

Zooplankton Community Genomics

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Zooplankton Diversity

◆ Difficulties in estimation

◆ Difficulties in samplings

◆ Difficulties in identification

◆ Cryptic species

As a part of CMarZ activity,

Exhaustive Analyses of Zooplankton Mitochondrial COI Genes

A pilot study

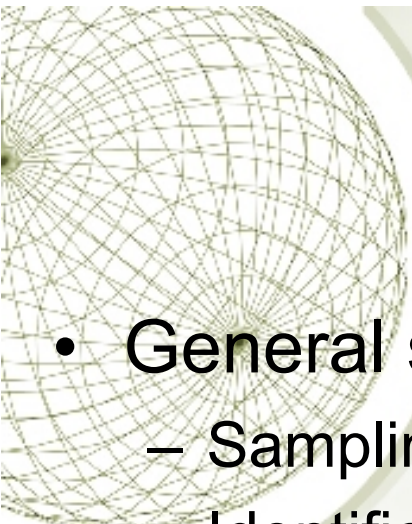
Site ; Off Pohnpei

Mouth opening; 2m²

Mesh size ; 0.69mm

Depth range ; 0-721m



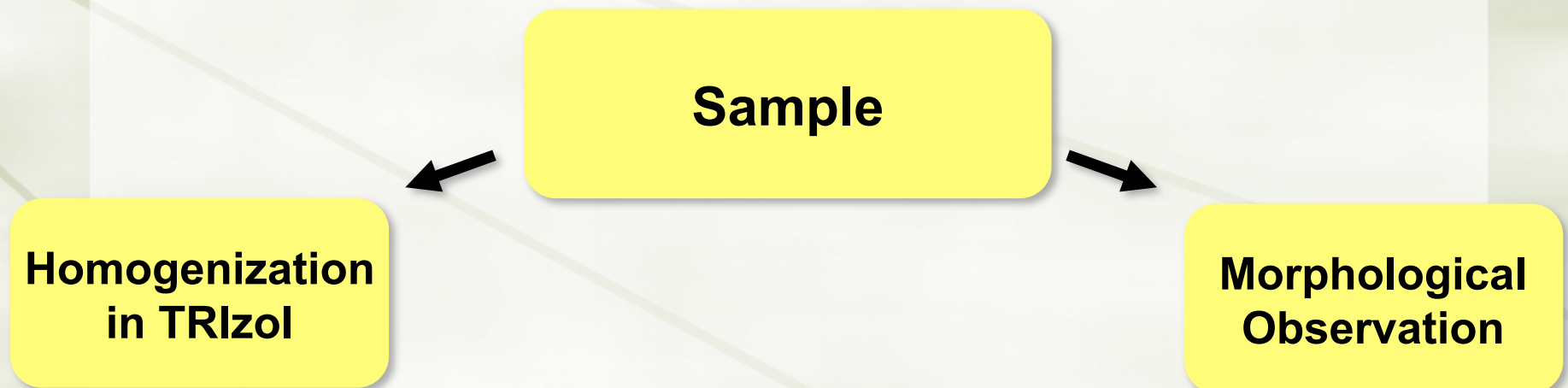


Zooplankton Community Genomics

- **General study**
 - Sampling
 - Identification
 - DNA extraction
 - PCR
 - Sequencing
 - **Present study**
 - Sampling
 - Homogenization
 - mRNA extraction
 - cDNA library construction
 - Screening of the COI genes by PCR
 - Exhaustive sequencing analyses
 - Inference of the original species
- Advantage**
- Almost all net collectable zooplankters
 - No pseudogene

Protocol of Zooplankton Community Genomics 1

- ★ Sample collection and homogenization on board
 - ★ Split sample into two fractions (one for mRNA extraction, the other for morphological observation).



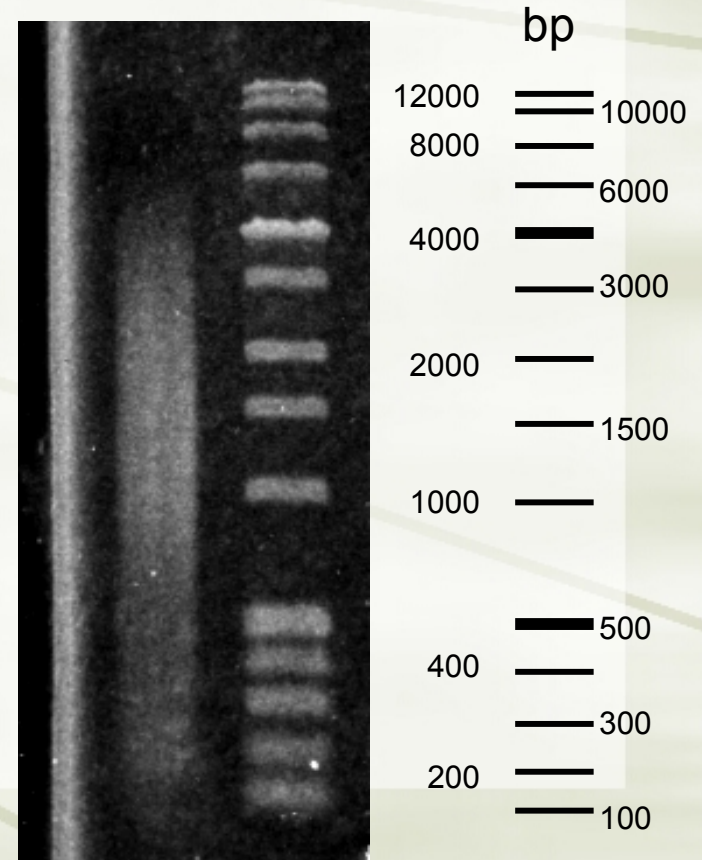
Protocol of Zooplankton Community Genomics 2

★ Construction of cDNA library

1. Extraction of total RNA from homogenized sample
2. Selection of mRNA
3. Reverse transcription of cDNA from mRNA

In this cDNA library, all expressed mRNA from all zooplankton are included.

Off Pohnpei

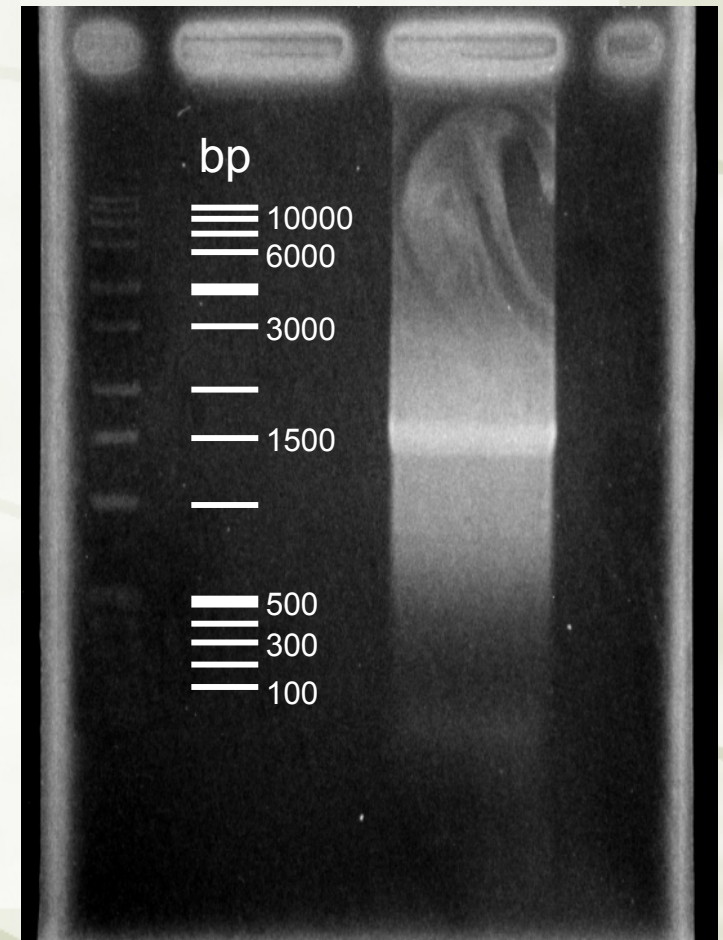


Protocol of Zooplankton Community Genomics 3

- ★ Selection of mitochondrial COI genes
 - ★ Using COI universal and poly (T) primers, mitochondrial COI genes are amplified from the constructed cDNA library.

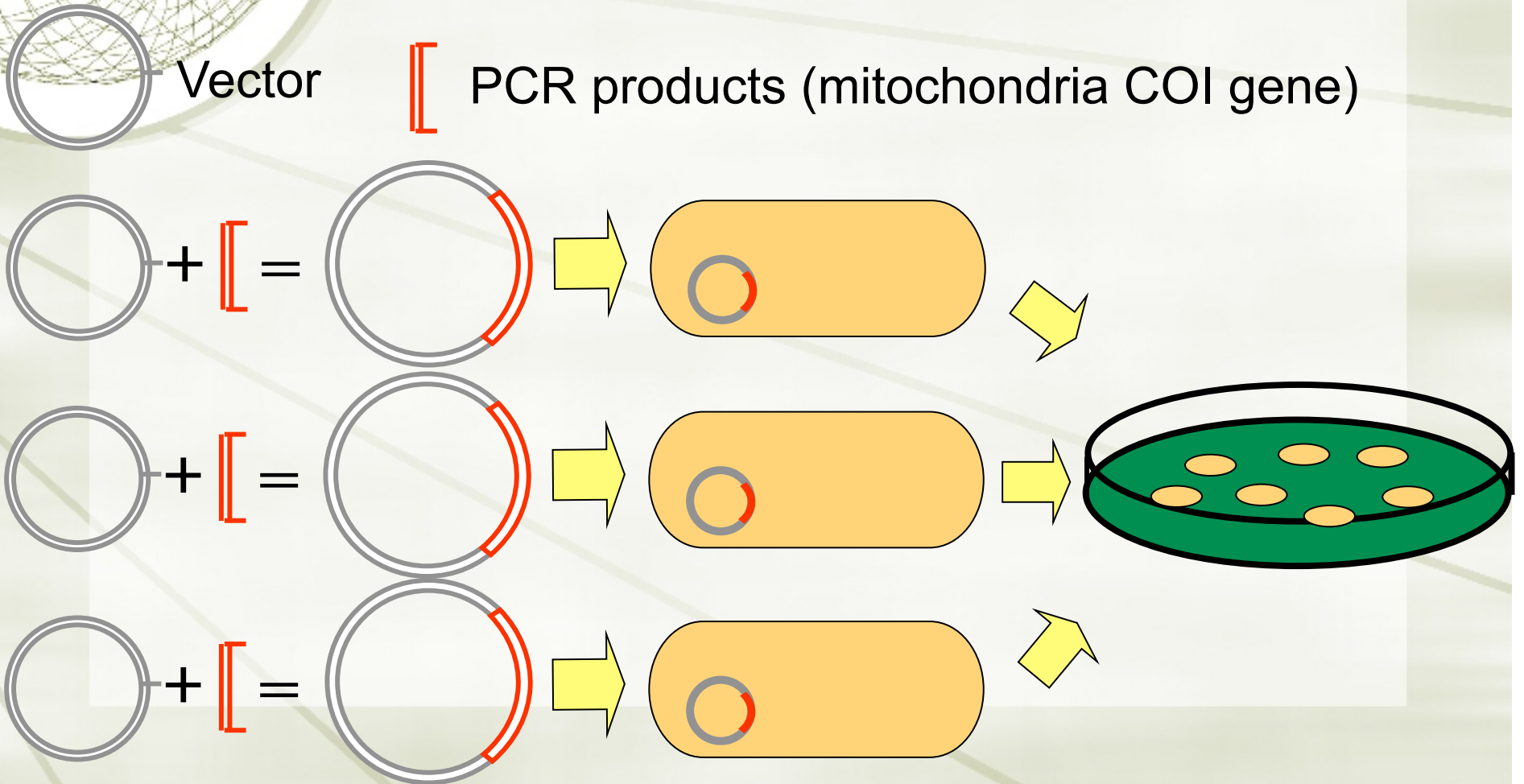
In this state, all zooplankton mitochondrial COI genes are included in this library.

Off Pohnpei



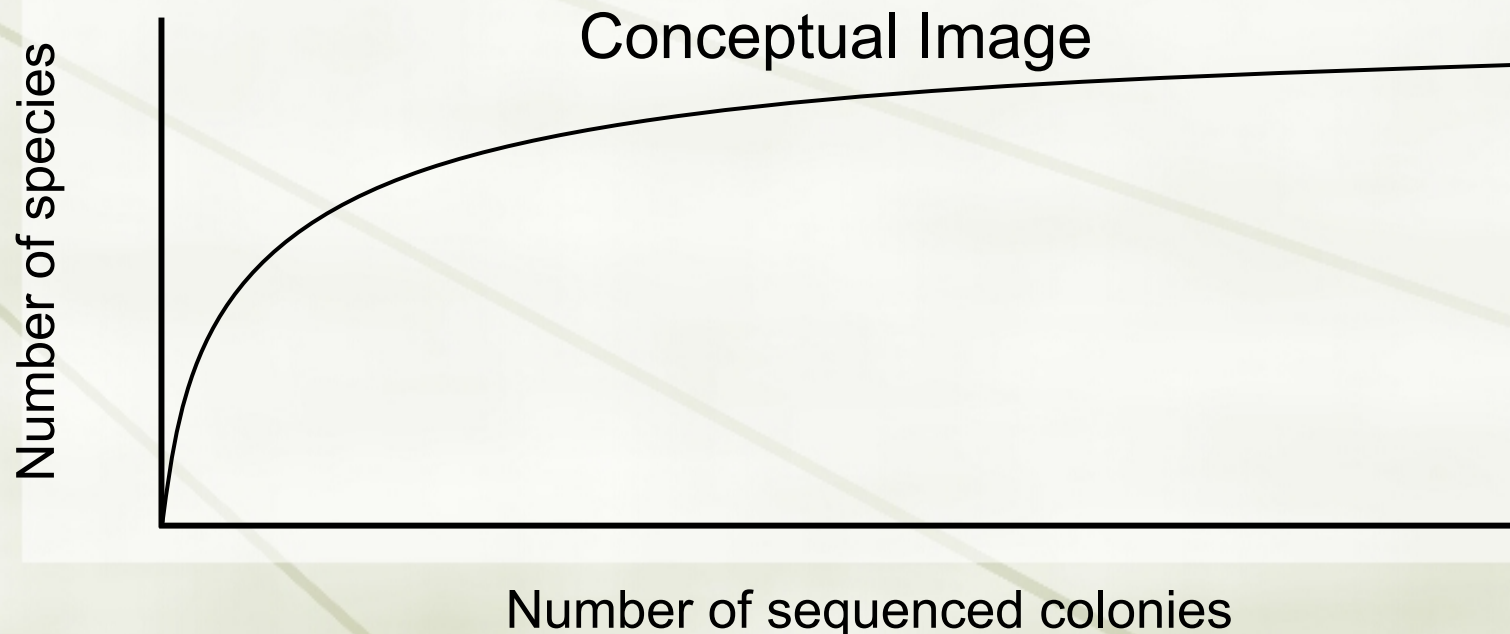
Protocol of Zooplankton Community Genomics 4

- ★ Cloning of the mitochondrial COI library
 - ★ Isolation of each mitochondrial COI molecule



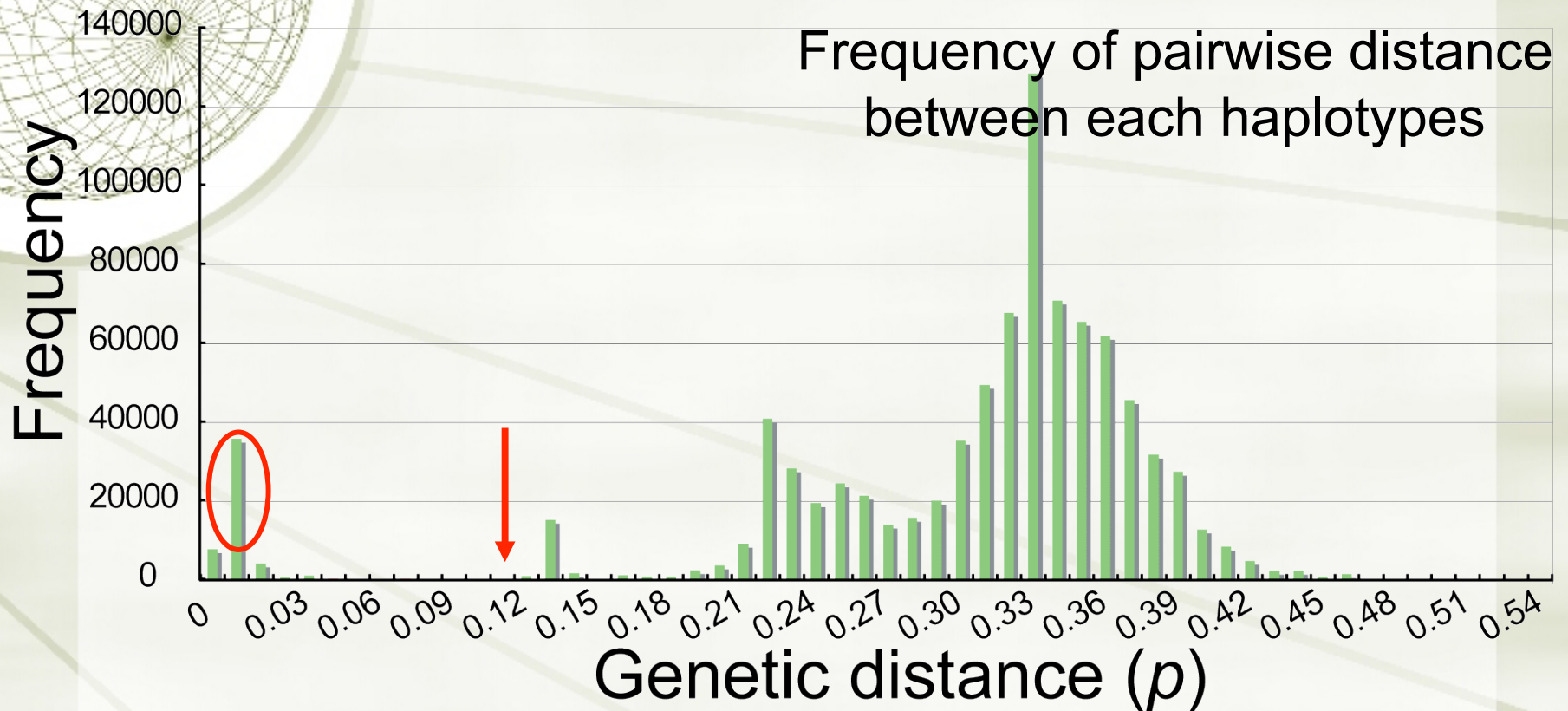
Protocol of Zooplankton Community Genomics 5

- ★ Exhaustive analyses of those clones
 - ★ PCR amplification of the inserted COI genes.
 - ★ Determination of those COI sequences.
 - ★ Continue sequencing until no new sequence will appear.



Result - Off Pohnpei 1

★ 1336 colonies have been sequenced.



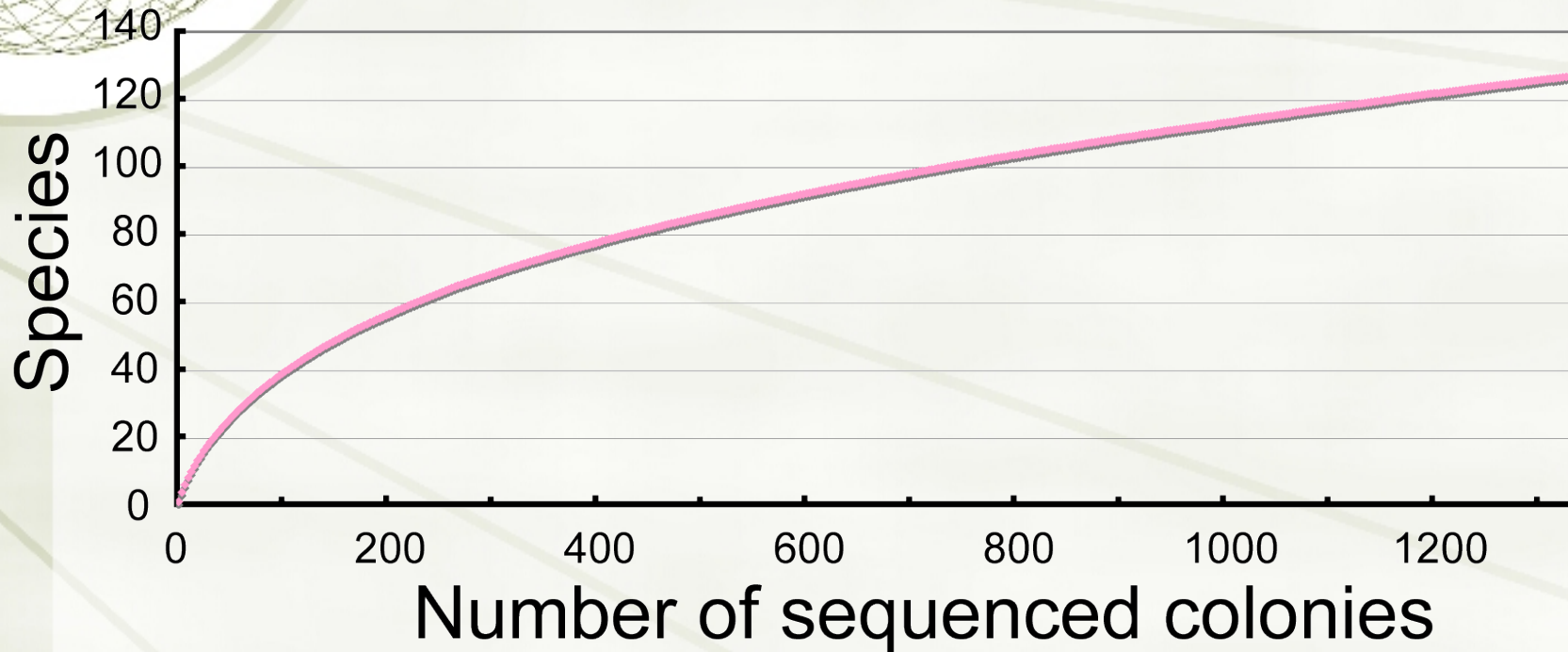
Minimum value was observed in 0.12

Sequences distance is less than 0.12 = same species

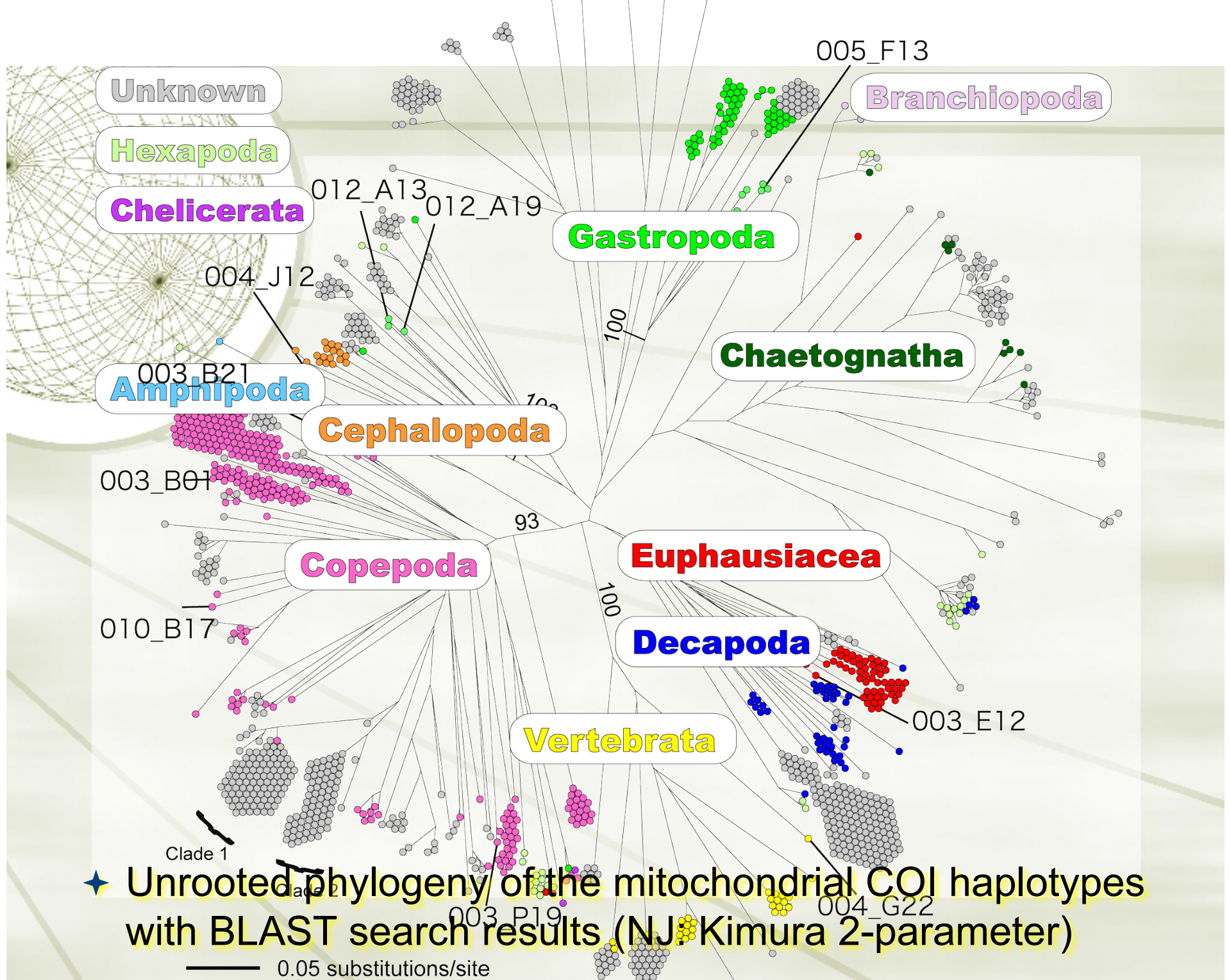
more than 0.12 = different species

Result

- ◆ Estimated number of species with greater sequence depth



189 species were estimated from 1336 sequences (Chao1)



Result - Off Pohnpei 4

- BLAST search results

<i>Sequence ID;</i>	<i>Species</i>	<i>BLAST score</i>	<i>Similarity (%)</i>
<i>Vertebrate</i>			
<i>004_g24</i>	<i>Coryphaena hippurus</i>	<i>1100</i>	<i>99</i>
<i>Krill</i>			
<i>003_e12</i>	<i>Stylocheiron carinatum</i>	<i>944</i>	<i>98</i>
<i>Mollusca</i>			
<i>005_f13</i>	<i>Clio pyramidata</i>	<i>898</i>	<i>98</i>
<i>004_j12</i>	<i>Sthenoteuthis oualaniensis</i>	<i>967</i>	<i>99</i>
<i>012_a13</i>	<i>Strombus mutabilis</i>	<i>938</i>	<i>99</i>
<i>012_a19</i>	<i>Strombus wilsoni</i>	<i>906</i>	<i>98</i>
<i>Copepoda</i>			
<i>003_p19</i>	<i>Candacia longimana</i>	<i>959</i>	<i>99</i>
<i>003_b21</i>	<i>Cosmocalanus darwinii</i>	<i>902</i>	<i>98</i>
<i>003_l24</i>	<i>Neocalanus robustior</i>	<i>940</i>	<i>99</i>
<i>010_b17</i>	<i>Rhincalanus rostifrons</i>	<i>597</i>	<i>99</i>

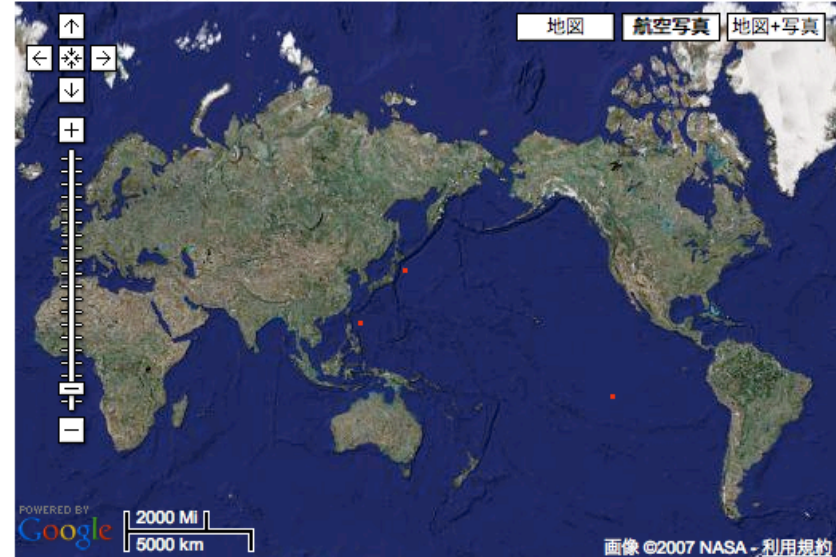
BLAST Search Database *will be linked from CMarZ homepage*

- ★ This database can identify the most similar sequences between the sequences derived from the zooplankton community genomic analysis and your species sequence data.

BLAST Search

Picking Point (Latitude, Longitude)

Point find sample.



180.00.00.0W	83.06.39.9N	180.00.00.0E
Map Range		
64.28.22.1S		


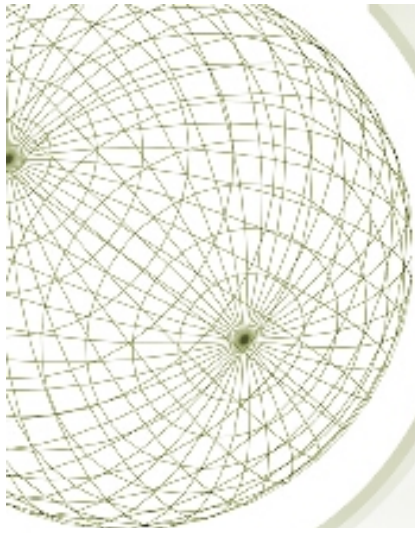
Database

Species Genes and Genomes

Sequence

Enter sequence below in FASTAformat
(ex:aagcttttgatttctacacctgcacttatcatgctttgt)

Search Clear



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