MetaZooGene SCOR WG157
2020 Annual Meeting Report

Corona Room, Hilton Gaslamp Quarter Hotel
401 K St, San Diego, CA 92101 USA
Sunday, February 16, 2020

Welcome and Introductions

All members and guests introduced themselves, briefly describing their interests. The meeting began with a review the Agenda (Appendix I) and overview of the MetaZooGene plan of work. In all, 14 people joined the meeting, plus one via SKYPE (see Appendix II, III). Most unfortunately, we were not able to get the GoToMeeting interface working for other members who had planned to join us online. Several WG157 members and guests joined a lunchtime meeting on Thursday, Feb. 20, where we met our new SCOR reporter, Enrique Montes Herrera (Appendix IV).

These notes from our discussions largely follow the Agenda for the meeting:

MetaZooGene 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. How to publish? Should it be published?

3) Develop best practices for DNA metabarcoding of marine zooplankton biodiversity.

MetaZooGene Deliverables:

1) Project website, web portal, and database:

2) Publications in peer-reviewed journals:

Review papers:
- DNA barcoding and DNA reference sequence database for marine zooplankton;

Best Practices documents:
- DNA barcoding: “bug to sequence” pipelines
- DNA metabarcoding

3) Capacity Building: Four workshops are proposed:

- Best practices for multi-gene DNA barcoding pipelines
- Use of DNA reference sequence databases for analysis of zooplankton diversity
- Hands-on ‘DNA-to-data’ training workshop in DNA barcoding and metabarcoding
- Best practices for metabarcoding analysis of marine zooplankton biodiversity

Overall, Ann stated that she thinks that WG157 is on-track for successful completion of our Terms of Reference, and that most deliverables are both realistic and likely of success. For Capacity Building in particular, Ann is planning to explore options for online workshops and virtual participation. Discussion on all topics is a primary purpose of the meeting.

Updates from MetaZooGene members and Guests

Leo Blanco-Bercial: Described paper recently published, which has acknowledgement for SCOR WG157. Study focused on protist plankton seasonality and community structure in the epi- and mesopelagic near BATS. Leo showed a slide with oxic-but-low oxygen minimum zone, that is very different from other protists between 700 and 900 m at the BATS site in North Atlantic.

Katja Peijnenburg: Described her studies using both DNA barcodes and population genomic SNPs to describe genetic structure of marine zooplankton, especially pteropods. She described results from her long-term collections and analysis of samples along on the Atlantic meridional transect.

Le Qin Choo: Dissertation research addresses question of whether planktonic gastropods can adapt to OA. Her studies use an evolutionary framework, she is analyzing genomic and phenotypic variability, using reference DNA barcode databases. She reviewed the use of COI for analysis of diversity and phyogeography of pteropods COI: Wall-Palmer analyzed 21 species of heteropods, finding 33 clades. Another study of Hyperiid amphipods by Tump et al. (unpubl) detected many new (cryptic) species based on COI. She mentioned similar findings for Chaetognaths by Peijnenburg et al. (unpubl).

*Junya Hirai***:** Using metabarcoding for ocean monitoring, including DNA sampling and analyses to map community structure, and understand and forecast response to change. Using direct RNA sequencing on Nanopore platform, to create reference library database, which is low quality and has low mapping rate. He will present at OSM 2020 on Monday @ 1145 on *Pleuromamma abdominalis*.

*Erica Goetze***:** Exploration of biodiversity in the deep ocean, mining manganese nodules and analyzing eDNA. Using a lander DeepCC project. Meroplankton of the BBL is a species-rich assemblage, representing 12 Phyla, 23 classes, 46 orders, 65 families. Widespread assemblage of parasitic/commensal taxa. Heterogeneity in the larval pool at scales of 10 to 50 km. She is talking about eDNA at OSM 2020 on Wednesday @ 1430. Upcoming cruise Midwater sediment plumes. Discussion about reference library of COI not being able to identify all the copepods present. Leo suggested using V1-V2 for species ID. Katja asked whether possible to avoid PCR; Erica said this was not feasible yet.

Stephanie Matthews: Currently examining vertical habitats of California Current, using CalCOFI time series collections. Samples are collected during nighttime tows across CC with MOCNESS to 400 m. Morphological counts, as well as metabarcoding to analyze diversity using 18S sequences and OTUs.

*Ryuji Machida***:** new way to get growth rates using Ribosome RNA and Protein. Used Daphnia magna to do experiments. Extrat total RNA do reverse transictiopn of cDNA. Ribosomal RNA and growth correlated. Good under low food conditions, but not higher. Ann asked why he was so interested in RNA. He asked asked about pseudogenes in DNA.

*Ann Bucklin***:** Gut contents of salps and fish as part of the WHOI Ocean Twilight Zone project. Also mentioned publication on diet of copepod Calanus finmarchicus, with samples from EuroBASIN cruise, GO Sars 2013.

SCOR overviews from Ed Urban and Patricia Miloslavich

SCOR expects each Working group to:

- Achieve its terms of reference in 3-4 years
- Plan activities in such a way to stay within the group's total budget (US$45,000)
- Help SCOR make the group's work visible to the broader ocean science community
- Cooperate with related groups sponsored by other organizations
- Build capacity within their area of science through specific capacity-development activities

After introductions by all members and guests, Ed and Patricia gave short presentations about SCOR. SCOR started in 1957 and currently receives funding from NSF and 31 international agencies. A primary focus is the working groups, with 2 new groups funded each year. In response to questions about SCOR's goals for capacity building, Ed stated that SCOR seeks to reach out to students world-wide. SCOR has a committee on capacity building and there is a website with suggested approaches (see https://scor-int.org/work/capacity/). Recognizes that funds are limited for capacity building and some groups raise additional funds for this effort.

MZGdb: Project website, web portal, and database (Todd O'Brien)

Todd gave an overview of his ongoing effort for the MetaZooGene barcode atlas and database. Patricia asked about the amount of data being produced today and how does Todd seeing the data management going. Todd described the project website, MetaZooGene.org, and the current status of MZG data portal and database. He reported that there are maps of global distributions for 20 taxa, but not forams. The MZGdb now includes ~186,000 barcodes; he is working with Jenn Questel (UAF) to format the database for use as a reference for metabarcoding using the Mothur pipline. The MZG database can be updated regularly from GenBank and BOLD. Todd noted problems with the GenBank nomenclature for the barcode gene region. Other problems are that GenBank and BOLD have many duplications. Some discussion on whether BOLD or GenBank has cleaner data / fewer errors; there was no consensus view! Todd is open to creating databases for other barcode genes after COI, probably the full 18S rRNA gene sequence. He also suggested that MZG consider including ichthyoplankton. And raised a question about including protists. The group consensus was that the database should include only mesozooplankton. Ryuji said that GenBank can flag bad sequences; he has found 2,000 bad sequences. Todd can ask GenBank to tag them. Erica recommended using the Razoul's Database for copepods (see https://copepodes.obs-banyuls.fr/en/).

Ann raised a question of whether Todd's efforts for the MZGdb should be the basis of a Best Practice paper about reference database? Ryuji thought it was a good idea. Erica asked if Todd was duplicating what Ryuji was doing. Patricia said that Frontiers will accept Best Practices manuscripts. Patricia said that this could contribute to capacity building as well. People would assist in the writing. This topic was deferred to a future meeting, while we focus on completing the already-promised deliverables.

Special journal issue of papers by MZG members and colleagues (Ann Bucklin & Ryuji Machida)

Ann reported that there are 8 manuscripts promised for the special issue. She will propose the issue to the Editor of the ICES Journal of Marine Science soon. Ann and Ryuji are the proposed Guest Editors, and will promise expeditious review for all submitted manuscripts, which are published online by ICES as soon as they are accepted. Open Access is available for purchase, but not required. Target date for submissions is April 1, 2020. Ann will ask the journal about the window for acceptances, to provide a final date by which manuscripts must be submitted for consideration for inclusion in the special issue. Manuscripts promised to date are:

- Carolina González, Ruben Escribano - Metabarcoding zooplankton diversity of South Atlantic
- Junya Hirai, Satoshi Nagai, Kiyotaka Hidaka - Characterization of diversity and community structure of small planktonic copepods in the Kuroshio region off Japan using a metabarcoding approach
- Chaolun Li, Yanjun Liu, Minxiao Wang - DNA barcode analysis of zooplankton diversity in the Northwest Pacific Ocean.
- Jon Corell, Xabier Irigoien, Naiara Rodriguez-Ezpeleta - Metabarcoding unveils global oceanic zooplankton distribution

- Ann Bucklin, Paola Batta-Lona, et al. - Time-series metabarcoding analysis of copepod diversity on the NW Atlantic continental shelf
- Joëlle van der Sprong, Katja Peijnenburg, Debbie Wall-Palmer, et al. - What do we know about cryptic diversity in marine zooplankton?
- Astrid Cornils et al. - Phylogeography in copepods in the Southern Ocean from different vertical habitats; or Cryptic diversity in small mesopelagic copepods - the case of Microcalanus spp.
- Marloes Tump, Alice Burridge, Ronald Vonk, Katja Peijnenburg, Erica Goetze et al. - Species diversity and phylogeography of hyperiid amphipods along a meridional transect in the Atlantic Ocean.

Best practices document for DNA barcoding

- Optimal pipeline for DNA barcoding marine zooplankton (Leocadio Blanco-Bercial)

Leo reported that he had sought for existing documents with optimal protocols for sample collection and preservation for DNA barcoding, but that he could not find any documents; in particular, no museum had written guidelines for DNA barcoding zooplankton. Discussion about the need for specific protocols for the individual taxonomic groups. Katja agreed there was no written document that covers all groups. To move forward, Leo will have to do it himself. Leo asked Katja about protocols for chaetognaths. Ryuji said drying will preserve the DNA the longest, better than cold conditions. Biometrica is a company that does this.

Best practices document for DNA metabarcoding

- OCB NA'Omics Workshop Report: Intercalibration vs standardization (Ann Bucklin)

Ann described the results of discussions at this workshop on metabarcoding, including molecular approaches and protocols, sequence platforms, bioinformatics pipelines, and reference databases. Erica urged the group not to focus on bioinformatics pipelines, but rather on which gene regions to use as markers. Ann noted that Todd is working with MZGdb formatting for the Mothur pipeline, but he can do other formats, once the MZGdb is deemed ready for distribution. Ann agreed that the issue of "standardization" – i.e., the choice of specific analytical and bioinformatics approaches – is likely hopeless. She noted that at the OCB Omics Workshop there was a focus on the use of "standard samples" and the development of "mock communities" as bases for intercalibration of genomics analyses, with presentations by David Emerson and others. Ann wondered whether MetaZooGene could do this? Would we need to distribute reference field or mock samples or just the DNA? Would we need reference samples from each geographic region or ocean depth? Someone mentioned the Continuous Plankton Recorder (CPR) ring test, which entails distribution of a zooplankton sample to labs doing morphological identifications, so the accuracy can be cross-checked.

More discussion of standardization versus intercalibration. Erica said doing multiple pipelines now, but we need to agree on same markers and primer sites. Erica suggested WG157 effort that was organized by the different analytical steps, so that each lab did the cross-comparisons of protocols (reagents, kits, etc) for a given step. Ann agreed that this was a sound experimental approach and could be suitable for publication. She suggested that the group might seek funding for this intercalibration experiment. Leo suggested the International Japan US program. All of the steps that will be used in the proposal is needed to be prepared right away. Ann agreed to prepare a draft list of the critical metabarcoding protocol steps, and send it around to WG157 members, with a request for them to indicate their interest, willingness, and ability to carry out the testing particular protocol steps.

Review paper: DNA Barcoding of Marine Zooplankton (Ann Bucklin, Katja Peijnenburg, et al.)

Working title: *Toward a global reference database of COI barcodes for marine zooplankton*. The co-authors have agreed to provide sections either by taxonomic group or ocean regions. A primary goal of the review paper is to answer the question of completeness of the barcode database for zooplankton. This information will also be used to identify and recommend priorities for next steps toward completion of a global database for all marine zooplankton. Ann showed a draft manuscript with what people had signed up for. Todd showed the MZGdb barcode atlas, with the example of amphipods. A primary task now if for all co-authors to submit any unpublished data; these can be added to the database and review paper only if they are publicly release or if authors provide Todd with access to the submission entries on GenBank or GOLD.

Capacity Building

- Hands-on 'DNA-to-data' training workshop in Taipei, Taiwan (Ryuji Machida)

Workshop at Academia Sinica (Taipei, Taiwan) during summer 2021. He wants to know what things to barcode, primers, etc. Ann wondered how to support the workshop? PICES has money to support travel of students to workshops. Could put together a cookbook of protocols and practices used by the members of this group that Ryuji can use for his workshop. He is happy to try things. How many students? Depends on available funding, but Ryuji estimates that 10 students would be cost effective. If a lot of interest break workshop in to several week long sessions.

- Online bioinformatics workshops for metabarcoding (Ann Bucklin)

Ann is exploring this with colleagues at UConn, which has excellent bioinformatics and computing facilities and expert staff (see https://bioinformatics.uconn.edu/). Ed suggested that users of the database might offer to teach a day course, with demonstrations by Todd on use of the MZGdb and atlas. Peter suggested that the OCB workshop, which is held in Woods Hole during June, might be a good place to have such a tutorial. Todd might be able to do this with Leo to show how to analyze COI data. Ed mentioned that SCOR can provide visiting scholars with travel support ($2,500 for air travel).

Plans for the 2021 WG157 Meeting

- ASLO 2021 Aquatic Sciences (Feb. 28 – Mar. 5, 2021; Palma de Mallorca, Spain). Ann suggested that this would be a useful opportunity to discuss WG157 progress and plans. Travel would be relatively inexpensive for WG157 members from Europe, but expensive for members from Asia. When asked, Ryuji said he would attend; Junya said he might attend. Ann said that she will now propose this as the third annual meeting for MetaZooGene.

She cautioned that this would likely mean that WG157 would not have sufficient funding for a meeting in association with the 2022 Ocean Sciences meeting in Hawaii. Erica suggested that WG157 member submit a OSM session proposal, to encourage members to attend the meeting using their own resources.

WG157 Timeline for Year 2 (Jan. 1 – Dec. 31, 2020)

Ann will prepare and distribute a timeline with a set of deadlines for 2020, based on the WG157 plans and deliverables discussed on this meeting, including the special issue, review paper, and database updates.

Appendix I. Agenda for MetaZooGene WG157 2020 Annual Meeting.

MetaZooGene SCOR Working Group 157
2020 Annual Meeting
Sunday, February 16, 2020

Corona Room, Hilton Gaslamp Quarter Hotel
401 K St, San Diego, CA 92101 USA

Agenda

0830 Arrival and continental breakfast in meeting room

0900 Welcome and Introductions

0915 Updates from MZG members and Guests

1030 Coffee break

1045 SCOR overview (Ed Urban and Patricia Miloslavich, past and new SCOR Executive Directors)

1100 WG157 Terms of Reference and Deliverables (Ann Bucklin)

1115 MZGdb: Project website, web portal, and database (Todd O'Brien)

1145 Special journal issue of papers by MZG members and colleagues (Ann Bucklin & Ryuji Machida)

1230 Lunch provided in hotel restaurant

1400 Best practices document for DNA barcoding

- Optimal pipeline for DNA barcoding marine zooplankton (Leocadio Blanco-Bercial)

1430 Best practices document for DNA metabarcoding

- OCB NA'Omics Workshop Report: Intercalibration vs standardization (Ann Bucklin)

1500 Coffee break

1515 Review paper: DNA Barcoding of Marine Zooplankton (Ann Bucklin, Katja Peijnenburg, et al.)

1600 Capacity Building

- Hands-on 'DNA-to-data' training workshop in Taipei, Taiwan (Ryuji Machida)
- Online bioinformatics workshops for metabarcoding (Ann Bucklin)
- Workshops to develop "best practices" documents

1630 Plans for the 2021 WG157 Meeting

- ASLO 2021 Aquatic Sciences (Feb. 28 – Mar. 5, 2021; Palma de Mallorca, Spain)

1700 WG157 Timeline for Year 2 (Jan. 1 – Dec. 31, 2020)

1730 Wrap-Up and Adjourn

Appendix II. Participant list for MetaZooGene WG157 2020 Annual Meeting.

Chair and Vice-Chairs	***Email***
Ann Bucklin	ann.bucklin@uconn.edu
Katja Peijnenburg	K.T.C.A.Peijnenburg@uva.nl
Full Members	
Leocadio Blanco-Bercial	leocadio@bios.edu
Junya Hirai	hirai@aori.u-tokyo.ac.jp
Ryuji Machida	ryujimachida@gate.sinica.edu.tw
Todd O'Brien	Todd.OBrien@noaa.gov
Associate Members	
Erica Goetze	egoetze@hawaii.edu
Guests	
Christy Acabado	cristy.acabado@gmail.com
Paola G Batta-Lona	paola.batta_lona@uconn.edu
Melissa Wojcicki	melissa.wojcicki@uconn.edu
Peter Wiebe	pwiebe@whoi.edu
Stephanie Sommer Matthews	sasommer@ucsd.edu
Le Qin Choo	chooleqin@gmail.com
Lisette Mekkes	lisettemekkes@gmail.com
Via SKYPE	
Jennifer Questel	jmquestel@alaska.edu

Appendix III. MetaZooGene WG157 2020 Annual Meeting photos.

Appendix IV. Notes from Lunchtime Meeting for MetaZooGene SCOR WG157

MetaZooGene Lunch
Thursday February 20, 2020
Broken Yolk Café, San Diego

Members present: Ann Bucklin, Katja Peijnenburg, Leocadio Blanco-Bercial, Ryuji Machida, Todd O'Brien, Erica Goetze. Guests: Le Qin Choo, Lisette Mekkes. SCOR representatives: Patricia Miloslavich, Enrique Montes Herrera.

After introductions, Enrique described his role as the WG157 SCOR Reporter and his research interests.

There followed a discussion about funding for SCOR WGs. Partricia noted that each WG was allotted $45K total and was usually expected to spend the total amount over 3 years. Todd has been a member of several SCOR WGs, and provided some insights from his experience. He mentioned that WG137, which focused on phytoplankton, was able to continue work for additional years by securing additional funding from another source. He noted that group members can change over the years. From his perspective MetaZooGene seems to be in good shape in this respect, with good engagement from all members.

Ann commented that she encourages members to invite guests to WG157 meetings and activities, especially students and early-career scientists with related interests. Enrique suggested reaching out to Anni Djurhuus at the Univ. of South Florida, and Ann agreed to do so.

One discussion topic focused on SCOR Best Practices documents. Leo is leading efforts for a planned WG157 Best Practices document on DNA barcoding. Enrique suggested that we review the best practice documents from the OVP. He noted that IOC UNESCO is gathering Best Practices, including protocols, in all fields. They have created a thematic repository, with open access, with all entries assigned a DOI for citation. Patricia noted that plankton diversity is one of the core variables (EOVs) for ocean observation.

Another alternative for Best Practices documents is publication in Frontiers in Marine Sciences, which now has a special section for these documents led by editors Johannes Carstenson and Jay Perlman. Ann noted that POBS SCOR WGxxx) published an article in L&O Methods, and that this seemed to her to be a very good way to get the word out and introduce the WG goals and approaches.

Todd helped staff the SCOR Exhibit Booth at OSM 2020, where he demonstrated the MZGdb DNA barcode atlas. He said he got lots of interest from many people who visited the booth. The database is linked to OBIS Ver. 2. Todd has downloaded over 250,000 sequences from GenBank and BOLD, which were distilled down to 183,000 with confirmed species ID. The resulting file (formatted for the Mothur pipeline, with fasta format sequence files, is ~200 MB.

Ann mentioned that the MetaZooGene WG157 approaches and methodologies, including the reference barcode database, will have direct relevance and potential applications for both ocean observation and ecosystem assessment / fisheries management. Rapid analysis of zooplankton diversity is central for these activities. Similar molecular / metabarcoding approaches can be used for microbes to mammals, and the WG157 focus on zooplankton could easily be expanded to include ichthyoplankton.

The group discussed plans for the MZG special issue, which is planned for the ICES Journal of Marine Science. The consensus was to advertise and encourage authors beyond WG157, but keep it to zooplankton. Patricia confirmed that broader participation is OK with SCOR.

Ongoing and planned special issues on related topics were discussed, including a special issue in the journal Genes that Carol Stepien mentioned in her talk at OSM 2020. Enrique mentioned that MBON is assembling two special issues focused on the intergovernmental organization, GEOBON (Global Earth Observation – Biodiversity Observing Network).

Katja mentioned that she is now one of 3 SCOR committee members for the Netherlands. She is planning to go to the SCOR Annual Meeting in Ecuador, and will plan to give the presentation and annual report for on WG157.