

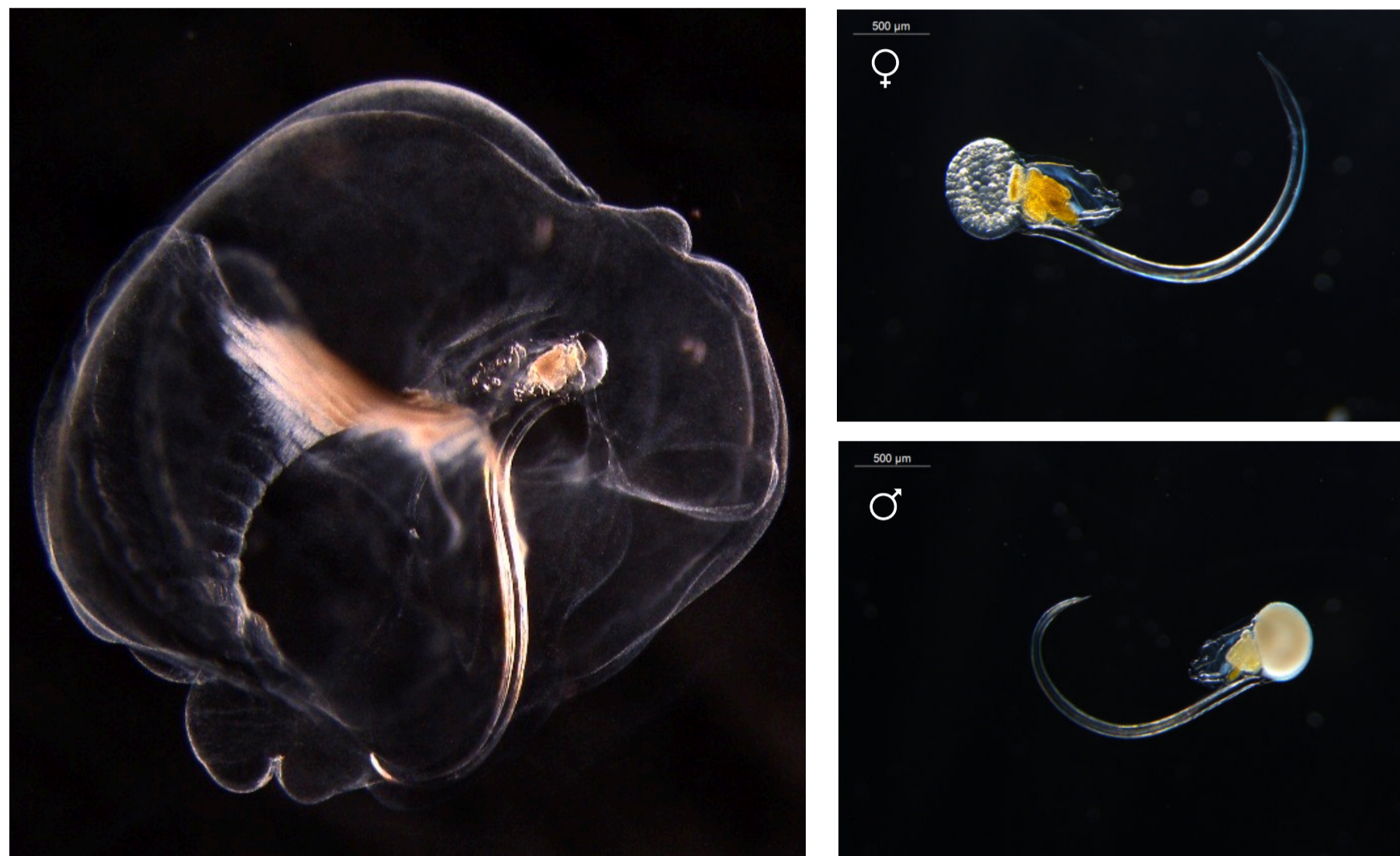


# Uncovering speciation and population structures in the marine zooplankton, *Oikopleura dioica*

Aki Masunaga<sup>1</sup>, Aleksandra Bliznina<sup>1</sup>, Andrew W. Liu<sup>1</sup>, Charles Plessy<sup>1</sup>, Michael Mansfield<sup>1</sup>, Yongkai Tan<sup>1</sup>, Nicholas M. Luscombe<sup>1</sup>  
1. Genomics and Regulatory Systems Unit, Okinawa Institute of Science and Technology Graduate University, Japan; aki.masunaga@oist.jp

## BACKGROUND

- **Larvaceans** – second most abundant zooplankton
  - **Easy sampling** – shore sampling
  - **Established culture** – natural and artificial seawater<sup>3</sup>
  - **Short lifecycle** – 4 days at 23 degrees
  - **High fecundity** – 100 - 400 eggs / female
  - **Marine snow** – vital role in vertical carbon flux<sup>1</sup>
  - **Chordate** – closest relative to vertebrates
  - **Tiny genome** – smallest among chordates, ~70Mb<sup>2</sup>
- A good model organism for marine ecological studies, evolutionary developmental biology, and genomics.



## METHODS

### Sample collection & culturing

The animals were collected with a modified plankton net from Kin bay, Okinawa and Sakoshi bay, Hyogo. They were fed three times a day and maintained at 20 degrees.

### Crossing experiment

10 pairs of Okinawa x Osaka animals were randomly selected for in vitro fertilization.

### DNA extraction and sequencing

Genomic DNA was extracted from a single male using a modified salting-out method and sequenced with ligation kit using Nanopore technology.

### ITS phylogeny

Maximum likelihood was used to estimate a phylogenetic tree from 18S and ITS sequences.

### Oikoplasmic epithelium (OE)

OE was stained with DAPI and Phalloidin and imaged using a confocal microscopy.

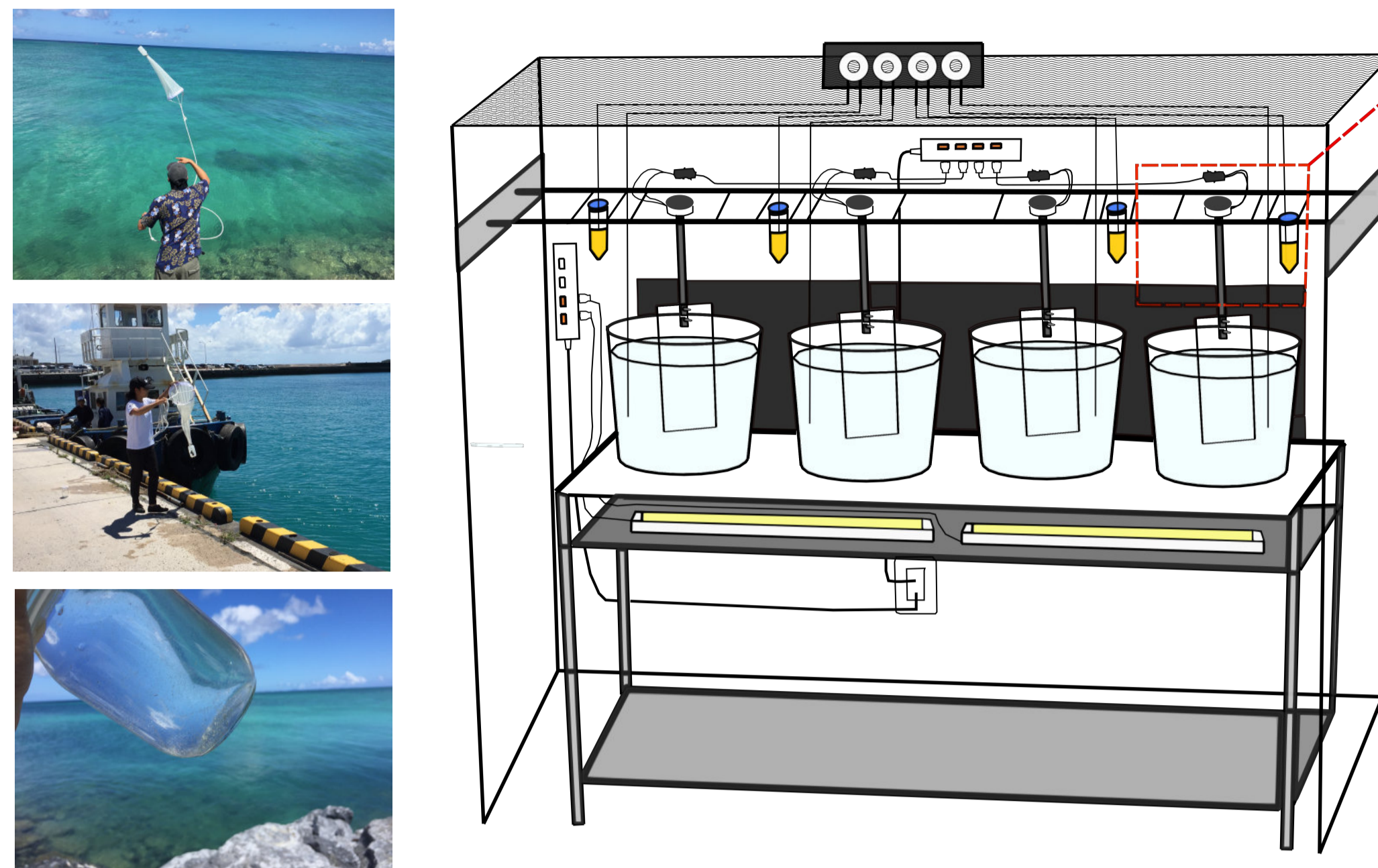
## RESULTS

- Two populations of dioecious *Oikopleura* from Okinawa and Osaka are **reproductively isolated**.
- Comparisons of whole genome alignments showed **significant rearrangement** at genomic level.
- Genome comparison and ITS sequence analysis indicated **three groups of dioecious *Oikopleura*** from the Atlantic, the North Pacific, and the Ryukyu Archipelago.
- Overall cellular arrangements and numbers of OE are conserved among genetically distinct populations.

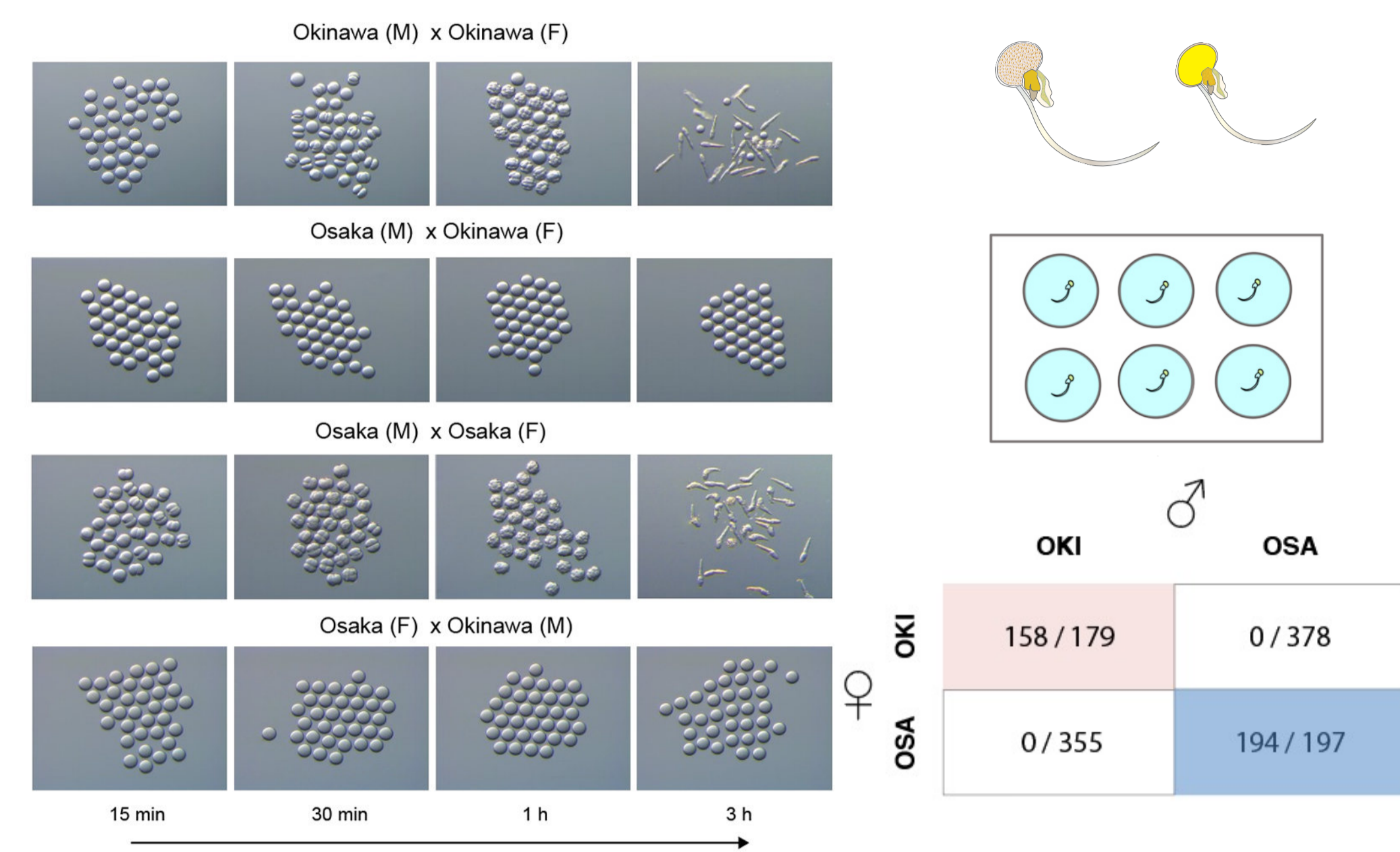
## DISCUSSIONS

*O. dioica* maintains strong morphological similarities among populations worldwide; however, further investigation is necessary for detailed morphological comparison between biologically and genetically distinct populations. Combined investigation of *O. dioica* from multiple aspects in biology will help us grasp the scope of complexity in community structure, biological as well as genetic diversity of this ecologically important pelagic tunicate, which once thought was a single species.

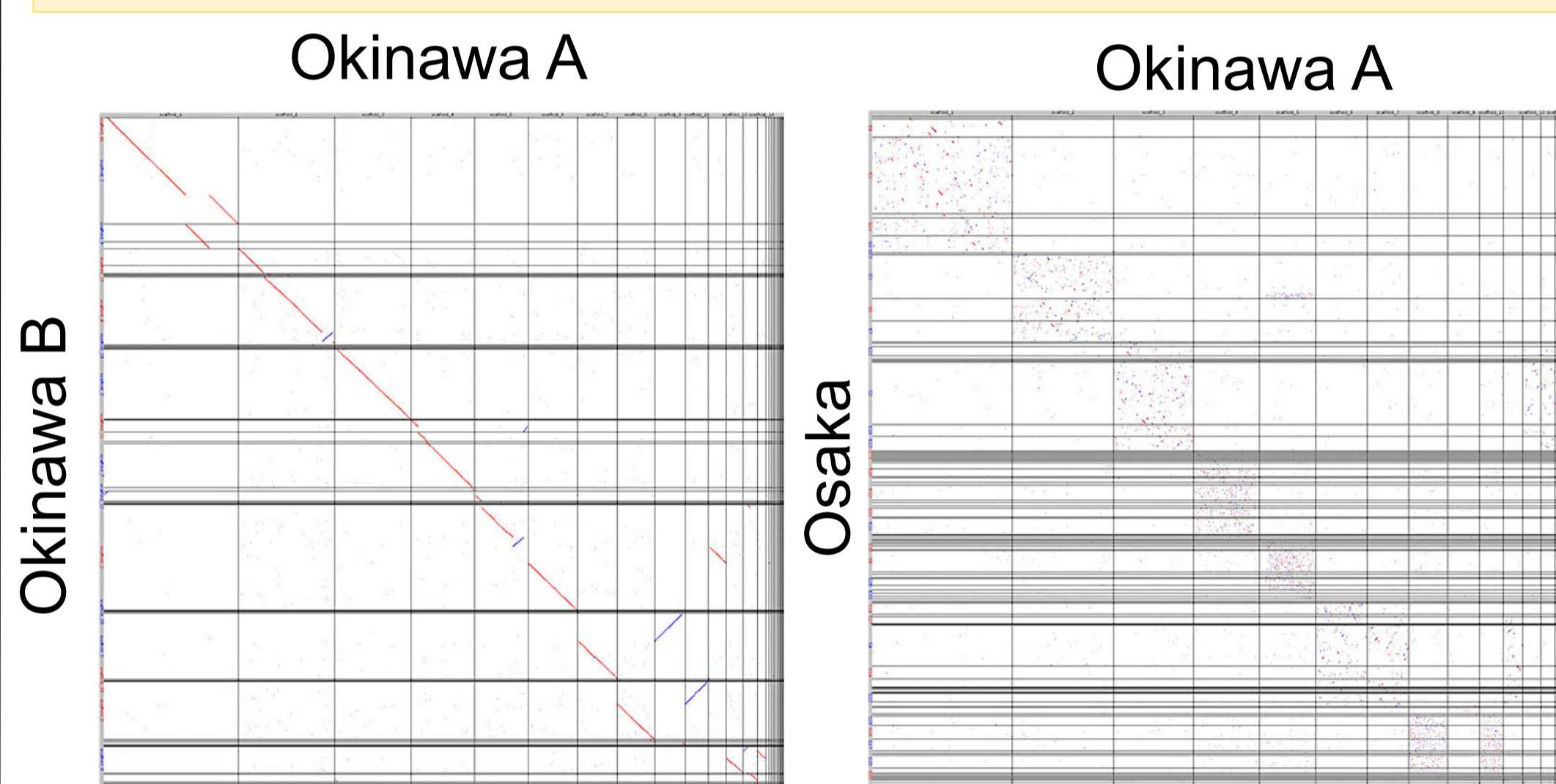
## Sampling & Culturing



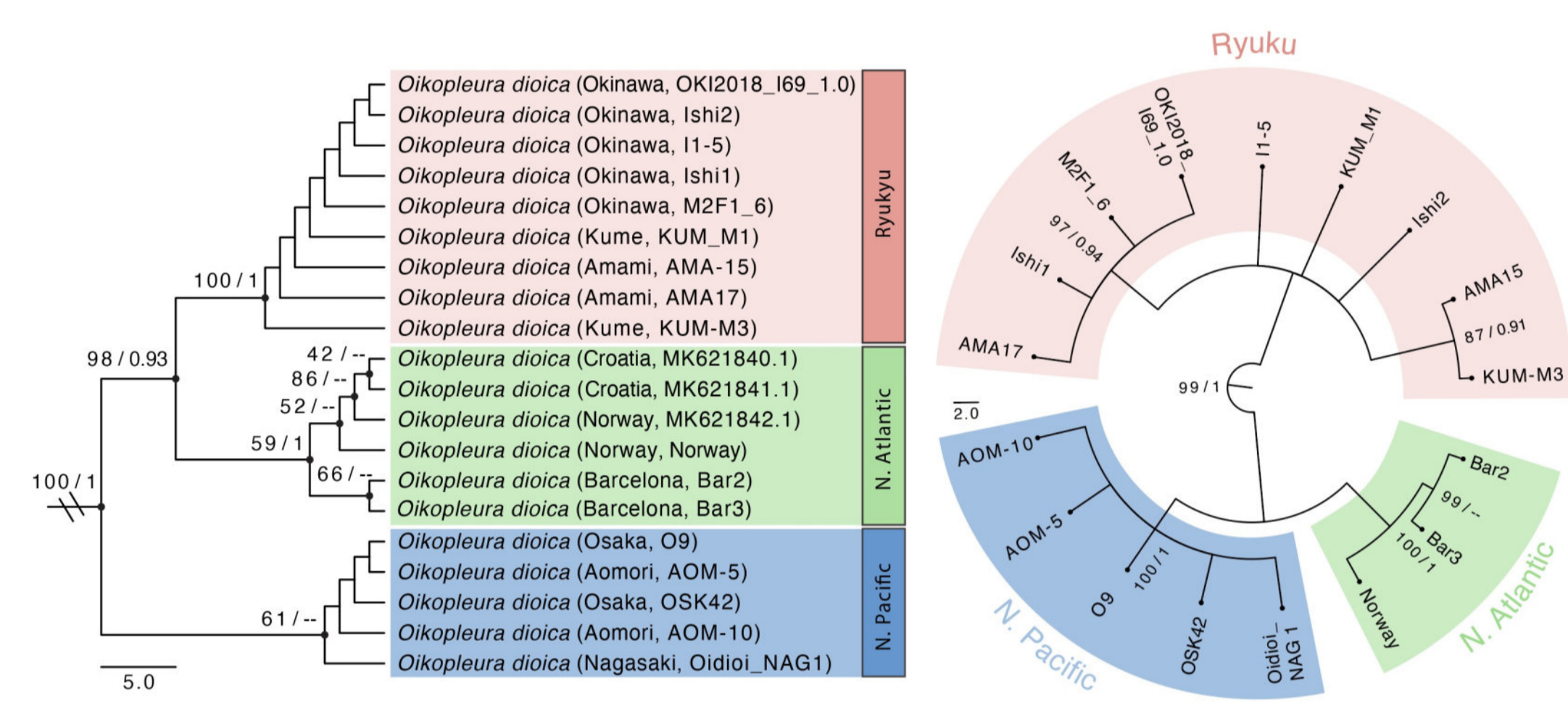
## In vitro fertilization



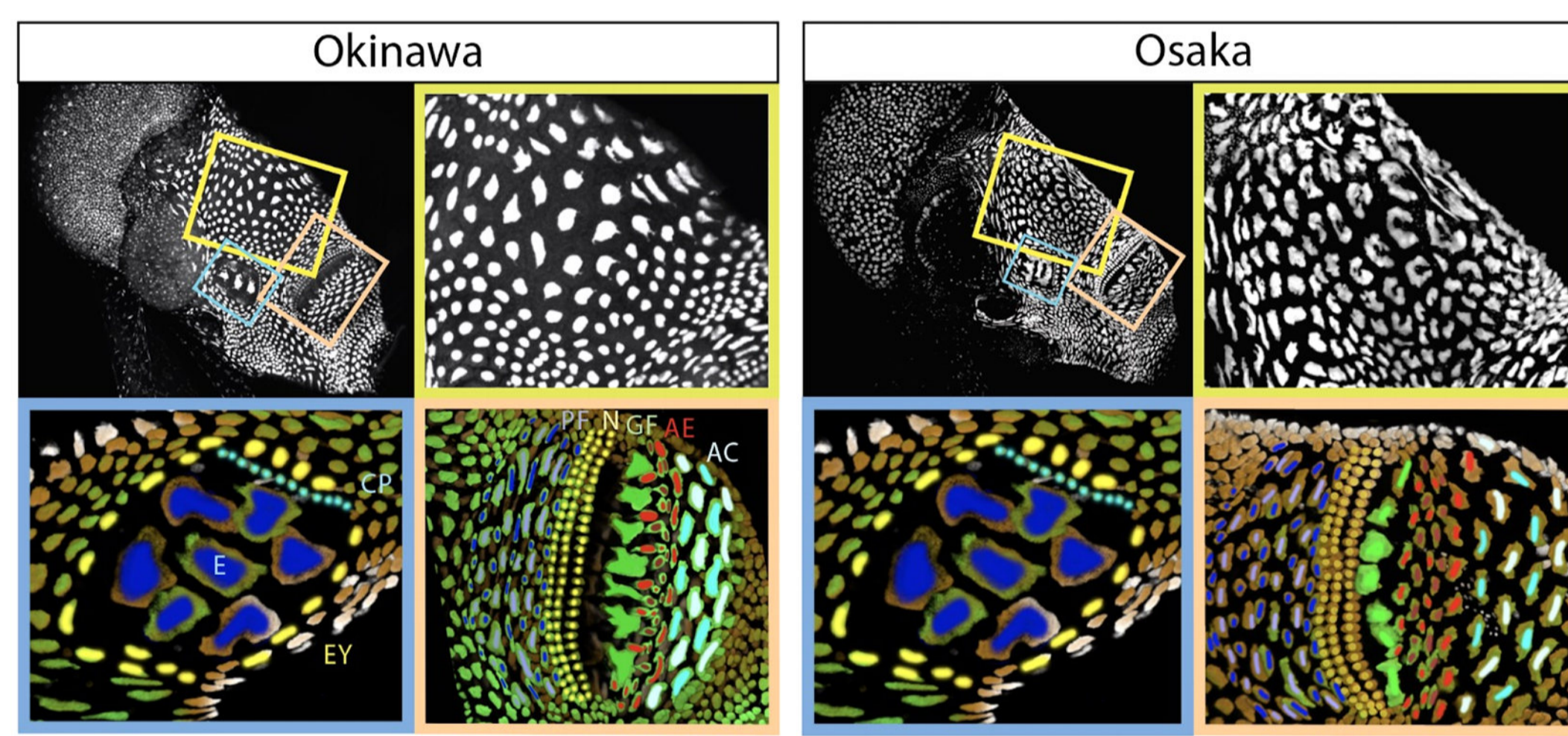
## Genome alignments



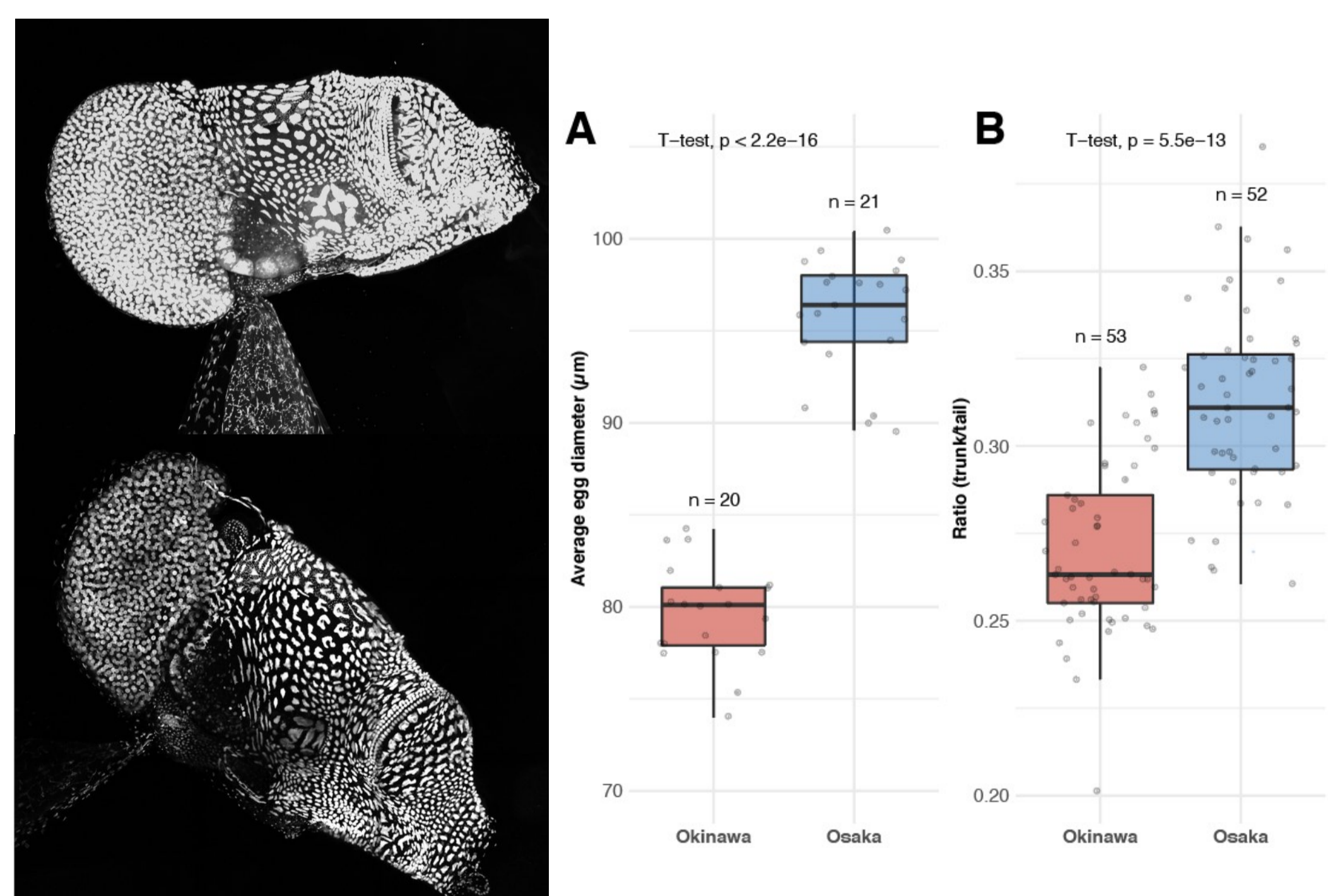
## 18S and ITS phylogeny



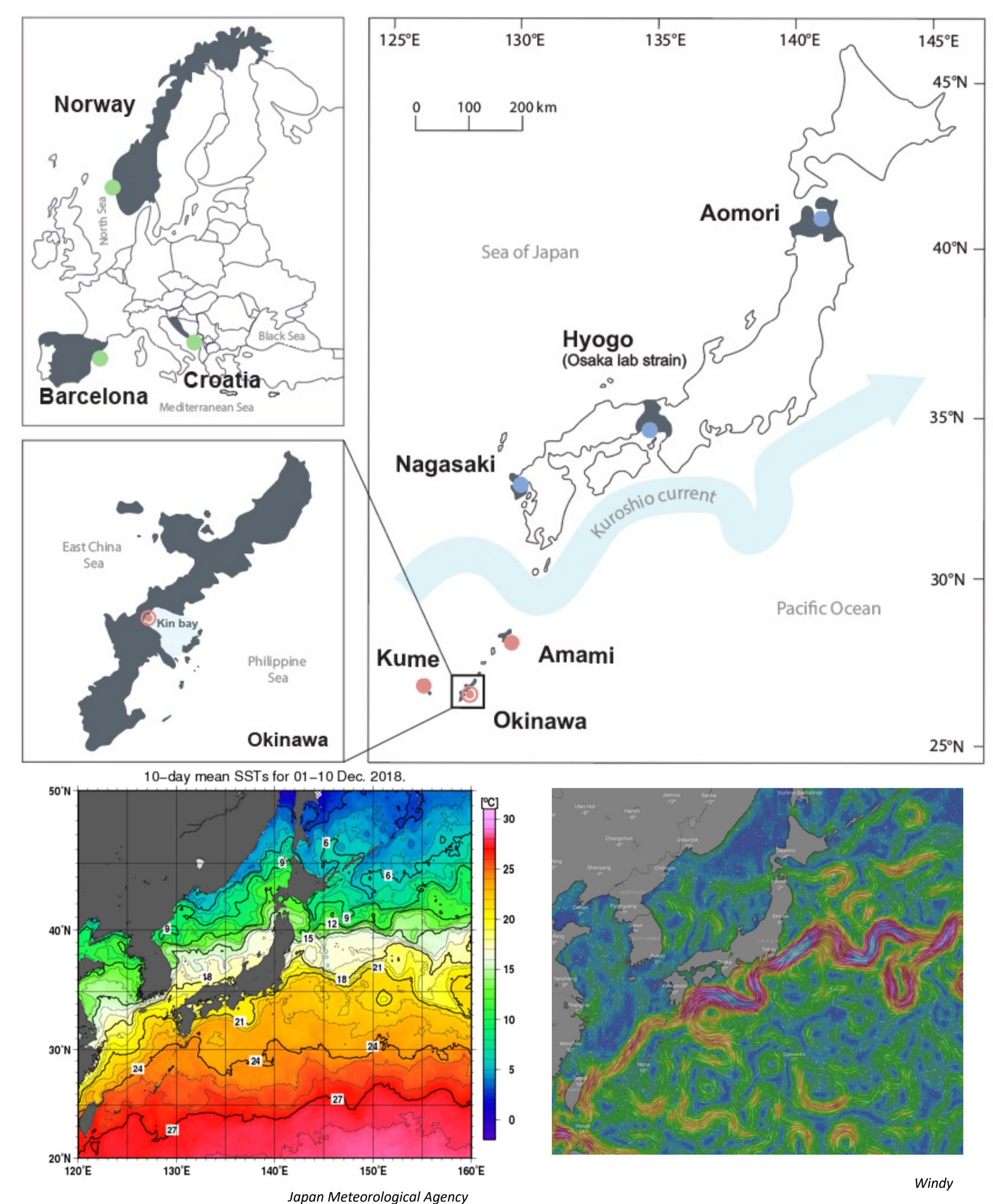
## Morphology



Confocal image of trunk epidermis by Shinya Komoto & Paolo Barzaghi



## Population structure



## Future work

- Investigate larvacean diversity with eDNA
- More sampling to better assess population structure
- Explore species boundaries using publicly available data like TARA oceans



### References:

1. Alldredge, A (2005) In response of marine ecosystems to global change: ecological impact of appendicularians. *Contemporary Publishing International* 45-57.
2. Seo, HC, et al. (2001) Miniature genome in the marine chordate *Oikopleura dioica*. *Science* 294:2506
3. Bouquet, J-M., Spriet, E., Troedsson, C., Ottera, H., Chourrout, D., Thompson, E.M. (2009) Culture optimization for the emergent zooplanktonic model organism *Oikopleura dioica*. *Journal of Plankton Research*. 31 (4), 359-370.
4. Onuma TA, Isobe M, Nishida H (2017) Internal and external morphology of adults in the appendicularian, *Oikopleura dioica*: an SEM study. *Cell Tissue Res* 367:213-227

### Acknowledgement:

We are thankful for G.R. Ilesley for the initial support in establishing the culture system. We thank J. Jolly, R. Suyama, and S. Guillot for the sampling effort and species identification. We are grateful for H. Nishida, T. Onuma, and T. Omotezako for their generous support and guidance for the initial establishment of our culture and providing us animals and microalgal culture and genome of Osaka *O. dioica*.