

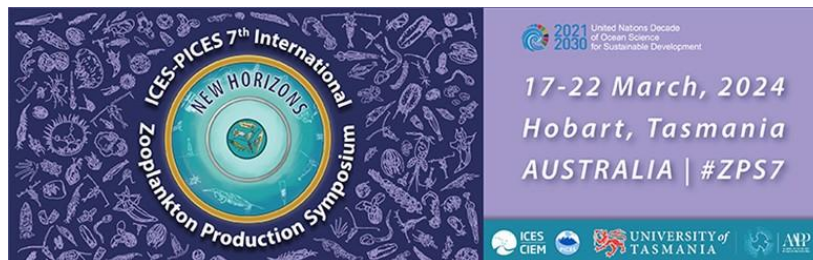
Metabarcoding Zooplankton Diversity: MetaZooGene Intercalibration Experiment (MZG-ICE)

Ann Bucklin¹, Leocadio Blanco-Bercial², Ruben Escribano³, Tone Falkenhaus⁴, Junya Hirai⁵, Jenny Huggett⁶, Pedro Martinez⁷, Katja Peijnenburg⁸, Leonie Suter⁹, Agata Weydmann-Zwolicka¹⁰, Paola Batta-Lona¹, Stacey Dubbeldam⁸, Elizaveta Ershova⁴, Carolina E. Gonzalez⁷, Ashrenee Govender¹¹, Johan Groeneveld¹¹, Sahar Khodami⁷, Anna MacDonald⁹, Monika Mioduchowska¹⁰, Andrea Polanowski⁹, Jasmin Renz⁷, Peter Wiebe¹², Todd O'Brien¹³

¹University of Connecticut (USA), ²Bermuda Institute of Ocean Sciences – Arizona State University (BM), ³Universidad de Concepción (CH), ⁴Institute of Marine Research (NO), ⁵Atmosphere and Ocean Research Institute, University of Tokyo (JP), ⁶South African Association for Marine Biological Research (SA), ⁷German Center for Marine Biodiversity Research (DE), ⁸Naturalis Biodiversity Center (NL), ⁹Australian Antarctic Division (AUS), ¹⁰University of Gdańsk (PL), ¹¹Oceans and Coasts, Department of Forestry, Fisheries and the Environment (SA), ¹²Woods Hole Oceanographic Institution (USA), ¹³NOAA Fisheries Office of Science & Technology (USA)



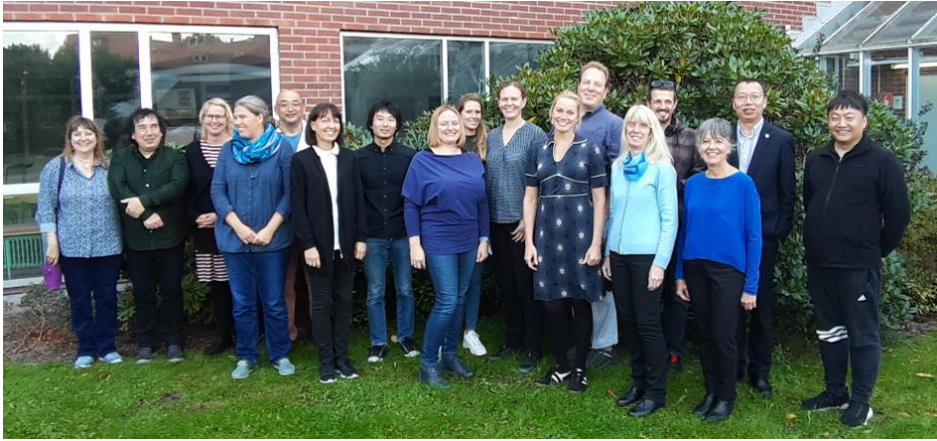
Zooplankton Production Symposium *Hobart, Tasmania (AUS)* #ZPS7 Session 4, March 20, 2024





SCOR WG157

MetaZooGene



2019 – SCOR WG157 Meeting @ Global Biodiversity Centre
(Gothenburg, Sweden)



2022 - MetaZooGene Symposium for Early Career Scientists
(Dublin, Ireland) <https://metazoogene.org/symposium2022>

SCOR WG157 Members: 23 members / 19 countries

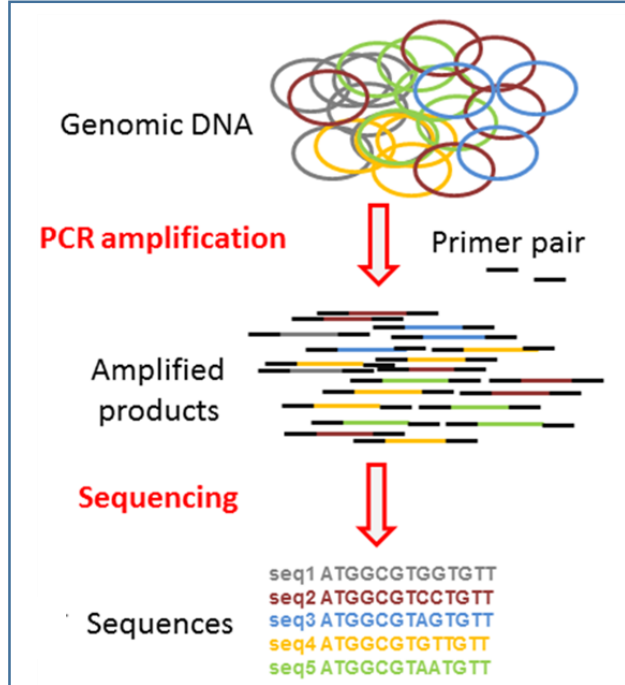
Primary focus: ~8,000 species of holozooplankton (15 phyla, 32 classes) of animals that drift with ocean currents

Primary goal: Integrative molecular – morphological taxonomic analysis of marine zooplankton biodiversity throughout global ocean

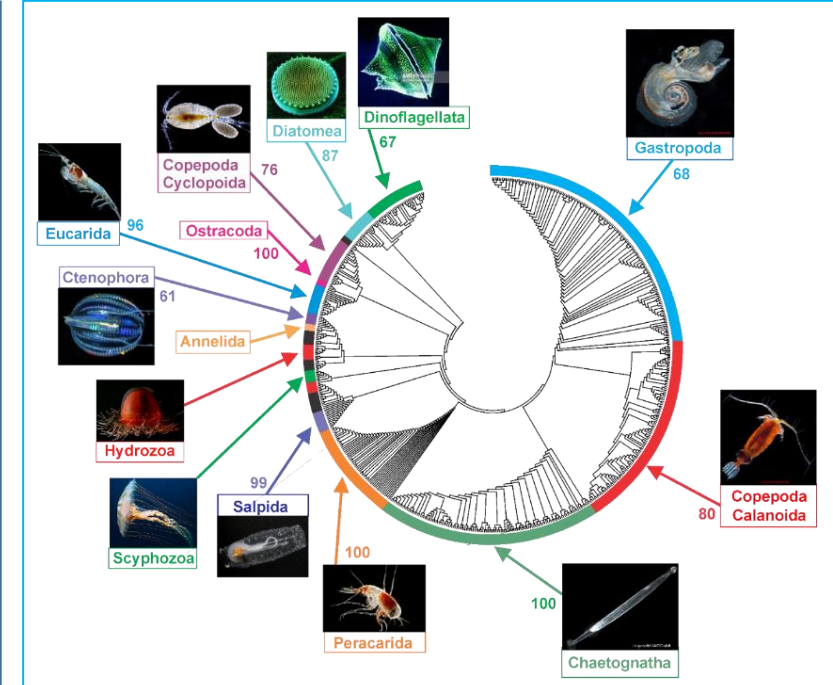
Terms of Reference:

- 1) Create an open-access web portal for DNA barcodes for marine zooplankton**
- 2) Design an optimal DNA barcoding pipeline for marine zooplankton**
- 3) Develop best practices for DNA metabarcoding of marine zooplankton biodiversity**

DNA Metabarcoding of Marine Zooplankton



Corell and Rodríguez-Ezpeleta (2014)



Bucklin et al. (2019)

- Genomic DNA from zooplankton or water samples
- PCR amplification of short gene regions: COI; 12S, 16S & 18S rRNA
- High-throughput DNA sequencing
- Identification requires reference DNA sequence database
- Bioinformatics & statistical analysis of taxonomy & biodiversity



MetaZooGene Intercalibration Experiment MZG-ICE



SCOR WG157 Deliverable 3:

Develop best practices for DNA metabarcoding of marine zooplankton biodiversity

Ocean Best Practices (Przeslawski et al., 2023)

- a) fit-for-purpose with clearly defined scope
- b) representative & inclusive of potential users
- c) accurate & effective, reflecting emerging technologies & programs
- d) supported & adopted by users

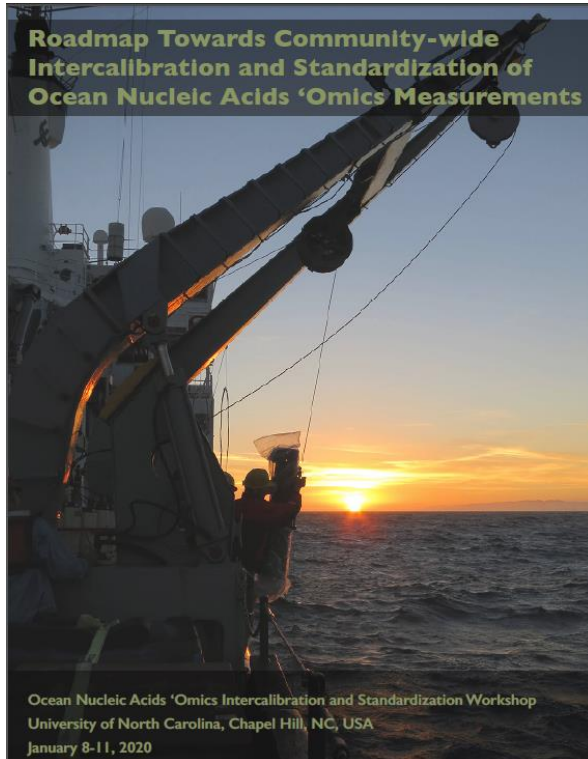


<https://www.oceanbestpractices.org/>

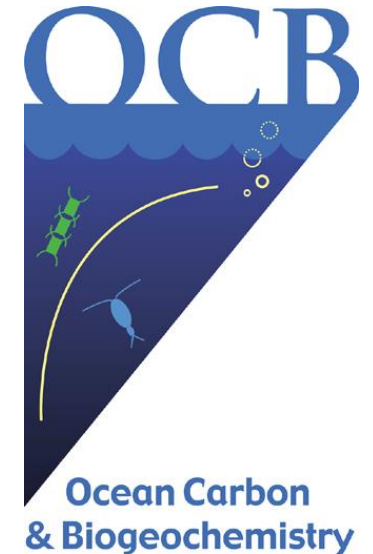
*Przeslawski, R. et al. (2023) Developing an ocean best practice: A case study of marine sampling practices from Australia. Front. Mar. Sci. 10 DOI: 10.3389/fmars.2023.1173075



MetaZooGene Intercalibration Experiment MZG-ICE



- OCB 'Omics workshop report recommending “intercalibration & standardization” (Berube et al., 2022)
- Re-consideration of SCOR WG157 deliverable of “best practices” for DNA metabarcoding of marine zooplankton biodiversity
- Design of MZG-ICE included some – but not all – metabarcoding analytical steps



Berube, P., S. Gifford, B. Hurwitz, B. Jenkins, A. Marchetti, A. E. Santoro (2022) *Roadmap Towards Communitywide Intercalibration and Standardization of Ocean Nucleic Acids 'Omics Measurements*. DOI 10.1575/1912/28054



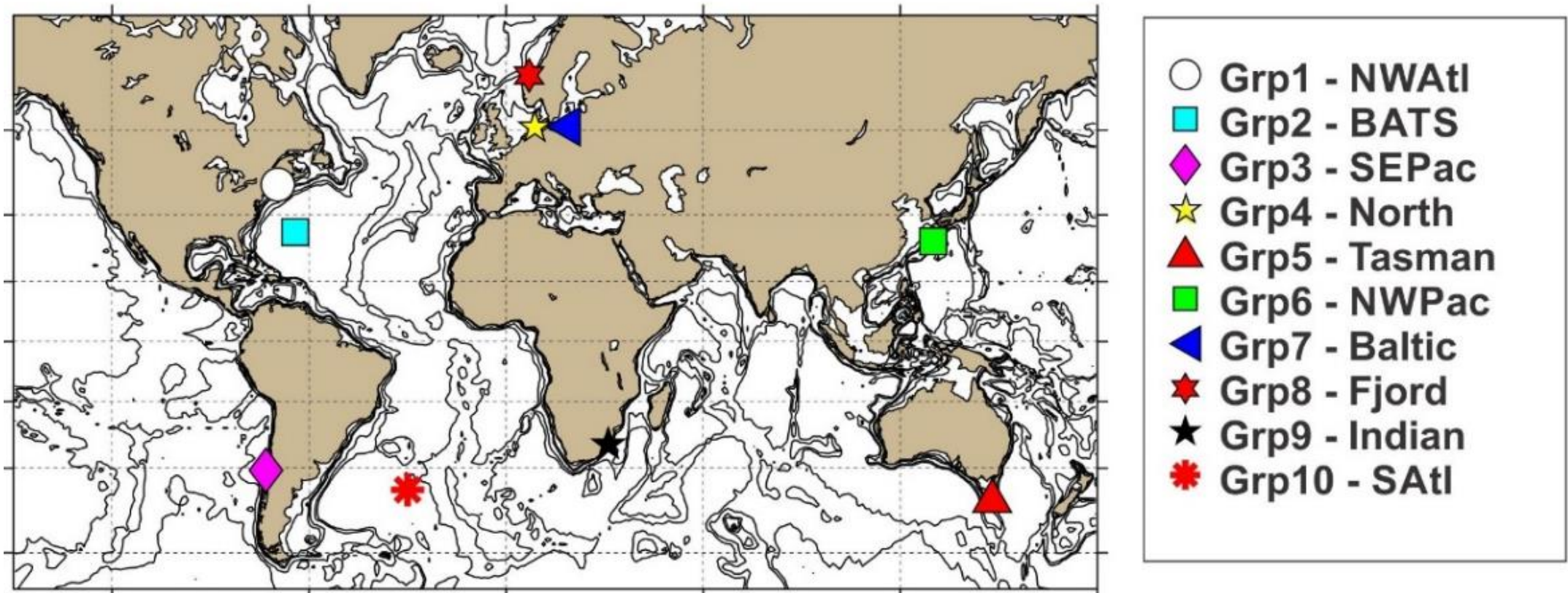
MetaZooGene Intercalibration Experiment (MZG-ICE)

Global Exchange of DNA from Zooplankton Samples

Leo Blanco-Bercial (BATS, ASU) - Bioinformatics & Statistics Lead



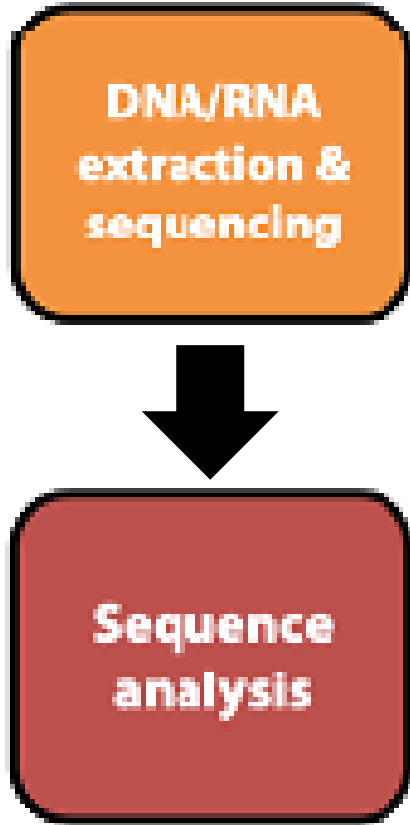
- 10 WG157 teams chose samples to compare zooplankton diversity of global ocean





MetaZooGene Intercalibration Experiment

MZG-ICE Project Design



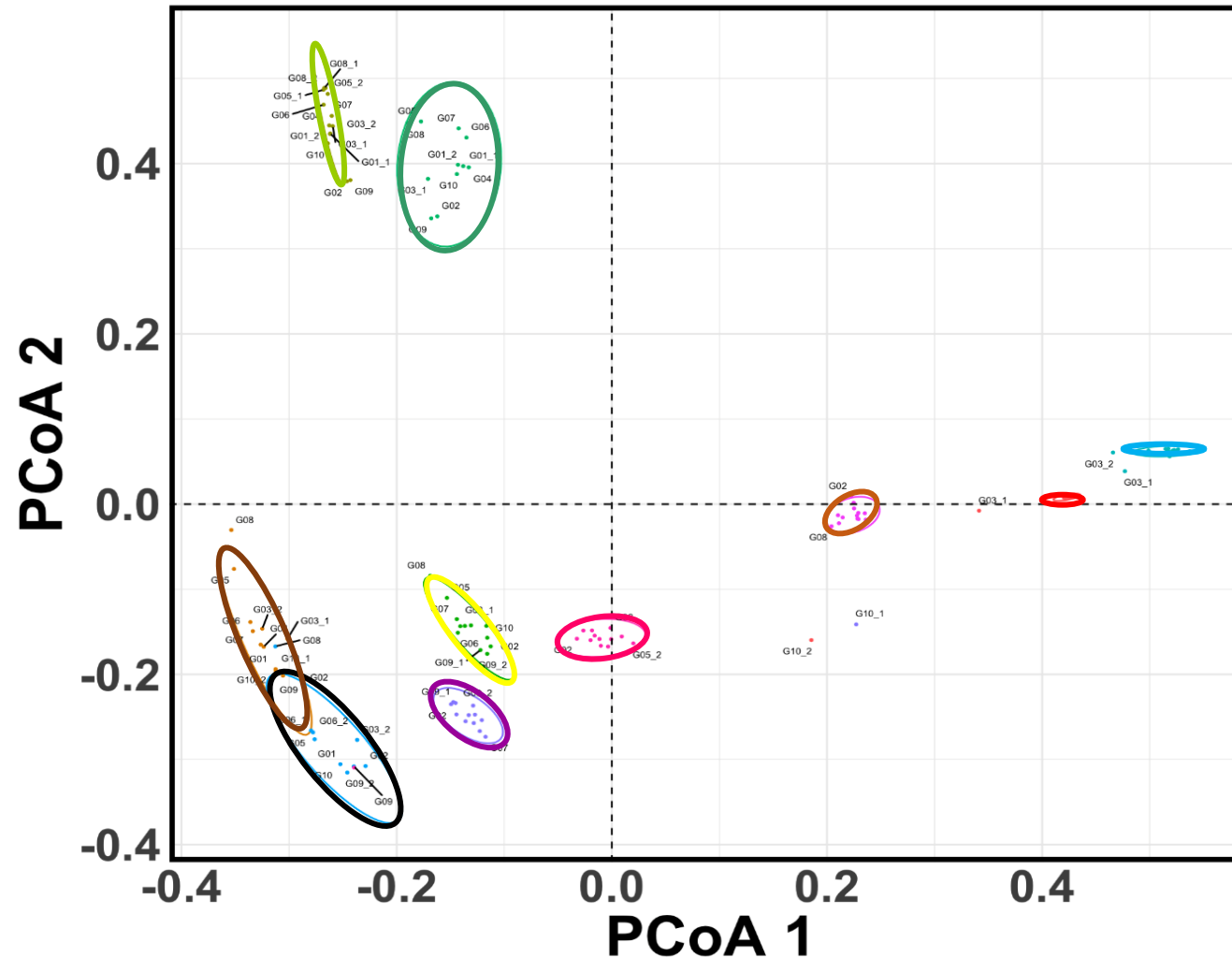
- WG157 teams extracted & shared DNA
- Metabarcoding of 4 gene regions:
 - Mitochondrial COI
 - V1-V2, V4 & V9 18S rRNA
- QA/QC of sequence data by each research group
- Sequence data uploaded to MZGdb shared / private website
- Centralized QA/QC; sampling re-naming as needed
- Bioinformatics & statistics by Leo Blanco-Bercial et al. (ASU)



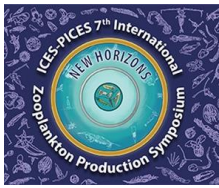
MetaZooGene Intercalibration Experiment (MZG-ICE) Principal Component Analysis for V9



PCoA analysis
of V9 data
also showed
pattern of
higher
replicability
with lower
diversity



- Legend
- NWAtl
 - BATS
 - SE Pac
 - North
 - Tasman
 - NWPac
 - Baltic
 - Fjord
 - Indian
 - SATl



SCOR WG157

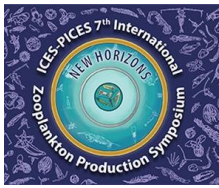
MetaZooGene



Conclusions & Future Directions

- **Metabarcoding methods & protocols differ among research groups & institutions.**
- **Universal or consensus agreement on “best practices” may not be realistic or possible.**
- **Intercalibration will allow comparison of results based on methods for all analytical steps: preservation, DNA extraction, PCR, sequencing, bioinformatics & more.**
- **Intercalibration may not lead to standardization, but will enable accurate interpretation of results from different research groups.**



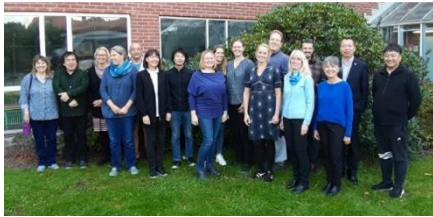


SCOR WG157

MetaZooGene

MetaZooGene Intercalibration Experiment (MZG-ICE)

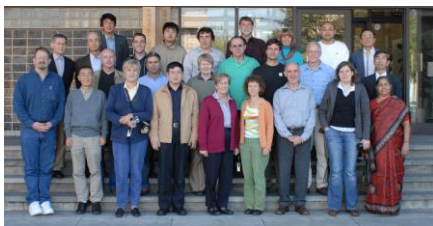
Acknowledgements



*MetaZooGene, SCOR WG157
Gothenburg, Sweden (2019)*



*ICES WGIMT
Boulogne-sur-Mer, France (2017)*



*CMarZ Steering Group
Tokyo, Japan (2006)*

Scientific Committee for Oceanic Research (SCOR)

- SCOR WG157 MetaZooGene

UN Decade of Ocean Science for Sustainable Development

- MetaZooGene UN-OD Action No. 102.2

International Council for the Exploration of the Sea (ICES)

- ICES WG for Integrative Morphological and Molecular Taxonomy (WGIMT)

NOAA COPEPOD: Coastal and Oceanic Plankton Ecology, Production, and Observation Database

University of Connecticut Institute for System Genomics

- Center for Genomic Innovation (CGI)

Census of Marine Life

- Census of Marine Zooplankton (CMarZ) Steering Group

Funding provided by:

- Scientific Committee for Oceanic Research (SCOR)
- NOAA Office of Ocean Exploration
- NSF Biological Oceanography Program

