International Census of Marine Microbes

(ICoMM)

Science Plan

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1. Executive summary:

Microbes of untold diversity in marine environments are the primary catalysts of energy transformation, and are responsible for > 98% of the carbon and nitrogen cycling [1]. An estimated 3.6×10^{30} microbial cells with cellular carbon of $\sim 3 \times 10^{17}$ grams may account for more than 90 percent of the total oceanic biomass [2]. The number of bacteriophage and viruses may be one hundred-fold higher. With such enormous populations, the accumulation of mutations should lead to very high levels of genetic diversity and phenotypic variation. Yet, traditional microbiological methods have described only 30,000 protists [3-5] and fewer than 5000 kinds of prokaryotes [6].

Today we are witness to a revolution in microbiology. Just as the first microscopes unveiled an unseen microbial world, the use of molecular techniques to enumerate different kinds and numbers of single-cell organisms has forever changed perceptions of the natural world. Microbial diversity is at least 100-1000 times greater than estimates based upon cultivation-dependent surveys [7]. Comparisons of genome sequences from cultivated and naturally occurring microbial populations reveal unanticipated levels of metabolic diversity and suggest new modes and mechanisms for evolutionary change. Microbes account for the preponderance of life's genetic and metabolic variation, but our understanding of microbial diversity and the evolution of its population structures in the oceans is only fragmentary.

To develop a full description of biodiversity in the oceans, the Census of Marine Life (COML) must look beyond metazoans and plants. It must develop a strategy to (1)

catalogue all known diversity of single-cell organisms inclusive of the Bacteria, Archaea, Protista and associated viruses, (2) to explore and discover unknown microbial diversity, and (3) to place that knowledge into appropriate ecological and evolutionary contexts. Several existing or proposed COML field projects including CeDAMar, ChEss, MAR-ECO, GoMA, NaGISA, CMarZ, Reefs, Arctic, Antarctic, Sea Mounts etc. either have microbial initiatives or the potential to develop microbial-based projects. Yet, there is no global effort to acquire information about diversity and distribution of microbes and associated viruses from the three domains of life in the World's oceans. This proposal describes an **I**nternational **C**ensus **o**f **M**arine **M**icrobes (**ICoMM**). It will advocate for and coordinate investigations of microbial diversity (Bacterial, Archaeal, Protistan and Viral) and their population structures in marine environments. **ICoMM** will have five major activities. The first is to support scientific working groups. These will focus on (1) open ocean and coastal systems, (2) benthic systems, and (3) technology that is specifically required for a microbial census. The second is to develop the data-base resource **MICROBIS**, which will organize morphological, molecular and contextual information for marine microbial diversity within a framework that integrates into OBIS. The third is to provide resources that can facilitate and coordinate requests for research support from governmental and private foundations. The fourth is to facilitate education and outreach of **ICoMM** to make it visible to the general public and raise awareness of its goals. Finally, **ICoMM** will support pilot projects that have the potential to shape larger-scale research initiatives in marine microbial diversity.

To be successful, **ICoMM** must promote international cooperation and forge linkages with existing and new COML field projects for collecting samples, contextual

information and new technologies. At the same time, **ICoMM** must engage the broader community of microbiologists with collateral interests in microbial diversity, evolution, biogeography and their functional roles in marine systems.

2. Uncharted Diversity of Marine Microbes: *The Known, Unknown and Unknowable.*

Communities of Bacteria, Archaea, and Protists account for greater than 90 percent of oceanic biomass and 98 percent of primary production [1, 2]. Stable isotopes studies reveal that for more than three billion years, these microscopic factories –initially anaerobic and later aerobic– mediated biogeochemical processes that shaped planetary habitability [8]. Today the oceans world-wide are teeming with microscopic and macroscopic life forms. Rich, chemosynthetic microbial communities thrive at deep-sea hydrothermal vents [9]. Abundant Archaea populate oceanic midwaters [10]. Very large populations of picoplankton including diatoms, dinoflagellates, picoflagellates and cyanobacteria are the primary catalysts in carbon fixation [11], orchestrate the cycling of nitrogen [12] and form the base of the traditional marine food web. Heterotrophic SAR11 represents the dominant clade in communities of ocean-surface bacterioplankton [13] while nonphotosynthetic protists of unknown diversity control the size of picoplankton populations and regulate the supply of nutrients into the ocean's food webs.

Amazing advances in microbiology over the past fifty years force us to think in terms of ever shifting boundaries between what is known, unknown and unknowable about single-cell organisms. In the late 60's, microbiologists had lost hope of constructing a robust natural system for microbial taxa. New molecular techniques developed during the 1970's opened pathways for establishing microbial phylogenetic relationships that were unknowable using traditional techniques (comparisons of phenotypic characters such as

morphology, staining properties, metabolic capabilities, and physiology). Modern technologies (molecular techniques, automated fluorescence cell sorting, etc) have demonstrated the great abundance and diversity of microbial life forms in the oceans, and DNA sequencing of environmental genomes (metagenomics) provides evidence of hitherto unrecognized physiological categories among the planktonic microbes. With the acceptance of the significance of microbial food webs in the 1980s [14, 15] and discoveries of microbial mediated biogeochemical cycles, oceanographers recognized the pivotal role of microbial communities as catalysts in oceanic processes. Biologists reached the profound conclusion that the continued survival of all multi-cellular life is contingent upon complex microbial communities of under-described and possibly unknowable diversity.

If we are to assemble a comprehensive description of marine biodiversity and the processes that shape habitats for multi-cellular life, we must determine what kinds of microorganisms occur in benthic and planktonic open ocean and coastal systems. For the traditional alpha taxonomist, a “kind” of organism is comparable to the concept of OTUs (Operational Taxonomic Units) for describing animal and plant species. Based upon traditional methods, the number of recognized microbial OTUs is almost trivial when compared to estimates of 10^6 to 10^8 species for marine fauna. This modest assessment of microbial diversity is not consistent with a 3.5 billion-year evolutionary history during which microbes have developed an enormous metabolic repertoire to cope with Earth’s dynamic environment. In contrast, culture-independent descriptions for the microbial world, which rely upon comparisons of homologous genes (phylotypes), reveal a much richer diversity. Sequence comparisons of polymerase chain reaction products (PCR

amplicons) that target phylogenetically conserved regions of ribosomal RNA (rRNA) coding regions, demonstrates that microbial diversity ranges from 10^5 to greater than 10^7 kinds of organisms [7]. Traditional microbiology has failed to culture more than 99.9 percent of these newly discovered "phylotypes" from marine environments. Using this powerful technology, the microbiologist can also make distinctions between cells with identical morphologies and enumerate differences in community structure between microbial populations. Despite the impact of new information provided by the molecular biology toolbox, traditional techniques must not be abandoned since it is within this context that our understanding of marine microbial ecology has developed.

The hallmark of microbial diversity is biochemical innovation that single-gene studies cannot fully describe. Within the next few years, molecular biology will allow us to incorporate a definition for functional capacity or inducible phenotype in descriptors of microbial diversity [16, 17]. Microbiologists are able to identify the occurrence of a particular functional or structural gene and exploit it as a marker of diversity within an isolate or for members of a naturally occurring microbial population. In a similar manner, post genomic technology can measure gene expression patterns as a means to differentiate between "kinds" of microorganisms. As a direct consequence of increased activity in marine metagenomics, the combination of high-throughput DNA sequencing, expression profiling and proteomics can describe new traits, novel functions, and unusual enzymes in microbial populations. In some cases, entirely new phyla with novel functions are being discovered [18]. These aid in understanding the evolution of life in this ancestral habitat and lead to sounder descriptions of new communities and species. Sequencing data will also be wedded to newly emerging molecular assays that

incorporate automated sampling technologies and which will lead to finer temporal and spatial resolution of molecular diversity. If advances in genome technology and bioinformatics continue on the current trajectory, sequence scans of entire genomes or communities of genomes [19] coupled with high-throughput gene expression or proteomic profiles may become the standard for defining diversity and monitoring distribution patterns for microbial species.

To fully understand microbial marine diversity it is important to integrate sequence-based studies with phylogenetically-rich information from isotopic analyses and characterizations of metabolic and biosynthetic products. For example, isotopic analyses have pinpointed lipids produced by novel Archaea that oxidize methane anaerobically [20]. Follow-up investigations at sites rich in these products have revealed abundant new phylotypes that are related to methanogens [21]. The abundance of carbon-14 and carbon-13 in lipids produced by planktonic Archaea [22] proves that those organisms are assimilating large amounts of inorganic carbon from the ocean's midwaters and must be growing as autotrophs. Unprecedented lipid structures have been traced to previously unknown planctomycetes and the long-sought capability for anaerobic oxidation of ammonia. These are just a few examples of the novel insights that can be achieved when molecular and biochemical information are combined.

3. International Census of Marine Microbes:

3.1 Objectives.

This proposal implements recommendations that are relevant to COML objectives as outlined in the document **Unveiling the Ocean's hidden majority: a roadmap**. The most general statement of **ICoMM**'s goal is to develop a highly-resolved biodiversity

data-base for marine microbes and to understand how these populations evolve and redistribute on a global scale. Beginning with Haeckel's reports from the Challenger expedition of over 100 years ago [23], traditional microbiological approaches have made important contributions to our knowledge of microbial eukaryotes too numerous to recount here, but little about Bacteria or Archaea. Most of what we must learn about microbial diversity in the oceans will depend upon the application of molecular techniques. Early molecular studies of marine microbial diversity only considered the Archaea and the Bacteria [24-27]. Recent molecular-based searches have already identified novel eukaryotic lineages in the water column and in warm anoxic sediments [28, 29]. Combined with fluorescent *in situ* hybridization technologies, it is already possible to associate novel, molecular-based lineages with specific morphologies. Efforts should be made to bring newly discovered key taxa into culture for more detailed investigations. One of our challenges is to create a bridge to expertise of the past.

Knowing what "kinds" of organisms exist within a marine microbial population and how community structure changes in response to environmental shifts are high priorities for **ICoMM**. Sampling strategies and the collection of contextual information will be important elements of this census. For example, culture – independent surveys reveal unanticipated numbers of distinct phylotypes in the benthos and plankton of open ocean and coastal waters. In contrast, deep-sea vents separated by thousands of miles sometimes display lower levels of diversity [27] but often harbor anaerobic thermophiles that have nearly identical rRNA sequences, even though these organisms have not been detected in open ocean waters. Mechanisms that might explain this biogeographical distribution will require studies of chemically-similar vent environments and strategically located,

intermediate stations. The high-throughput DNA sequencing of environmental shotgun libraries from an oligotrophic, low diversity environment [19], provides another lesson about the importance of sampling strategies. This landmark study shows that current de-facto standards of a few hundred to a few thousand sequences for PCR amplicons of conserved genetic elements e.g. rRNA coding regions- cannot fully describe microbial diversity. A more complete accounting of diversity will dictate significant increases in data collection. But this comes at a considerable cost both in terms of reagents and in analytical efforts. To maximize the science return from such costly, high-throughput studies, marine microbiologists must identify the most important questions to be addressed and the best study sites and strategies for obtaining unambiguous answers.

The historical events and underlying mechanisms that led to contemporary microbial diversity are mostly uncharted (exceptions might include the marine foraminifera). The goals of **ICoMM** include cataloguing and discovery, but must extend to an understanding of the processes by which marine microbial diversity has been created and is maintained. Genome-based studies suggest that large-scale genetic exchange corresponding to tens of thousands of base pairs from unknown genetic sources can occur over timescales required by microbes to adapt to shifts in environmental chemistry. Stunningly, we have only scratched the surface of marine environments but already learned that the correct conceptual framework for describing the dynamics of metagenome evolution and shifts in diversity might not yet be known. Some of the fundamental questions that we must address and molecular approaches make this possible include:

1) How many kinds of microorganisms exist in marine environments and what governs the evolution of microbial lineages within complex microbial communities?

2) Why do complex microbial consortia retain functionally equivalent but genetically distinct lineages rather than selecting for a single "winner" with an optimal suite of metabolic activities?

3) Does the diversity of a microbial guild relate to the stability of its functioning?

4) Is there a biogeography for distinct microbial lineages and, if so, what are the principal drivers or restrictors? What genomic changes, if any, are associated with relocation of dormant organisms over large distances?

5) How widespread is horizontal gene transfer and does it completely obliterate phylogenetic patterns for microbes? Do viruses mediate this process?

6) Do chemical environments select for lineages endowed with particular metabolic capabilities, or does the unit of selection correspond to individual genes that can transfer particular metabolic functions between lineages?

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7) What accounts for large-scale genetic variation in microbial genomes that share a very recent common ancestry? Is there a cryptic source of genetic information that selectively invades microbial genomes, or are there undocumented mechanisms that can rapidly generate novel coding capacity within a bacterial chromosome?

8) How does genotypic diversity shape phenotypic diversity, and how does this diversity influence the functioning of ecosystems?

When coupled with a larger genomic context, the interpretation of data from molecular-based field studies will challenge even the most advanced genetic algorithms and evolutionary theory. This enterprise will demand interdisciplinary efforts to explore the dynamics of microbial population biology, genome diversity, and the metabolic basis of biogeochemical processes.

3.2 Strategy

Unlike COML initiatives that focus upon geographical locations (e.g. Arctic, Antarctic, GoMA, MAR-ECO, NaGISA, POST, TOPP etc.), or restricted environments (e.g. ChEss, Seamounts, CeDAMAR, etc.), **ICoMM** will embrace a world-wide strategy to explore the diversity and distribution patterns of all kinds of single-cell organisms in marine environments. Understanding the diversity of marine microbes is a mega-science problem that requires new approaches to mapping diversity, grand strategies, integration of diverse communities, and enabling studies that will explore processes – whether ecological or evolutionary. The community of marine microbiologists that need to be involved is diverse and do not yet form a unified community. A problem of this magnitude requires careful planning and international cooperation. Because we know so little about the limits of microbial diversity or whether biogeographical distribution patterns exist for microorganisms, major advances will occur by 2010 albeit complete descriptions may require decades of research.

To address the key scientific questions outlined above (**3a. Objectives**), **ICoMM** must seek community consensus about research priorities and an integrated experimental plan. Unification of this discipline will require the development of shared, enabling technologies and standardized measurements in the same way that DNA sequencing and

"bar coding" has provided a common means to index metazoan and plant biodiversity. Constituents of **ICoMM** must agree upon sampling regimes and mechanisms for sharing samples, contextual information and new data with the scientific community. We must determine how to bring together the existing molecular data into a single framework/synthesis or establish coding standards that promote electronic exchange of information including close ties with OBIS. An important goal will be to make data from ICoMM readily accessible to process oriented interest in microbial oceanography. It will be especially important to form alliances with relevant COML and other marine microbiology initiatives. For example, **ICoMM's** advisory board and working groups include participants from ChEss, CeDAMar and GoMA. Because of overlapping interests in certain protist groups, **ICoMM** has agreed to cooperate with CMarZ in development of programmatic infrastructure. Preliminary discussions are also underway to establish a Protistan focus Group at the interface of both programs. Other collaborative activities include participation in the NSF Research Coordination Network application "Seamount Biogeosciences Network" submitted by Scripps Institution of Oceanography, formation of a partnership with the Woods Hole Oceanographic Institution in an NIH/NSF funded Center or Oceans and Human Health, and a collaboration between the MBL and the Alfred Wegener Institute to develop the WEB resource **plankton*net**. Finally, **ICoMM** must set an agenda to guide the development of funding strategies and provide support for pilot projects that have the potential to generate additional support from governmental agencies and private foundations.

3.3 Organization of ICoMM

The MBL will be the lead organization and will support a Secretariat, a small administrative staff, and a computational biology group charged with development of the **ICoMM** data base **MICROBIS** (see below). NIOZ & NIOO-CEME in The Netherlands will fund a European coordinator and will employ a data base specialist who will integrate data from our international collaborators into **MICROBIS**. The MBL and NIOZ & NIOO-CEME formed a partnership in the preparation of this proposal. The Secretariat will coordinate ICoMM activities including setting agendas, developing a community-driven data base, and providing support (financial and organizational) for meetings of **ICoMM**'s constituency.

ICoMM will coordinate scientific activities through a multi-tiered interface that will engage the general marine microbiology community, **ICoMM**'s specialized working groups and its **S**cientific **A**dvisory **C**ommittee (**SAC**). Three working groups **(Open ocean and coastal systems, Benthic systems, Technology)** will consider the science questions posed under **3.1 Objectives** as they develop a plan to address the challenges outlined under **3.2** The working groups for Benthic systems and for the Water column will consider the current status of the field, the most promising approaches for exploring marine microbial diversity, sampling requirements and potential obstacles. The Technology working group will be cross-cutting and will consider issues that overlap with the other two working groups. Their primary charge is to determine what kinds of methods and which targeted genes will be most appropriate for meeting ICoMM's scientific objectives. They will also evaluate alternative methods for sample processing, standards for data collection and data sharing.

Collectively, the three working groups will propose objectives, agendas and resource requirements for consideration by the **SAC**, which will guide and monitor development of ICoMM activities. These interactions will provide guidance for a broader community of representative marine microbiologists who will meet at least annually in order to move **ICoMM**'s agenda forward. Members of the **ICoMM** Secretariat and the **SAC** will review funding requests associated with the preparation of research proposals including either financial support or DNA sequencing support for small-scale pilot projects. Examples of four such projects are provided in the Appendix.

The division of labor into the three working groups allows us to be inclusive of the taxa to be studied and addresses fundamental differences between the benthos and the water column that will impact experimental design and processing of data. Separate working groups for the Benthos and the Water Column face different challenges in surveys of microbial diversity. The communities of organisms that inhabit these environments have different compositions and structures. The physical environments are dissimilar and different nutrient and energy pathways drive each of these systems. Chemosynthetic energy and heterotrophy dominate the Benthos, whereas photosynthesis drives Open ocean and coastal water systems. There are fundamental differences in the physical stability, scale and patchiness and therefore sampling protocols for the two types of habitats will be different. Even the extraction of biopolymers requires alternative technologies for samples collected from the benthos versus open ocean and coastal waters (water column samples). In general, we have a clearer understanding of the microbiology and physical parameters of open ocean and coastal waters, where the systems complexity is lower and the technology demands are better developed. The evaluation of benthic

diversity poses special problems associated with differentiating between organisms that are endemic versus the introduction of cells that normally live closer to the surface via sedimentary processes.

3.4 Membership of Secretariat, SAC and Working Groups.

Secretariat			Scientific Advisory Council (SAC)	
PI:	Mitchell L. Sogin	MBL	John Baross	Univ. Wash.
Co PI:	Jan W. de Leeuw	NIOZ	Robert Anderson	Bigelow
Secretariat /EPO	Linda Amaral-Zettler	MBL	Edward DeLong	MIT
Co-I	Stefan Schouten	NIOZ	Victor Ariel Gallardo	Univ. of Conc.
Co-I	Gerhard Herndl	NIOZ	Antje Boetius	MPI
Co-I	Lucas Stal	NIO	Carlos Perdos-Alio	ICM
Co-I	David J. Patterson	MBL	Francisco Rodriguez-Valera	**UMH**

Working Groups:

Open ocean and coastal systems	**Benthic systems**	**Technology**
David Karl	Andreas Teske	Rudi Amann
Steve Giovanonni	Katrina Edwards	Chris Scholin
Daniel Vaulot	Steve D'Hondt	Eric Mauther
Curtis Suttle	David M. Patterson	Robert Friedman
Peter Burkhill	Jim Prosser	Michael Kuhl
Penny Chisholm	Anna-Louise Reysenbach	

4. Data base development: MICROBIS

ICoMM will support the development and maintenance of **MICROBIS**, which is a distributed knowledge resource that provides systematic and biogeographic information

for marine viruses, archaebacteria, bacteria, photosynthetic eukaryotes and heterotrophic protists. The design of **MICROBIS** allows it to integrate seamlessly with OBIS and it takes advantage of the MBL's development effort for construction of the image-rich WEB resource, **micro*scope** (http://www.mbl.edu/microscope). Using the MBL's star*model for sharing distributed information about microbial diversity between different WEB portals, **micro*scope** currently integrates information from **plankton*net**, a network of distributed information that includes collaborators in Japan, Australia, Germany, France, Norway, Denmark and the US. **Plankton*net** seeks to develop encyclopedic knowledge resources for marine phytoplankton (e.g. http://e-bck.rd.awi-bremerhaven.de/protist/baypaul/microscope/general/page_01.htm or http://www.sb-roscoff.fr/baypaul/microscope/general/page_01b.htm). Web sites using the star*template derived from micro*scope are assembled quickly and allow distributed teams to work co-operatively to create resources of a grand scope and scale. The Data Model meets inter-operability requirements of OBIS and of other major databases (e,g., TreeBase, GenBank, the Ribosomal Database Project, the European RISSC, MIRACLE etc.). Records will include names and latitude and longitude information, will be annotated with Dublin core, ISO and TDWG-SDD - metadata standards, and incorporate DiGIR and SOAP-based protocols to promote cross-resource indexing, search and retrieval.

MICROBIS will employ a Distributed Workgroup Environments to enable a diverse community of users to manage unprecedented volumes of largely molecular data; as well as developing scaleable and flexible internet services that will allow many users to contribute to, access, organize and package information to suit the needs of a diverse

community of users. Integration relies heavily on the TNS system developed at the MBL/WHOI library to emulate taxonomy within internet services. TNS exploits the universal system of metadata – the names and the classification of organisms – that has been applied to most biological information, and uses this to organize and index information locally and remotely, to create taxon-specific links between data sources, to promote inter-operability by standardizing the names in previously independent databases, or to provides services that will mark up documents with taxonomic metadata and catalogue the resources. TNS is developed in close compliance with the International Union of Biological Sciences Taxonomic Database Working Group (TDWG) (http://efgblade.cs.umb.edu/twik/bin/view/SDD/WebHome).

To enable the international community to contribute descriptive information into a communal knowledge repository about marine microbes, the repository will include, the names, synonyms, taxonomic authorities, descriptions, images, references, web sites, distribution, ecology, dynamic links on all marine microbes. This system will share resources with micro*scope and plankton*net.

5. Education and Outreach

The outreach and education components of **ICoMM** are important. The lack of familiarity with the diversity and significance of microbial communities demands that we make a strategic and targeted commitment to education and outreach. Our proposed Education and Outreach activities include two objectives: 1) to raise community awareness of **ICoMM**; 2) to provide resources that will underpin the education of marine microbiology in schools and universities. We will work closely with the Office of Marine Programs at the University of Rhode Island (URI_OMP) and draw on their experience of

existing COML projects to implement the **ICoMM** education and outreach strategy. That strategy will take advantage of new informatics initiatives. We will use **MICROBIS** to open up access to resources across the **ICoMM** program narrowing the gap between researchers and consumers of knowledge. Working with the MLER (Microbial Life Educational Resources) project that has been funded through the NSF National Science Digital Library program, we will generate a library of digital educational resources with models for how those resources may be embedded in K-12 and undergraduate educational packages. This will be based on the model already developed for the geosciences (http://serc.carleton.edu/introgeo/index.html).

We will provide to URI_OMP the necessary imagery, content, text, and out-link bundles for CoML portal subprojects. Our outreach liaison officer (Linda Amaral-Zettler) will become a member of the COML Education and Outreach network and has already developed contacts with Sara Hickox. Our budget will ensure attendance at annual meetings. We will add customized access to the resources of the micro*scope, plankton*net, and MLER web-sites to each area of the CoML portal. The web-based knowledge environments **micro*scope** and **plankton*net** are discussed above while MLER is summarized below. We will add educational resources and special navigational pathways to **MICROBIS** to the 'Partner Resources' page. Finally we will hold our own facilitation workshops, and/or link with existing workshops being developed at the MBL in the context of other programs.

We are well positioned to do this. The team is committed to outreach and education includes participation in the Microbial Diversity course at the MBL, teacher education workshops (MBL) and the Astrobiology Education and Outreach program. We have

biodiversity informatics initiatives that will improve access to resources; and we are actively involved in educational research programs funded by the NSF.

6. ICoMM's Pogress (June, 2004).

The process of preparing an application to the Alfred P. Sloan foundation to support **ICoMM** activities has already influenced funding decisions. Several of **ICoMM**'s organizers (Sogin, Schouten, Patterson, Stal) participated in an international meeting of marine microbiologists that was organized by M.L. Sogin and supported by Alfred P. Sloan and the Gordon B. Moore Foundations. This meeting produced the white paper: *Unveiling the Ocean's Hidden Majority: a Roadmap, December 17, 2003, Guidelines from a November, 2003 strategic planning workshop MBARI / Moss Landing, CA.* The Agouron and Gordon B. Moore Foundations used our guidelines to make strategic decisions about funding activities in microbial oceanography that will impact activities relevant to **ICoMM**'s primary objectives. In the case of The Agouron Foundation, the document served as a framework for a meeting held in January 2004 in San Diego. Although they have yet to determine the exact areas of marine microbiology to fund, the Agouron Foundation will support an annual course in microbial oceanography similar to their offering in geo-microbiology. At least one significant module of their course will cover molecular techniques that **ICoMM** considers to be important for a census of marine microbes. David Kingsbury of the The Gordon B Moore Foundation used the planning document to convince his Board about the wisdom of investing in microbial oceanography. In April, the Moore foundation decided to invest approximately 143 million dollars in microbial oceanography over the next ten years. The program will initially fund twelve fellows in marine microbiology with direct cost awards that will

range from ~$300,000 to $800,000/year. Dr. David Karl of the University of Hawaii is the first recipient of the Moore funding, but other fellows to be announced in the near future have interests in marine microbial diversity issues that are directly relevant to **ICoMM**'s objectives. Several have agreed to participate in our working groups. The Moore foundation has also committed nearly five million dollars to support Craig Venter's ongoing expedition that will sample globally distributed marine environmental genomes. This project will revolutionize what we know about marine microbial diversity, but it is only the tip of the iceberg. The Moore foundation will consider other large-scale projects over the next ten years and one of ICoMM's tasks will be to develop competitive proposals. Finally, several other activities have commenced at the MBL that converge with the objectives of **ICoMM**. The first is a molecular based microbial population and genome study of microbes from effusive flows of hydrothermal vents. The National Research Council has awarded a grant to Julie Huber and she will carry out the work in M.L. Sogin's laboratory. The second is the recent expansion of **MICROBIS** to support **plankton*net** as described above. Finally, M.L. Sogin and L. Amaral-Zettler are Co-investigators in a new project that will study marine microbial population diversity in the context of a Center for Oceans and Human Health. We anticipate that the activities of ICoMM will stimulate additional funding.

6. Appendix:

6.1 Provisional schedule.

For the first two years of the project, we have assembled a schedule that is consistent with goals and objectives of ICoMM. Each year we anticipate at least two opportunities for the Organizing committee, SAC and working groups to meet. In addition we anticipate the development of MICROBIS will require interactions with other COML programs. Once ICoMM becomes established, we anticipate that ideas worthy of additional pilot project funding will develop. This may require shifting of some funds for meeting activities into support for those projects. For that reason, we have not committed to a rigid schedule for year 3.

6.1.1 Year 1.

1. **October 2004.** Establish secretariat at the MBL in Woods Hole with a European office at NIOZ.
2. **October 2004.** Initiate development of MICROBIS.
3. **October 2004.** Organizing committee will review final composition of each working group, identify science and technology goals to be explored by the working groups. This will be accomplished through e-mail and a series of video conferences between NIOZ and the MBL.
4. **November-December 2004.** Assemble each of the working groups and charge them with addressing science and technology questions outlined in this proposal. They will develop a draft strategy document that also makes

recommendations about pilot projects. Each of these meetings will be attended by a member of the organizing committee.

5. **January-February 2005.** Scientific Advisory Panel to meet in Amsterdam with ICoMM organizers and chairs of working groups to review documents generated by the working groups and to develop a plan for a broader community meeting. This plan must discuss opportunities for interactions with other CoML initiatives including opportunities to acquire samples and linking information content of MICROBIS to other WEB portals.
6. **April-May 2005.** Host a meeting for representatives of the marine microbial community to address funding opportunities, identify sampling opportunities within and external to existing COML projects, describe common technology issues, data base content including community input, and confirm potential pilot projects that could shape future funding initiatives.

6.1.2 Year 2.

1. **October 2005-September 2006**. Initiate and complete pilot projects
2. **October 2005**. **MICROBIS** to be subject to critical review by working groups, SAC, OBIS, and collaborators.
3. **October 2005-September 2006**. Identify and pursue strategic funding allies.
4. **November 2005.** Education and outreach resources to be developed, EPO strategy to be communicated to working groups, ICoMM organizers and SAC

5. **January-February 2006**. Working groups, ICoMM organizers and SAC to meet to review progress

6. **April-May 2006**-Synthesize a final strategy document addressing funding opportunities, technical issues relating to sampling, sample processing, MICROBIS standards, scientific priorities, and long term funding opportunities.

6.2 ICoMM Budget:

	Year 1	**Year 2**	**Year 3**	**Total**
Salaries & Fringe (33.6%) (MBL)	$107,422	$139,502	$143,687	$390,611
Meeting Expense				
Working Groups	$32,400	$32,400	$32,400	$97,200
SAC (inc. Organizers & Chairs	$27,000	$27,000	$27,000	$81,000
General Meeting	$138,600	$138,600	$138,600	$415,000
Total Meeting Expense	**$198,000**	**$198,000**	**$198,000**	**$594,000**
Pilot Projects (Subawards)	$50,000	$50,000	$50,000	$150,000
Total Direct Costs (MBL)	**$355,422**	**$387,502**	**$391,687**	**$1,134,611**
Salaries & Fringe (48%) (NIOZ)	$53,126	$54,720	$56,361	$164,207
Total Direct Costs (NIOZ)	**$53,126**	**$54,720**	**$56,361**	**$164,207**
Total Project Direct Costs	**$408,548**	**$442,222**	**$448,048**	**$1,298,818**
Indirect Costs	$61,282	$66,333	$67,207	$194,822
Total Project Budget	**$469,830**	**$508,555**	**$515,255**	**$1,493,640**
Cost Share	$139,884	$99,830	$102,826	$342,540
Total Project (w/Cost Share)	**$609,714**	**$608,385**	**$618,081**	**$1,836,180**

6.2.1 Budget Justification:

MBL Salary and Fringe. Dr. Sogin will contribute up to two months of time during year 1 and has requested an estimated 1.5 months of support in years 2-3 (Base salary $164,078/yr). Funds are requested in Years 1-3 to support a half time Secretariat, **Linda Amaral Zettler** (Base salary 49,768/yr), 1 month per year for **D.J. Patterson** who is the primary author of MICROBIS (Base salary at 92,028/yr) and a full time Data base programmer, **Richard Fox** (Base Salary 47,853/yr). The current MBL **fringe rate is 33.6%.** Dr. Sogin is the PI of ICoMM. He has maintained a long-standing interest in eukaryotic evolution and microbial diversity. Dr. Sogin is the Principal Investigator on the *Giardia lamblia* genome project and an NSF funded effort, *Microsporidia and the next generation of genome scientists***,** which supports a comprehensive course in genome science titled "Advances in Genome Technology and Bioinformatics" and a small genome project for the microsporidian *Nosema locustae*. His molecular phylogenetic work with ribosomal RNAs has altered our view of eukaryote evolution. Over the past fifteen years, Dr. Sogin's group has sequenced ribosomal RNA genes from more than 1000 taxa as well as a number of actin, tubulin, and P450 genes. Dr. Sogin is also a Co-I on the Woods Hole Center for Oceans and Human Health initiative and he is the PI of the MBL Astrobiology program, which supports studies of microbial diversity in extreme environments. He has experience in organizing courses and meetings, and has served as the Director of the Bay Paul Center, which has reached a steady-state population of 60 research scientists since its founding in 1997. Dr. Amaral Zettler is a Staff Scientist II in the Bay Paul Center and she is a co-Investigator on an NSF LExEn grant, the Woods Hole Center for Oceans and Human Health and the MBL's Astrobiology Initiative. She is

trained in marine microbiology with expertise in molecular phylogeny and protist diversity. She is particularly interested in the diversity and evolution a amoeboid species including Acantharians, Foraminifera and the Radiolaria. She has directed the activities of several technicians and post doctoral as part of our Astrobiology program, and her multi-lingual capabilities will facilitate interactions with our international partners. Dr. Patterson is internationally recognized as an authority on the systematics of protists with special interests in heterotrophic flagellates. He invented the WEB site mcro*scope and has transformed it into a portal that can serve taxonomic requirements for a broad range of systematic biology. Rich Fox is a scientific programmer with a strong backgound in data base management and systems design. He will work full time on the technical development of MICROBIS over the next three years. The Marine Biological Laboratory is a "soft-money research institute" and all personnel, including principal investigators, must obtain their salaries from research grants or foundation support.

Meeting Expenses. Based upon our experience with planning the meeting *Unveiling the Ocean's hidden majority: a roadmap* held at MBARI in California, we estimate international travel and meeting expenses should not exceed **$1,800/conferee**. Any unexpended funds in our budget will be used to expand the scope and size of our general meetings or they will be used to support additional pilot projects. Funds are requested in year 1 and 2 to support meetings for each of the three working working groups. We estimate that 5-6 people will attend each of these meetings or a total of 18 conferees ($32,400). The SAC plus ICoMM organizing committee will meet with the working group chairs at least once (15 conferees or $27,000) per year. A larger community

meeting will host at least 50 representatives of the marine microbiology community plus members of the working groups, SAC and ICoMM organizing committee ($138,000).

Pilot Projects. Funds have been reserved for pilot projects ($50,000/yr) and will be available to marine microbiologists who wish to initiative new investigations of microbial diversity or who need support in the preparation of competitive research proposals. We anticipate that it may be advantageous in years 2 and 3 to shift money from meeting expenses to support especially meritorious pilot projects or preparation of research applications to governmental agencies or private foundations. Examples of possible pilot projects are provided below.

NIOZ Salary and Fringe. Professor Jan de Leeuw will contribute one month per year to the ICoMM project at no charge to the Sloan Foundation. Funds are requrested to support a half time Data Base expert at NIOZ (Base salary $71,792/yr) who will coordinate European activities associated with MICROBIS. The current NIOZ fringe rate is 48%.

Indirect Costs: Indirect costs are computed at 15 percent of direct costs for both NIOZ and the MBL. Separate awards will be made to each institution.

Cost Sharing: Dr. Sogin has already contributed approximately two months of time over the past year to this project and will contribute an additional 1.5 months during year one. In addition, the MBL will provide support for 6 months of administrative support each year as well as computer facilities as required. Dr. Jan de Leeuw will contribute one month per year and NIOZ will support 6 months of administrative support. The combined cost share from both institutions for this three year project will be ~$342,000.

6.3 Literature Cited:

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6.4 Curriculum vita and Biographical Information:

6.4.1 PI. Mitchell L. Sogin, Ph.D.

Director, Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA

Positions and Employment

1967	University of Illinois, Urbana	B.S. Chemistry and Microbiology
1969	University of Illinois, Urbana	M.S. Industrial Microbiology
1972	University of Illinois, Urbana	Ph.D., Microbiology and Mole.
Bio. 1972-1976	National Jewish Center, Denver	Postdoctoral NIH Fellowship
1976-1989	Senior Staff Scientist, National Jewish Center, Denver, CO	
1980-1986	Assist. Professor, University of Colorado Health Sciences Center	

1987-1989 Assoc. Professor, Univ. of Colorado Health Sciences Center

1986-1999 Associate Fellow, Canadian Institute for Advanced Research

1997-1998 Visiting Miller Research Professor, UC Berkeley

1989-present Senior Scientist, Marine Biological Laboratory, Woods, Hole, MA

B. Honors

9/96-present Fellow of the American Academy of Microbiology

1998-present Fellow of the American Academy of Arts and Sciences

1998 present Fellow of the American Association for the Advancement of Science

C. Publications (164 TOTAL)

Teske, A., K-U. Hinrichs, V. Edgcomb, A. de Vera Gomez, D. Kysela, S.P. Sylva, M.L. Sogin & H.W. Jannasch. Microbial Diversity of Hydrothermal Sediments in the Guaymas Basin: Evidence for Anaerobic Methanotrophic Communities. *AEM* **68**(4): 1994-2007 (2002).

Edgcomb, V.E., D.T. Kysela, A. Teske, A. de Vera Gomez, and M.L. Sogin. Benthic eukaryotic diversity in a hydrothermal vent. *Proc. Natl. Acad. Sci*. **99**(11):7658-7662 (2002).

Amaral Zettler, L.A., F. Gomez, E. Zettler, B.G. Keenan, R. Amils and M.L. Sogin. Heavy-metal, acid-loving eukaryotes from Spain's "River of Fire". *Nature* **417**:137 (2002).

Dhillon, A., A. Teske, J. Dillon, D.A. Stahl and M.L. Sogin. Molecular Characterization of Sulfate Reducing Bacteria in the Guaymas Basin. *AEM* **69**(5):2765-2772 (2003).

D. Thesis Advisor or Postgraduate-Scholar Sponsor to (past 5 years):

Total Postdoctoral Advisees: 11 Total Graduate Students: 7

E. Active Research Support (Budgets shown for one year)

NNA04CC04A From Early Biospheric Metabolisms to the Evolution of Complex Systems. Project Period: 11/1/03 – 10/31/08 Award Amount: $683,132

NSF-DEB0085486 Adaptation of Unicellular Eukaryotes to Extremely Acidic Environment. Project Period: 2/1/01 – 1/31/05 Award Amount: $109,693

NSF-MCB-0135272 Microsporidia and the Next Generation of Genome Scientists Project Period:10/1/01 – 9/30/05 Award Amount: $143,959

NIH 1-R01 AI058054-01 Molecular Evolution of Eukaryotes: a protistan emphasis Project Period:12/1/03 – 11/30/08 Award Amount: $348,104

NSF / NIH 1 P50 ES012742-01 Woods Hole Center for Oceans and Human Health Project Period 2/16/04 – 12/31/08 Award Amount: $167,493

6.4.2 Co-PI.– Jan W. de Leeuw, **Prof.dr..**

Director, Royal Netherlands Institute for Sea Research, Texel, The Netherlands;

A. Positions and Employment

1965	University of Amsterdam, BSc degree Chemistry
1968	University of Amsterdam, MSc degree Chemistry with Synthetic Organic Chemistry, Physical Organic Chemistry, Chemical Technology, Electronics, Microbiology, Biochemistry
1971	University of Amsterdam, PhD degree in Natural Sciences
1971-1976	Delft University of Technology, Junior scientist
1974-1975	University of California, Berkeley, Visiting Research Professor

1982	University of Melbourne, Australia, Visiting Research Professor
1976-1986	Delft University of Technology, Senior scientist, Dept of Chemistry and Chemical Engineering,
1986-1992	Delft University of Technology, Ass. Research Professor in Organic Geochemistry, Dept. Chemistry & Chemical Engineering.
1989-1995	University of Utrecht, Reader, Dept. of Earth Sciences
1992-present	University of Cataluña, Spain, Professorship in Geochemistry
1993-1995	Netherlands Institute for Sea Research, Texel, Head, Dept of Marine Biogeochemistry
1995-present	University of Utrecht, Professor, Dept. of Earth Sciences
1995-1996	Netherlands Institute for Sea Research, Texel, Director a.i and Head Department of Marine Biogeochemistry & Toxicology
1996-Present	Netherlands Institute for Sea Research, Texel, Director
2003-present	University of Utrecht, Professor, Dept. of Biology

B. Honors

1989	Best Paper Award 1988 - Geochemical Society
1989	Most productive scientist 1987 of the Delft University of Technology
1991	Treibs Award of the Organic Geochemistry Division of the Geochemical Society
1992	Doctor Honoris Causa of University of Cataluña
1993	Dr. P.H. Given Lecturership award from Pennsylvania State University
1995-present	Member Royal Dutch Academy of Sciences (KNAW)
1997-present	Geochemistry Fellow

C. Publications (437 in total, 20 of which in Nature and 2 in Science)

Van der Meer M.T.J., Schouten S., Sinninghe Damsté J.S., De Leeuw J.W. & Ward D.M. Compound-specific isotopic fractionation patterns suggest different carbon metabolisms among *Chloroflexus*-like bacteria in hotspring microbial mats. *Appl. Environm. Microbiol.* **69:** 6000-6006 (2003

Grutters M., Van Raaphorst W., Epping E., Helder W. & De Leeuw J.W. Preservation of amino acids from in situ-produced bacterial cell wall peptidoglycans in northeastern Atlantic continental margin sediments. *Limnol. Oceanogr.* **47**: 1521-1524 (2002

Ward D.M., Bateson M.M. and De Leeuw J.W. Use of 16SrRNA, lipid and naturally preserved components of hotspring mats and microorganisms tohelp interpret the record of microbial evolution. *In: Thermophiles: Biodiversity, Ecology, and Evolution* (Reysenbach et al. eds.; Kluwer Academic/Plenum Publishers, New York) pp. 167-181 (2001)

Passier H.F., Bosch H-.J., Nijenhuis I.A., Lourens L.J., Bottcher M.E., Leenders A., Sinninghe Damsté J.S., De Lange G.J. & De Leeuw, J.W. Sulphidic Mediterranean surface waters during Pliocene sapropel formation. *Nature* **397**: 146-149 (1999)

D. Thesis Advisor or Postgraduate-Scholar Sponsor to (past 5 years):

Total Postdoctoral Advisees: 10 Total Graduate Students: 50

E. Active Research Support (Budgets shown for one year)

Funded proposals over the last 30 years: about 40, ca. 20 of which funded (total budget: ≈ M€ 3,5

Other ICoMM Co-Investigators (Organizing committee members):

6.4.3.1 Secretariat: Linda Amaral Zettler, Ph.D. Staff Scientist II, Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA; **Research interests**: Diversity and Phylogenetic relationships of amoeboid protists in marine environments, Eukaryotic diversity in extreme environments. **Active Research Support:** NNA04CC04A From Early Biospheric Metabolisms to the Evolution of Complex Systems: 11/1/03 – 10/31/08 ($684,178); NSF-DEB0085486 Adaptation of Unicellular Eukaryotes to Extremely Acidic Environments: 02/01/01 – 1/31/05 ($109,693); NIH 1 P50 ES012742-01 Woods Hole Center for Oceans and Human Health 02/16/04 – 12/31/08 ($19,511); NSF-OCE-0430724 Woods Hole Center for Oceans and Human Health ; 05/01/04 – 04/30/09 ($27,647); NSF- MCB-0084224 "A Tropical Microbial Observatory: Collaborative Research on Microbial Diversity in Caterpillars"

01/01/01 – 09/30/04

6.4.3.2 Co-I David J. Patterson - MICROBIS, Ph.D., D.Sc**.,** Senior staff scientist, Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA. **Research interests**: Alpha taxonomy and phylogeny of protists, Role of heterotrophic flagellates in marine environments, Development of WEB resources for descriptions of microbial diversity. **Active Research Support**: NSF Digital Educational Resources in Microbial Ecology, Evolution and Diversity: 11/01/03 – 10/31/05 ($485,054); NSF From Early Biospheric Metabolisms to the Evolution of Complex Systems; 11/01/03 – 10/31/08 ($683,132); NSF - NSDL: The Tree of Life Database: A Digital Library of Biodiversity Information: 01/01/041 – 12/31/04 ($42,247); NSF Collaborative Research: Microbial Observatory in the Cariaco Basin – Dynamics of protistan diversity across time, space, and chemical gradients: 10/1/04 – 12/31/08 ($127,183); NASA Earth's Earliest

Ecosystems in the Classroom: 01/01/04 – 12/31/06 ($12,033).

6.4.3.3 Co-I. Stefan Schouten, Ph.D.**,** Senior researcher, Royal Netherlands Institute for Sea Research, Texel, The Netherlands. **Research interests:** Bacterial and Archaeal diveristy in marine environments, Diversity and distribution of Lipid biomarkers in marine microbes, Microbial mediated biogeochemical processes in the benthic environments. **Active Research Support:** ALW 805.47.097: Biophysical properties of newly discovered membrane lipids: 5/1/04 – 4/30/07 (€ 236,714); ALW 854.00.003 A new sea surface temperature proxy based on archaeal membrane lipids: 04/1/03 – 3/30/08 (€134,417).

6.4.3.4 Co-I. Lucas J. Stal, Ph.D., Head of Department, Marine Microbiology, Netherlands Institute of Ecology – KNAW, Yerseke, The Netherlands. **Research interests:** Structure and dynamics of cyanobacterial population structures in the oceans, Primary production and Nitrogen Fixation in marine environments, **Active Research Support:**

EU QLRT-2001-02132 Microalgae as Cell Factories for Chemical and Biochemical Products: 03/01/2003 – 09/01/2004 (€ 27,600); EU EVK3-CT-2002-00087 Microbial marine Communities Diversity: from culture to function: 11/01/2002 – 11/01/2005 (€ 273,960); ALW – VLANEZO 832.11.003 Diversity – Productivity Relationships in Microphytobenthos: 03/01/2002 – 12/31/2006 (€ 300,000); IOP-Senter IZW99121B New antifouling coatings with natural biocides for ships: 11/16/2000 – 11/16/2004(€ 180,586).

6.4.3.5 Co-I. Gerhard J. Herndl. Ph.D. Professor of Biological Oceanography at the University

of Groningen (The Netherlands) and Head of Department of Biological Oceanography at the Netherlands Institute for Sea Research (NIOZ). **Research interests**: Marine Microbial and Molecular Ecology with emphasis on structure and function relationships in microbial communities. Biotic and abiotic transformation of dissolved organic matter in the sea. Role of virioplankton in structuring the bacterioplankton community. Microbial Oceanography (linking the thermohaline circulation to the biogeography of prokaryotes and to biogeochemical cycling of organic matter)

Active Research Support: Dutch Science Foundation (NWO-ALW) 2004. (€ 235,015)

6.5 Example Pilot Projects.

6.5.1 ICoMM pilot project 1: Haptophyte diversity and distribution in the Oceans.

The Haptophyta (Prymnesiophyta) is a group of eukaryotic algal protists that is almost completely restricted to marine environments. The bodies of many haptophytes are surrounded by a layer of calcium carbonate scales - coccoliths. Some species, such as Emiliana huxleyi, occur in massive blooms, and as the blooms terminate so vast quantities of coccoliths precipitate through the water column to form the ooze which coats much of the ocean floor. This process sucks sequesters large quantities of carbon dioxide, and is believed to be the principal pathway by which carbon is removed from the biosphere and transferred to the geosphere – thereby mitigating global warming. Consequently, our understanding of the diversity and ecology of haptophytes feeds directly to global carbon budgets. Traditional techniques reveal only several hundred species within this group. Because of the geochemical significance but modest diversity, this group has been identified as ideal test group with which to develop the protocols and

structure of the biocentric dimensions of MICROBIS. The goal would be to comprehensively populate the communal knowledge repository with biological and distributional data on all coccolithophores, and to create the bridges between molecular data environments and traditional data for these organisms. This work woiuld involve experts Ric Jordan (Kochi University, Japan, Ian Probert, University Caen, France) and the **plankton*net** consortium.

6.5.2 ICoMM pilot project 2: Linking the oceanic conveyor belt circulation to the composition and function of prokaryotic communities

Over the past 2 decades, research on marine biogeochemical cycles and in biological oceanography was dominated by the concept of the biological pump, i.e., the vertical flux of matter in the ocean and its transformation mediated by the different functional biotic groups. In contrast, one of the major paradigms in physical oceanography, the meridional ocean circulation (MOC), also known as the oceanic conveyor belt circulation, linking all the oceanic basins on a time scale of 1500 to 2000 years has barely been investigated for its biological or biogeochemical consequences.

During two cruises within the TRANSAT project, the succession of the prokaryotic and viral communities of the North Atlantic Deep Water (NADW) driving essentially the oceanic conveyor belt was investigated. Following the NADW over more than 4000 km corresponding roughly to the first 50 years of the MOC, distinct successions in the prokaryotic communities in the NADW and its adjacent water masses were detectable along with distinct changes in the concentrations of dissolved matter. Thus, deep water prokaryotic communities are obviously tightly linked to the

biogeochemistry of these water masses and are essentially independent of the processes in surface waters. More important than surface water processes and consequently, vertical transport, for the prokaryotic composition and biogeochemistry of the meso- and bathypelagic water masses are their source of origin. Once the deep water masses are formed they carry their signal for several decades. These water masses are only modified in regions of the MOC where other water masses mix with the NADW such as at the Charlie-Gibbs-Fractor Zone at the Mid-Atlantic Ridge where Labrador Seawater recharges the NADW and therefore, the conveyor belt circulation. It is likely that there are several 'hot spots' in the oceanic conveyor belt circulation where the gradual changes in the deep water microbial communities and biogeochemistry are interrupted by input of other water masses. Oceanic deep water prokaryotes are phylogenetically and functionally different from surface water prokaryotes. It was recently shown that unculturable pelagic Archaea, dominating the prokaryotic community in terms of abundance in the ocean's interior are actively metabolizing a range of substrates. To further elucidate the role of the prokaryotic community driving the biogeochemical cycles in the dark ocean, a combined multidisciplinary international research effort is required in which high-throughput, microbial diversity and population studies are combined with contextual measurements that reflect microbial mediated processes.

6.5.3 ICoMM pilot project 3: Lipids as markers of past and present microbial diversity:

The taxonomy of marine microbial organisms mainly relies on morphology and particularly gene composition. Identification of uncultivated microbes in the ocean,

specifically those with no morphological features, are primarily depending on several gene techniques and relating the sequences derived from these techniques to cultivated members. Another feature which can be used to group (classes of) organisms is the lipid composition of membranes. This composition is usually qualitatively determined by the gene composition of the microbe whilst the quantitative composition is mainly determined by expression of genes as induced by the environment and growth conditions. A number of lipids are of taxonomic value. For example, isoprenoid diether lipids are only biosynthesized by Archaea, particularly the Euryarchaeota. A specific isoprenoid tetraether lipid called crenarchaeol is biosynthesized only by uncultivated group I Archaea, the non-thermophilic crenarchaeota. Highly branched isoprenoids are biosynthesized only by two distinct phylogenetic clusters within the diatoms.

The membrane composition of marine microbes can thus be of assistance in making general taxonomic classifications. Importantly, these lipids can also be analyzed in sea water thereby assisting in the determination of the presence or absence of groups of marine microbes. Furthermore, in contrast to DNA, these components can be traced back into ancient rocks of up to 2 billion years old thereby allowing the reconstruction of the evolutionary history of marine microbes and the calibration of the molecular clock [22]. However, the widespread application of the combined lipid-gene sequence approach is hindered by the lack of a comprehensive database on the composition of membrane lipids in cultivated marine microbes and in sea water of different oceanic regimes. This potential pilot project, could provide an additional tool to recognize the diversity of past or present-day marine microbes.

6.5.3 ICoMM pilot project 3: Linking Cyst Morphology to Adult Stages of Acantharia in the World's Oceans

The biogeography of certain members of the plankton community that secrete carbonaceous or siliceous hard parts play a critical role in the movement of particulate organic carbon to depth and form the basis of silica-based versus carbonate-based oceans. The influence that other kinds of "ballast" materials may have has not been systematically explored in other groups of plankton that may also contribute significantly to the carbon cycle – particularly at depth. The acantharia represent such an unexplored group. These sarcodine amoebae, belonging to the class Acantharea, are ubiquitous inhabitants of the world's oceans, particularly in the high latitudes and are contributors to primary productivity through their algal endosymbioses. Being the only organisms to construct skeletons of celestite ($SrSO_4$), acantharia are the most significant group of plankton responsible for the global Sr cycle. Since acantharia are abundant and their celestite skeletons are dense (celestite is among the most dense biogenic minerals in the ocean; about 4.0), acantharian skeletons, and in particular, reproductive cysts that sink to a depth down to 400 m, most likely act as unique ballasts removing particulate organic carbon from the upper ocean and transferring it to the Mesopelagic Zone.

Studying acantharia has been difficult because they are fragile, cannot be reared in the laboratory through successive generations, and their celestite ($SrSO_4$) skeletons are highly soluble in seawater and generally disappear as soon as the cell dies. While about 150 species of acantharia have been described in total, many acantharian cysts identified by workers are described as new species based on cyst material alone. In general, cysts are not typed according to species of adult but instead based on artificial numbering or

lettering designations. Some species, however, have known cyst morphologies. These include: *Acanthicolla cruciata, Haliommatidium mülleri, Gigartacon sp., Stauracon pallidus, Amphiacon denticulatus* and *Heteracon biformis*. It is important to note that only three of the four orders of Acantharea form cysts and therefore only certain species can serve as ballast. In the cases where no information is known about the associated adult form of a cyst, comparative molecular techniques can be used to compare sequences derived from adults with those from cysts. There are currently 9 acantharian SSU rRNA gene sequences reported in GenBank. Of these 6 were derived from individual specimens identified at least to the taxonomic level of "order" and some to genus level. Since this represents a small fraction of the total number of acantharian species, the molecular diversity of this group is still largely unknown. Therefore, knowing the taxonomic order to which given acantharian species belong will help to predict the depth to which a given "bloom" may affect celestite concentrations. Furthermore, it is important to note that acantharian abundance may have an indirect impact on other plankton populations such as polycystine radiolaria, that often possess skeletons of silica. Skeleton-bearing and non-skeletal bearing polycystine radiolaria also have crystals of $SrSO_4$ associated with the central capsules of colonial forms and inside of swarmer cells produced by reproductive individuals. By increasing celestite concentrations in the surrounding seawater as their skeletons dissolve, acantharia may serve as a link to plankton that secrete SiO_2 and also require celestite.

This pilot project will provide a link to a possible CMarZ project and will form the basis of an electronic taxonomic key that will be incorporated into MICROBIS. It

will also serve as example for addressing similar life stage questions in other groups of plankton – such as the planktonic foraminifera.