims to delimit biodiversity from multiple and complementary perspectives: phylogeography, morphology, population genetics, ecology, development, behavior, etc.

-- Dayrat (2005) Biol J Linnean Soc

Applications of integrative taxonomy:

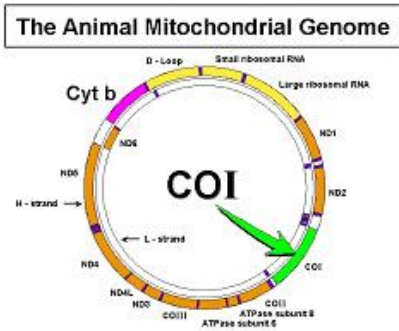
- 1) Biodiversity assessments of marine communities.**
- 2) Detection and identification of invasive non-indigenous species.**
- 3) Trophic relationships and food web structure.**



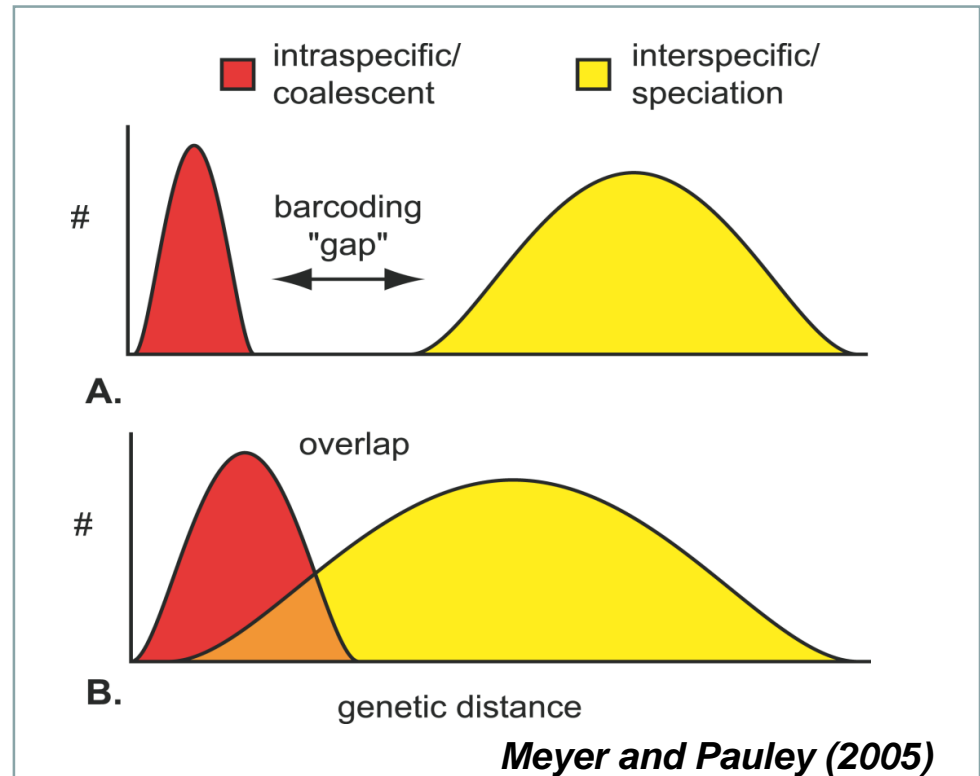
DNA Barcoding: COI

Barcodes are short DNA sequences that enable species recognition and discrimination.

For animals, usual barcode is 658 base-pair fragment of mitochondrial cytochrome oxidase I (COI).



- **Most invertebrate zooplankton groups show non-overlapping distances within-versus between-species.**
- **The “barcode gap” allows species identification**



Integrative Taxonomy in Practice

Identifying species at sea



PCR machines

Ship-board DNA sequencer



Analyzing DNA data at sea

Photos P. Wiebe

Barcoding at sea

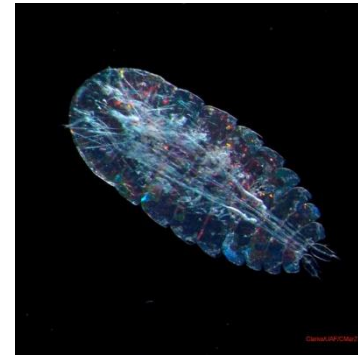
- Collection, identification, photography, DNA extraction, DNA sequencing.
- Carried out onboard the R/V *RH BROWN* in the Sargasso Sea (2006)



Scina crassicornis



Mollicia tyloda



Sapphirina metallina



Applications of COI Barcodes

Species identification: ‘Gold standard’ barcodes based on specimen identified by taxonomic expert, georeferenced, archived vouchers.

Discrimination of cryptic species: Barcodes can reveal cryptic, rare and unexpected species within known taxa.

Phylogeography: Barcodes used to describe geographic distribution of genetic lineages; large-scale population genetic structure, connectivity, and migration.

Phylogeny: Barcodes contribute to multi-gene phylogenetic analysis.

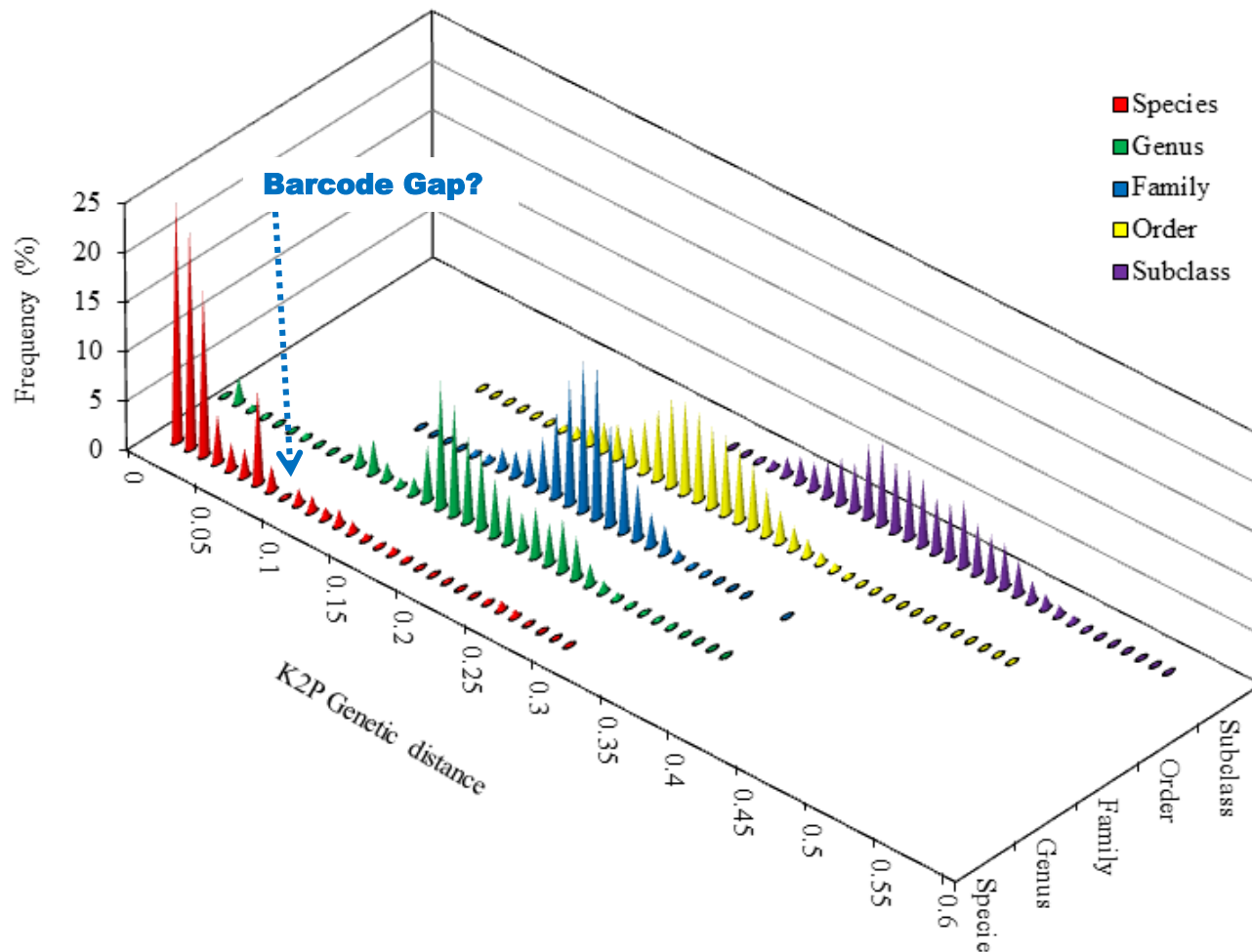
Diversity: Species diversity decoded by linking species names, morphology, and DNA sequence variation in reference database.



Barcoding Marine Copepods

Blanco-Bercial, Cornils, Copley, and Bucklin (2014) PLOS Currents - Tree of Life

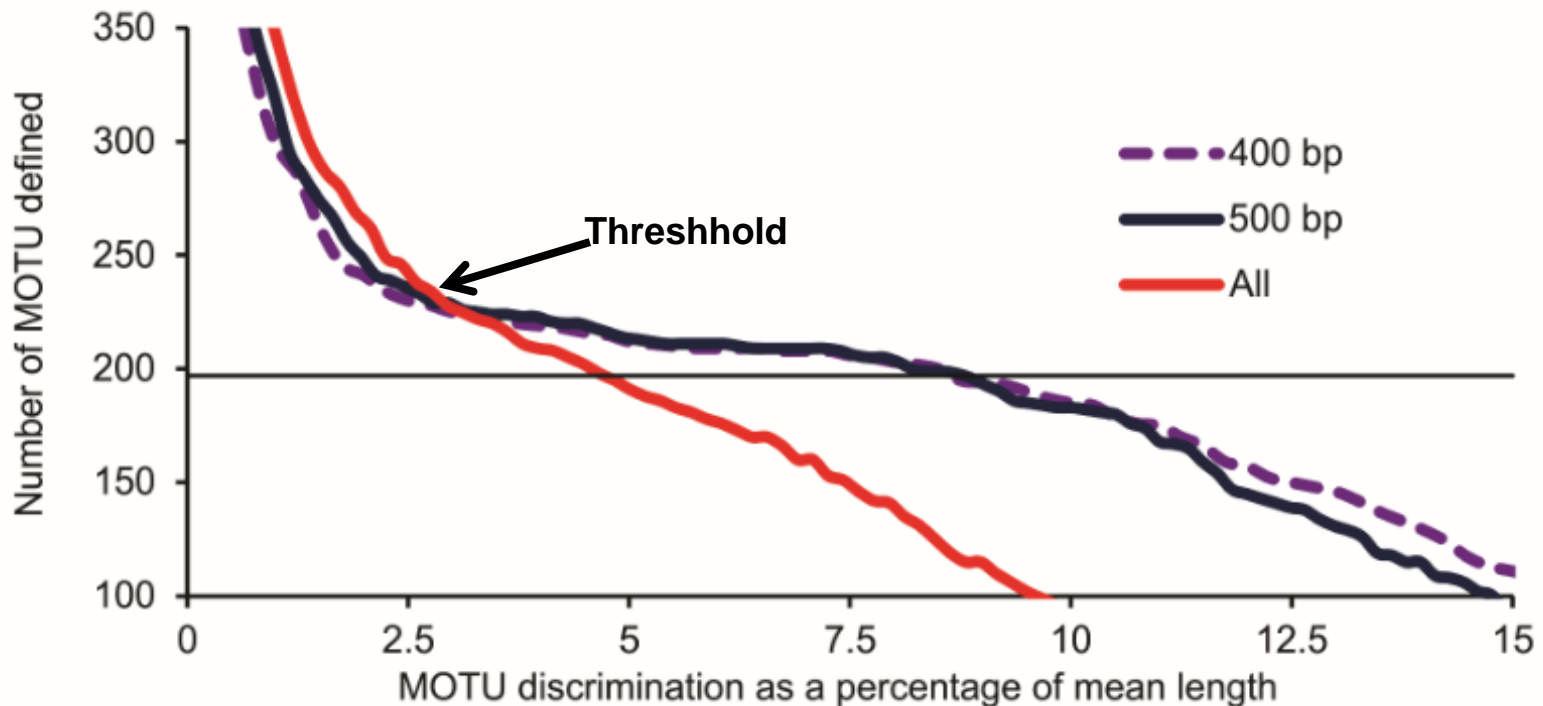
- Total of 1,381 sequences for 195 copepod species
- Kimura-2-Paramater (K2P) distances between > within species ($p < 0.001$)
- Some overlap within / between species due to geographic variation, cryptic species; no clear barcode gap.



Barcoding Marine Copepods

Blanco-Bercial, Cornils, Copley, and Bucklin (2014) PLOS Currents - Tree of Life

Discrepancies between MOTUs (~220 at thresholds) and OTUs (195 at line) due to geographic variation, cryptic species, etc



Data analysis: 1,381 barcodes for 195 species. Number of MOTUs inferred in jMOTU based on thresholds for species-recognition (% of mean barcode length) based on 3 alignments.

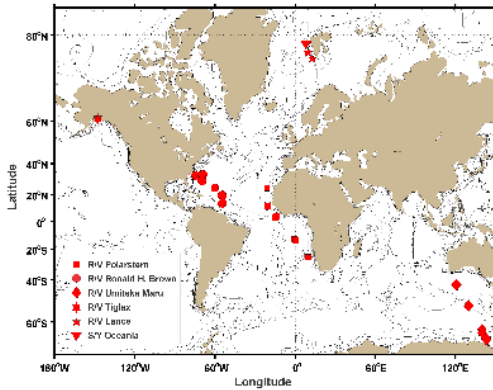
Barcoding Marine Ostracods

Nigro, Angel, Blachowiak-Samolyk, Hopcroft and Bucklin (2016) PLOS-One

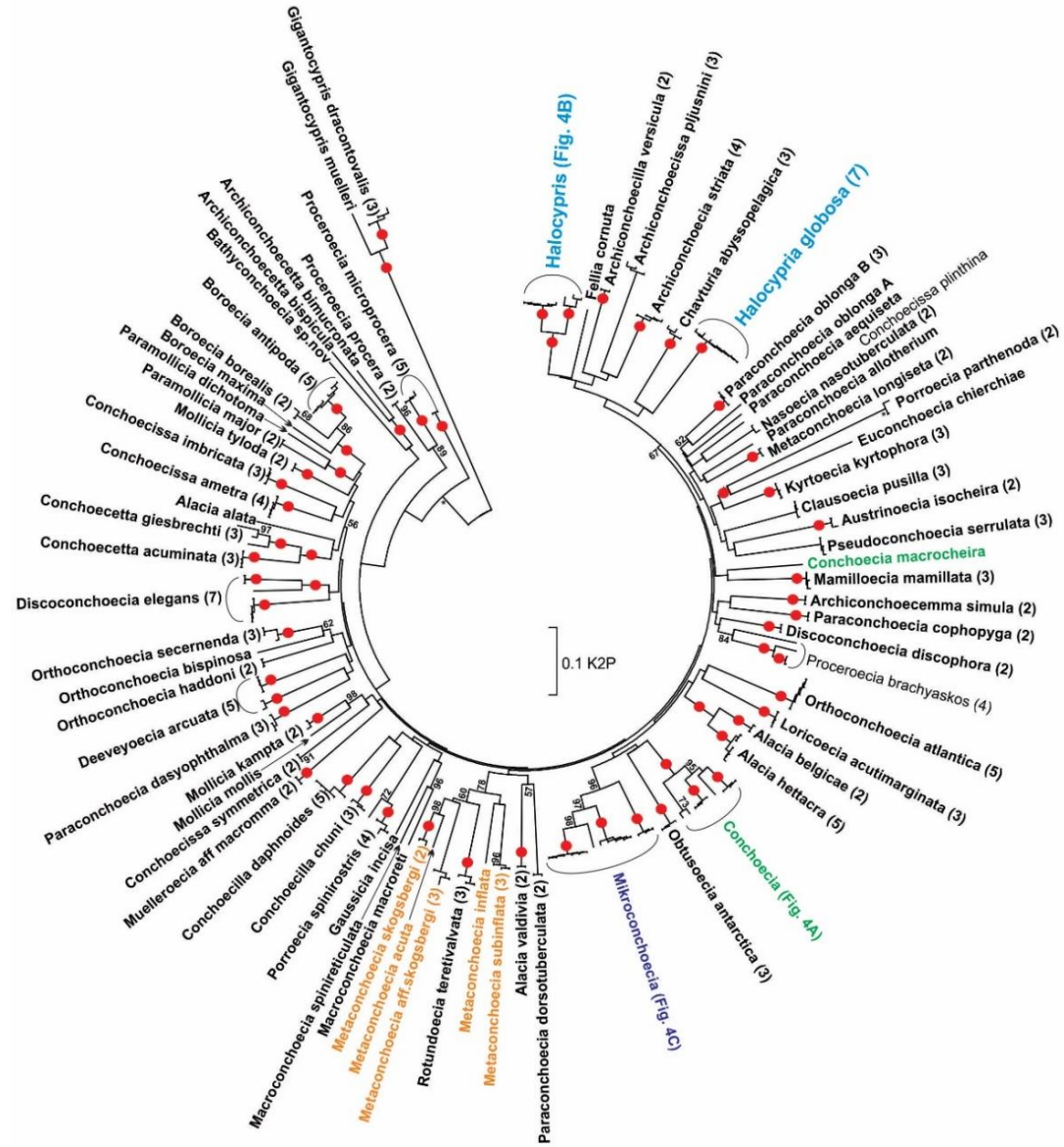


Barcodes for:

- 210 specimens
- 79 species



Specimens collected from Atlantic, Indian, Southern Oceans; Greenland Sea, Gulf of Alaska.



Neighbor Joining tree, K2P distances, 1000X bootstrap

Cryptic Speciation in *Paracalanus parvus* (Copepoda)

Cornils and Held (2014) *Frontiers in Zoology*

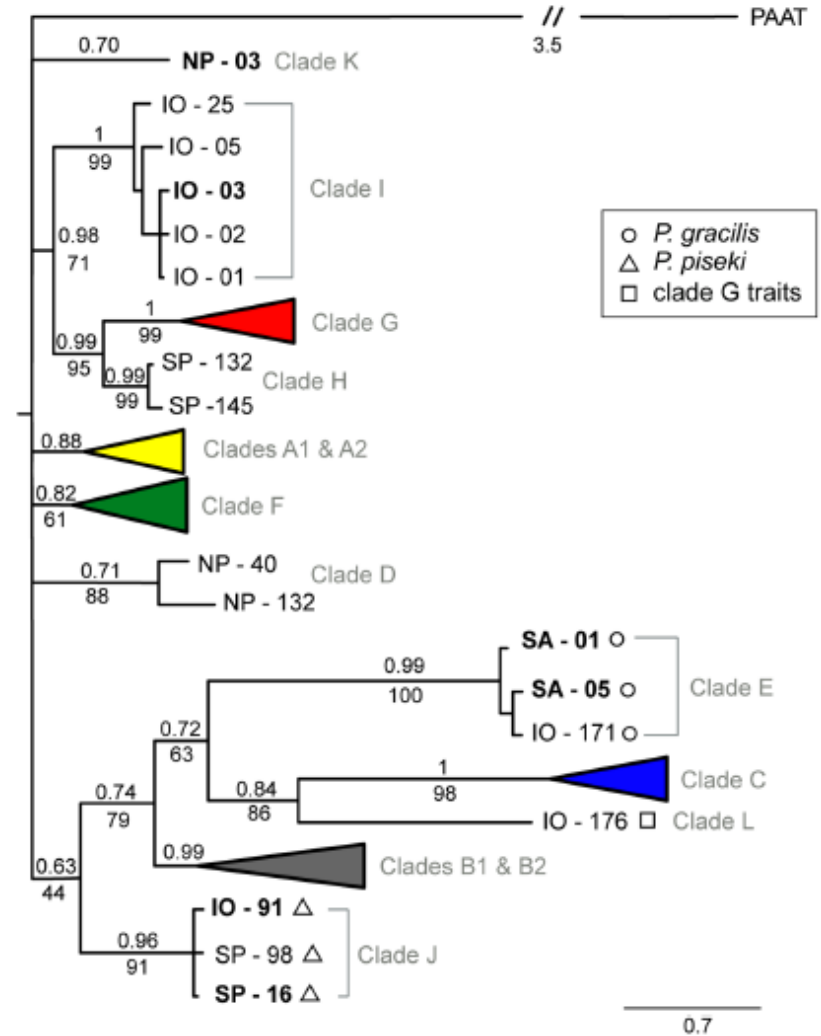
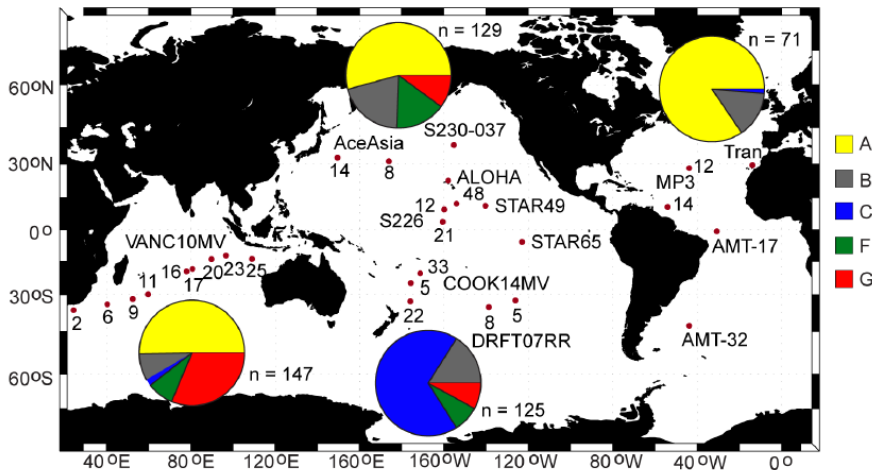
- COI and Cytochrome b sequences analyzed for 160 specimens of copepod *Paracalanus parvus* complex from all oceans
- COI is good indicator of specimen identification in *Paracalanus*.
- Species delimitation methods were largely congruent,
- Evidence of cryptic or pseudocryptic speciation, with 10-12 putative species.



Cryptic Speciation within *Pleuromamma* (Copepoda)

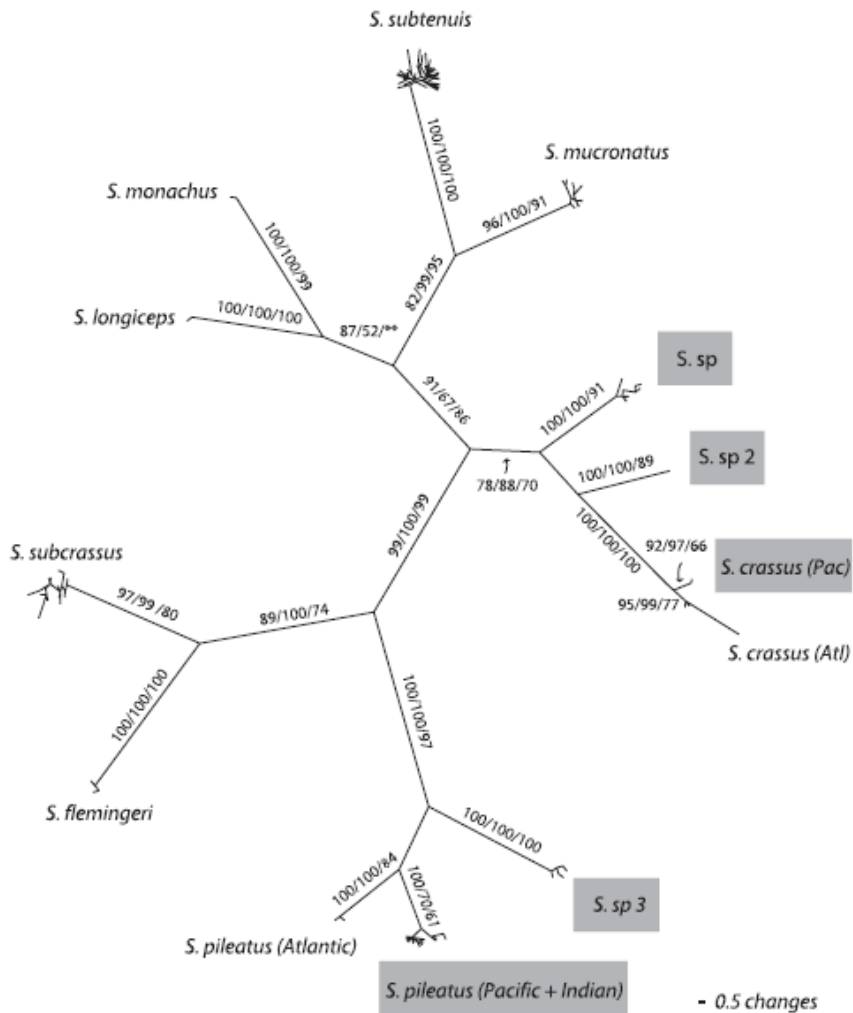
Halbert, Goetze, Carlon (2013) PLOS-One 8: e77011

- Analysis of *Pleuromamma* species complex in Atlantic, Pacific and Indian Oceans.
- Cytochrome Oxidase II barcode gene
- Cryptic clades within morphologically described species

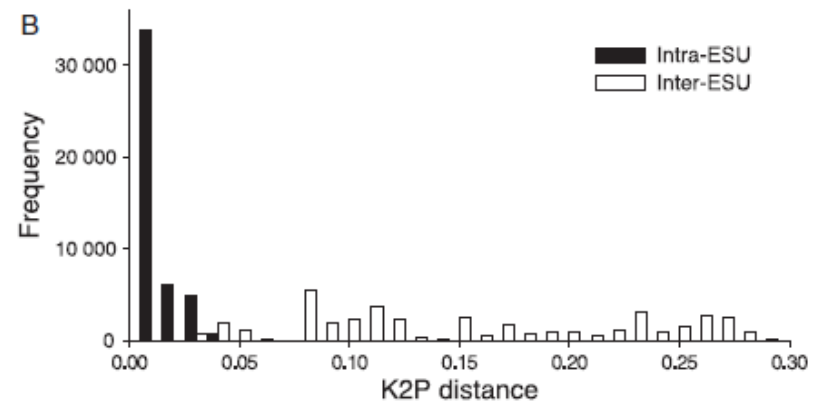


Cryptic Diversity of Eucalanid Copepods - 16S rRNA

Goetze (2010) Molecular Ecology 19: 952–967

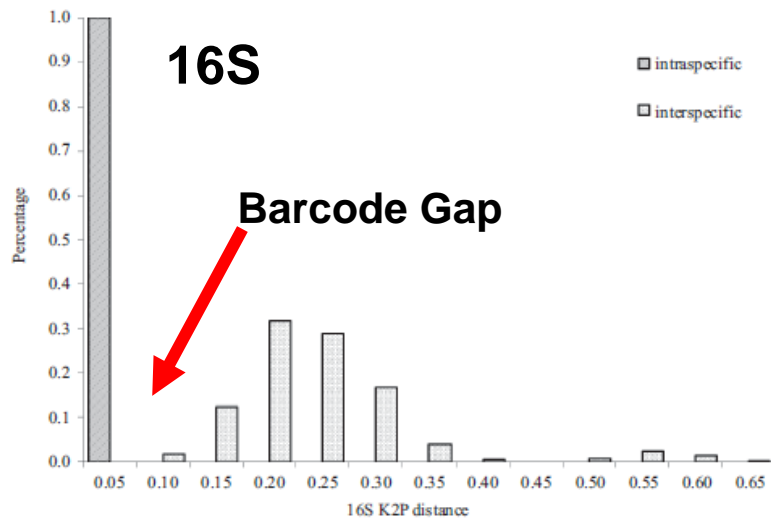
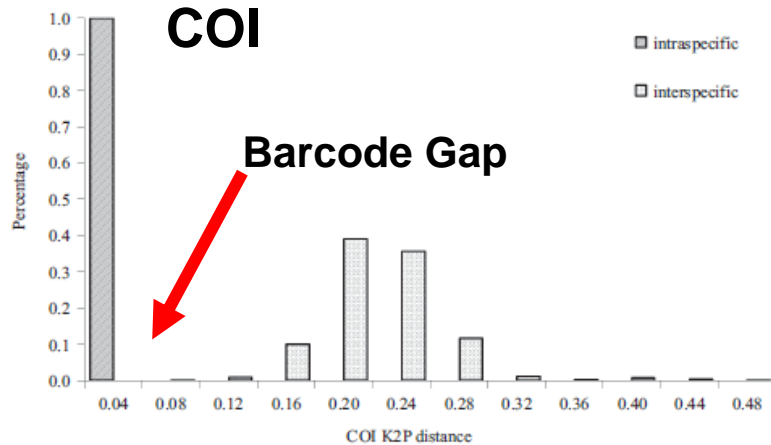


- Global sampling of 1,295 individuals of 22 described species.
- 16S rRNA showed barcode gap within and between evolutionarily significant units (ESUs).
- Relatively high phylogenetic signal at species and generic levels in the Eucalanidae



DNA Barcoding of Hydrozoa: COI versus 16S

Zheng, He, Lin, Cao and Zhang (2014) Acta Oceanol. Sin. 33: 55–76

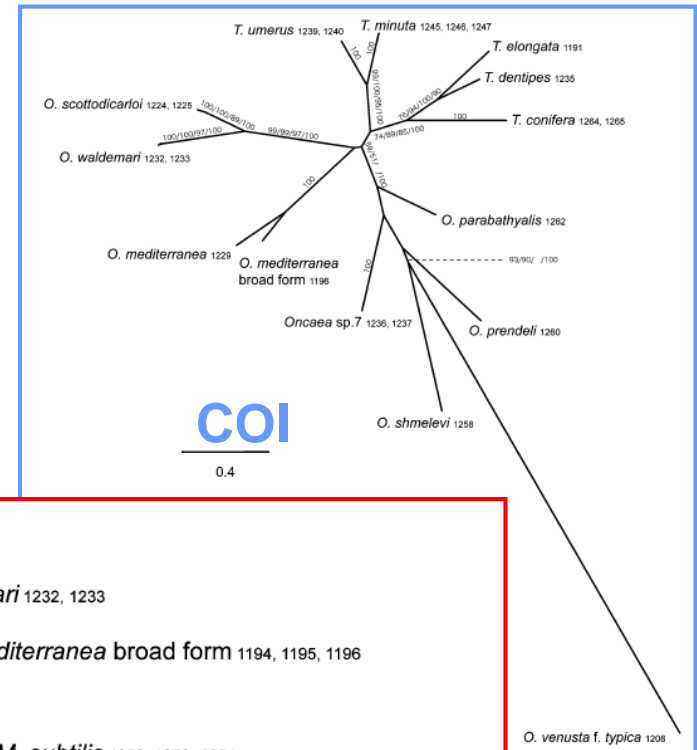


- Clear barcode gap for both COI and 16S
- Strong recommendation for 16S as barcode gene for Hydrozoa.
- Advantages of 16S:
 - 1) Better resolution of genera and sometimes families.
 - 2) More 16S sequences: 1,566 16S sequences (596 species) versus 621 COI sequences (169 species)
 - 3) 16S more easily amplified and sequenced across hydrozoan taxa (Moura et al., 2011).

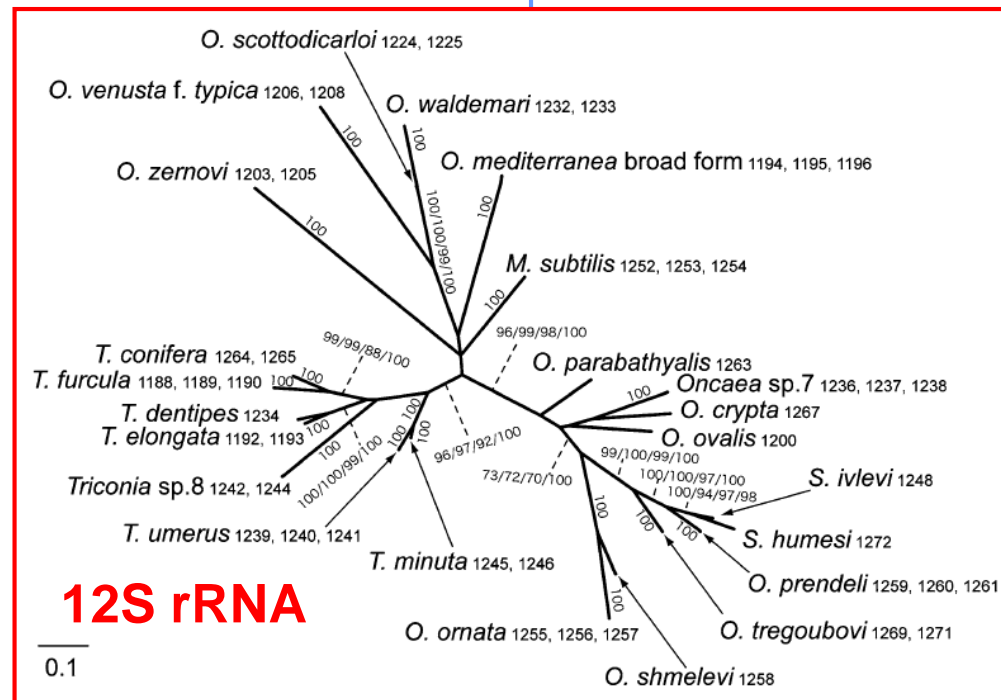
Species Identification of Oncaeidae (Copepoda)

Boettger-Schnack and Machida (2011) Hydrobiologia 666: 111–125

- Integrative taxonomy of Oncaeidae in Mediterranean Sea.
- All morphospecies investigated were genetically distinct and no cryptic species were found.

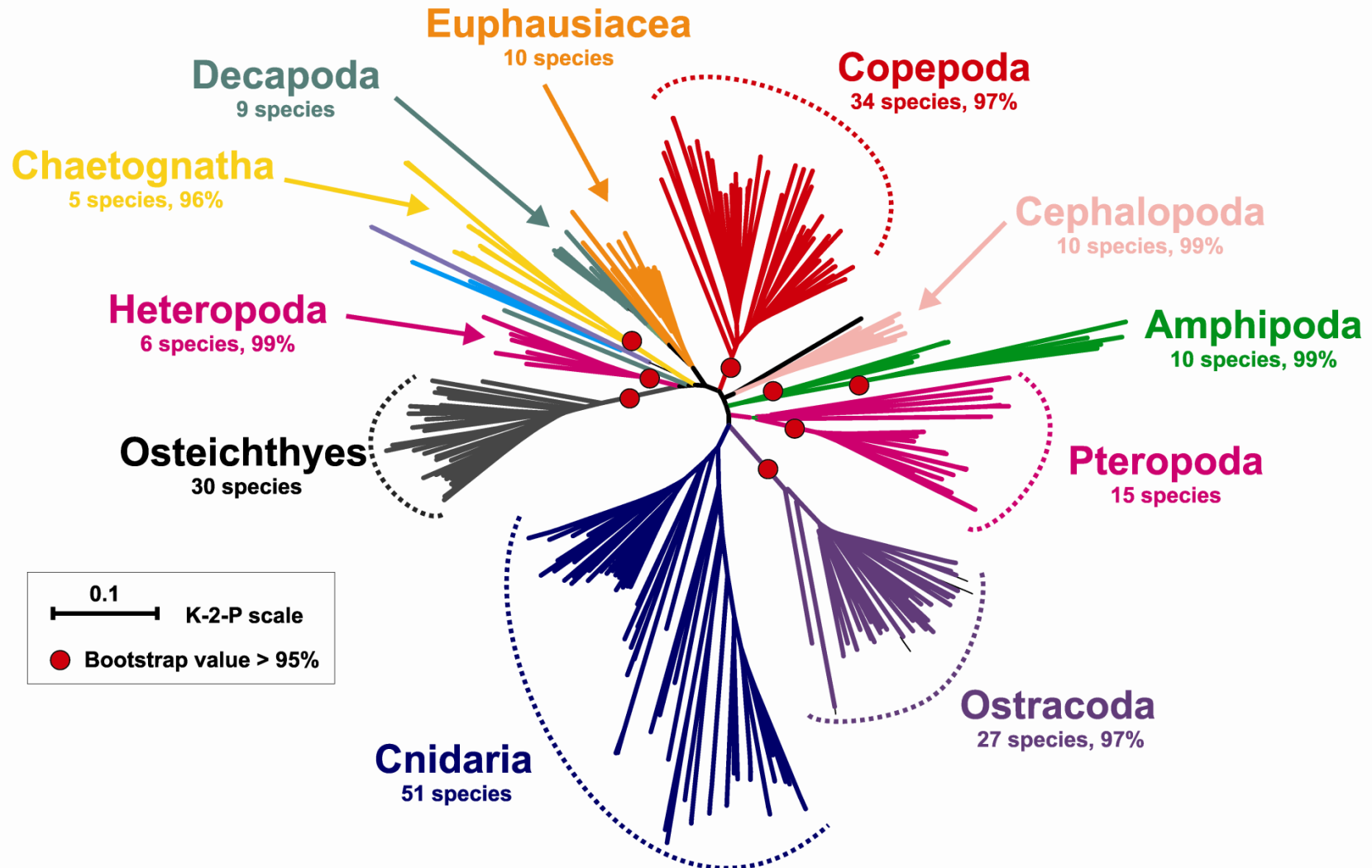


- 12S rRNA better barcode gene for oncaeid copepods



Barcoding Zooplankton Communities

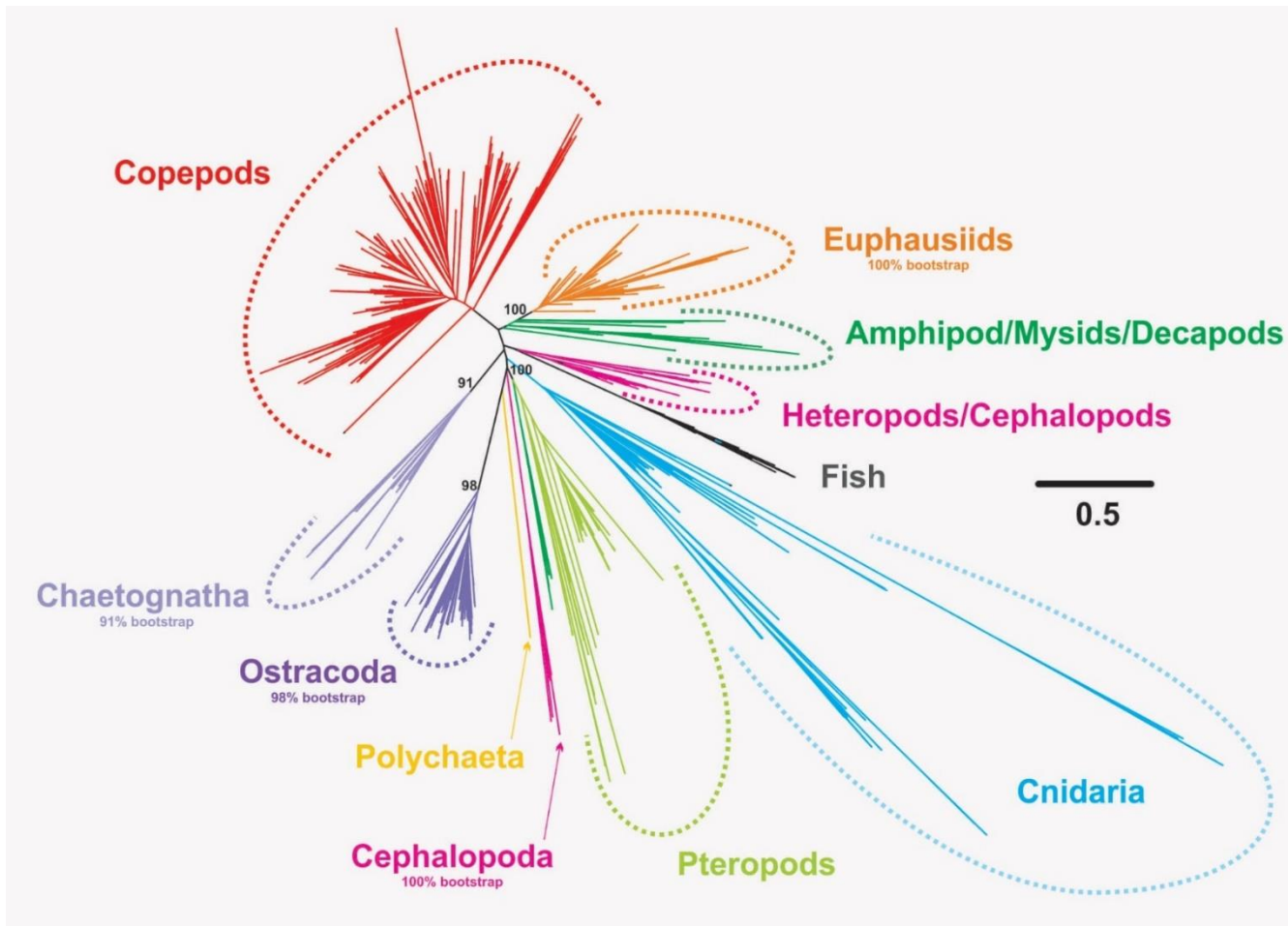
Bucklin, Ortman, Jennings, Nigro, Sweetman, Copley, Sutton, Wiebe (2010) Deep-Sea Res. II



Data analysis: 327 COI barcodes for 207 species; Neighbor Joining tree, Kimura-2-Parameter distances, 1000X bootstrapping

Zooplankton COI Barcode Sweep-up

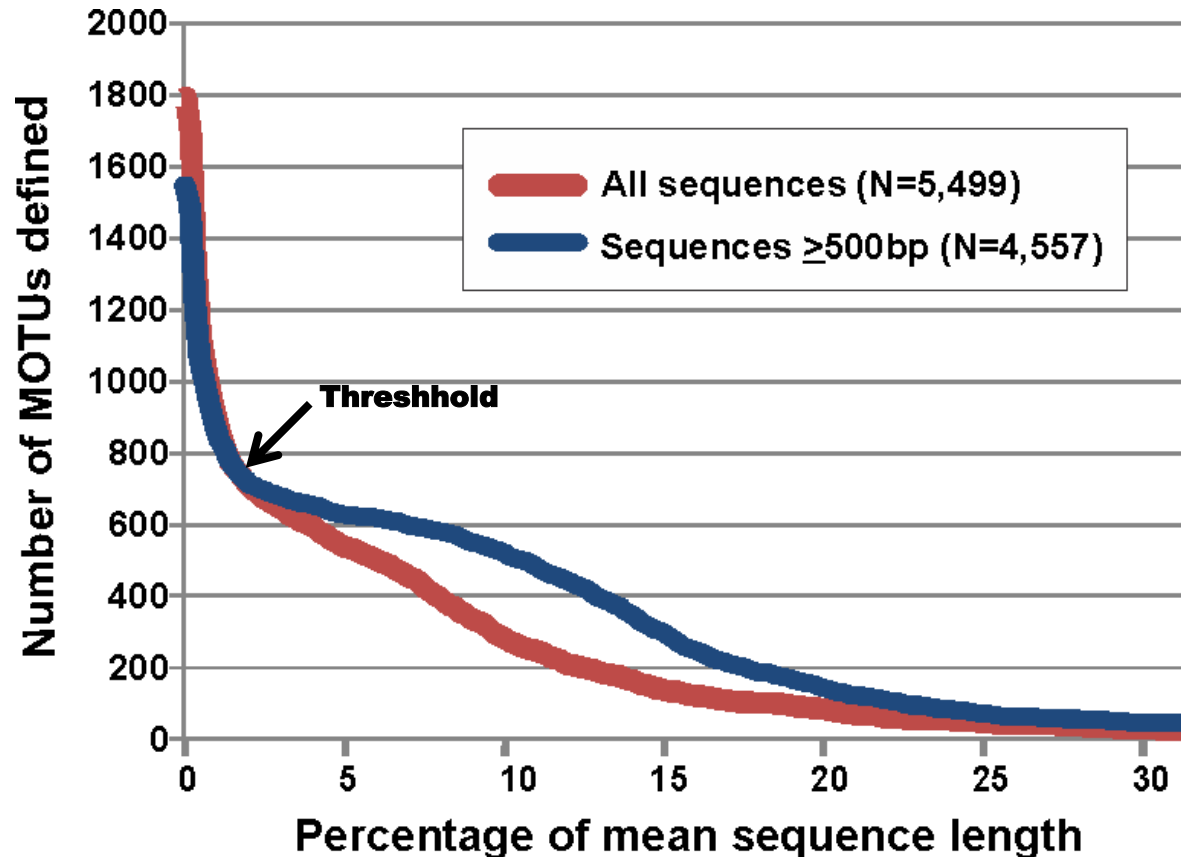
- Total of 4,557 COI sequences of zooplankton and fish collected 1992-2011
- Specimens identified to species by taxonomic experts
- Species are discriminated; major groups cluster, but not all are well-resolved.



Data analysis: MEGA Ver. 6; sequence alignment in MAFFT; Maximum Likelihood (ML) tree in RAxML Ver. 7.2.8. - Bucklin et al. (2014) OSM Poster

Zooplankton COI Barcode Sweep-up

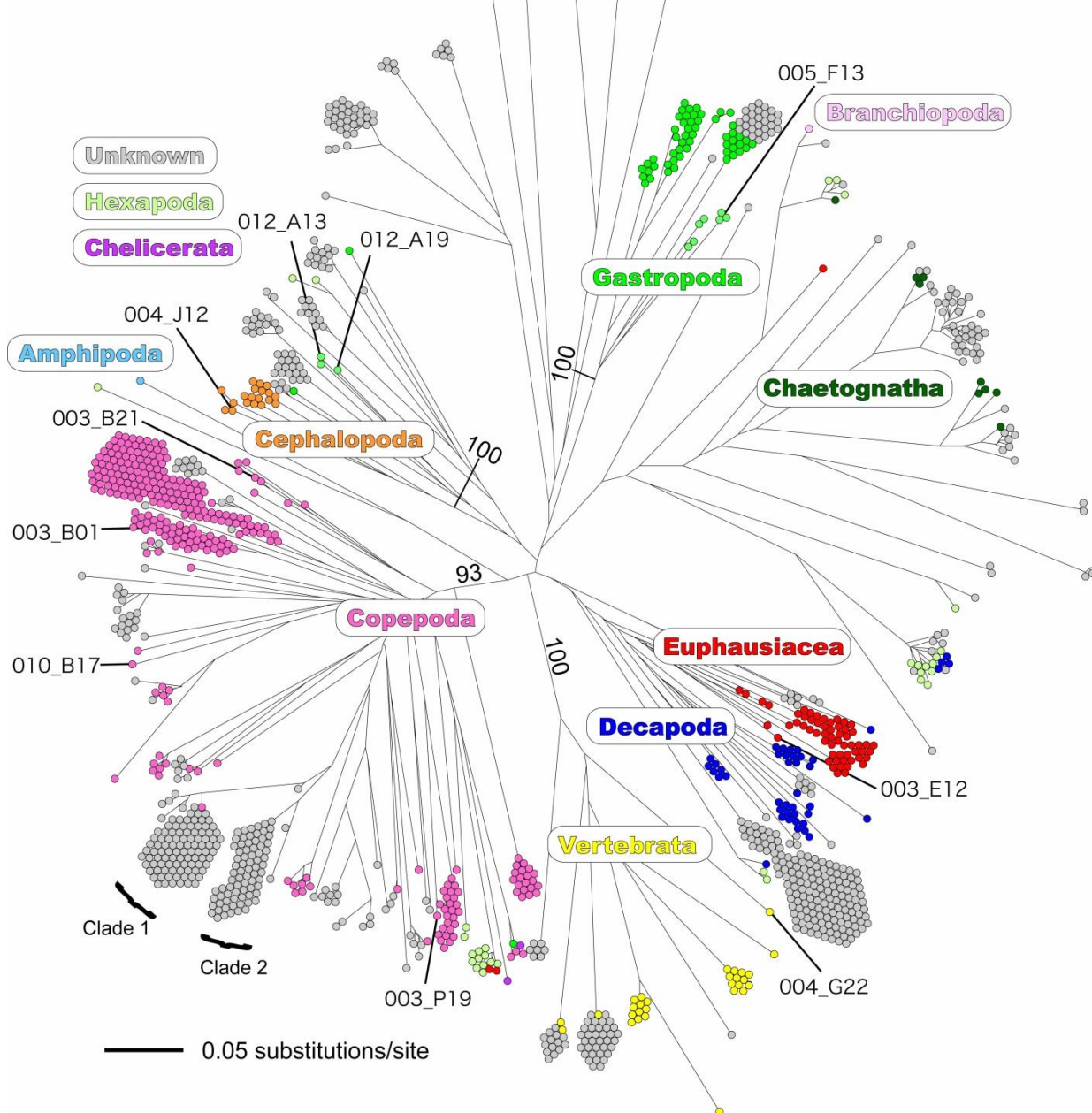
Threshold indicates 2% COI sequence difference for MOTU differentiation



Data analysis: Numbers of MOTUs (Molecular Operational Taxonomic Units) resolved as a function of percentage of mean sequence length in jMOTU. Diversity and species identification using automated Basic Local Alignment Search Tool (BLAST).

Metagenetic Analysis of Zooplankton Diversity

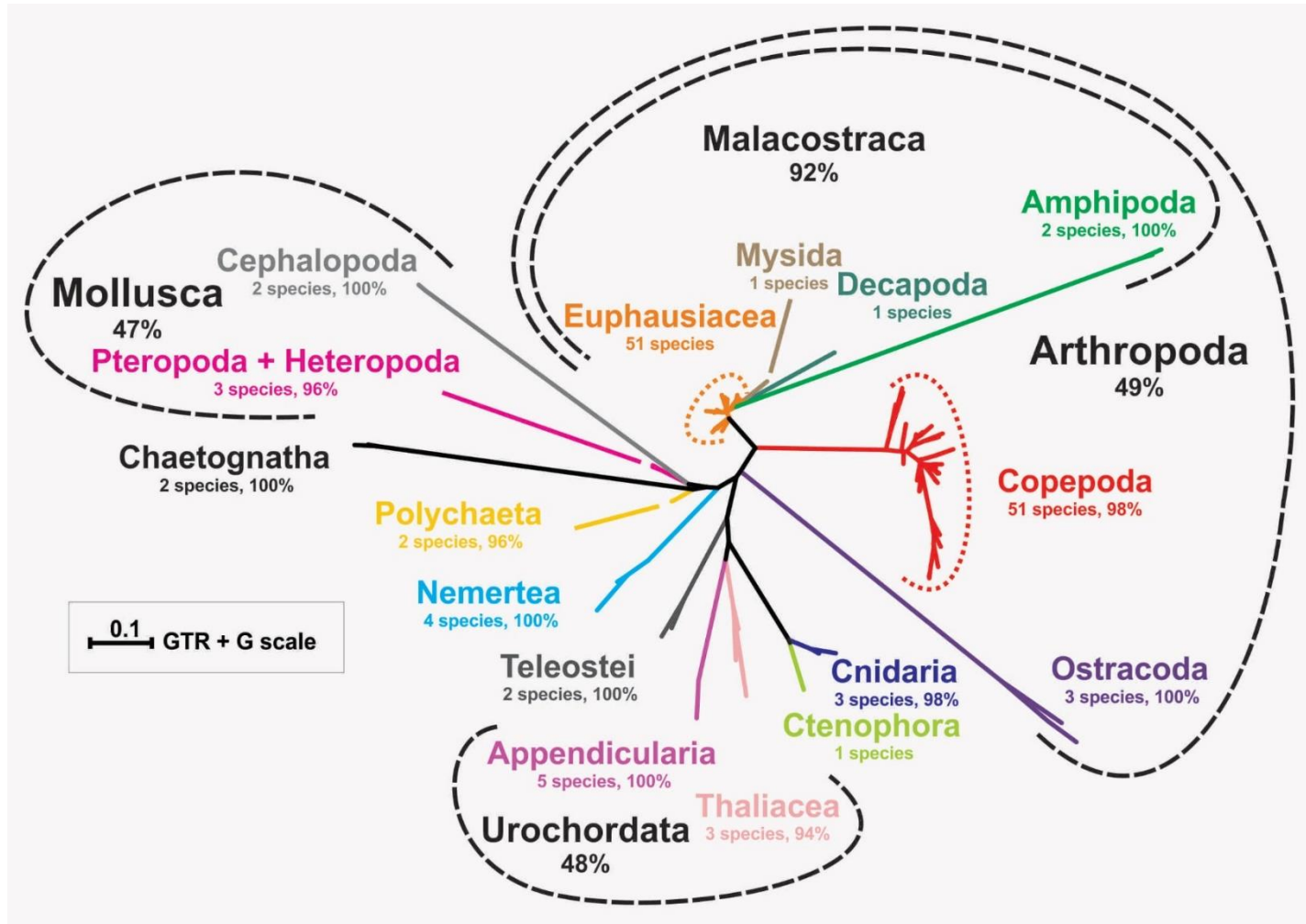
Machida et al. (2009) BMC Genomics



- Zooplankton net sample from Equatorial Pacific Ocean.
- Total of 1,336 COI sequences resolving 189 species.
- NJ tree; K2P distances (0.12 species threshold); 1000X bootstrap.
- Species identification requires COI reference database.

Zooplankton 18S rRNA Barcode Sweep-up

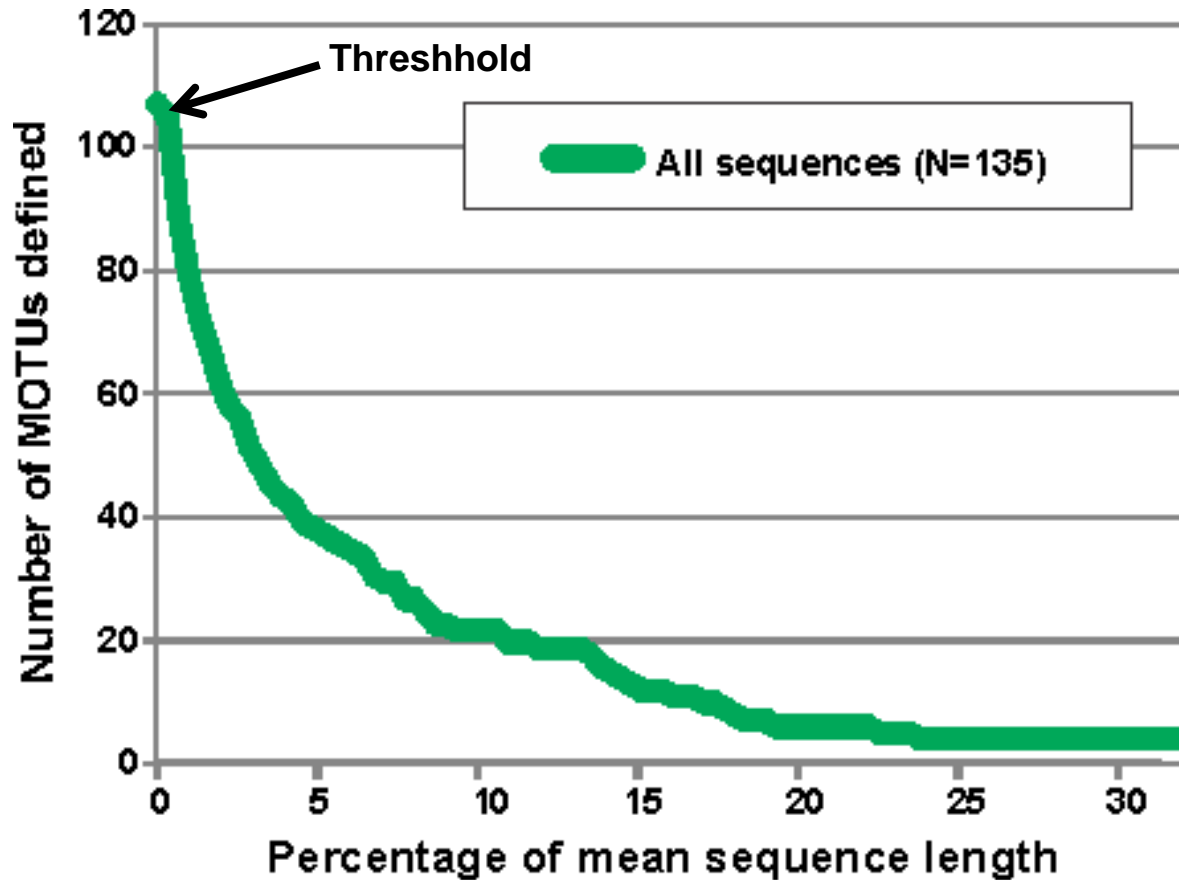
- Consistent divergence across taxa
- Accurate classification of novel sequences
- Resolution of most groups, not species



Data analysis: 136 species; 8 phyla; ML tree; GTR+G distances 1000X boot-strapping; 18S primers: Fonseca et al. (2010). - Bucklin et al. (2014) OSM Poster

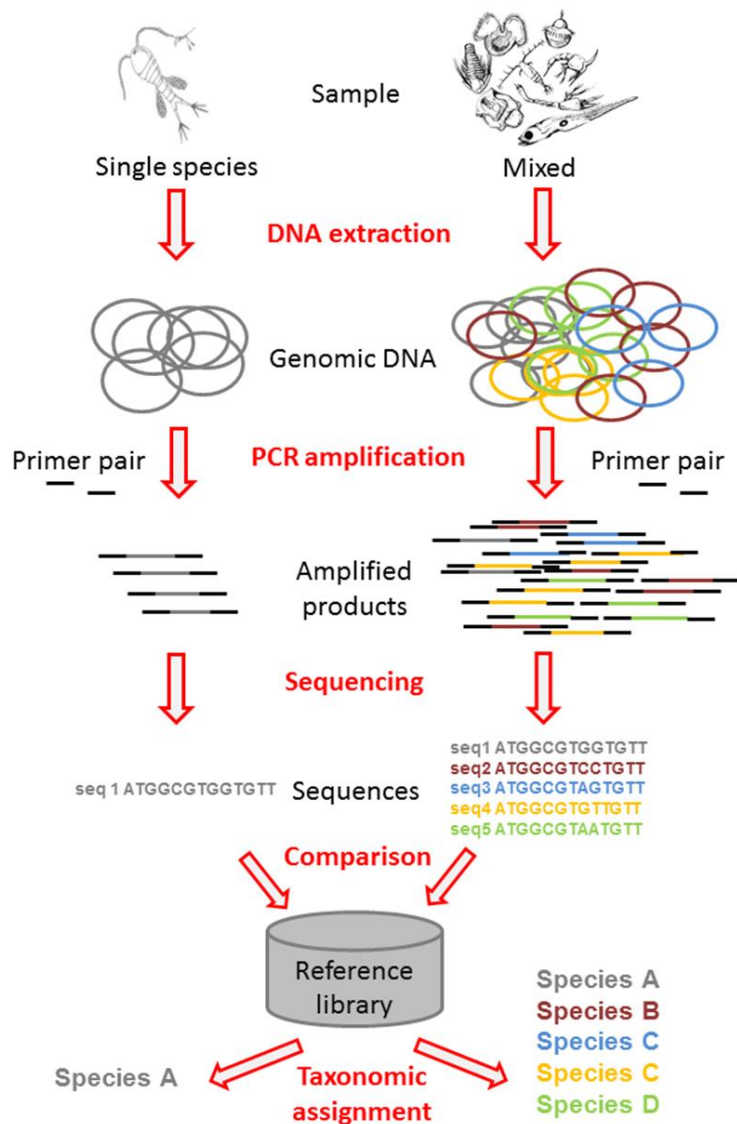
Zooplankton 18S rRNA Barcode Sweep-up

Threshold indicates <1% 18S sequence difference for MOTU differentiation



Data analysis: Numbers of MOTUs (Molecular Operational Taxonomic Units) resolved as a function of percentage of mean sequence length in jMOTU

Metabarcoding Analysis of Zooplankton Diversity



- **Metagenetics:** large-scale high-throughput DNA sequencing of target genes from environmental samples for analysis of taxon richness.
- **Metabarcoding:** analysis of a barcode gene using metagenetics.
- **Integrative taxonomic analysis:** metabarcoding analysis that matches MOTU to DNA sequence for morphologically-identified species in reference database.

Figure: Corell and Rodríguez-Ezpeleta (2014)

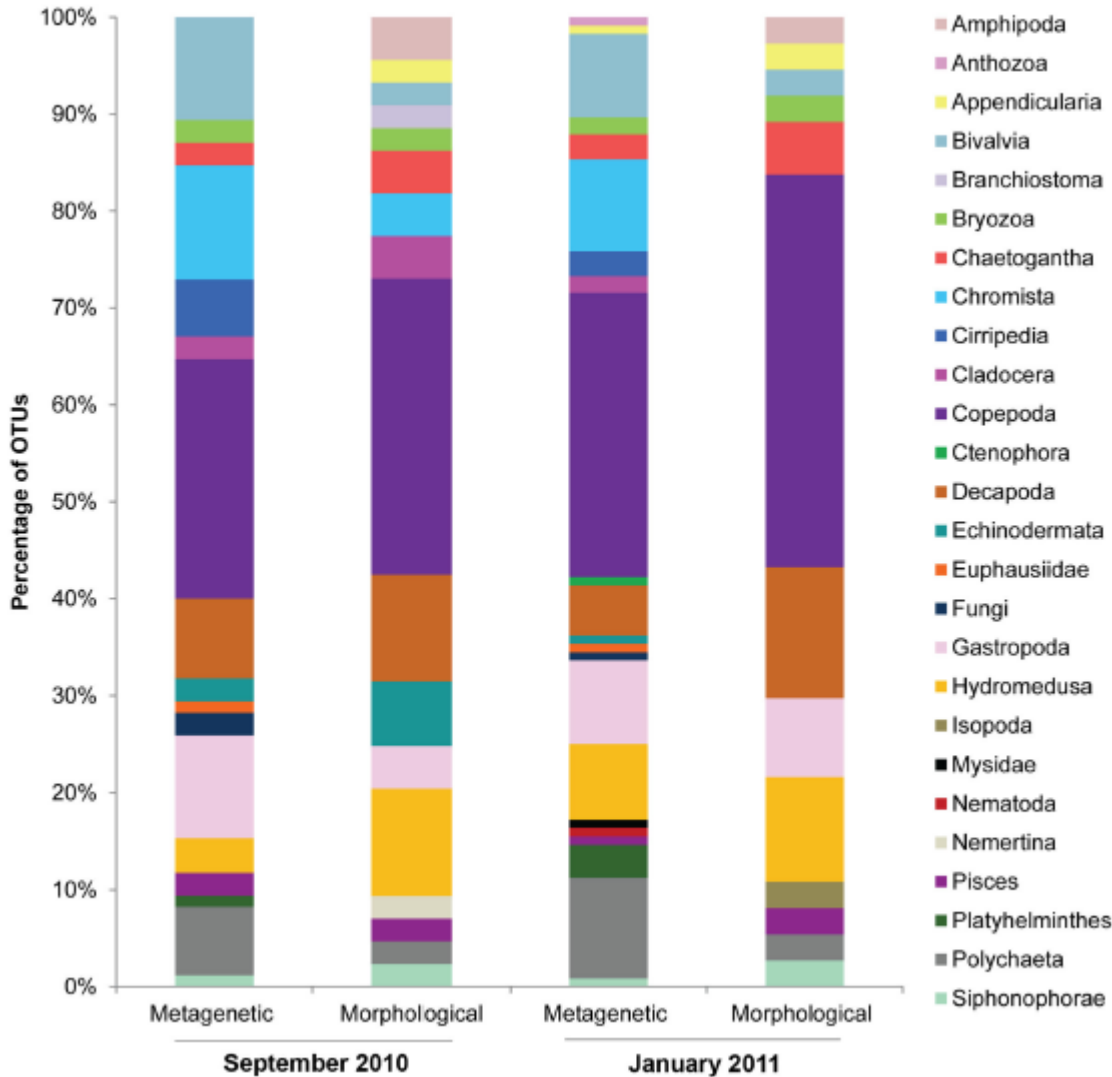
Prospects and Pitfalls of Metabarcoding

Bucklin, Lindeque, Rodriguez-Ezpeleta, Albaina, Lehtiniemi (2016) J. Plankton Res

- **Rapid monitoring and assessment of biodiversity, including hidden diversity associated with cryptic, rare, and invasive species.**
- **Coordinated analysis across broad array of taxa: holo- and mero-zooplankton, microbes to metazoans.**
- **Multiple genetic markers can be used to characterize diversity across a range of systematic levels.**
 - **Conserved genes for higher PCR success rates, may underestimate species diversity.**
 - **Variable genes may cause PCR artefacts, allow detailed assessment of species diversity.**

Metagenetic Analysis of Zooplankton Diversity

Lindeque, Parry, Harmer, Somerfield, Atkinson (2013) PLoS ONE 8: e81327.

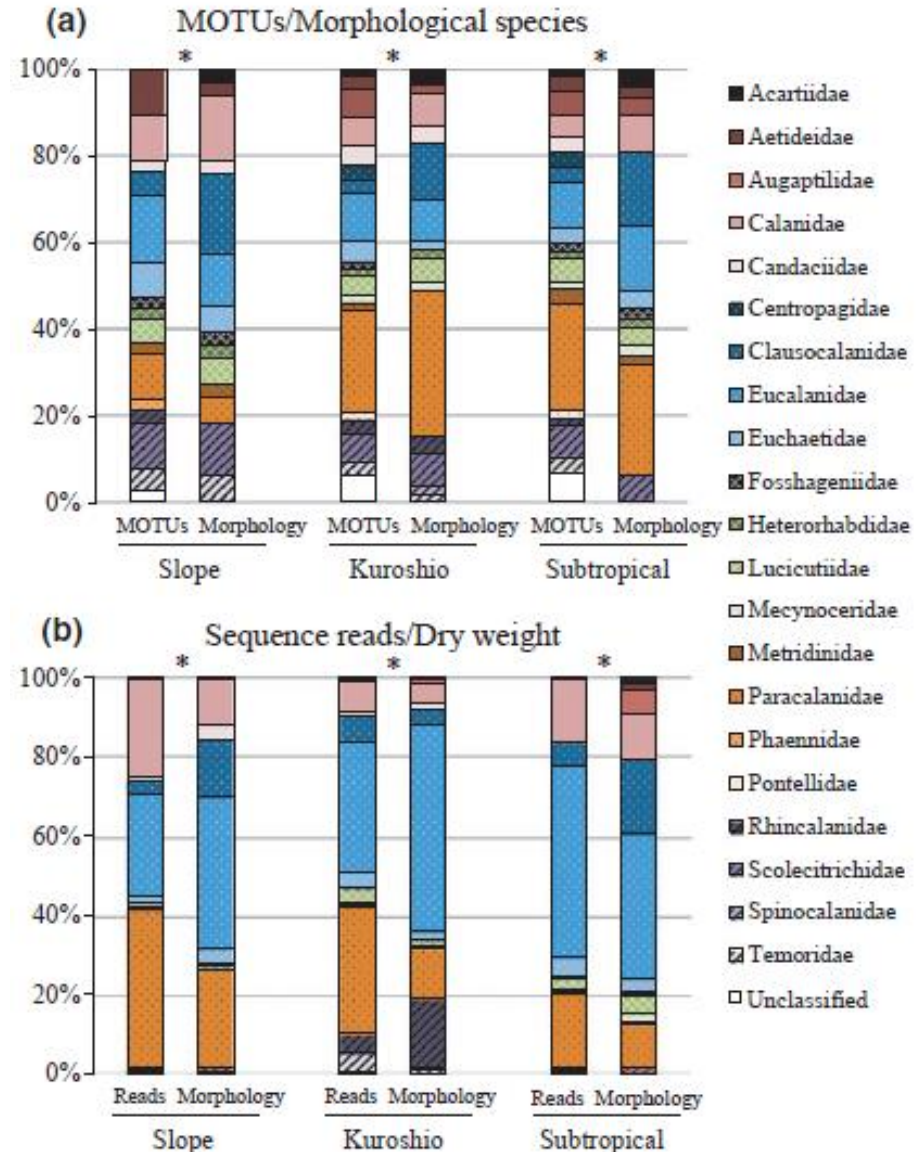


- **Metagenetic analysis reveals “hidden diversity” of marine plankton.**
 - nuclear 18S rRNA
 - 419,041 sequences
 - 205 OTUs (clusters)
 - 58 taxa
- **Need complete reference libraries of sequences for accurately-identified individuals.**

Metagenetic Analysis of Copepod Community Structure

Hirai, Kuriyama, Ichikawa, Hidaka, Tsuda (2014) Molecular Ecology Resources

- Calanoid MOTUs (97% similarity) showed significant correlation between metagenetic and morphological analyses.
- Family-level composition of molecular operational taxonomic units (MOTUs) and morphological species.
- Family-level composition of sequence reads and dry weight.



Barcoding and Metabarcoding

- **Species-level analysis of pelagic biodiversity is critical for understanding impacts of climate change, detecting invasive species, and management and assessment objectives.**
- **Metabarcoding analysis of zooplankton samples allows rapid characterization of zooplankton diversity; usual marker gene regions do not discriminate species.**
- **Species identification by barcoding or metabarcoding requires matching DNA sequence to barcode for morphologically-identified specimen in a reference database.**





Call to Action

- **Comprehensive reference databases are needed for all barcode genes.**
- **Coordinate taxon- and region-specific barcoding efforts.**
- **Collaborate with morphological taxonomic experts, share archived DNA and specimens from previous field programs, and sequence multiple barcode regions for identified specimens.**
- **Species-level protocols for metagenetic analysis of zooplankton diversity.**

ICES WGIMT

Working Group on Integrated Morphological and Molecular Taxonomy



WGIMT Goals

- Promote and provide new tools for species-level taxonomic analysis of the pelagic ecosystem.
- Focus on species recognition, discrimination, and identification of marine metazoan zooplankton, detection of cryptic species, evolutionary / systematic relationships of species.
- Contribute to the ICES mission to analyze, recognize, and understand changes in community structure, species diversity, and species phenology and productivity.

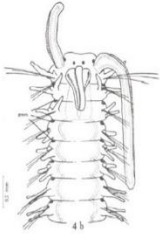
WGIMT Web Portal: Resources to Support Integrative Taxonomy of Zooplankton

Morphological Methods

Links to taxonomic resources for species identification of marine zooplankton

ICES Zooplankton Identification Leaflets

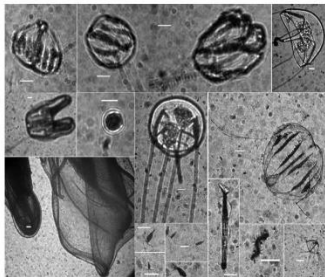
ICES Plankton Identification Leaflets, 1939-2001 (including Fiches d'identification du Zooplancton and ICES Identification Leaflets for Plankton, 1-187, and Fiches d'identification des Oeufs et Larves de Poissons, 1-6)



Click on the image above to access the interactive PDF file

Optical Methods

- Promote integrated optical, molecular, and morphological approaches to zooplankton species identification.
- Resource for training of image recognition software.
- Similar portal for phytoplankton operated by ICES WGPME.



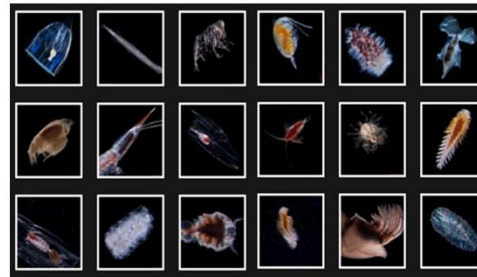
WGIMT.net

Main Menu

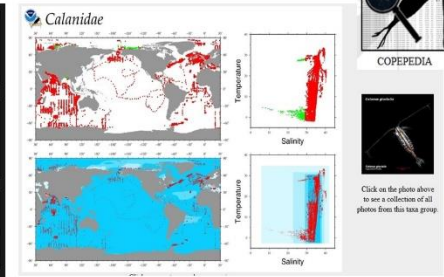
- ★ About WGIMT
- ★ Morphological Methods
 - Identification Keys
 - Photos
 - Optical Methods
 - Literature
- ★ Molecular Methods
 - Primers & Protocols
 - Literature
- ★ Topics of Interest
 - Workshops & Events
 - News
- ★ Related Links

Photo Gallery

High-quality images of living zooplankton. Software will allow posting and organizing images for easy access and viewing. Left: Photos from the Census of Marine Zooplankton (CMarZ) and other participating programs will be reproduced. Right: Images will be cross-linked to WGIMT/WGZE taxonomic database entries, with alternate access via the NOAA Copepod website and Copepedia (now in development by Todd O'Brien, NOAA).



<http://www.cmarz.org/>

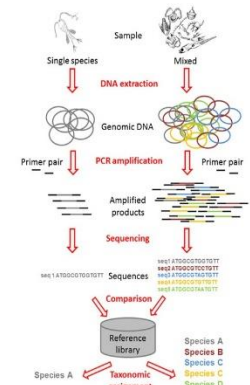
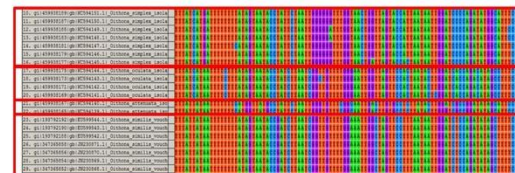


<http://www.st.nmfs.noaa.gov/copepod/index.html/>

Molecular Methods

- Listing of PCR and sequencing primers for zooplankton.
- Various marker genes: mitochondrial (COI, 16S rRNA) and nuclear (18S rRNA)
- Universal (conserved), group-specific and species-specific primers.

GENE	TAXON	PRIMER	PRIMER SEQUENCE	REFERENCE
MtCOI	Copepods	Cop-COI-2105R	R CGRCHGTHARNARYATDGTAAATDGC	Bucklin et al. 2010a
MtCOI	Copepods	Crus-COI-2198R	R CCHACDGTAAAYATRTGRIG	Bucklin et al. 2010a
MtCOI	Copepods	Crus-COI-2428R	R TTAATHCCHGTDGGNACVGC.AAT	Bucklin et al. 2010a
MtCOI	Copepods	HCO-Co-2358	R CCHACDGTAAAYATRTGRIG	Bucklin et al. 2010b
MtCOI	Calanoida	LCO-1703	F CTATTGATTGGAGGATTGG	Hill et al. 2001
MtCOI	Calanoida	LCO-1719	F GGATTGGTAACTGATTAATGCCC	Hill et al. 2001
MtCOI	Calanoida	H2612-COI	R AGGCCTAGGAAATGTATAGGGAAA	Figueroa 2011
MtCOI	Calanoida	L592-RCOI	F AACCTAATACATCTTTTATGATG	Figueroa 2011
MtCOI	<i>C. helgolandicus</i>	ChelgCOI-F	F GGCCAAAACAGSGAGAGATA	Papadopoulos et al. 2005
MtCOI	<i>C. helgolandicus</i>	ChelgCOI-R	R CGGGACTCAGTATAATTTCGTCTA	Papadopoulos et al. 2005



- Potential and promise of metabarcoding for rapid detection of climate change, biotic indices, food webs, NIS. Figure from Corel & Rodriguez (2014)

Call to Action: Reference Barcode Database

Barcoding / Metabarcoding Studies at ZPS 2016

TAXONOMIC FOCUS

Astrid Cornils: Copepods

Tone Falkenhaug: Copepods / cladocerans (Norwegian Sea)

Sergio Hernandez Trujillo: Calanoid copepods (Gulf of California)

Junya Hirai: Copepods (Pacific Ocean)

Aino Hosia: Hydrozoans (Norwegian Sea)

Panagiotis Kasapidis: Copepods (Mediterranean / Black Seas)

Glafira Kolbasova: Scyphozoa

Sanna Majaneva: Ctenophores

Mary Mar Noblezada: Copepods

Stephanie Bush: Pteropod molluscs

Jasmin Renz: Copepods (benthopelagic)

Call to Action: Reference Barcode Database

Barcoding and Metabarcoding Studies at ZPS 2016

REGIONAL FOCUS

Paola Batta-Lona: Gulf of Mexico

Leocadio Blanco-Bercial and Samiah Alam: Sargasso Sea

Ann Bucklin: NW Atlantic continental shelf

Erica Goetze: North Pacific

Silke Laakmann: North Sea

Penelope Lindeque: North Atlantic

Ryuji Machida: Pacific

Inga Mohrbeck: North Sea

Naiara Rodriguez-Ezpeleta: Malaspina circumnavigation

Sergio Stefanni: Adriatic / Mediterranean Seas

Lidia Yebra: Alboran Sea

ENVIRONMENT / HABITAT FOCUS

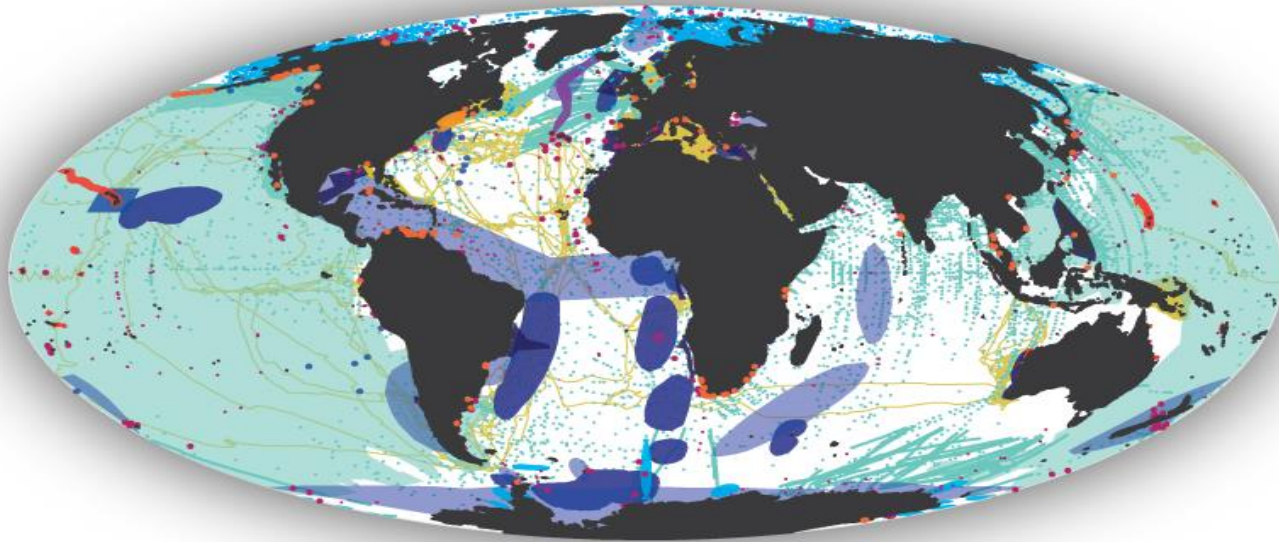
Holger Auel: Midwater / deep sea

David Abad: Estuaries

Oliver Kersten: Abyss

Call to Action: Census of Marine Life Projects

- Census of Marine Life (CoML) ocean realm field projects sampled throughout global ocean environments
- Barcoding in partnership with Consortium for Barcode of Life (CBOL)



Coastal

- Regional Ecosystems (GoMA)
- Near Shore (NaGISA)
- Coral Reefs (CReefs)

Polar

- Arctic Ocean (ArcOD)
- Antarctic Ocean (CAML)

Pelagic

- Top Predators (TOPP)
- Continental Shelves (POST)
- Zooplankton (CMarZ)

Deep Sea

- Vents and Seeps (ChEss)
- Abyssal Plains (CeDAMar)
- Seamounts (CenSeam)
- Continental Margins (COMARGE)
- Mid-Ocean Ridges (MAR-ECO)

Global Information and Analysis

- Oceans Future (FMAP)
- Information Systems (OBIS)
- Microbes (ICoMM)
- Oceans Past (HMAP)



Call to Action

- **Comprehensive reference databases are needed for all barcode genes.**
- **Coordinate taxon- and region-specific barcoding efforts.**
- **Collaborate with morphological taxonomic experts, share archived DNA and specimens from previous field programs, and sequence multiple barcode regions for identified specimens.**
- **Species-level protocols for metagenetic analysis of zooplankton diversity.**

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Census of Marine Zooplankton (CMarZ) 2004 - 2010

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CMarZ Steering Group Members

24 scientists from 14 countries



CMarZ Planning Group Meeting - 2003



CMarZ Steering Group Meeting - 2010

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ICES Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT)



WGIMT Members at the 2016 Annual Meeting, IPMAR, Lisbon, Portugal (March 20, 2016)

Thank you for your attention!

