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Institute of Marine Environment and Ecology

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Can *Exaiptasia pallida*, a sea anemone, become a key to understanding molecular/cellular/endocrine mechanisms of coral reproduction?

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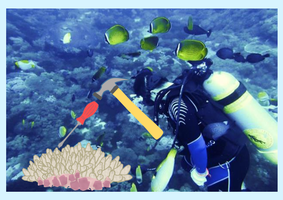
Introduction

Coral reefs ecosystem is one of the most highest marine biodiversity ecosystem on the earth. In decade, due to global climate change and human activity, coral reefs have been seriously damaged. If we could well-understand the mechanisms of sexual reproduction in coral, it would be helpful to the restoration of coral reefs.



Problems in research

- Large quantities of live coral are required for research and it will damage nature coral populations
- Collecting sample is laborious
- It is dangerous and costly to collect coral sample by scuba diving

We thought that *Exaiptasia pallida* is similar to coral, and might be used as an experimental model because *Exaiptasia* possesses many advantages as the table below

Coral polyp

Scleractinian coral	Characteristic	*Exaiptasia* sp.
Cnidaria	Phylum	Cnidaria
Mostly Anthozoa	Class	Anthozoa
Mostly Scleractinia	Order	Actinaria
Polyp	Morphology	Polyp
Present	Skeleton	Absent
Dinoflagellates	Symbiodinium	Dinoflagellates
Slow	Growth speed	Fast
Annual	Sexual reproductive cycle	Monthly
Difficult	Induction of spawning in aquariums	Easy
Tropical /Subtropical	Species distribution	Widely dispersed
Difficult, Expensive	States of aquaculture	Easy, cheap
Hard	Tissue dissection	Easy
Yes	Genomic or transcriptome databases	Yes

Exaiptasia polyp

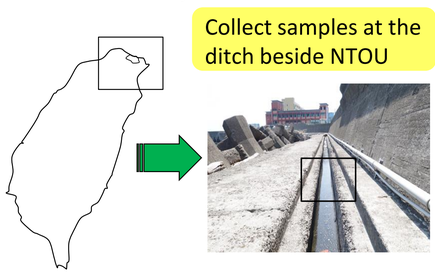
Objective

To establish research bases of *E. pallida* for revealing the molecular/cellular/endocrine mechanisms underlying sexual reproduction of cnidarian including corals

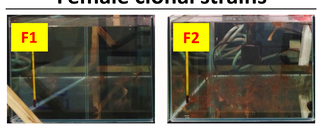
Experiment 1

➢ Establishment of *E. pallida* clonal strains

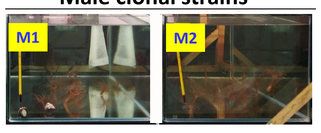
A. Clonal strains of *E. pallida*



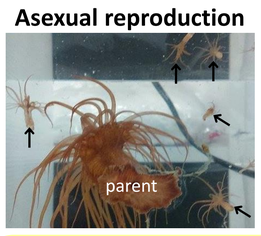
Female clonal strains



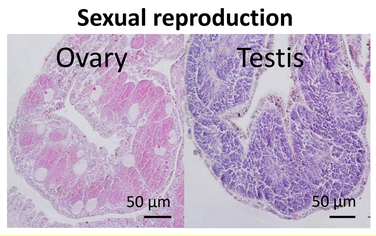
Male clonal strains



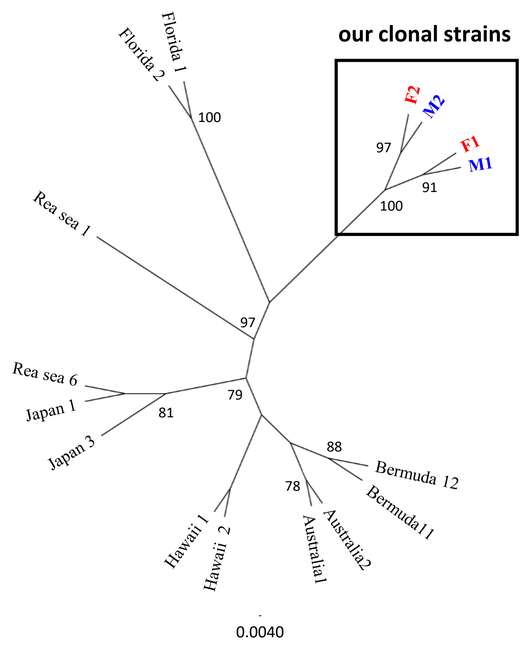
Asexual reproduction



Sexual reproduction



B. Phylogenetic tree of the clonal strains

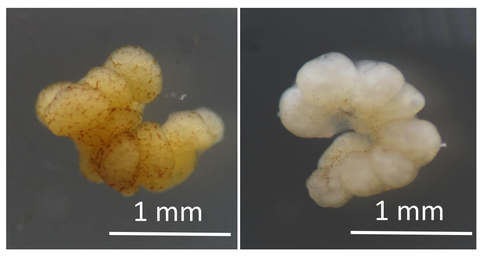


We successfully established clonal strains for our experimental materials

Experiment 2

➢ Transcriptome analysis on *E. pallida* ovary and testis

A. Isolation of gonad



Illumina Hiseq platform sequencing

de novo assembly

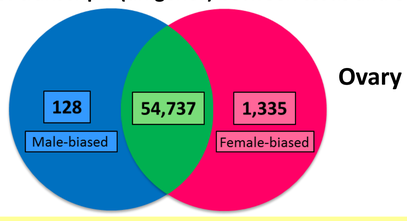
Contig expression analysis (RPKM) Testis vs. Ovary

Annotation

B. Summary statistics of the transcriptome assembly for *Exaiptasia* gonads (testis/ovary)

Sequencing	
Total QT reads	234,342,658
Total QT bases	28,153,894,821
Average QT length (bp)	120
Genome mapping	
Mean mapping of Exaipitasia genome	72.80%
(Male/Female)	(72.1% / 73.4%)
De novo assembly of Unmappable Reads	
Total unigene sequences	26,932
Number of base pairs	33,022,712
N50 of unigene sequences (bp)	1,660
Average length of unigene sequences (bp)	1226
Number of unigene sequences >= 500pb	21469
Combined transcriptome (Exaiptasia ref + de novo unique sequences)	
Total unigenes	56200
(Aiptasia Ref/De novo)	(29,268 / 26,932)
Number of base pairs	109,492,074
(Aiptasia Ref/De novo)	(76,469,362 / 33,022,712)
Mean mapping rate of unigenes	98.70%
(Male/Female)	(98.6% / 98.8%)
Average FPKM of unigenes	12.2
(Male/Female)	(10.5 / 13.9)

C. The distribution of transcripts (unigenes) between testis and ovary

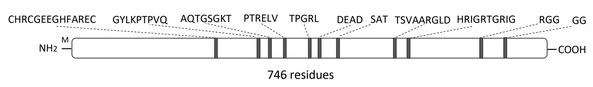


- Reproduction related gene could be identified in the transcriptome database
- Sexually dimorphic expressed genes were also identified

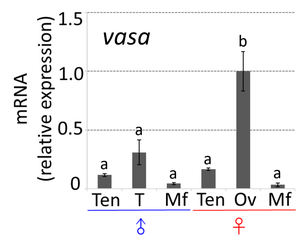
Experiment 3

➢ Identification of marker gene for germ cells: *E. pallida* vasa

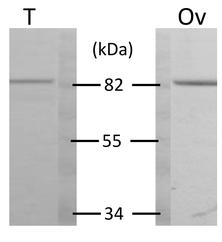
A. Sequence of *E. pallida* vasa



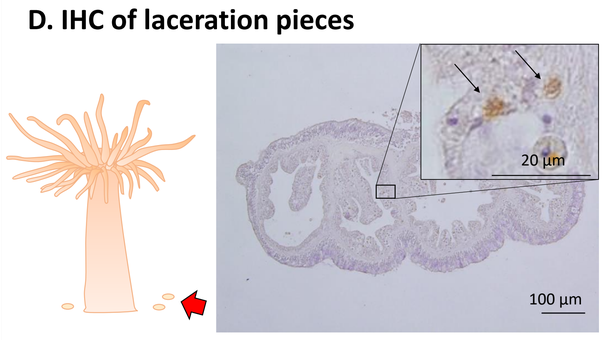
B. Quantitative RT-PCR



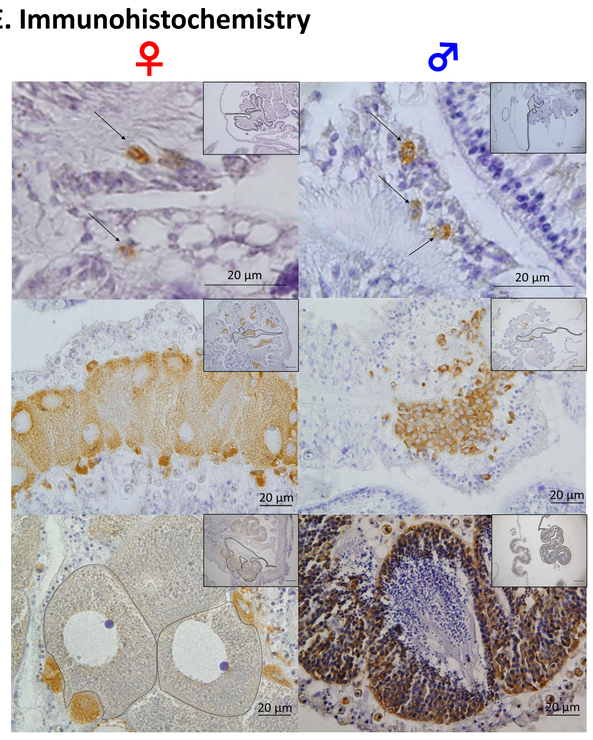
C. Western blot



D. IHC of laceration pieces



E. Immunohistochemistry

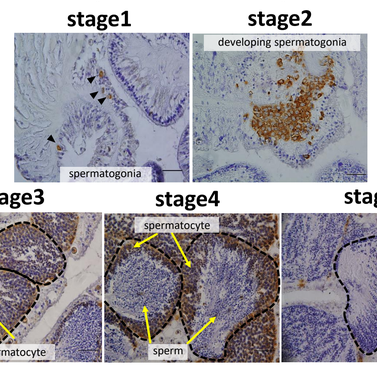


E. pallida vasa was identified as a marker gene for germ cells

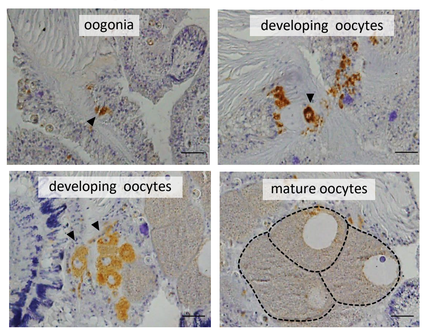
Experiment 4

➢ Identification of the process of germ cell development

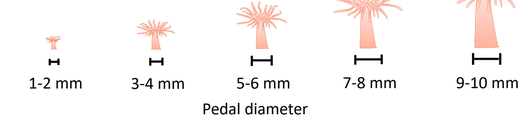
A. Germ cell development in male *E. pallida*



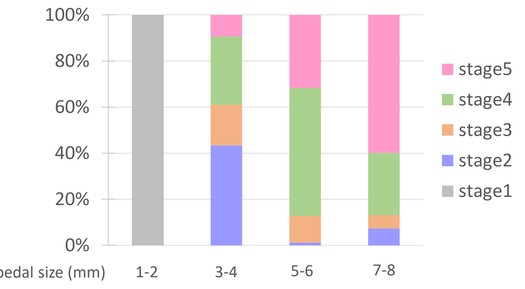
B. Germ cell development in female *E. pallida*



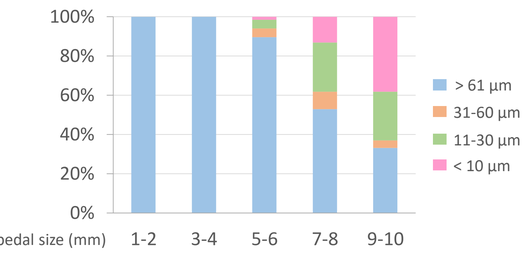
All scale = 20 μm



C. Percentages of different developmental stage



D. Percentages of different sizes of ooctye



- The process of germ cell development were revealed
- The sizes of *Exaiptasia* that begin gametogenesis were revealed

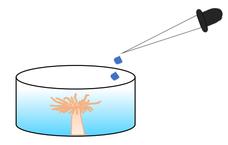
Experiment 5

➢ Establishment of gene knockdown technology (RNAi)

A. RNAi related-proteins found in *E. pallida* transcriptome

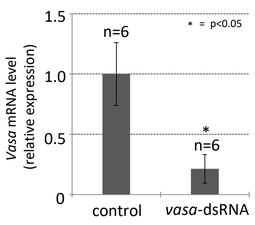
Gene	*E. pallida*	Predicted sequence ID	Reference
Drosha	✓	denovo.id409.tr11045	Song and Wessel, 2007 Owens and Malham, 2015
DGCR8/Pasha			Song and Wessel, 2007 Owens and Malham, 2015
Dicer	✓	denovo.id1615.tr28418 (Dicer1) denovo.id251.tr21029 (Dicer2)	Song and Wessel, 2007 Owens and Malham, 2015
TRBP			Song and Wessel, 2007
Argonautes 1	✓	denovo.id866.tr16367	Song and Wessel, 2007
Argonautes 2	✓	denovo.id4726.tr10895	Song and Wessel, 2007 Owens and Malham, 2015
Exportin-5			Song and Wessel, 2007
Piwi	✓	denovo.id1539.tr32691	Song and Wessel, 2007
HIV-TRBP = loquacious			Owens and Malham, 2015
Interferon Regulatory Factor	✓	denovo.id13767.tr43007	Owens and Malham, 2015
Toll-like Receptor 3			Owens and Malham, 2015

B. *Vasa*-dsRNA treatment of *E. pallida*

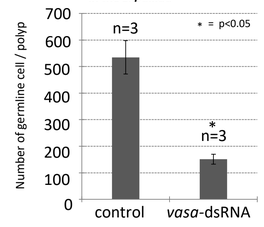


Control : *luciferase*-dsRNA
Treatment : *vasa*-dsRNA
Concentration : 50 ng/μl
Volume : 100 μl

◆ Expression level of the *vasa* in the *E. pallida*



◆ The number of the germline cell in the *E. pallida*



The established RNAi technology can be used as an important tool to investigate gene functions of *E. pallida*

Summary

- We successfully established new materials, database, tools and techniques to study molecular/cellular/endocrine mechanisms of *E. pallida* sexual reproduction
- Using our transcriptome database, we were able to identify genes regarding germ cell development, mitosis and meiosis, hormones and hormone receptors that are expressed in the gonads