A decorative graphic on the left side of the slide depicts a blue river or stream flowing downwards. The water is represented by a blue gradient with white highlights. Numerous colorful copepods, including Oithona attenuata, are scattered throughout the water. The copepods are shown in various colors such as orange, purple, green, red, and blue. The background of the entire slide is a light gray grid.

MORPHOLOGICAL AND GENETIC ANALYSIS OF *OITHONA ATTENUATA* (COPEPOD, CYCLOPOIDA) POPULATIONS IN THE COASTAL WATERS OF SOUTHEAST ASIA, JAPAN AND PACIFIC OCEAN

Mary Mar P. Noblezada¹

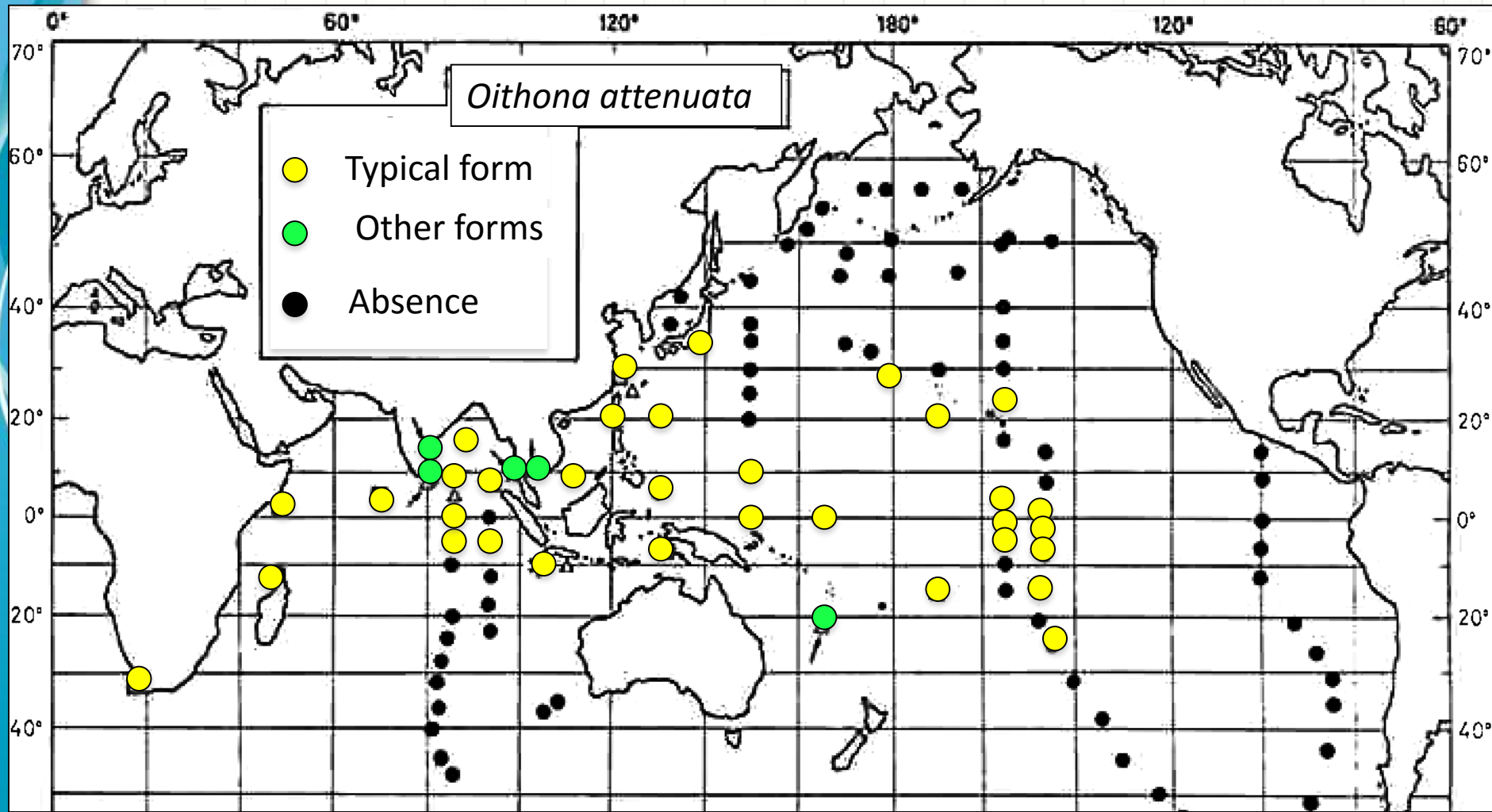
Shuheii Nishida²

13 May 2016

¹Marine Science Institute, University of the Philippines Diliman, Philippines

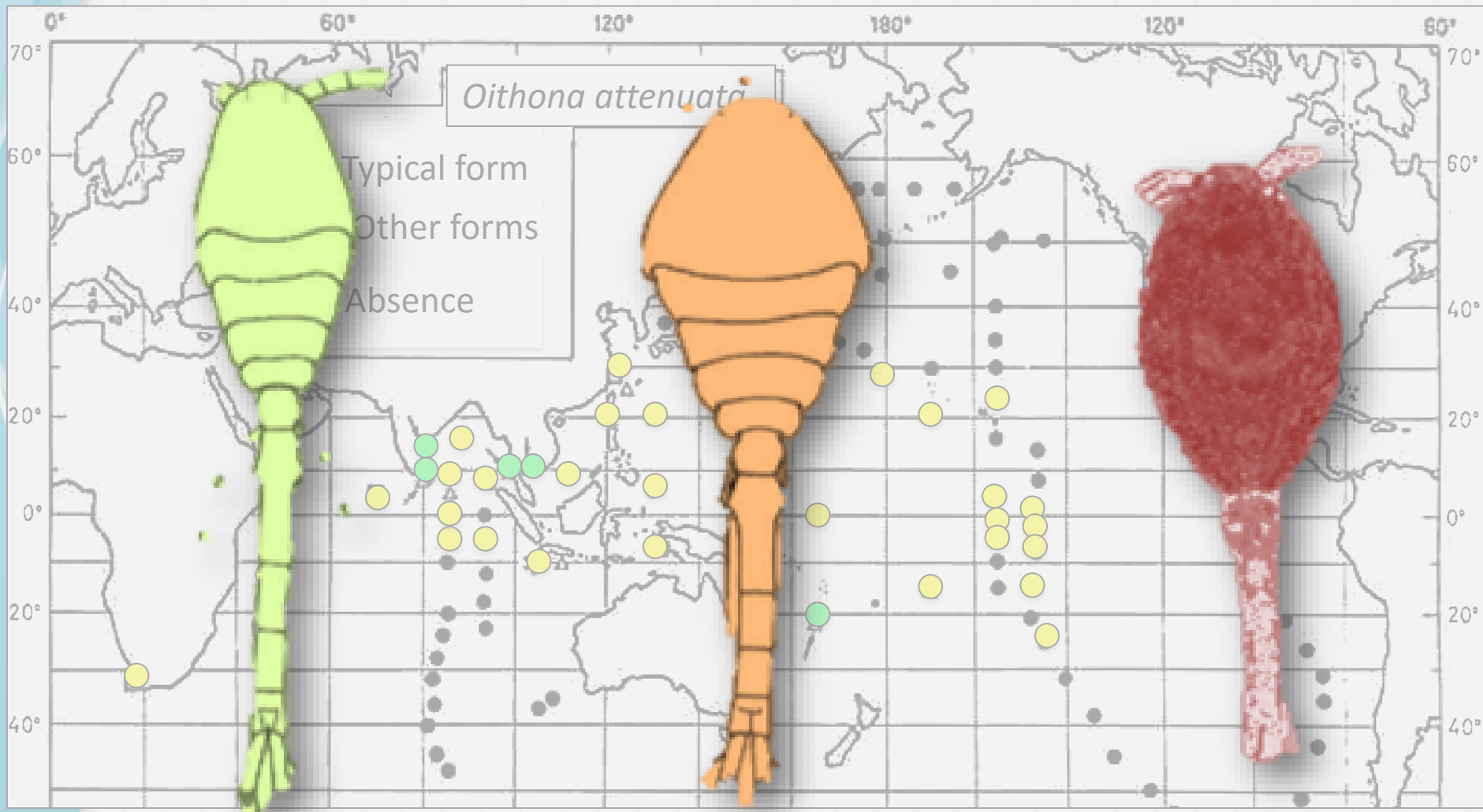
²Atmosphere and Ocean Research Institute, University of Tokyo, Japan

Geographical distribution of *Oithona attenuata*



Nishida, 1985

Intraspecific forms of female *Oithona attenuata*

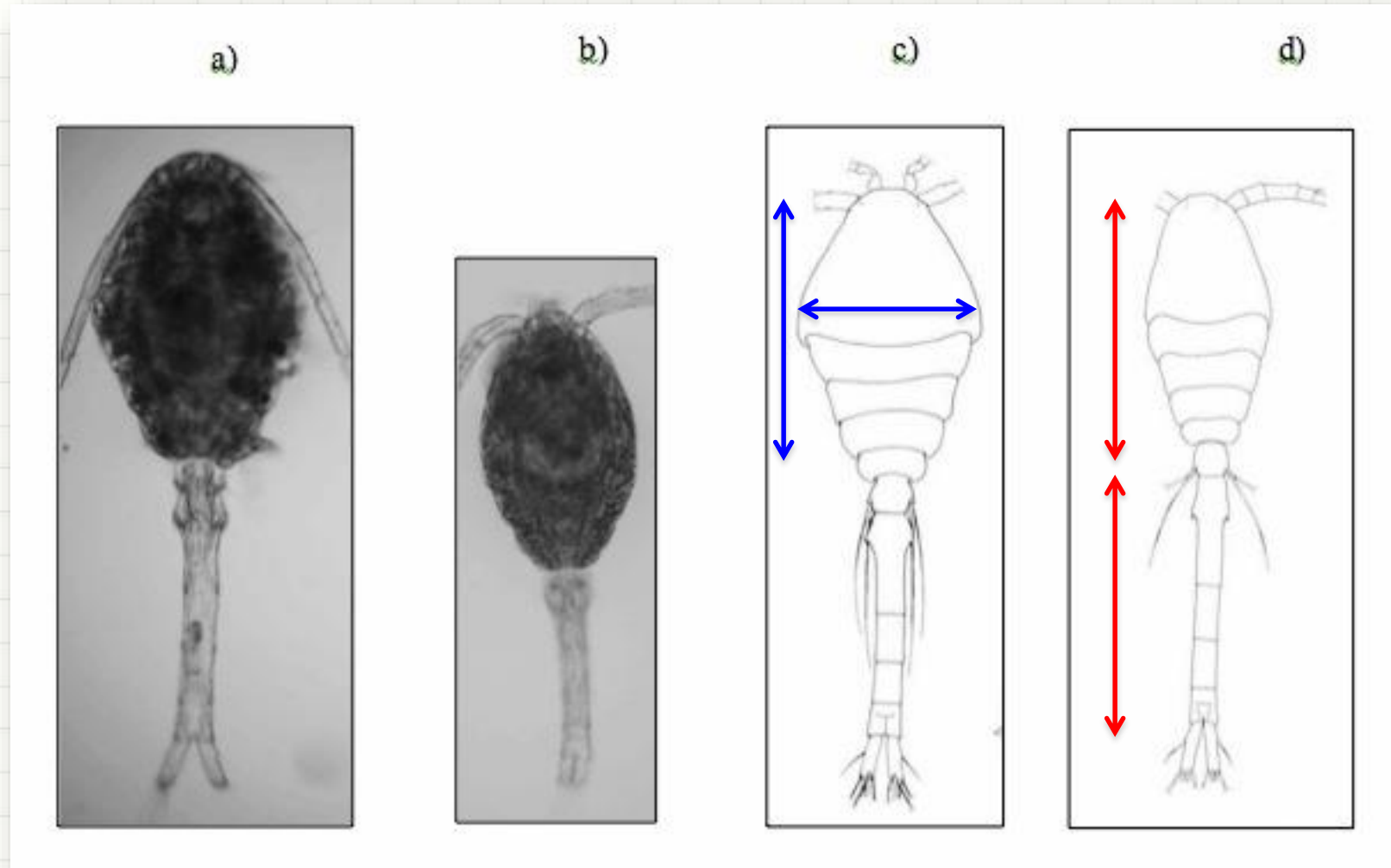


Typical

Stocky

Small

Intraspecific forms of female *Oithona attenuata*



a and c: stocky form. b: small form. d: typical form. a and b are from coastal waters of Malaysia (this study), c and d are from Nishida (1985)

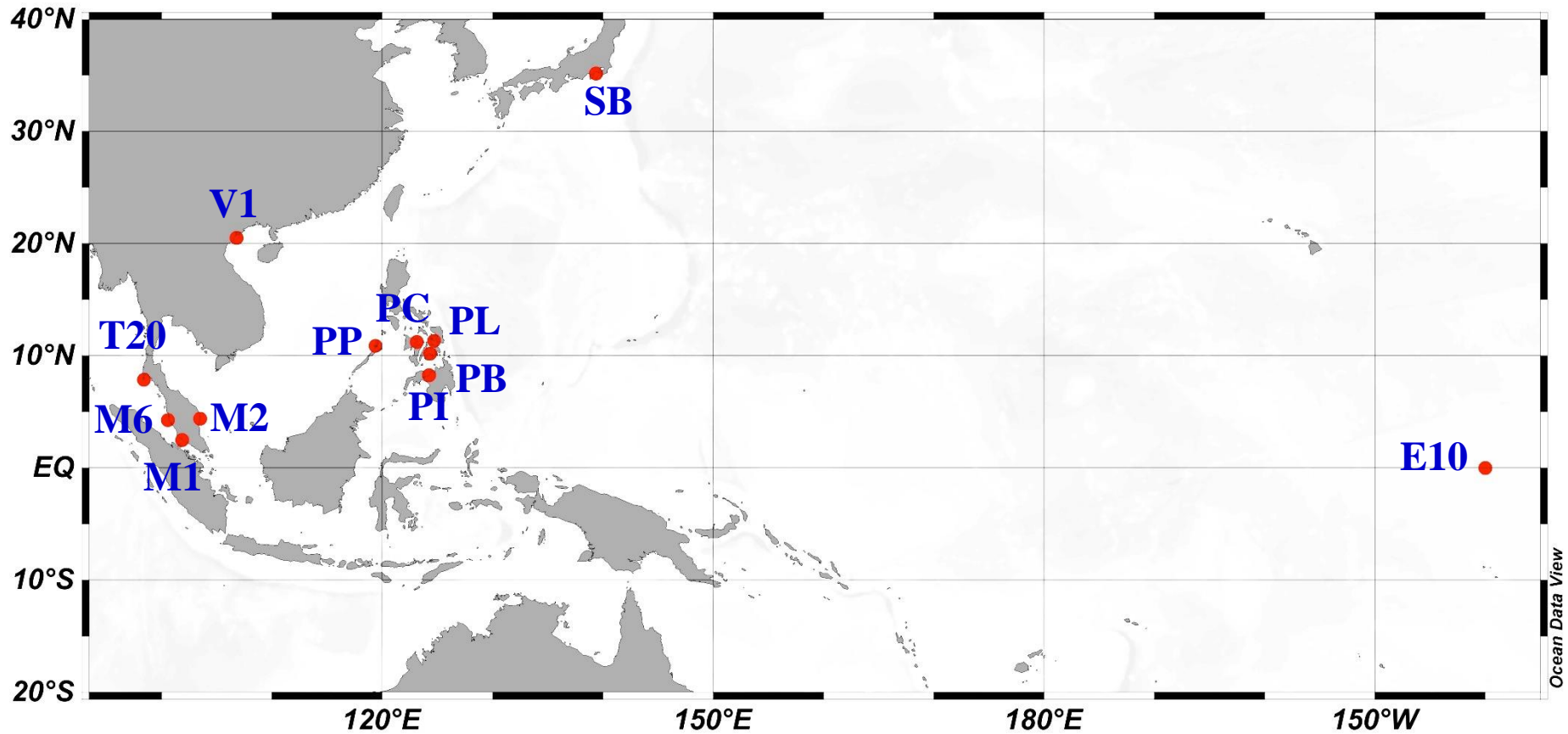
OBJECTIVE

- examines the genetic relationships and morphological variations among populations (small and stocky + typical forms) of *Oithona attenuata* in the Southeast Asia, Japan and Central Pacific

Contribute to our understanding of the mechanisms for generation of species diversity in the pelagic realm

- focusing on zooplankton in coastal waters of Southeast Asia

Collection sites of *Oithona attenuata*



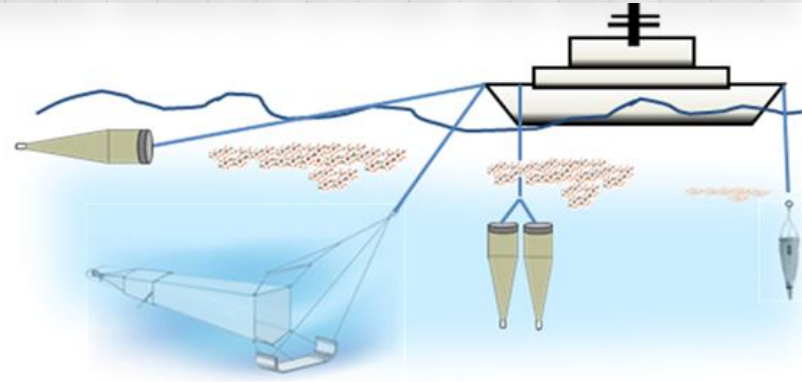
Collection sites of *Oithona attenuata*

| Sample site | Station code | Latitude | Longitude | 12S rRNA | 12S rRNA | Measurements |
|-----------------------------|--------------|---------------------|----------------------|----------|----------|--------------|
| Malaysia | M1 | 2.5 ⁰ N | 101.8 ⁰ E | 11 | 12 | 5 |
| | M2 | 4.4 ⁰ N | 103.5 ⁰ E | - | - | 9 |
| | M6 | 4.2 ⁰ N | 100.5 ⁰ E | - | - | 5 |
| Philippines | PC | 11.2 ⁰ N | 123.1 ⁰ E | - | - | 6 |
| | PB | 10.2 ⁰ N | 124.3 ⁰ E | 2 | 2 | 9 |
| | PL | 11.3 ⁰ N | 124.7 ⁰ E | 2 | 1 | - |
| | PP | 10.9 ⁰ N | 119.4 ⁰ E | 3 | 2 | - |
| | PI | 8.3 ⁰ N | 124.2 ⁰ E | 2 | 2 | - |
| Thailand | T20 | 7.8 ⁰ N | 98.4 ⁰ E | - | - | 8 |
| Pacific Ocean | E10 | 0.0 ⁰ N | 140.0 ⁰ W | 2 | 2 | 1 |
| Sagami Bay | SB | 35.2 ⁰ N | 139.4 ⁰ E | 2 | 1 | - |
| Vietnam | V1 | 20.5 ⁰ N | 106.8 ⁰ E | - | 2 | - |
| Total | | | | 24 | 24 | 43 |
| bp | | | | 353 | 303 | |
| No. of small forms | | | | 7 | 6 | |
| No. of stocky/typical forms | | | | 17 | 18 | |

Materials and Methods

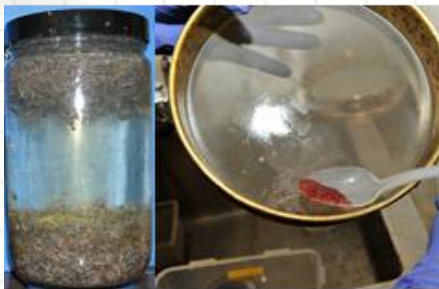
1.) Sample collection

- Hand net (scoop/tow)
- Horizontal/vertical tows (Bongo, NORPAC)
- 100-300 μm mesh
- Fixed in 5% seawater-buffered formalin and 99% ethanol



2.) Sorting & identification

- Oithonids were sorted and identified to species level under compound & dissecting microscopes



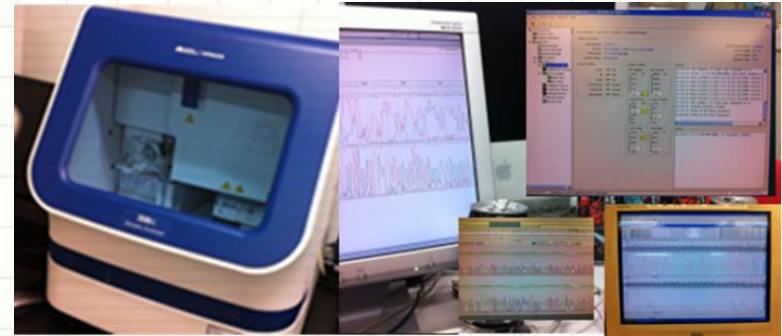
Materials and Methods

3.) DNA extraction, amplification (PCR) & sequencing

a.) Extraction: QIAGEN Dneasy blood & tissue kit



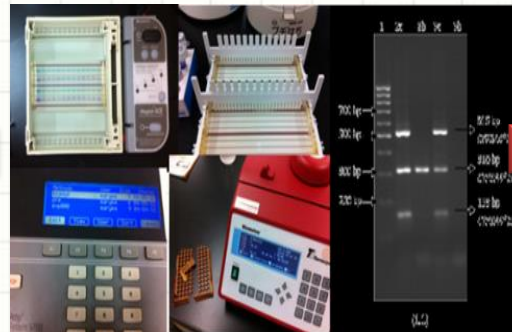
c.) Sequencing



- ✓ purified with Exosap-IT (GE Health BioScience)
- ✓ Dye-labeled terminators direct cycle sequencing
- ✓ 3130XL sequencer (Applied Biosystems)

b.) Amplification: PCR (9700 thermal cycler, Applied Biosystems Inc.)

| Reaction mixture | | Thermal cycle profile | |
|-------------------|---------------|-----------------------|---------------|
| | Standard PCR | | Standard PCR |
| | 15µl reaction | | Temp. °C Time |
| Reagent | µl | | |
| Stabilize Water | 7.62 | Initial denaturations | 94 5min |
| 2.5 µM dNTP | 1.20 | Denaturations | 94 5s |
| MgCl ₂ | | Annealing | 50 5s |
| 10x Buffer | 1.50 | | 35 cycles |
| Primer (5 µM) | 1.80 | Extension | 72 30s |
| Z Taq (Takara) | 0.08 | Final extension | 72 5min |
| Template | 1.00 | | |



PCR Products were electrophoresed

- 1% TBA agarose gel
- Stained with ethidium bromide (band characterization)
- Visualized by UV transillumination

PRIMERS

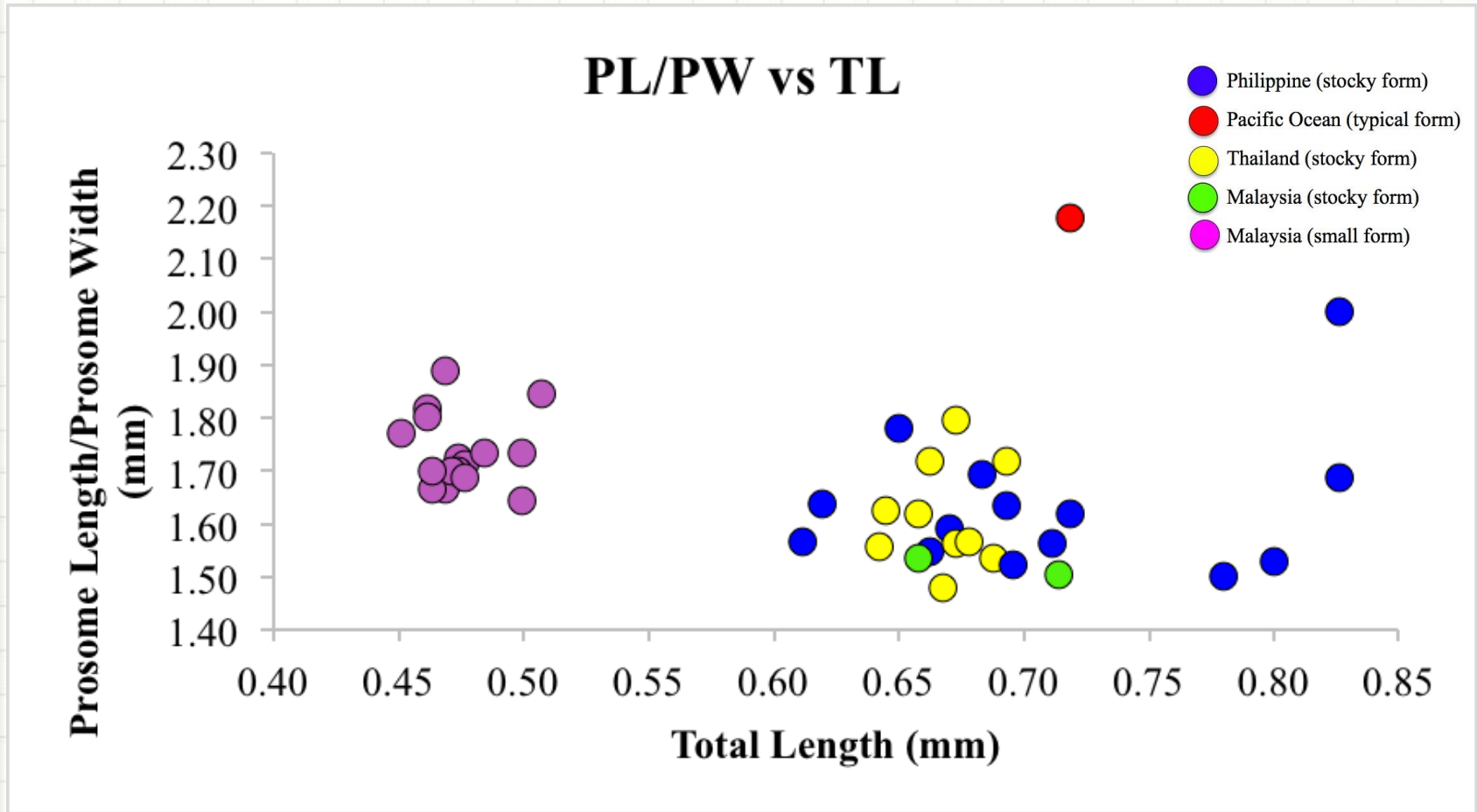
| Name of primer | Source |
|----------------------------------|-------------------------|
| Mitochondrial region 12S rRNA | Machida et al., 2002 |
| Nuclear region 28S rRNA | Machida & Knowlton 2012 |

Materials and Methods

Molecular Analysis

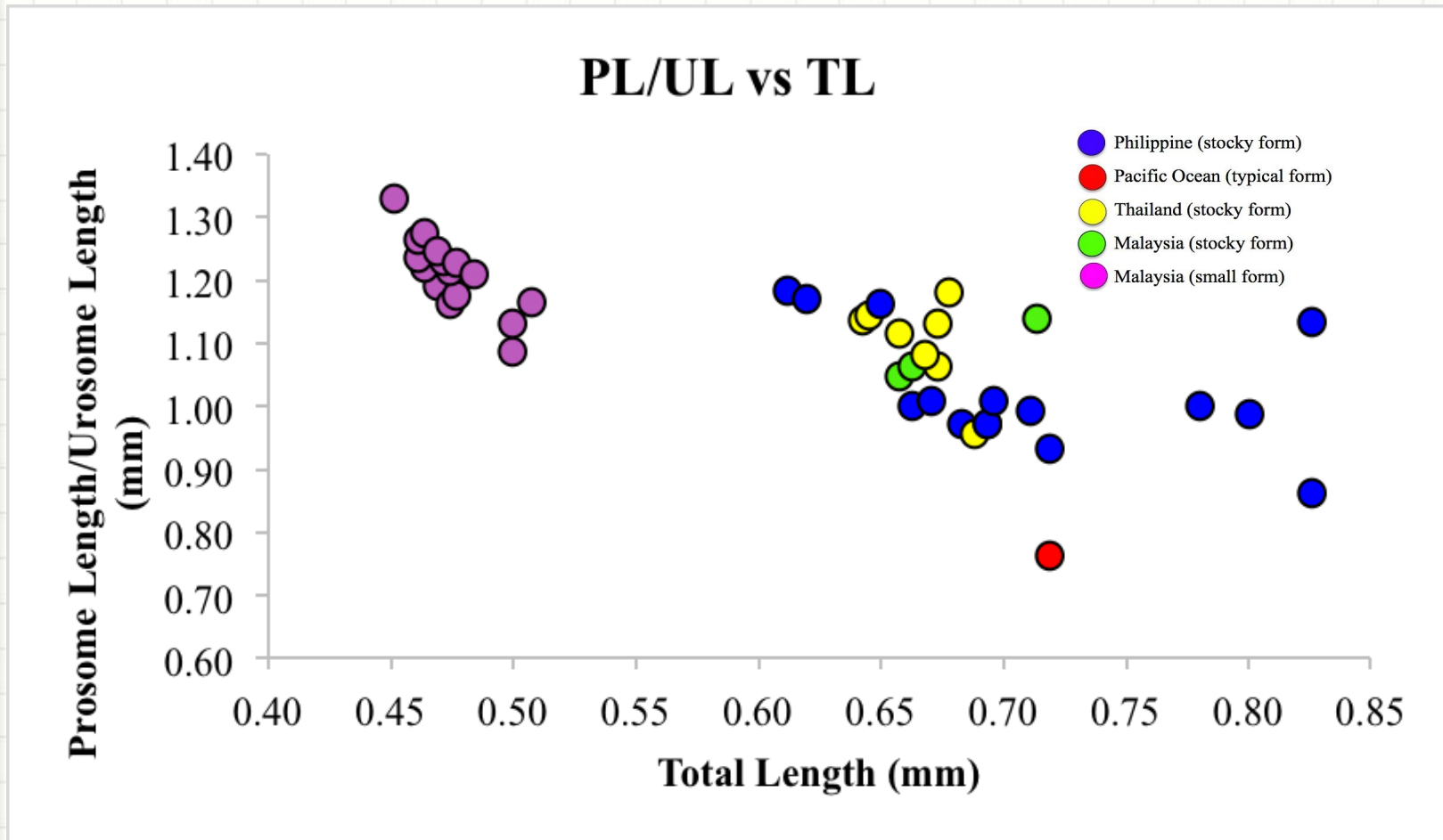
- ✓ MEGA Ver. 5
- ✓ Geneious Ver. 5.1.7
- ✓ Arlequin Ver. 3.5.1.2
- ✓ DnaSP Ver. 5 & 6
- ✓ Mr. Bayes

Body proportions of *Oithona attenuata*



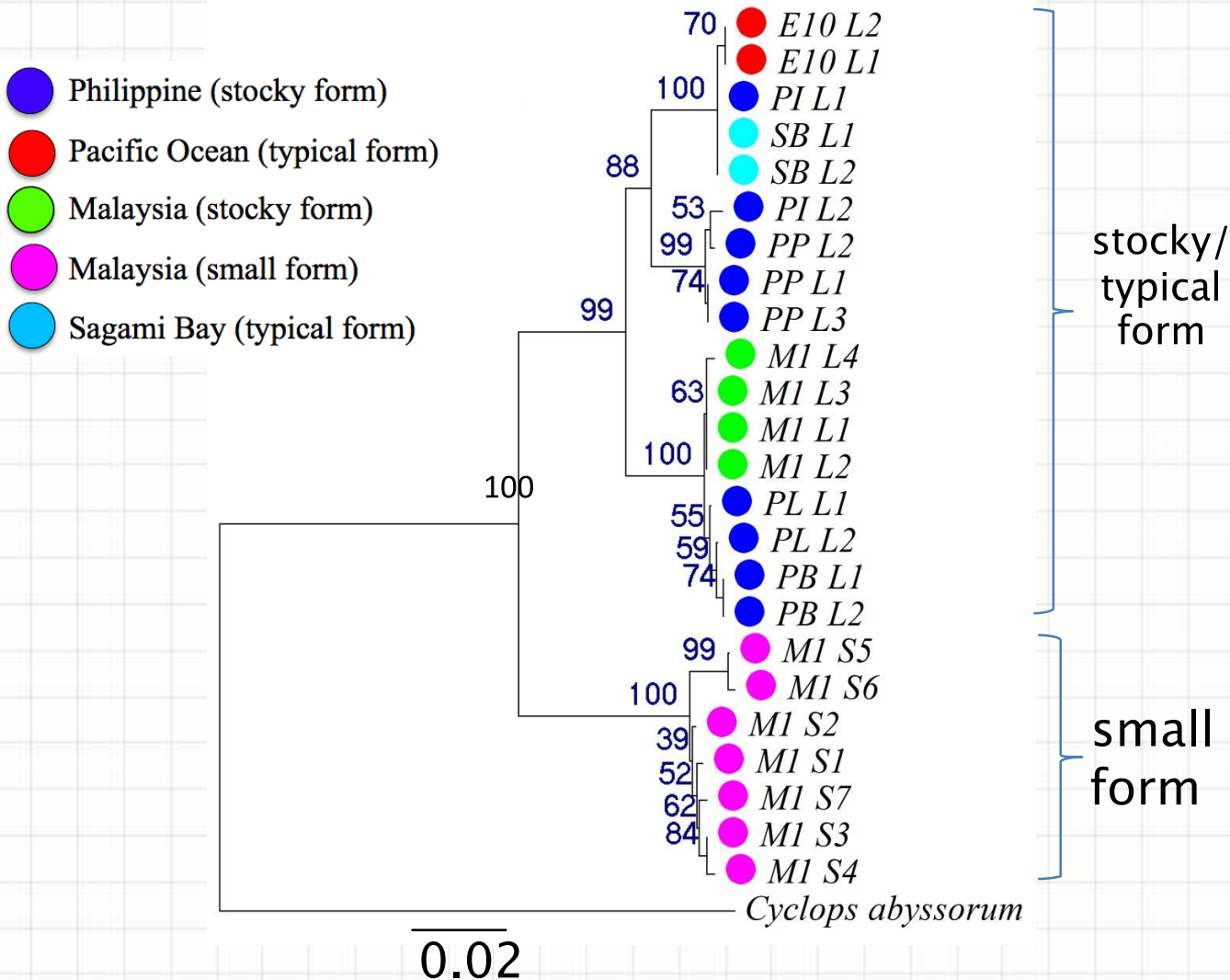
Ratio of prosome length (PL)/prosome width (PW) versus total length (TL)

Body proportions of *Oithona attenuata*



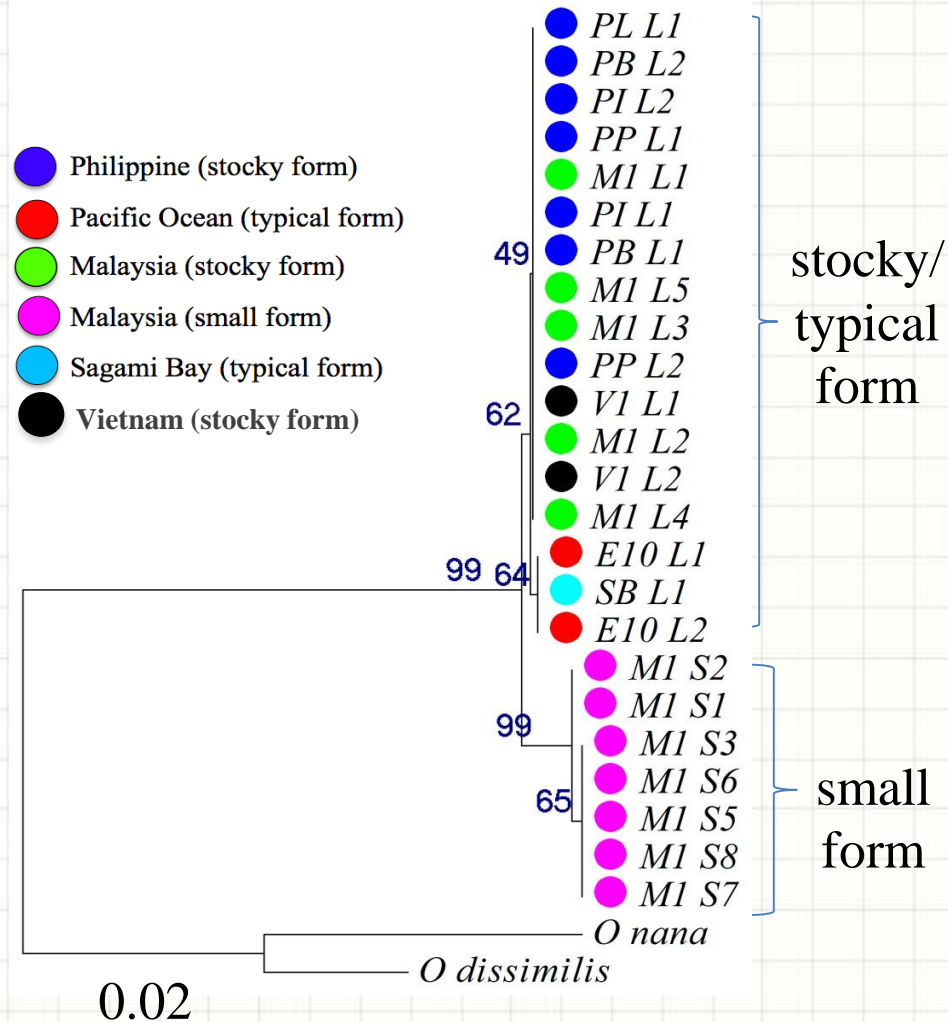
Ratio of PL/urosome length (UL) versus TL

Gene trees for mitochondrial 12s rRNA showing proportional differences between individual females of the large and small forms of *O. attenuata* from Southeast Asia and neighboring waters



Numbers at branch are bootstrap values (i.e. percentage of trees with branch point among 10000 subreplicates).

Gene trees for nuclear 28s rRNA showing proportional differences between individual females of the large and small forms of *O. attenuata* from Southeast Asia and neighboring waters



Numbers at branch are bootstrap values (i.e. percentage of trees with branch point among 10000 subreplicates).

Pairwise % difference for 12s rRNA. % values in bold indicate pair-wise comparisons between forms

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|-----------------------|------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----|-----|-----|
| 1 <i>C. abyssorum</i> | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 M1_S5 | 44.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 M1_S6 | 44.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | |
| 4 M1_S7 | 43.0 | 0.2 | 0.2 | | | | | | | | | | | | | | | | | | | | | | |
| 5 M1_S3 | 43.0 | 0.2 | 0.2 | 0.1 | | | | | | | | | | | | | | | | | | | | | |
| 6 M1_S4 | 44.0 | 0.3 | 0.3 | 0.1 | 0.0 | | | | | | | | | | | | | | | | | | | | |
| 7 M1_S1 | 44.0 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | | | | | | | | | | | | | | | | | | | |
| 8 M1_S2 | 44.0 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.0 | | | | | | | | | | | | | | | | | | |
| 9 M1_L4 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | | | | | | | | | | | | | | | | |
| 10 PB_L1 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 0.1 | | | | | | | | | | | | | | | |
| 11 PB_L2 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 0.1 | 0.1 | | | | | | | | | | | | | | |
| 12 PL_L2 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 17.0 | 16.0 | 16.0 | 16.0 | 0.1 | 0.1 | 0.0 | | | | | | | | | | | | | |
| 13 PL_L1 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 0.1 | 0.1 | 0.1 | 0.0 | | | | | | | | | | | | |
| 14 M1_L1 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 15.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | | | | | | | | | | | |
| 15 M1_L2 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 15.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | | | | | | | | | | |
| 16 M1_L3 | 44.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 15.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | | | | | | | | | |
| 17 E10_L2 | 44.0 | 19.0 | 19.0 | 18.0 | 18.0 | 18.0 | 17.0 | 17.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | | | | | | | | |
| 18 E10_L1 | 44.0 | 19.0 | 19.0 | 18.0 | 18.0 | 18.0 | 17.0 | 17.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 0.0 | | | | | | | |
| 19 SB_L1 | 44.0 | 19.0 | 19.0 | 18.0 | 18.0 | 18.0 | 17.0 | 17.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 7.0 | 7.0 | 7.0 | 0.0 | 0.0 | | | | | | |
| 20 PI_L1 | 44.0 | 19.0 | 19.0 | 18.0 | 18.0 | 18.0 | 17.0 | 17.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 7.0 | 7.0 | 7.0 | 0.0 | 0.0 | 0.0 | | | | | |
| 21 SB_L2 | 44.0 | 19.0 | 19.0 | 18.0 | 18.0 | 18.0 | 17.0 | 17.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 7.0 | 7.0 | 7.0 | 7.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | |
| 22 PI_L2 | 43.0 | 17.0 | 18.0 | 16.0 | 16.0 | 17.0 | 16.0 | 16.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | | | |
| 23 PP_L1 | 43.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 7.0 | 7.0 | 7.0 | 8.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 6.0 | 6.0 | 5.0 | 6.0 | 5.0 | 0.1 | | |
| 24 PP_L3 | 43.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 7.0 | 7.0 | 7.0 | 8.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 6.0 | 6.0 | 5.0 | 5.0 | 5.0 | 0.1 | 0.0 | |
| 25 PP_L2 | 43.0 | 17.0 | 17.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 7.0 | 7.0 | 7.0 | 7.0 | 6.0 | 6.0 | 6.0 | 5.0 | 6.0 | 0.1 | 0.0 | 0.0 |

small form vs. stocky form=15-19%
small vs. typical form=17-19%



stocky form vs. typical form=5-8%



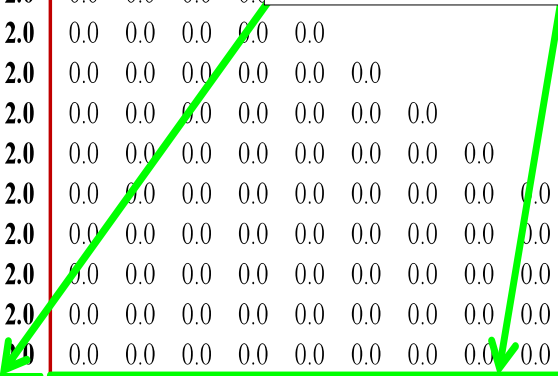
Pairwise % difference for 28s rRNA. % values in bold indicate pair-wise comparisons between forms

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|-----------------------------|------|------|------------|------------|------------|------------|------------|------------|------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 <i>O. nana</i> | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 <i>O. dissimilis</i> 14.0 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 M1_S1 | 29.0 | 26.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 M1_S2 | 29.0 | 26.0 | 0.3 | | | | | | | | | | | | | | | | | | | | | | | |
| 5 M1_S3 | 29.0 | 26.0 | 0.3 | 0.3 | | | | | | | | | | | | | | | | | | | | | | |
| 6 M1_S5 | 29.0 | 26.0 | 0.3 | 0.3 | 0.0 | | | | | | | | | | | | | | | | | | | | | |
| 7 M1_S6 | 29.0 | 26.0 | 0.3 | 0.3 | 0.0 | 0.0 | | | | | | | | | | | | | | | | | | | | |
| 8 M1_S7 | 29.0 | 26.0 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | | | | | | | | | | | | | | | | | | | |
| 9 M1_S8 | 29.0 | 26.0 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | | | | | | | | | | | |
| 10 PB_L1 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | | | | | | | | | | | | | | | | |
| 11 PI_L1 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | | | | | | | | | | | | | | | |
| 12 P_L1 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | | | | | | | | | | | | | | |
| 13 M1_L5 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | | | | | | | | | | | | | |
| 14 PL_L1 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | | | | | |
| 15 M1_L1 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | | | | |
| 16 M1_L3 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | | | |
| 17 M1_L4 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | | |
| 18 M1_L2 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | |
| 19 V1_L1 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | |
| 20 PB_L2 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | |
| 21 PP_L2 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | |
| 22 PI_L2 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | |
| 23 V1_L2 | 29.0 | 25.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | |
| 24 SB_L1 | 28.0 | 25.0 | 2.0 | 2.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| 25 E10_L2 | 28.0 | 25.0 | 2.0 | 2.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| 26 E10_L1 | 28.0 | 25.0 | 2.0 | 2.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.0 |

small form vs. stocky form=2-3%

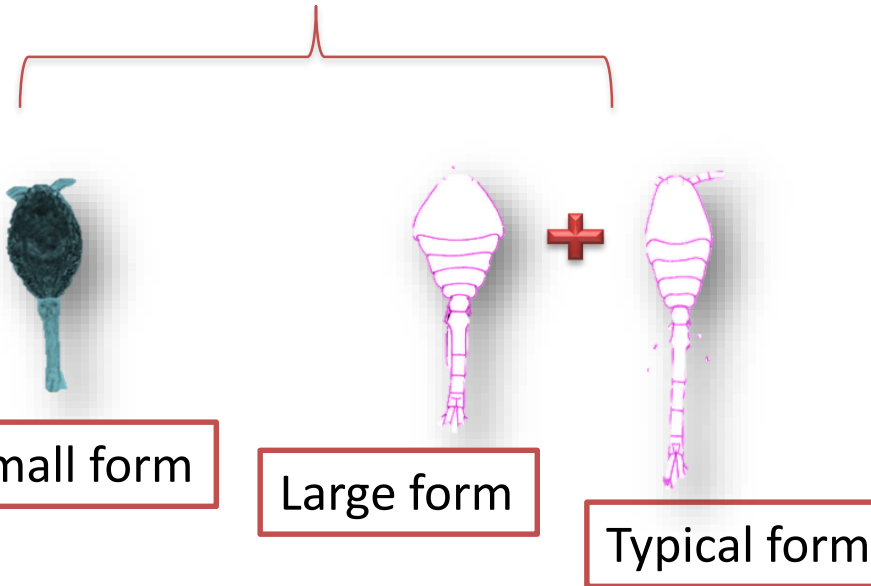


typical form vs. small form=3%
stocky form vs. typical form not significant



Summary

Oithona attenuata



- Reproductive isolation of *O. attenuata* = speciation with salinity gradients?
- stocky-coastal vs typical-oceanic
 - ✓ elusive
 - ✓ necessitating wider sample coverage
 - ✓ copepods with highest environmental adaptability = little genetic differentiation
- cryptic species in other inlet-oceanic species
 - ✓ *O. nana* & *O. simplex*,
 - ✓ integrative morphological- and molecular analyses

Thank You!
Tusen Takk!
Maraming Salamat!
ありがとうございました!
Merci!



JSPS 独立行政法人
日本学術振興会
Japan Society for the Promotion of Science



The University of the Philippines
Marine Science Institute



Atmosphere and Ocean
Research Institute
The University of Tokyo



North Pacific Marine
Science Organization (PICES)