

BARCODING MARINE LIFE

Report of a meeting organized by the Census of Marine Life in conjunction with the Consortium for the Barcode of Life

Amsterdam, 15-17 May 2006

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Introduction

The Census of Marine Life (CoML) is an international initiative dedicated to documenting the diversity, distribution and abundance of past and present life in the world's oceans, and to predicting the future state of marine life. CoML operates through a number of activities, including an ambitious set of field projects. "DNA barcoding" is a technique that several CoML field projects are using to assist their efforts to identify and document marine species. DNA barcoding uses a short gene sequence from a standard position in the genome as a diagnostic tool for species identification. The goal of this 2 ½ day meeting was to disseminate information on DNA barcoding to the leaders of CoML field projects, and to build collaborations between CoML and the DNA barcoding community.

The Consortium for the Barcode of Life (CBOL) is an international initiative hosted by the National Museum of Natural History, Smithsonian Institution. CBOL has more than 125 Member Organizations from 40 countries, and it is devoted to promoting DNA barcoding as a global standard for species identification. The workshop agenda was developed as a collaborative effort between CoML and CBOL.

Acknowledgments

Funding for this meeting was provided by the Alfred P. Sloan Foundation of New York. CBOL provided support for the participation of leaders from the barcoding community, and administrative support during the organizational stages leading up to the meeting. **The University of Connecticut provided...** The Scientific Committee on Oceanic Research (SCOR) provided support for several attendees. Local arrangements and meeting space were provided by the Netherlands Royal Academy of Arts & Sciences.

Workshop Structure and Content⁵

The workshop included four sections:

- A public symposium which introduced DNA barcoding, CBOL, and the application of barcoding to taxonomic research and applied problems such as marine invasive species. A presentation on biodiversity research in India sensitized workshop

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⁵ See meeting agenda, Appendix 1. Presentations linked to the agenda are available at http://www.barcoding.si.edu/coml_agenda_2006.htm

participants to the concerns of developing countries that are approached by international initiatives;

- A seminar on the DNA barcoding technique and the design and implementation of barcoding research projects. Participants discussed data standards for barcode data, strategies for obtaining and curating voucher specimens, laboratory protocols for extracting and amplifying DNA and obtaining sequences, intellectual property rights, and other topics related to the conduct of large-scale barcoding projects. The BOLD data system at the University of Guelph was described. BOLD is the workbench being used by all CBOL initiatives to assemble barcode data records for later transfer to GenBank;
- A series of 13 presentations on CoML's field projects and a presentation on CoML's Ocean Biogeographic Information System (OBIS), with special emphasis on the potential for adding value through barcoding. Two presentations on marine projects in the Assembling the Tree of Life (AToL) initiative and a presentation on Diversitas explored potential linkages to the Barcode of Life Initiative; and
- A whole-group discussion on the potential for synergy between the Barcode Initiative and CoML, some potential goals for the collaboration, and the resources and processes that would be needed to attain these goals.

Meeting Results

Participants agreed that adding DNA barcoding to CoML activities would enhance their impact significantly. Barcode data could be linked to data records in OBIS, which would increase the value of records generated by CoML field projects. Occurrences of species in other locations could then be compared genetically to earlier observations. Potential new species could also be compared with known species using standardized barcode data, thereby confirming or refuting differences. In these ways, barcode data could make CoML data records the basis for a more integrated, objective and repeatable analysis of marine biodiversity.

The participants agreed that setting the following goal for 2010 would be worthwhile:

- Establish a "reference library" of DNA barcodes for as many of the 200,000 known marine species as possible. For each species, representative voucher specimens would be obtained from:
 1. Existing collections and museum holdings. These specimens have the advantage of already being identified, for the most part. The disadvantage is that the DNA of many of these specimens is degraded, either because of original fixation in formalin and/or the aging process; and
 2. Recent and future CoML expeditions. These specimens provide intact DNA as well as fresh tissue that can be preserved in cryogenic repositories. Using these specimens as barcode vouchers will require identification by taxonomic specialists in that group.

Participants discussed the challenges associated with reaching this goal, and they identified a number of action items that should be undertaken:

- A. **Increase awareness of DNA Barcoding.** Develop an educational process on the potential value of DNA barcoding to CoML. This process should target the science

community, collectors, end-users (e.g., government agencies, industry, conservation groups), CoML scientists and other scientists such as AToL. This process should also enhance collaboration among CoML scientists.

- B. **Establish a data infrastructure for marine barcodes.** CBOL has established data standards for barcode records in GenBank, EMBL and DDBJ, with GPS locality data and linkages to databases of taxonomic names and on-line museum catalogs. CoML projects will need to encourage the managers of existing and new specimen collections to digitize their specimen records and put them online, so they can be linked to barcode records.
- C. **Agree on the standard barcode region to be used in marine groups.** The mitochondrial COI region is being used for most animal groups but it does not work for some marine taxa. CBOL anticipates that there will be barcode regions other than COI and is creating a protocol for reviewing proposals to formally adopt others. CoML projects should learn more about this process and submit proposals for suitable barcode regions in groups where COI doesn't work.
- D. **Consider costs and benefits.** Most CoML projects have not been designed and costed to include comprehensive programs of specimen curation and barcoding. They will need to consider the costs and benefits of adding a barcoding component. Alternatively, they could seek partnerships with taxonomic research programs that would benefit from the specimens collected by CoML projects and would obtain barcode data that could be linked back into OBIS.
- E. **Expand linkages between CoML field projects and specimen repositories.** CoML needs to develop and promote a tradition of specimen vouchering. Museums and other kinds of collections, especially those that are CBOL Member Organizations, should be approached about becoming repositories for new collections produced by CoML field projects.
- F. **Create barcoding and curatorial protocols for use by CoML field projects.** As the culture of specimen vouchering grows and linkages to specimen repositories develop, CoML projects will need to adopt standard curatorial practices. CBOL and the museum community could produce and distribute updated protocols for specimen collection, processing and analysis. These protocols would ensure preservation of tissue and DNA for molecular analysis as well as smooth transfer of information about specimens. CBOL's DNA working group might also be helpful in compiling laboratory protocols and in making them available to CoML participants. Some protocols have already been developed and are posted on the BOLD website. Other protocols have been developed by individual programs, and they need to be assembled and updated.
- G. **Conduct barcoding workshops** that would introduce barcoding to CoML projects and create working partnerships with barcoding labs; and
- H. **Develop sequencing capacity in developing countries.** CoML projects in developing countries will have less access to sequencing facilities. There needs to be an outreach effort to these countries that will result in training and capacity-building related to DNA sequencing.

The workshop participants articulated the following Next Steps that should be proposed to CoML's Scientific Steering Committee:

1. Adopt CBOL's data standards, policies, and protocols;
2. Develop and agree upon standard formats for visualization of specimens and data;
3. Design strategies for archiving tissue and/or DNA extracts resulting from CoML field projects;
4. Establish partnerships with large museums that would be willing to act as repositories of barcode voucher specimens collected by CoML projects;
5. Establish partnerships with other relevant organizations (e.g., Ocean Genome Legacy) and institutions
6. Develop and distribute integrated "workbench software" to track specimens from collection, through barcoding, to species page production.
7. Identify barcoding "service centers" for each taxon or guild; consider distributed versus centralized barcoding facilities
8. Try to influence equipment manufacturers in ways helpful to barcoding
9. better at-sea sequencing
10. Identify bottlenecks in CBOL protocol chain; address most serious bottlenecks

APPENDIX 1: Meeting Agenda



Barcoding Marine Life



DNA Barcoding for CoML Workshop
The Netherlands Royal Academy of Arts & Sciences
The Trippenhuis, No. 29 Kloveniersburgwal, Amsterdam

Monday, May 15th PUBLIC SYMPOSIUM

- 1:00 pm Welcome
- 1:30 pm DNA Barcoding in Broad View
 - Paul Hebert (University of Guelph, Canada)
- 2:00 pm DNA Barcoding: the CBOL Perspective
 - Freek Bakker (National Herbarium, the Netherlands)
- 2:30 pm Taxonomy and Species Discovery
 - James Hanken (Harvard University, USA)
- 3:00 pm Coffee Break
- 3:30 pm FISH-BOL: the Fish Barcode of Life Campaign
 - Bob Ward (CSIRO, Australia)
- 4:00 pm Barcoding for CoML: Assessing Zooplankton Diversity
 - Ann Bucklin (University of Connecticut, USA)
- 4:30 pm Using DNA Barcodes to Study Marine Invasions
 - Jon Geller (Moss Landing Marine Laboratories, USA)
- 5:00 pm Capacity Development and Education for the Barcode of Life: Meeting the Demand for Knowledge and Skills.
 - D. Chandramohan (National Inst. of Oceanography, India)
- 5:30 pm Summary
- 6:00 pm Adjourn
- 6:30 pm Welcome Reception

Tuesday, May 16th BARCODING PROTOCOLS and PRACTICES

- 9:00 am Overview of the barcoding protocol chain
- David Schindel (CBOL)
- 9:30 am Data management & data standards for barcoding
Moderator: Bob Hanner (FISH-BOL, CBOL)
- CBOL and NCBI data requirements and standards (Bob Hanner)
- 10:00 am Specimen sources, collection, and shipping
Moderator: Lee Weigt (Smithsonian)
- 10:30 am Fixing, preserving and curating specimens
Moderator: Christoffer Schander (MAR-ECO)
- The formalin question (Christoffer Schander)
- 11:00 am Coffee break
- 11:30 am Molecular protocols
Moderator: Keith Crandall (ATOL)
- Ribosomal genes as barcodes for microbial eukaryotes (Jan Pawlowski)
- Tag sequencing approaches for barcoding (Mitch Sogin)
- Exhaustive analysis of zooplankton mtCOI genes (Ryuji Machida)
- Multiple mitochondrial pseudogenes from a copepod (Ryuji Machida)
- 12:30 pm Lunch
- 2:00 pm Taxonomic issues: identifying, naming, describing species
Moderator: Rob de Salle (CBOL)
- Character-based diagnosis of species boundaries (Rob de Salle)
- Contributions from GBIF (Larry Speers)
- 2:30 pm Data analysis and display
Moderator: Endre Willassen (CoML)
- Identifying genes in cyberspace – Endre Willassen
- DNA-Surveillance tool – Shane Lavery
- 3:00 pm Infrastructure, coordination, and capacity-building
Moderator: Nancy Knowlton (CoML)
- Approaches to implementing a CoML barcoding effort (Nancy Knowlton)
- Outreach needs for barcoding marine life (James Woods)
- The MOOREA approach (Chris Meyer)
- Cephalopod Steering Committee (Bob Ward & Victoria Wadley)
- Zooplankton sampling for barcoding in Antarctic / South China Seas (Sun Song)
- Status and challenges of barcoding fish in Africa (Ernst Swartz)
- 3:30 pm Coffee break
- 4:00 pm Open discussion
Moderators: Paul Hebert (CBOL) & Ann Bucklin (CoML)
- 5:30 pm Summation: David Schindel (CBOL)
- 6:00 pm Adjourn

Wednesday, May 17th BARCODING FOR CoML

9:00 am DNA barcoding: opportunities for CoML (Jesse Ausubel)

9:30 am Barcoding for CoML: current activities and future plans

- ArcOD – Rolf Gradinger
- CAML – Victoria Wadley
- GOMA – Lew Incze
- NaGISA – Yoshihisa Shirayama
- CREEFS – Nancy Knowlton
- MAR-ECO – Endre Willasen
- POST – David Welch
- OBIS – Catherine Duckett

11:00 am Coffee break

- CeDAMAR – Pedro Martinez
- CoMarge – Lenaick Menot
- CenSeam – Malcolm Clark
- ChEss - Cindy van Dover
- ICOMM – Mitch Sogin
- CMarZ – Ann Bucklin

12:30 pm Lunch

2:00 pm Coordination with other programs and projects

- ATOL / decapods – Keith Crandall
- ATOL / cnidarians – Allen Collins
- DIVERSITAS – Carlo Heip

3:30 pm Coffee break

3:45 pm Goals for accomplishments for 2007 and 2010

4:30 pm Next steps and action items

5:30 pm Adjourn

APPENDIX 2: Meeting Participants

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