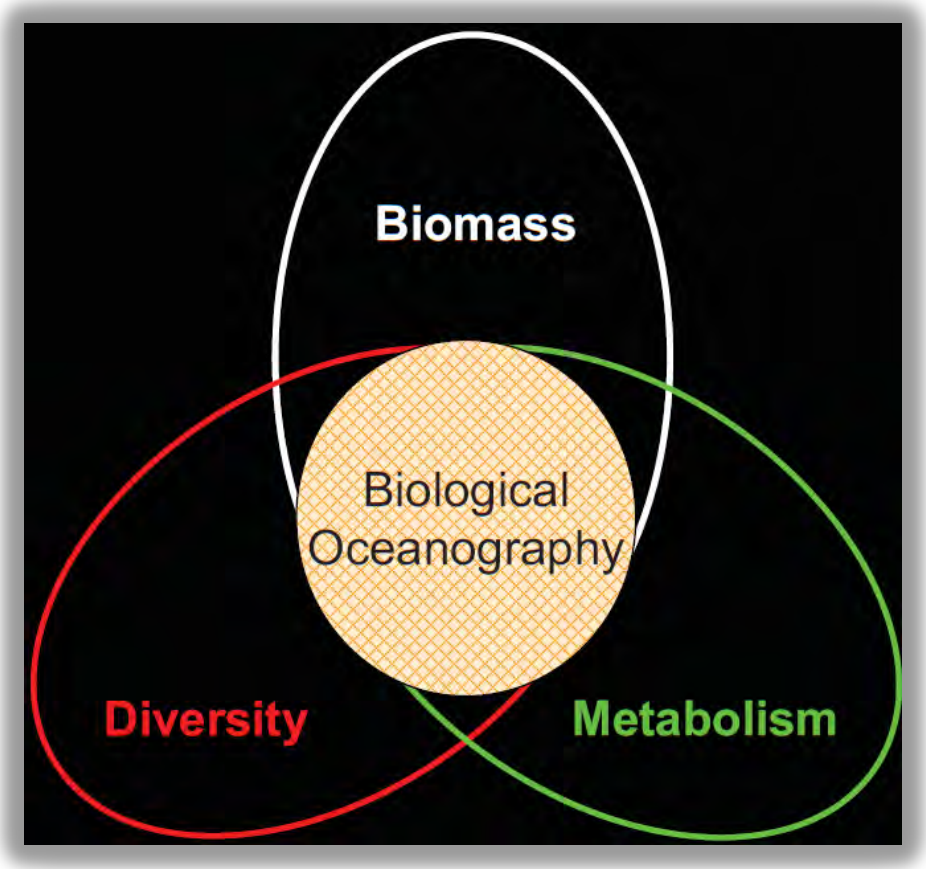
Metagenomic analysis reveals the cryptic plankton biodiversity in the Nakdong River Estuary in Korea

Sang-Rae Lee[1], Jee Eun Lee[2], Jung Hyun Oak[1], Jin Ae Lee[3] and Ik Kyo Chung[1,2]

[1]Marine Research Inst., PNU, Busan
[2]Div. Earth Environ. System, PNU, Busan
[3]Sch. Environ. Sci. Eng., Inje U, Gimhae
Republic of Korea

Species Identification, Biodiversity & Biological Oceanography

Solution ?



http://www.soest.hawaii.edu/oceanography/courses/OCN621/Spring2009/introduction_lecture.pdf

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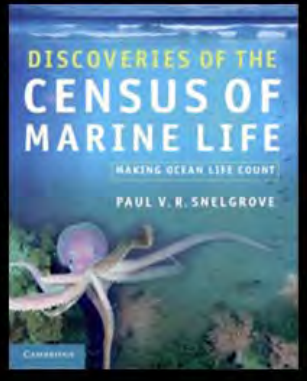

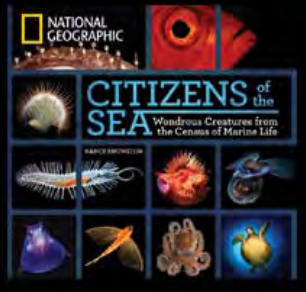

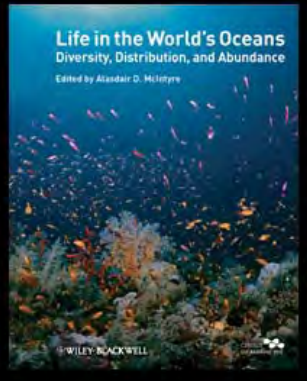

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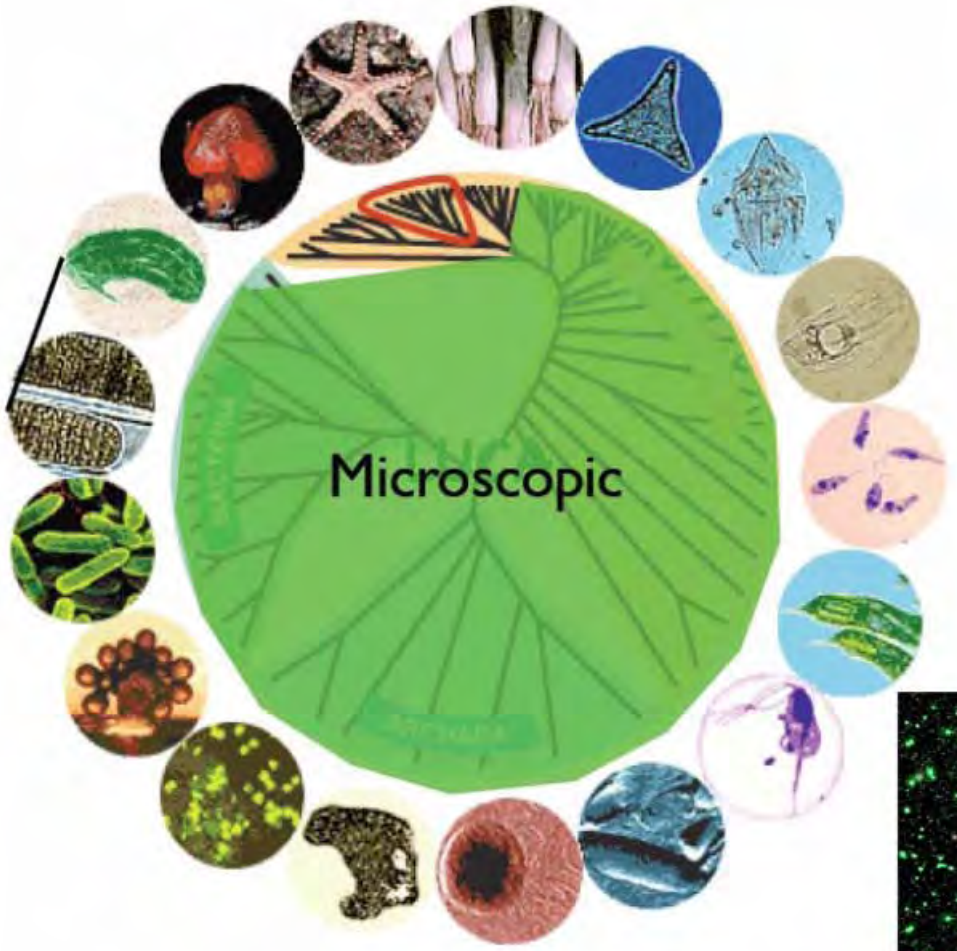
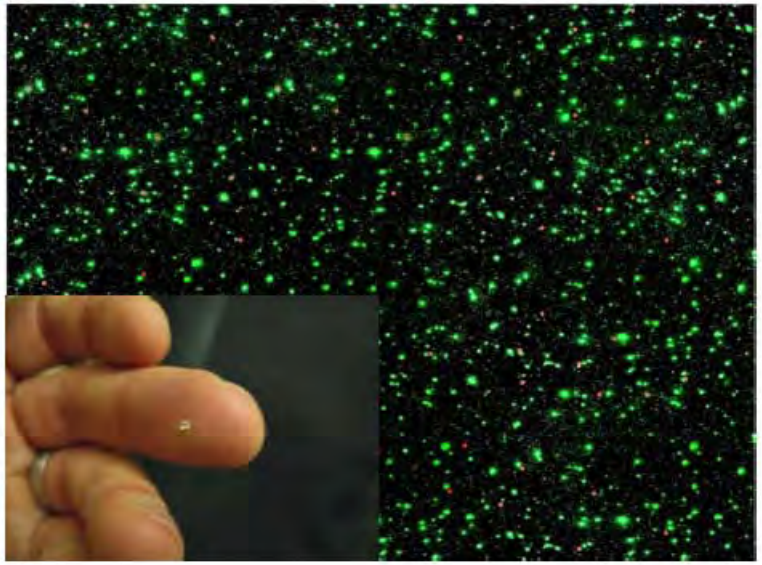
Image Gallery

Most life is microscopic





In the ocean, microscopic organisms account for >60% of the total living biomass.

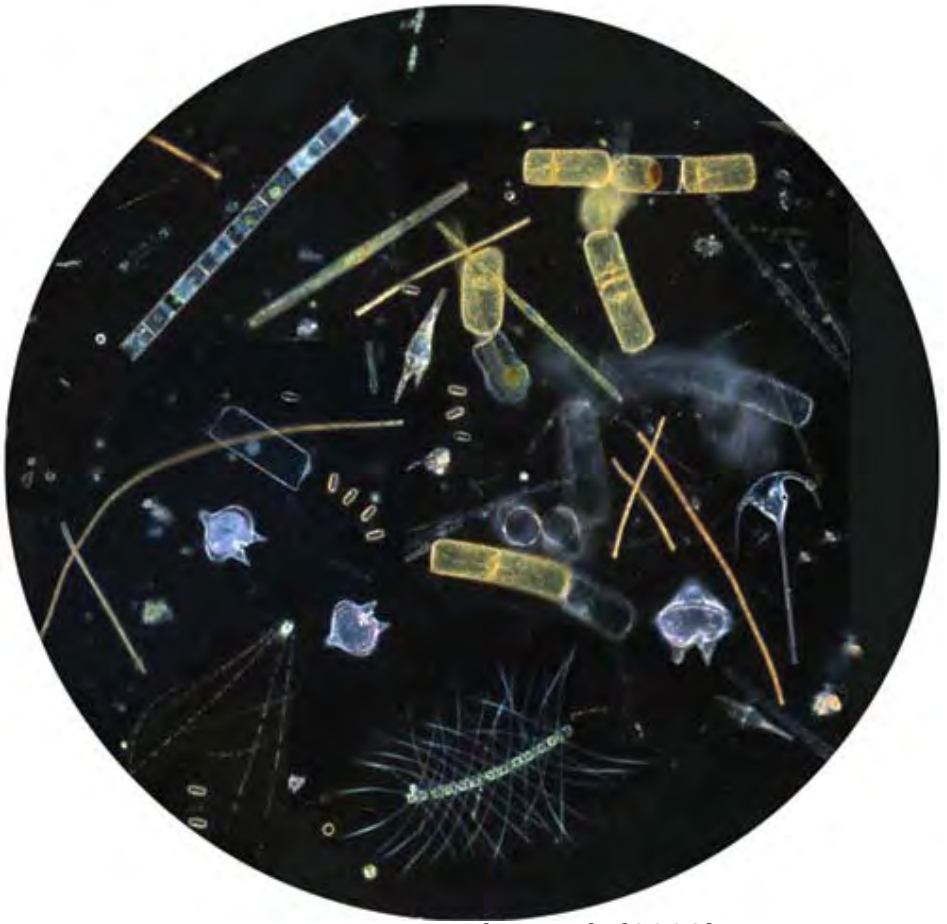
http://www.soest.hawaii.edu/oceanography/courses/OCN621/Spring2009/introduction_lecture.pdf

NATURE|Vol 459|14 May 2009|doi:10.1038/nature08056

Microbial oceanography in a sea of opportunity

Chris Bowler[1,2], David M. Karl[3] & Rita R. Colwell[4]

**Plankton use solar energy to drive the nutrient cycles t
We can now explore the diversity and functions of plar
associated with survival in the oceans. Such studies wi
and of the ocean's response to climate change, improv**



Bowler et al. (2009)

Where's Waldo?!

Traditional Morphological Approach?

http://www.flickr.com/photos/82519984@N00/158630569

Metagenomics

Molecular Biological Methods

- ….This relatively new field of genetic research *enables studies of organisms that are not easily cultured in a laboratory as well as studies of organisms in their natural environment*….
- …*The power of genomic analysis (the analysis of all the DNA in an organism) is applied to entire communities of microbes, bypassing the need to isolate and culture individual microbial species*

Nature (2001)

Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity

Seung Yeo Moon-van der Staay*†, Rupert De Wachter‡ & Daniel Vaulot*‡

** Station Biologique, UPR 9042 Centre National de la Recherche Scientifique et Université Pierre et Marie Curie, BP 74, 29682 Roscoff Cedex, France*
‡ Departement Biochemie, Universiteit Antwerpen (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium

Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton

Purificación López-García*, Francisco Rodríguez-Valera*, Carlos Pedrós-Alió† & David Moreira*

** División de Microbiología, Universidad Miguel Hernández, 03550 San Juan de Alicante, Spain*
† Institut de Ciències del Mar, CSIC, 08039 Barcelona, Spain

Venter et al. 2004. Environmental genome shotgun sequencing of the sargasso sea. Science, 304:66-74

Published Online March 4 2004
Science 2 April 2004:
Vol. 304 no. 5667 pp. 66-74

“whole-genome shotgun sequencing”

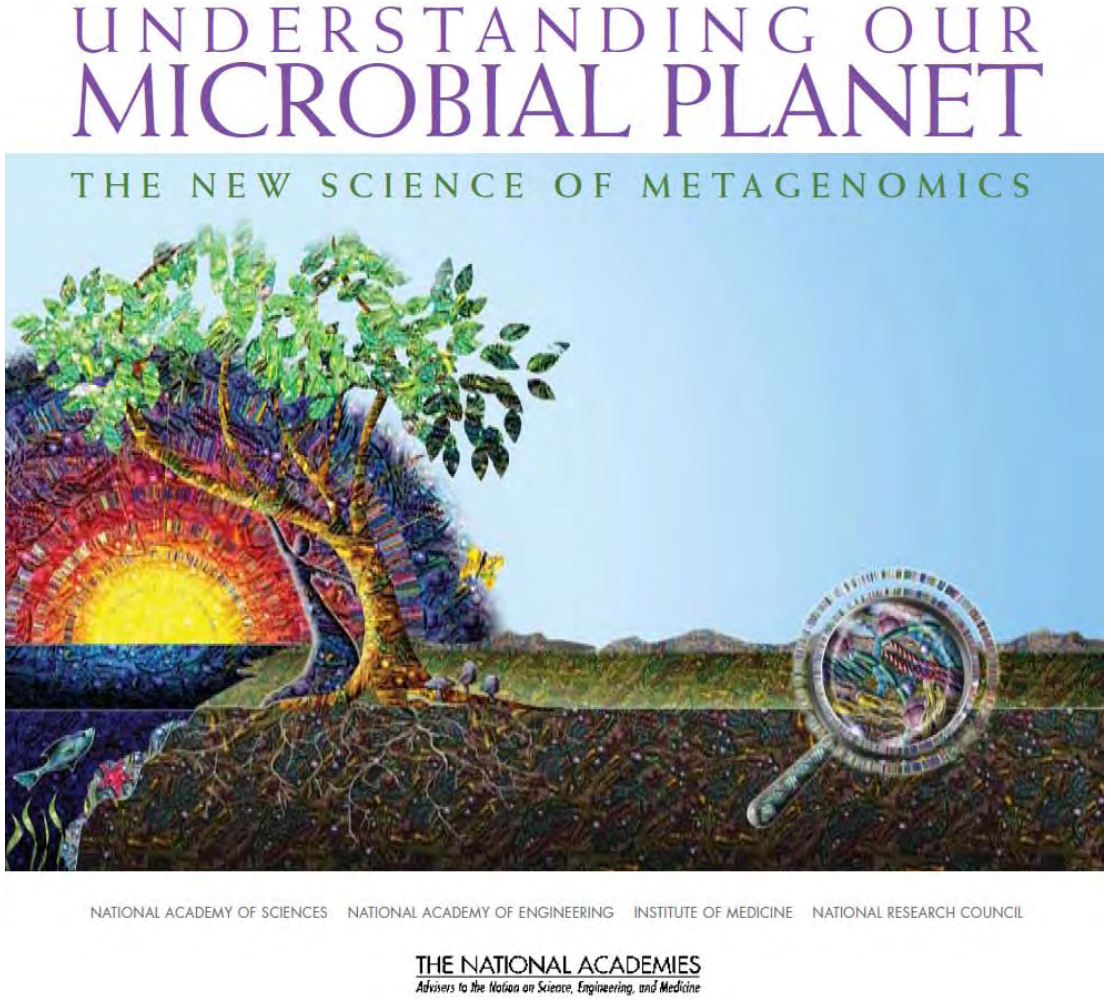
Environmental Genome Shotgun Sequencing of the Sargasso Sea

**1800 genomic species
based on sequence relatedness, including
148 previously unknown bacterial phylotypes
over 1.2 million previously unknown genes**

ABSTRACT

We have applied “whole-genome shotgun sequencing” to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1.045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.

The New Science of Metagenomics:
Revealing the Secrets of Our Microbial Planet (2007)



Tech News

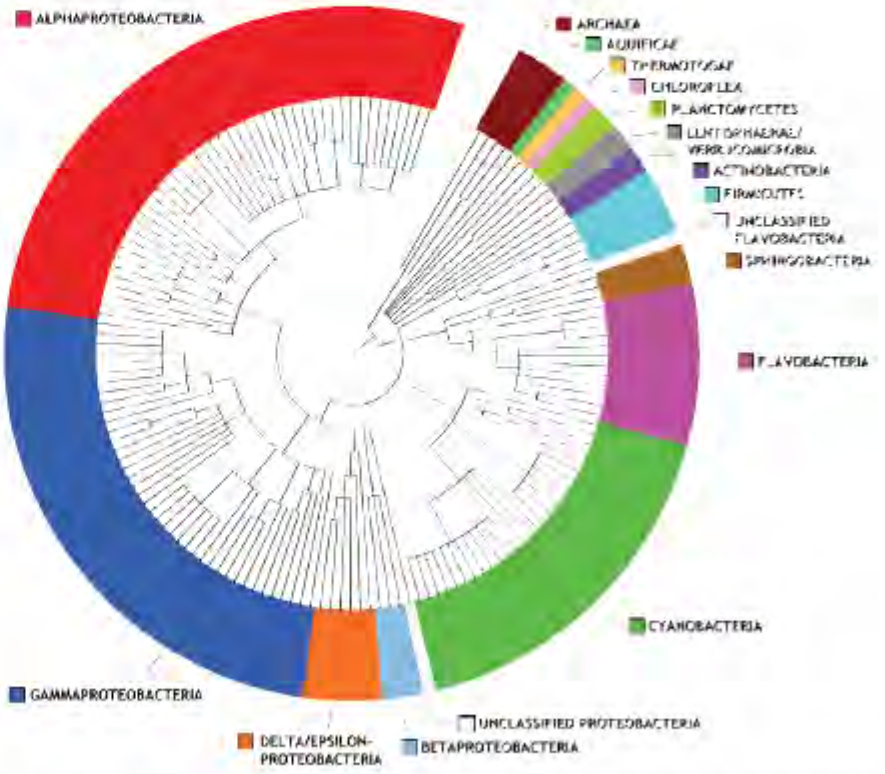
Marine Metagenomics

With oceans covering 70% of our planet and one milliliter of seawater containing 10,000–1,000,000 microbes (1), there is clear impetus for researchers to identify and access this plethora of genomic information, which could offer a better understanding of these microbes' role in regulating and responding to global climate changes and other critical ecosystem processes.

But as much as researchers have their eye on the yet-untapped genetic sequences of marine microbial species, these millions of unseen ocean inhabitants aren't exactly suited to conventional DNA sequencing, which requires a culture of identical cells. Fortunately for those interested in deciphering the ocean's metagenome—that is, the collection of microbial genes in this mixed environmental sample—recent advances in sequencing technology and computational power are starting to break below the surface on the importance of ocean microbes.

Trends in Expression

"We need a whole earth catalog of genes and genomes to help us describe processes driven



Jonathan Eisen and colleagues have compiled a phylogeny-driven genomic encyclopedia of prokaryotes. This will aid researchers in identifying novel genetic sequences. Phylogenetic tree courtesy of Patrick Chain, Gordon and Betty Moore Foundation.

Diana Gitig, BioTechniques, Vol. 48, No. 5, 2010, pp. 361–365

http://metagenomics.anl.gov/

OPEN ACCESS Freely available online

PLoS one

New Insights into the Diversity of Marine Picoeukaryotes

Fabrice Not[1,2]*, Javier del Campo[1], Vanessa Balagué[1], Colomban de Vargas[2], Ramon Massana[1]

1 Institut de Ciències del Mar, CSIC, Barcelona, Spain, **2** Station Biologique de Roscoff, UMR7144, Evolution du Plancton et PaléoOcéans (EPPO), Centre National de la Recherche Scientifique (CNRS) et Université Pierre et Marie Curie (UPMC), Place George Teissier, Roscoff, France

Abstract

Over the last decade, culture-independent surveys of marine picoeukaryotic diversity based on 18S ribosomal DNA clone libraries have unveiled numerous sequences of novel high-rank taxa. This newfound diversity has significantly altered our understanding of marine microbial food webs and the evolution of eukaryotes. However, the current picture of marine eukaryotic biodiversity may be significantly skewed by PCR amplification biases, occurrence of rDNA genes in multiple copies within a single cell, and the capacity of DNA to persist as extracellular material. In this study we performed an analysis of the metagenomic dataset from the *Global Ocean Survey* (GOS) expedition, seeking eukaryotic ribosomal signatures. This PCR-free approach revealed similar phylogenetic patterns to clone library surveys, suggesting that PCR steps do not impose major biases in the exploration of environmental DNA. The different cell size fractions within the GOS dataset, however, displayed a distinct picture. High protistan diversity in the <0.8 μm size fraction, in particular sequences from radiolarians and ciliates (and their absence in the 0.8–3 μm fraction), suggest that most of the DNA in this fraction comes from extracellular material from larger cells. In addition, we compared the phylogenetic patterns from rDNA and reverse transcribed rRNA 18S clone libraries from the same sample harvested in the Mediterranean Sea. The libraries revealed major differences, with taxa such as pelagophytes or picobiliphytes only detected in the 18S rRNA library. MAST (Marine Stramenopiles) appeared as potentially prominent grazers and we observed a significant decrease in the contribution of alveolate and radiolarian sequences, which overwhelmingly dominated rDNA libraries. The rRNA approach appears to be less affected by taxon-specific rDNA copy number and likely better depicts the biogeochemical significance of marine protists.

Citation: Not F, del Campo J, Balagué V, de Vargas C, Massana R (2009) New Insights into the Diversity of Marine Picoeukaryotes. PLoS ONE 4(9): e7143. doi:10.1371/journal.pone.0007143

Editor: Ryan L. Earley, University of Alabama, United States of America

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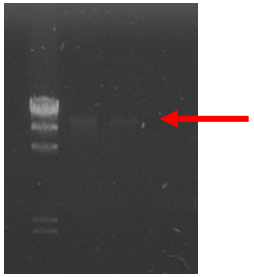
Competing Interests: The authors have declared that no competing interests exist.

* E-mail: not@sb-roscoff.fr

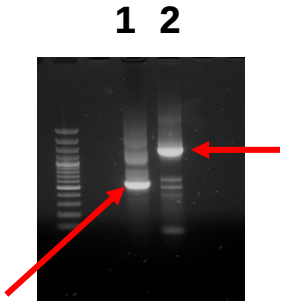
Molecular Analyses

Filtering system

250 ml seawater
Filters

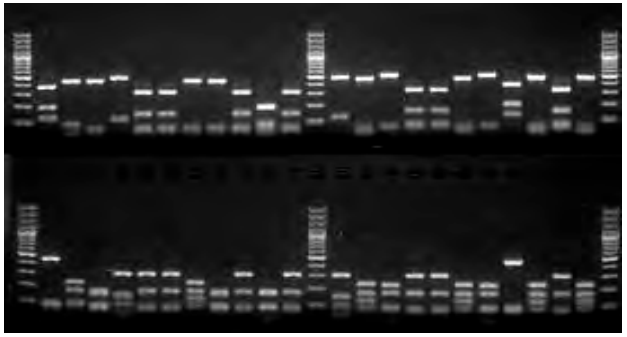


DNA extraction
from environmental sample

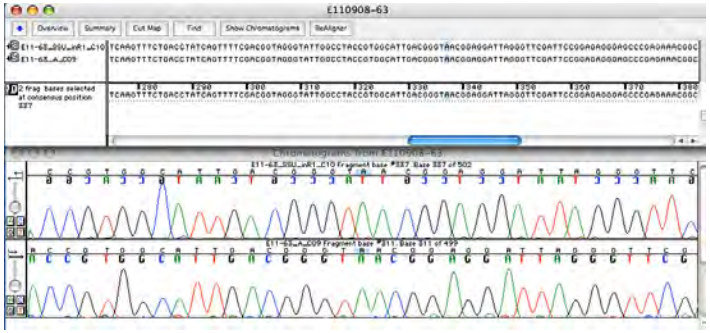


Environmental PCR
1; Primer pair A & SSU-inR1
2; Primer pair A & B

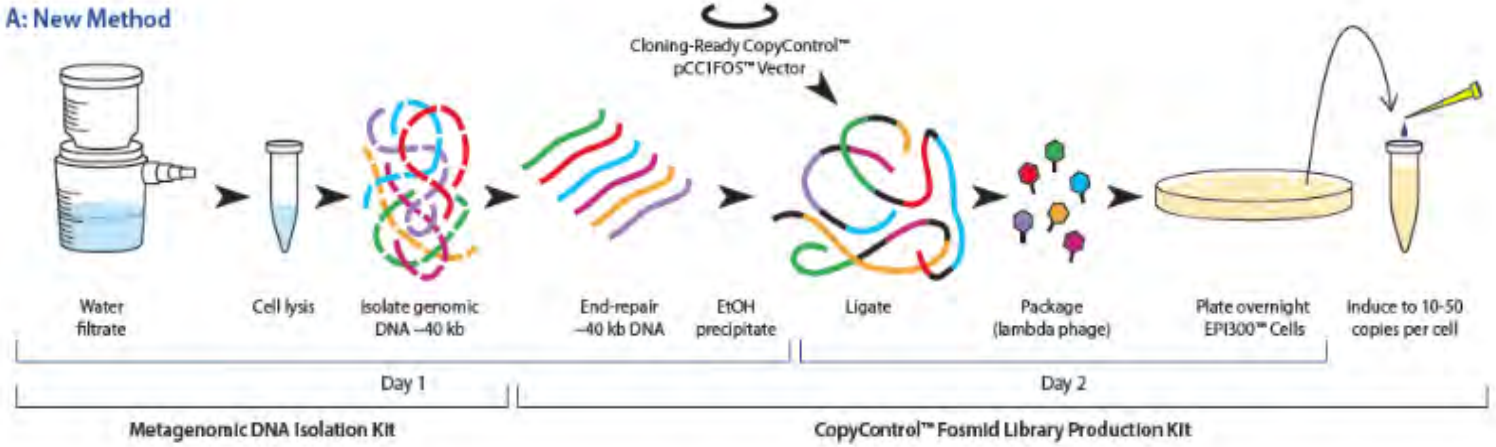
18S rDNA clone library

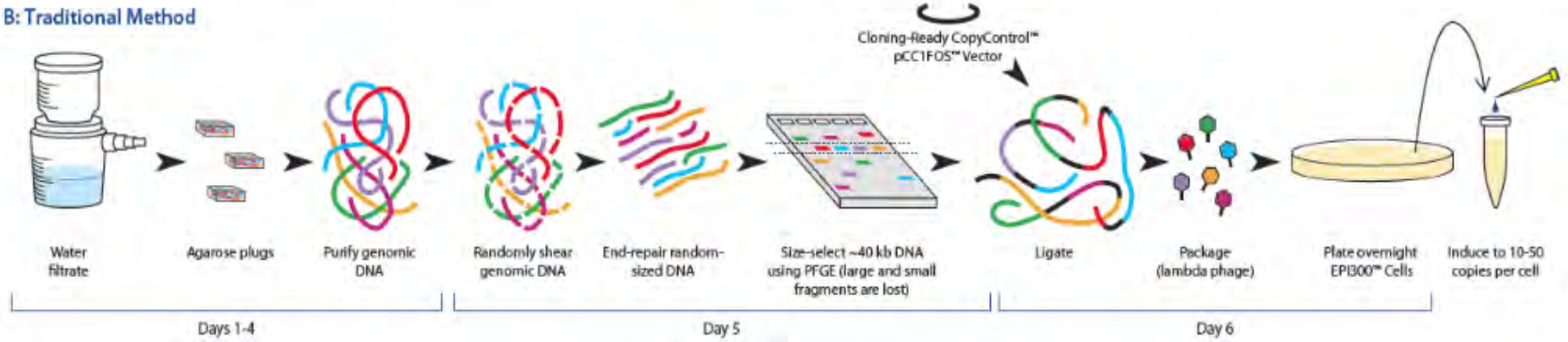


PCR-RFLP pattern analyses

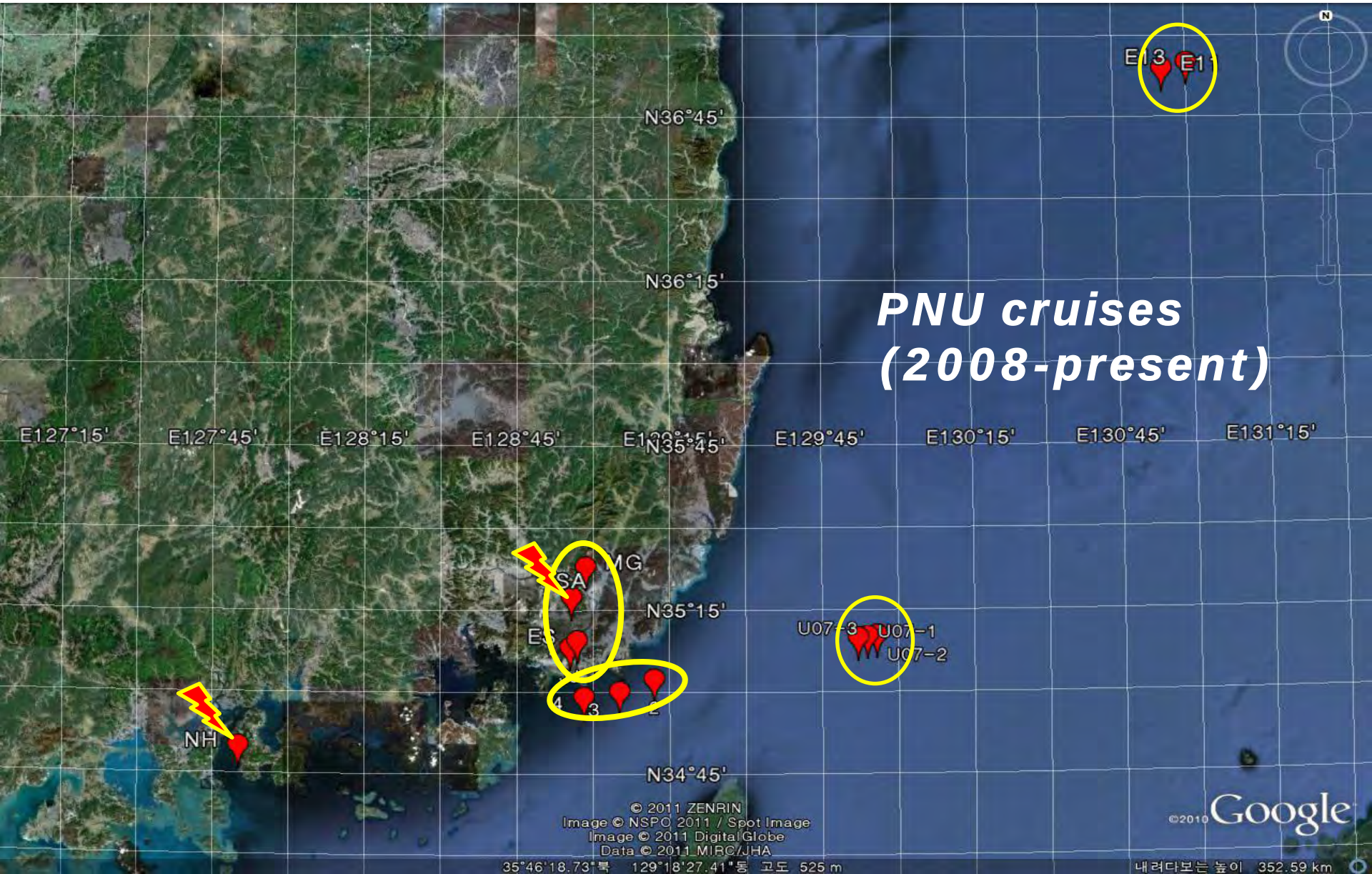


Sequence analyses


A: New Method
Cloning-Ready CopyControl™
pCC1FOS™ Vector
Water filtrate
Cell lysis
Isolate genomic DNA ~40 kb
End-repair ~40 kb DNA
EtOH precipitate
Ligate
Package (lambda phage)
Plate overnight EPI300™ Cells
Induce to 10-50 copies per cell
Day 1
Day 2
Metagenomic DNA Isolation Kit
CopyControl™ Fosmid Library Production Kit


B: Traditional Method
Cloning-Ready CopyControl™
pCC1FOS™ Vector
Water filtrate
Agarose plugs
Purify genomic DNA
Randomly shear genomic DNA
End-repair random-sized DNA
Size-select ~40 kb DNA using PFGE (large and small fragments are lost)
Ligate
Package (lambda phage)
Plate overnight EPI300™ Cells
Induce to 10-50 copies per cell
Days 1-4
Day 5
Day 6

Analyzed Clone Libraries



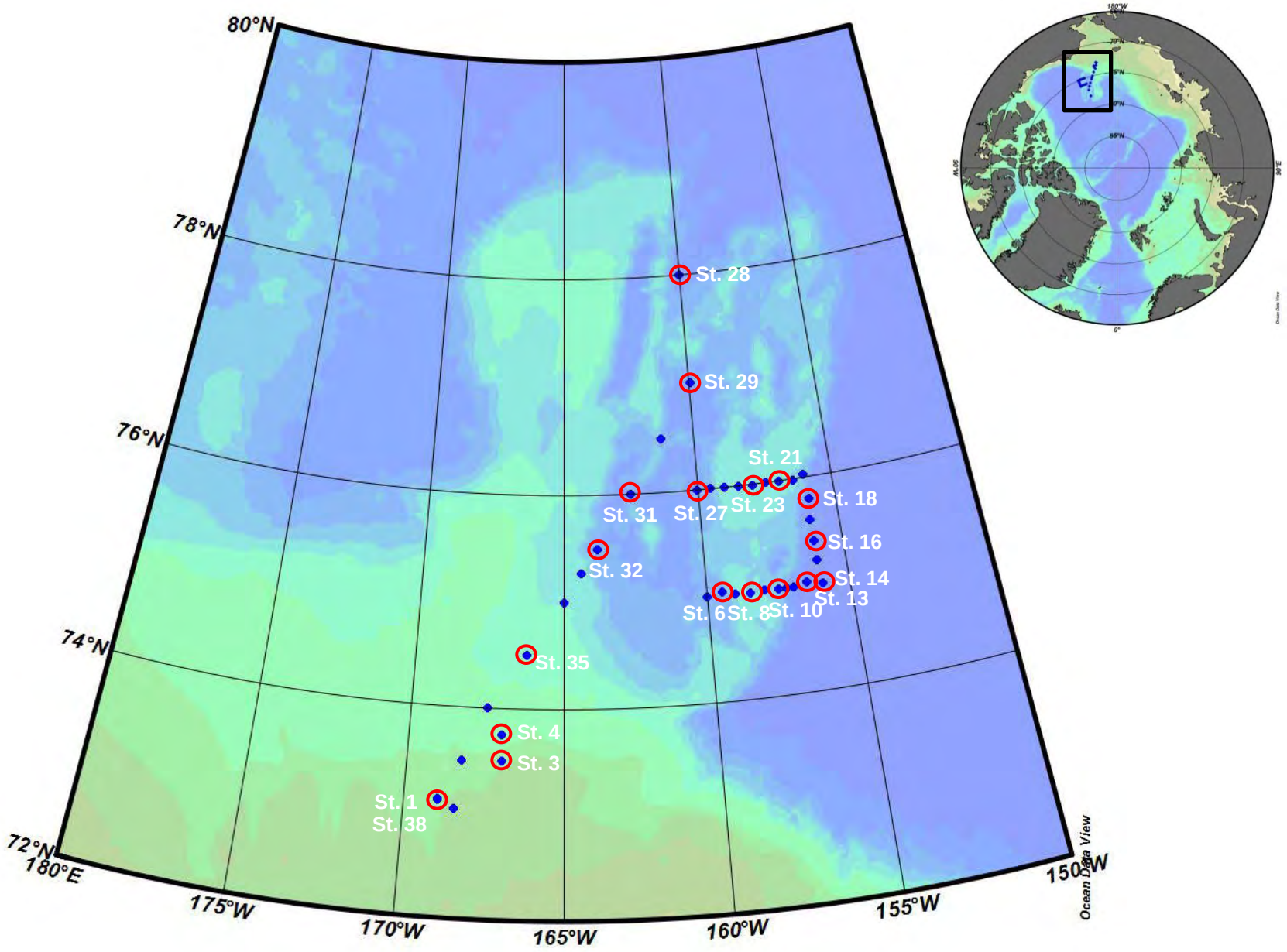
Phylotypes diversity

- ✓ revealed from environmental samples
- ✓ Diverse taxonomic lineages were reported by the metagenomic method

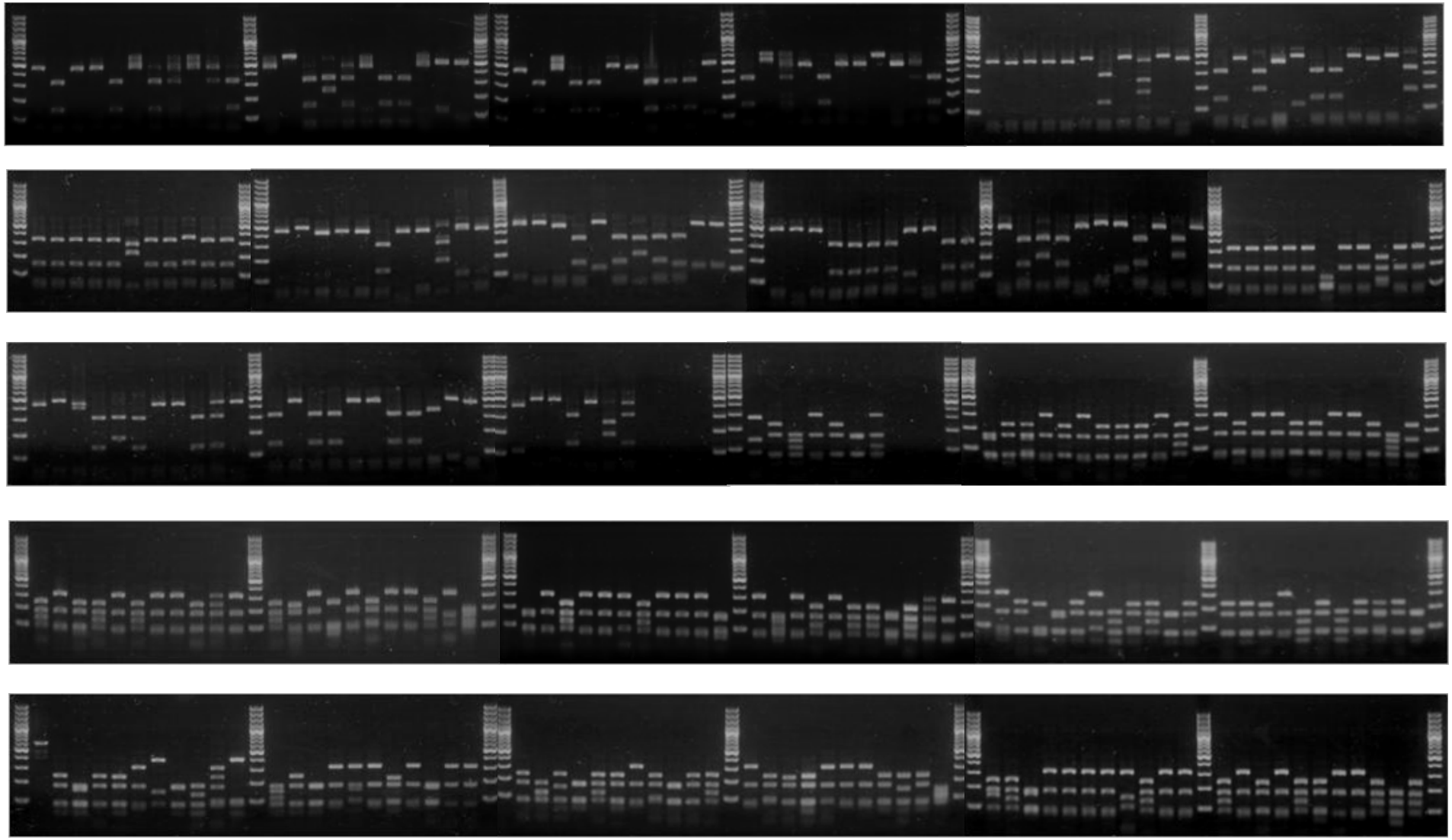
- **Freshwater (Nakdong River: SA, MG)**
- **Estuary (Nakdong River Estuary: JJ, ES)**
- **Coastal water (NH, NKC2, NKC3, NKC4)**
- **The Korea Strait (U07)**
- **East/Japan Sea (E11, E13)**
- Chukchi sea (arctic region)

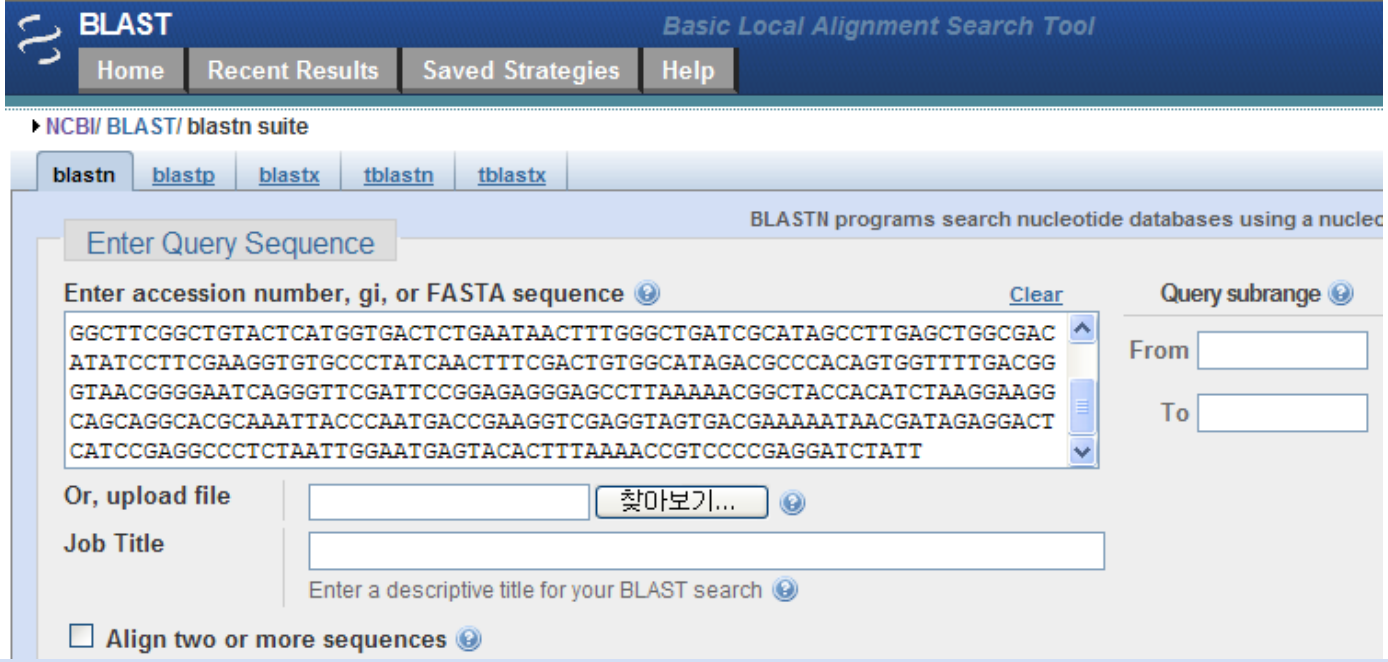
- ✓ total **500+** phylotypes / **1738** clones / **15** clone libraries

Study Locations in 2010



PCR – RFLP Analysis





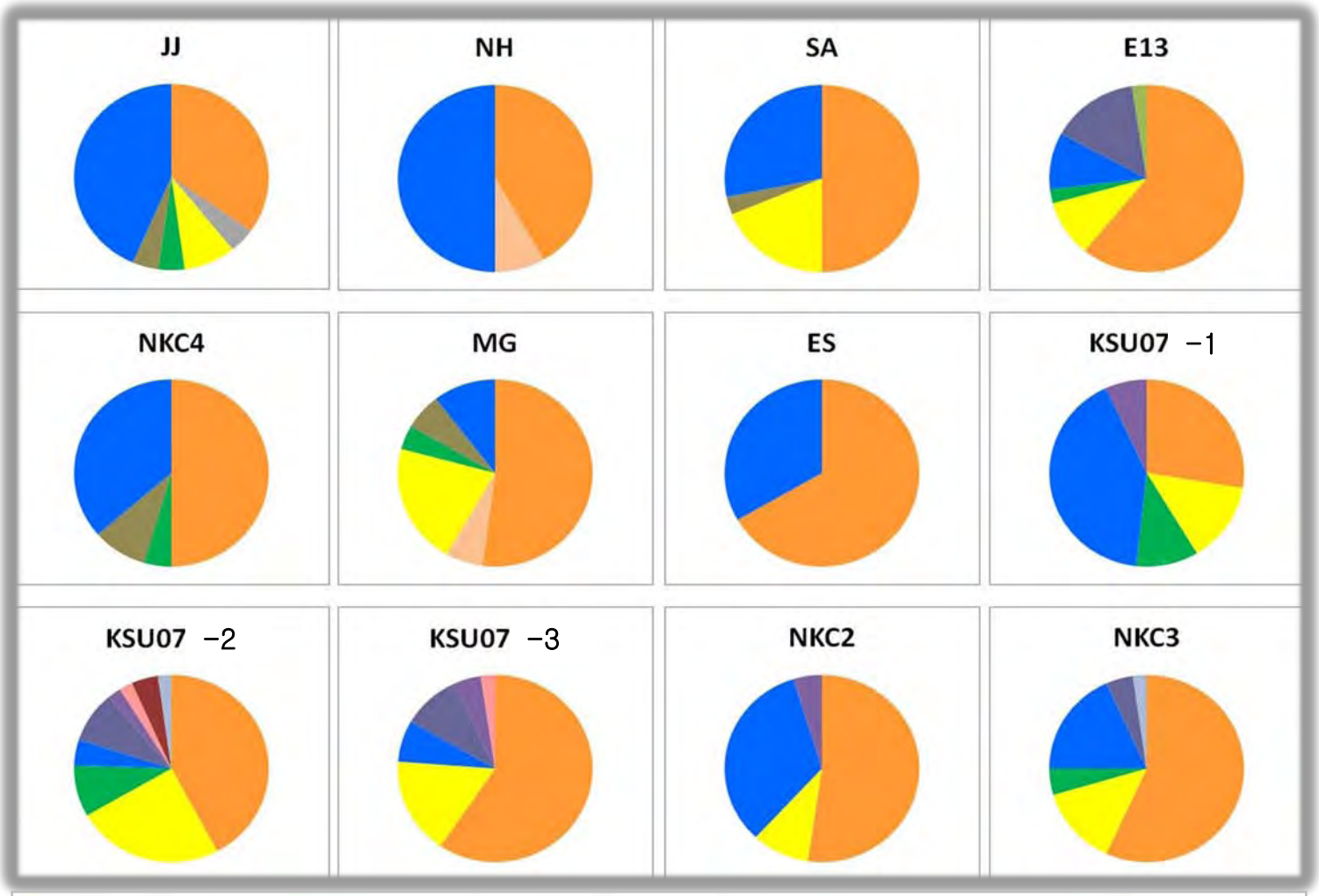
Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

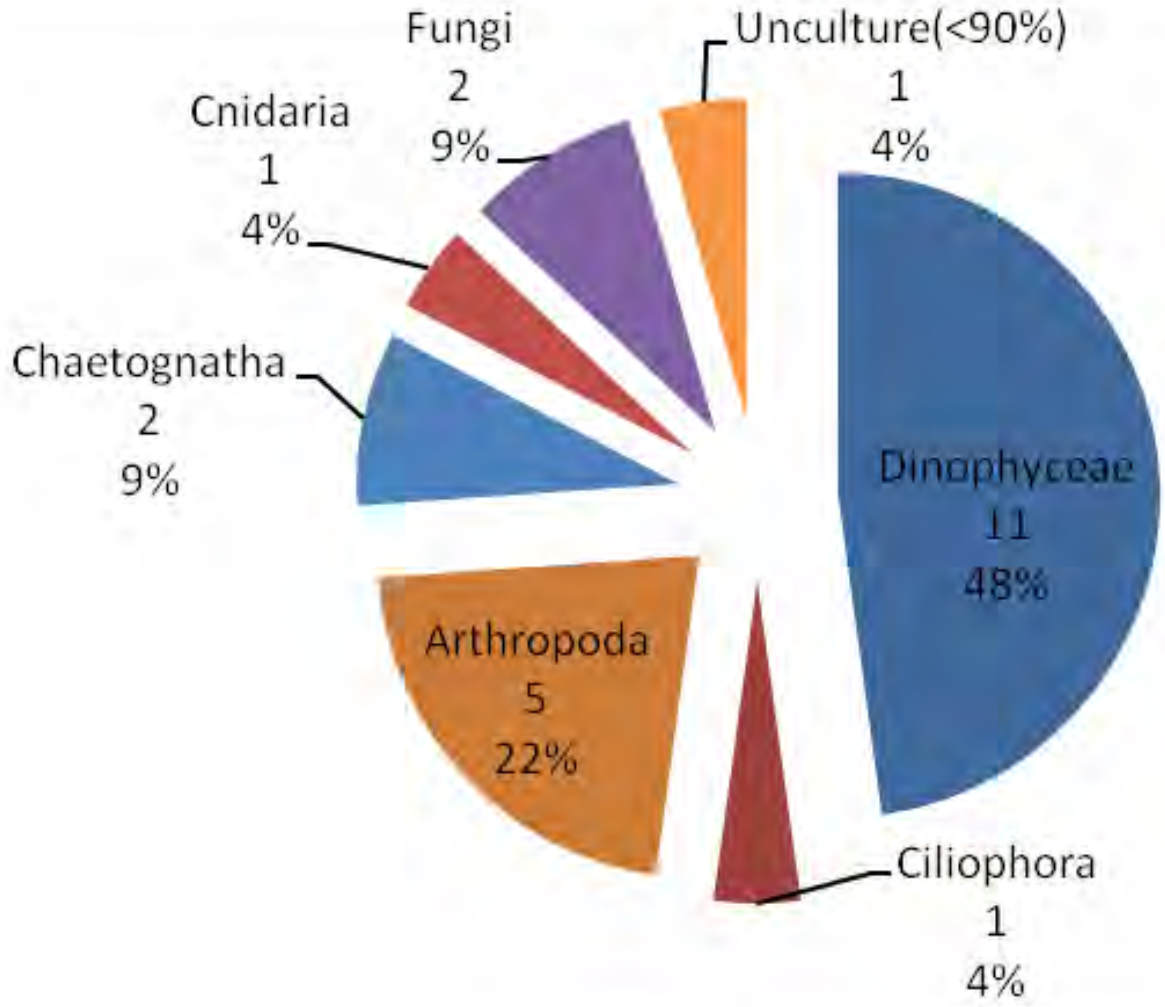
Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
GQ899151.1	Uncultured eukaryote clone JJ18S_8 18S ribosomal RNA gene, partial	966	966	99%	0.0	99%
GU474374.1	Uncultured eukaryote clone NH18S_6 18S ribosomal RNA gene, partia	966	966	99%	0.0	99%
AY665127.1	Uncultured eukaryote clone SCM38C38 18S ribosomal RNA gene, part	963	963	100%	0.0	99%
GQ899165.1	Uncultured eukaryote clone JJ18S_64 18S ribosomal RNA gene, partia	961	961	99%	0.0	99%
GU474382.1	Uncultured eukaryote clone NH18S_110 18S ribosomal RNA gene, par	961	961	99%	0.0	99%
GU474377.1	Uncultured eukaryote clone NH18S_10 18S ribosomal RNA gene, part	961	961	99%	0.0	99%
GU474373.1	Uncultured eukaryote clone NH18S_4 18S ribosomal RNA gene, partia	961	961	99%	0.0	99%
AY665126.1	Uncultured eukaryote clone SCM38C9 18S ribosomal RNA gene, parti	957	957	100%	0.0	99%
GU474375.1	Uncultured eukaryote clone NH18S_7 18S ribosomal RNA gene, partia	953	953	99%	0.0	99%
DQ667609.1	Uncultured eukaryotic picoplankton clone XMAB05 18S ribosomal RNA	946	946	99%	0.0	99%
AY145104.1	Uncultured marine eukaryote isolate JPeuk-27 18S ribosomal RNA ger	942	942	98%	0.0	99%
DQ667657.1	Uncultured eukaryotic picoplankton clone XMCC5 18S ribosomal RNA	920	920	100%	0.0	97%
DQ352849.1	Uncultured eukaryotic picoplankton isolate DGGE gel band X2 18S rib	907	907	95%	0.0	99%
GU969181.1	Paracalanus parvus 18S ribosomal RNA gene, partial sequence	900	900	92%	0.0	99%
AY665124.1	Uncultured eukaryote clone SCM27C27 18S ribosomal RNA gene, part	898	898	100%	0.0	97%
DQ667651.1	Uncultured eukaryotic picoplankton clone XMCC10 18S ribosomal RNA	896	896	100%	0.0	97%
DQ667667.1	Uncultured eukaryotic picoplankton clone XMCE10 18S ribosomal RNA	894	894	99%	0.0	97%

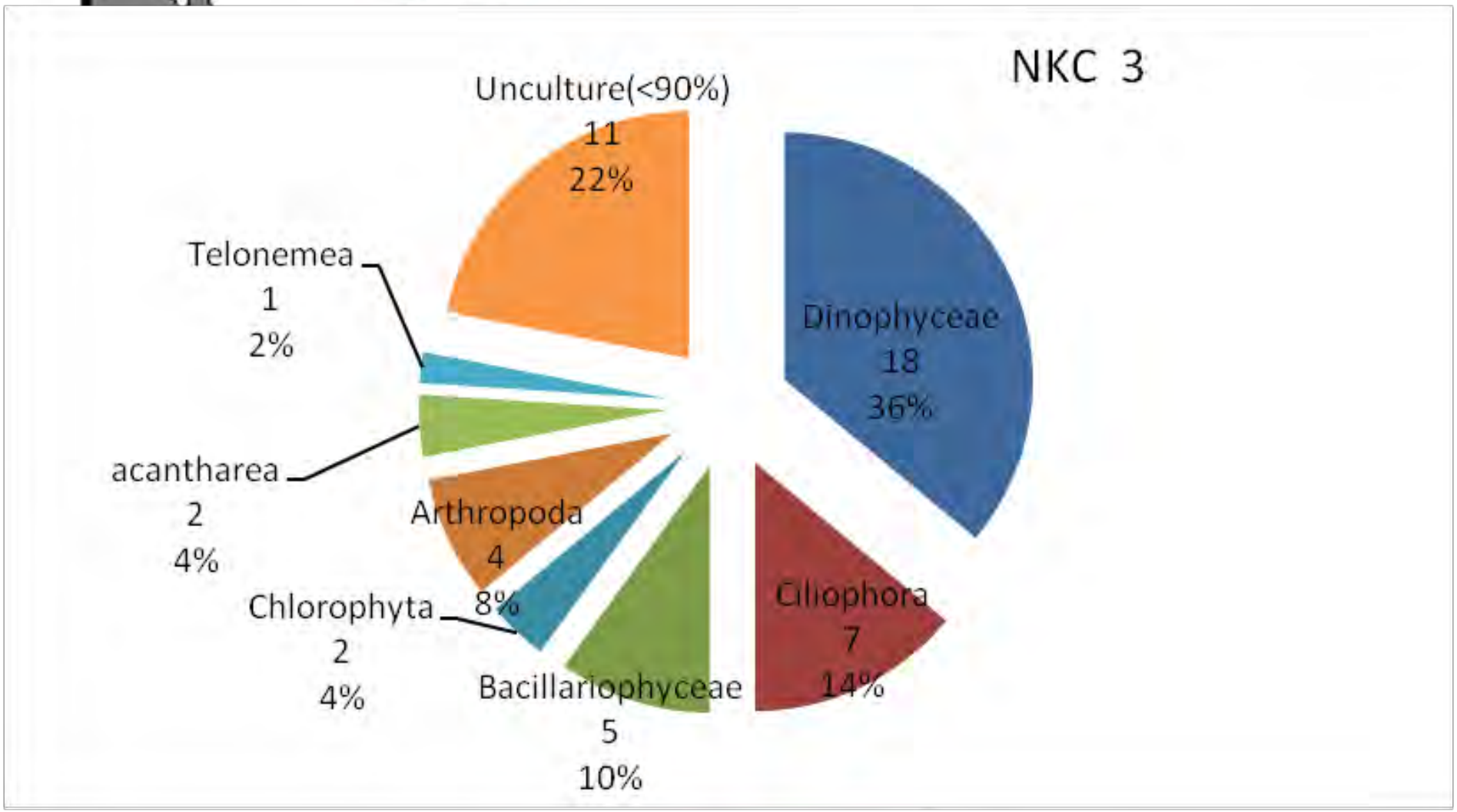
Taxonomic lineage of phylotypes



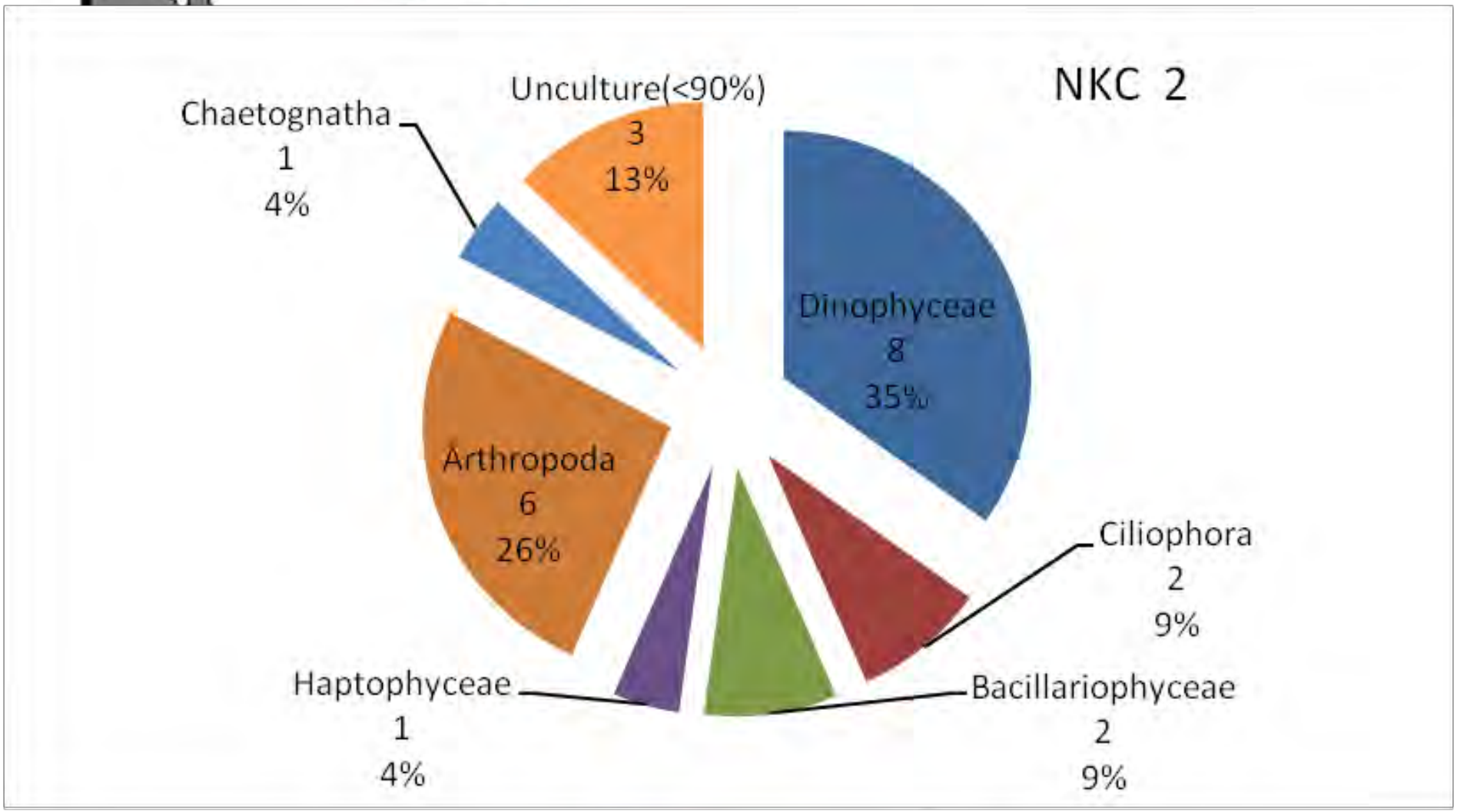
35°N
NKC 2
NKC 3
NKC 4


NKC 4
Fungi
2
9%
Unculture(<90%)
1
4%
Cnidaria
1
4%
Chaetognatha
2
9%
Dinophyceae
11
48%
Arthropoda
5
22%
Ciliophora
1
4%

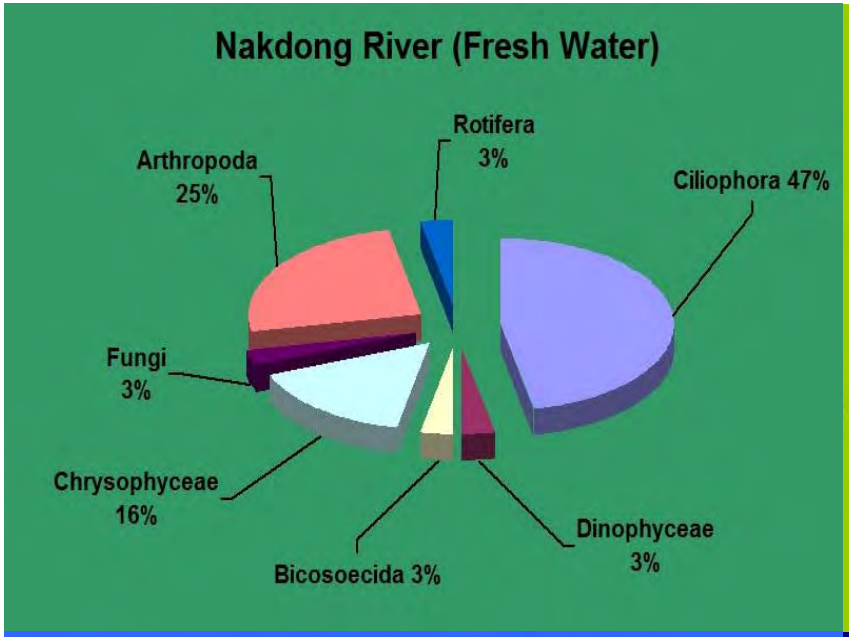
35°N
NKC 2
NKC 3
NKC 4

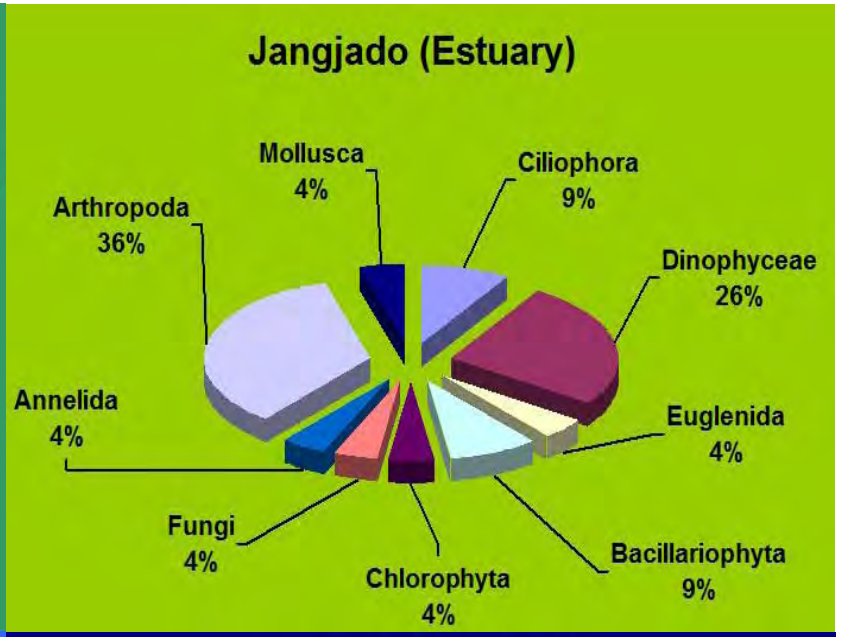

NKC 3
Unculture(<90%)
11
22%
Telonemea
1
2%
acantharea
2
4%
Arthropoda
4
8%
Chlorophyta
2
4%
Bacillariophyceae
5
10%
Dinophyceae
18
36%
Ciliophora
7
14%

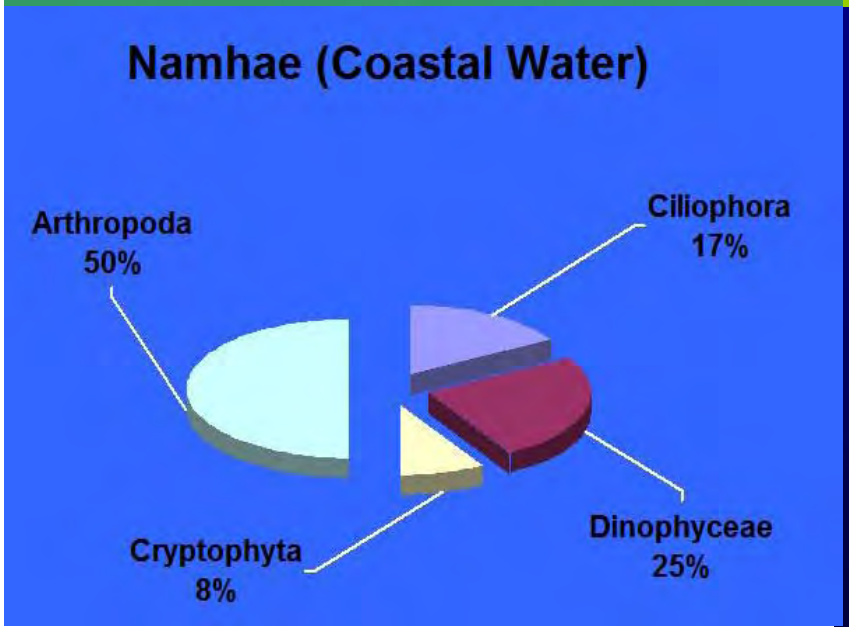
35°N
NKC 2
NKC 3
NKC 4

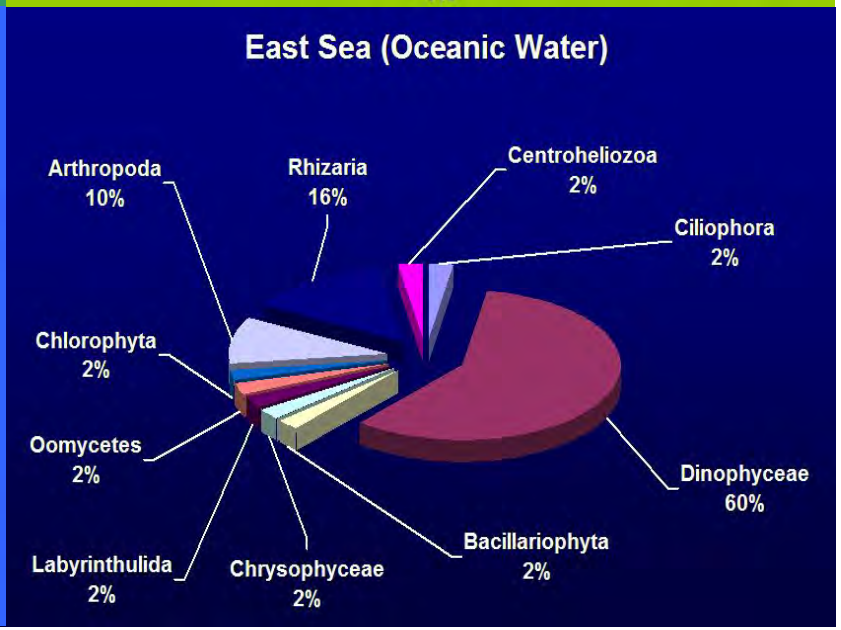

NKC 2
Unculture(<90%)
3
13%
Chaetognatha
1
4%
Dinophyceae
8
35%
Arthropoda
6
26%
Ciliophora
2
9%
Haptophyceae
1
4%
Bacillariophyceae
2
9%

Plankton species diversity









J Appl Phycol
DOI 10.1007/s10811-010-9509-7

Effective molecular examination of eukaryotic plankton species diversity in environmental seawater using environmental PCR, PCR-RFLP, and sequencing

Sang-Rae Lee · Jung Hyun Oak · Ik Kyo Chung · Jin Ae Lee

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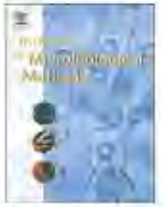


Monitoring of phytoplankton community structure using terminal restriction fragment length polymorphism (T-RFLP)

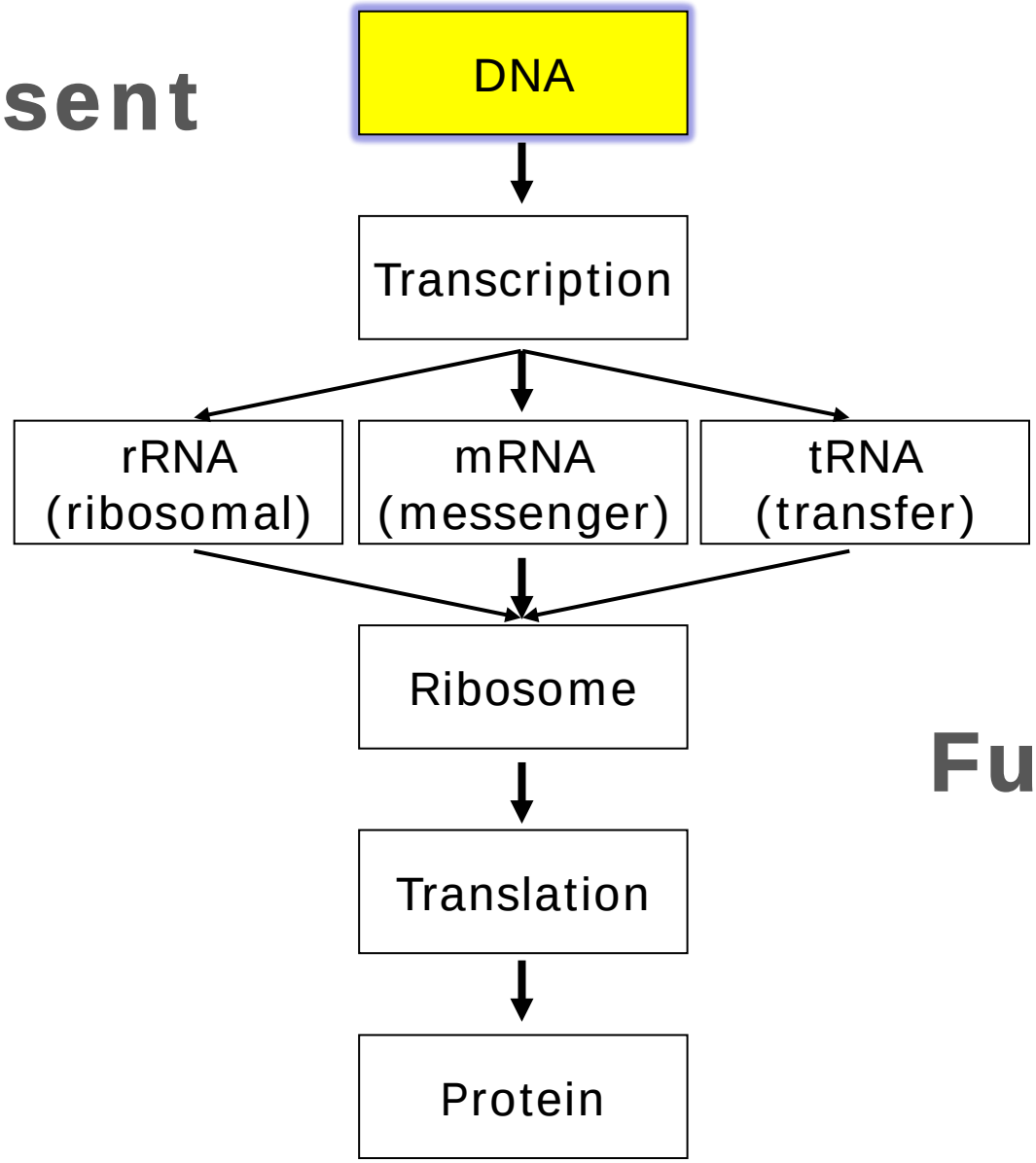
Sungbae Joo [a], Sang-Rae Lee [b], Sangkyu Park [a,*]

[a] Department of Biological Sciences, Ajou University, Suwon, 443-749, South Korea
[b] Marine Research Institute, Pusan National University, Busan, 609-735, South Korea

Applications

- Species identification
- Biodiversity; How many species is
- The relationship between species diversity & the characteristics of water masses
- Bio-indicator species to trace water masses
- Monitoring
- Food web
- Modeling

Present



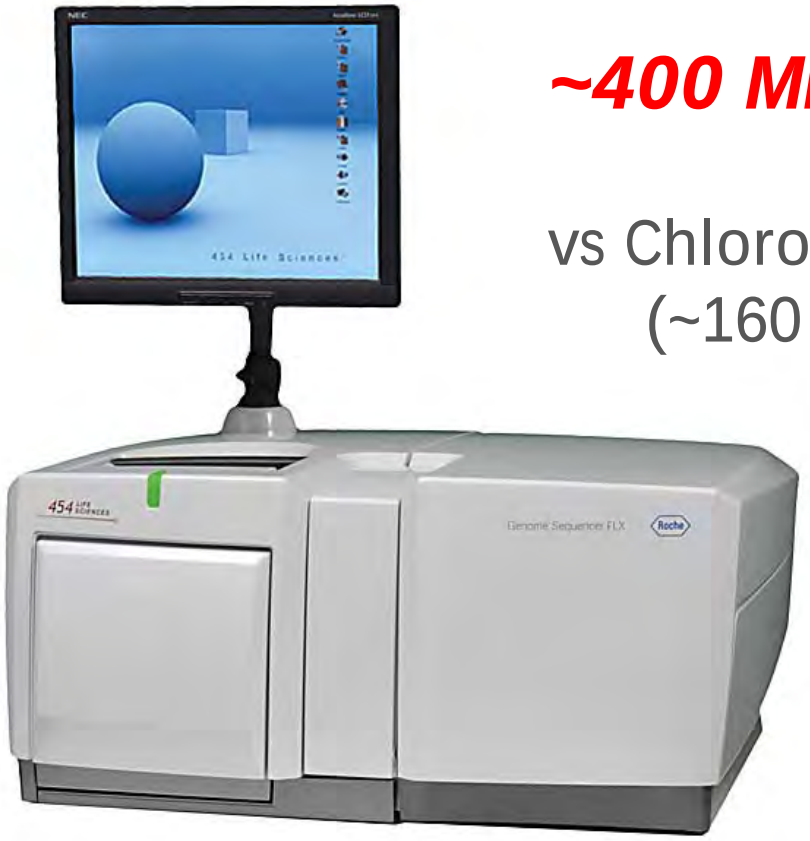
Future

NGS *(Next Generation Sequencing system)*

Tremendous data in short time

~400 MB yield/run

vs Chloroplast genome
(~160 kb)

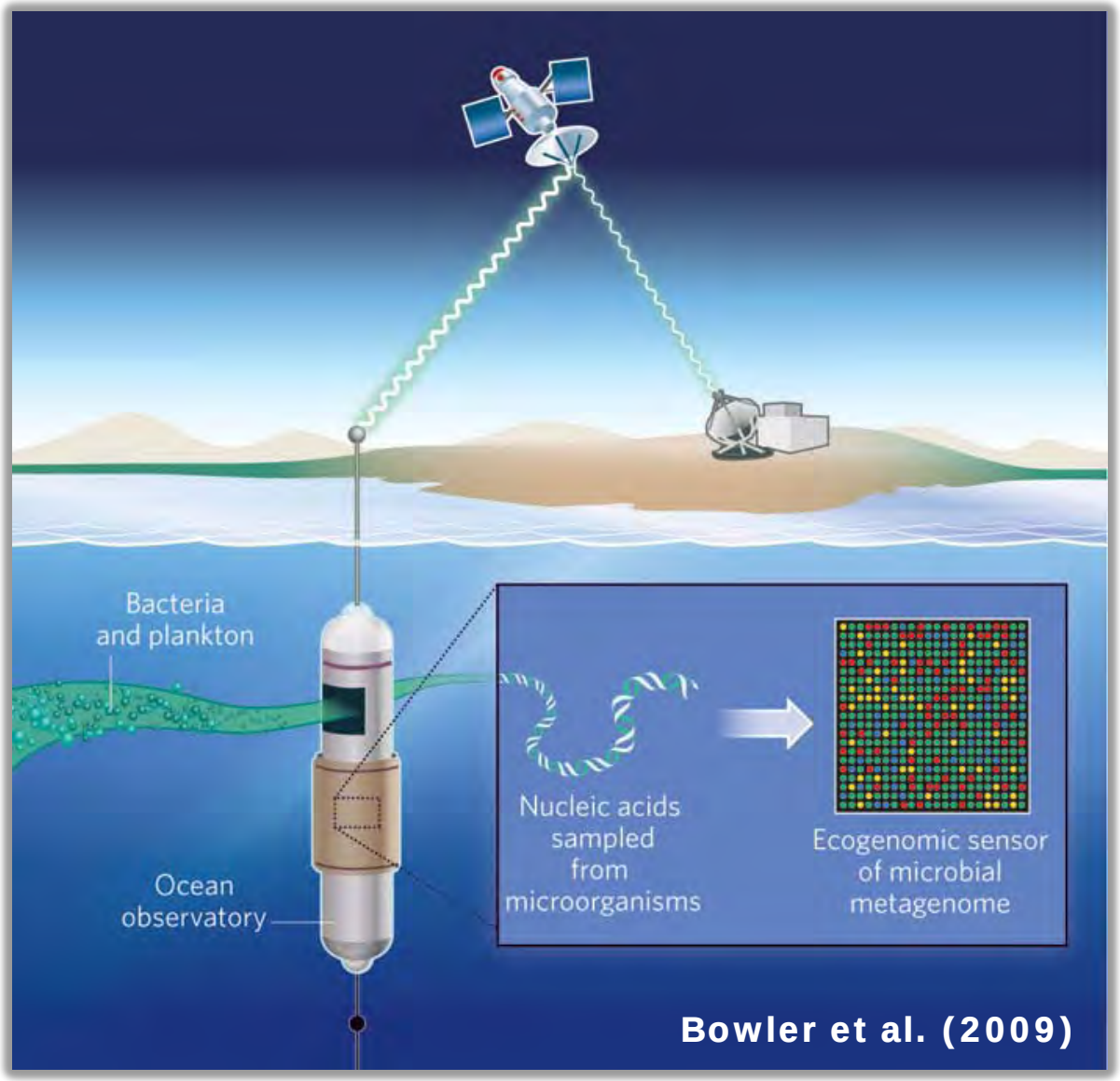


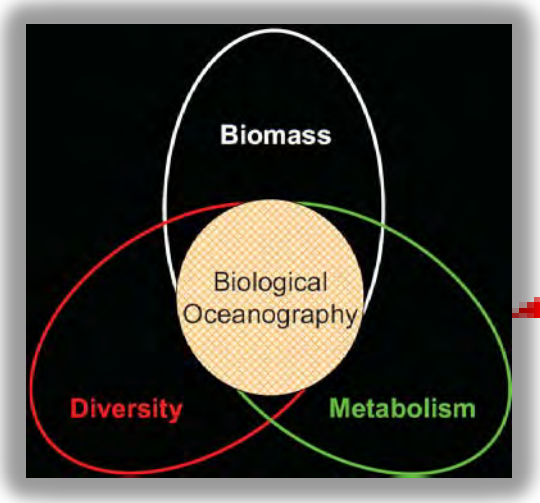
Genome Sequencer FLX system (Roche, 454 Life Sciences)

http://www.454.com/

Labor, Cost & Time

Miniaturized ecogenomic sensors





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