

PICES-2019 Annual Meeting:  
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# Studies on *Prorocentrum* (Dinophyceae) in the coastal water of China

Pengbin Wang, Ruoyu Guo, Xinfeng Dai, Douding Lu

Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou, 310012, China

2019-10-23

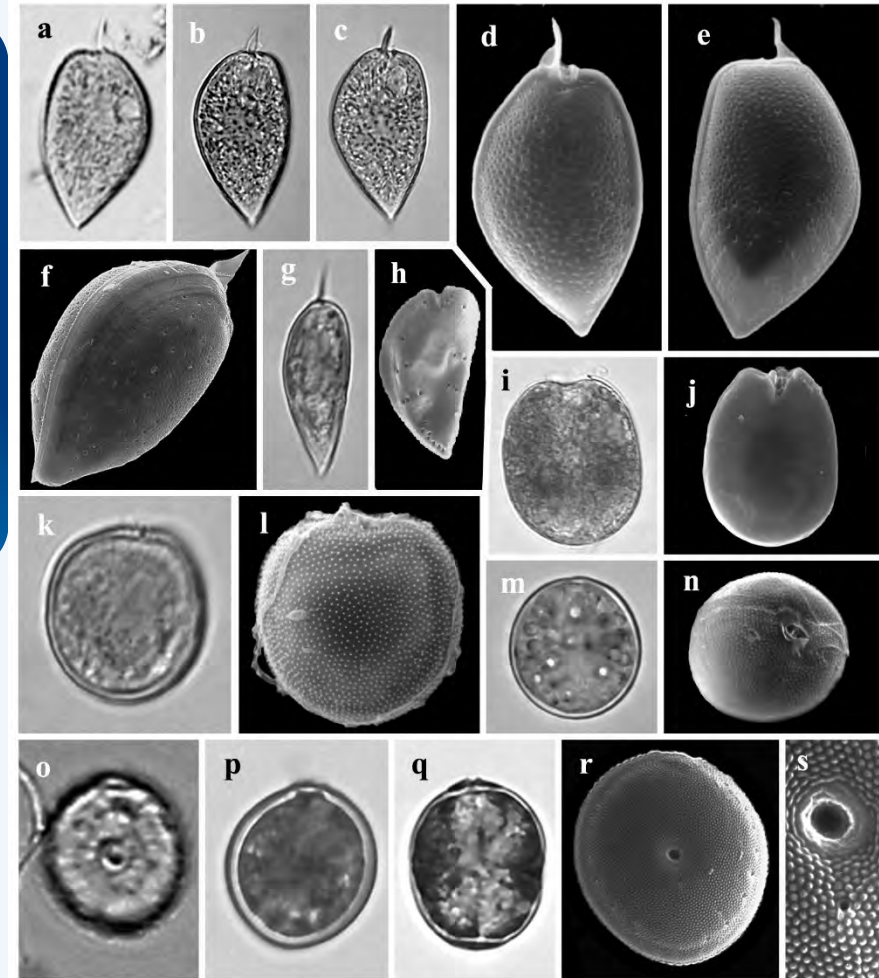
# Introduction

# ★ Who is *Prorocentrum*?

- *Prorocentrum* is a cosmopolitan dinoflagellate genus, distributed world widely, with divers morphonology. (Croome and Tyler 1987)



*Prorocentrum* widely distributed in coastal areas, estuaries and oceanic waters, which is one of main causative species of Harmful Algal Blooms.



World wide:

***Pelagic Prorocentrum***



***Benthic Prorocentrum***



In China : Only  
Reported

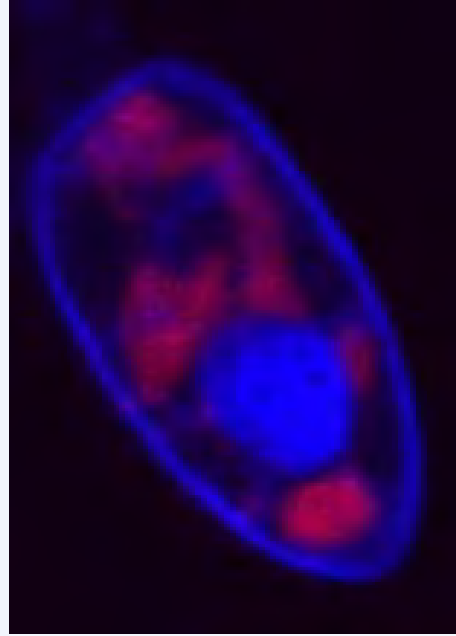
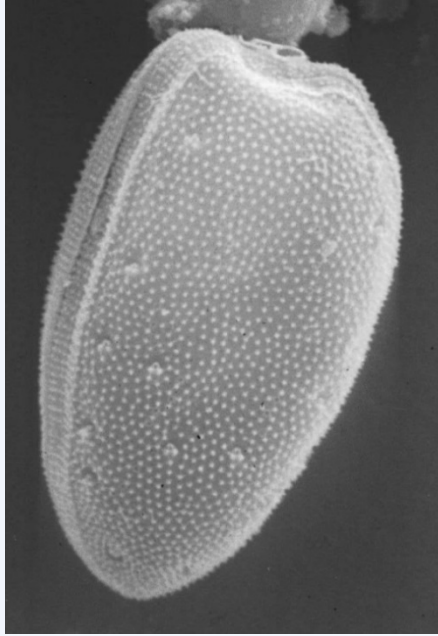
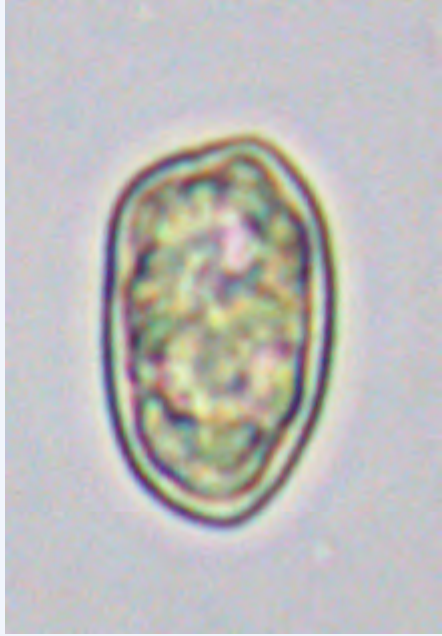


**R-1**

# Introduction

Current research of *Prorocentrum* in China

## *Prorocentrum donghaiense*



Prof. Douding LU



# Causative species——*Prorocentrum donghaiense* Lu

2000~2017,

□ 278 times / 86 382 Km<sup>2</sup> were recorded.

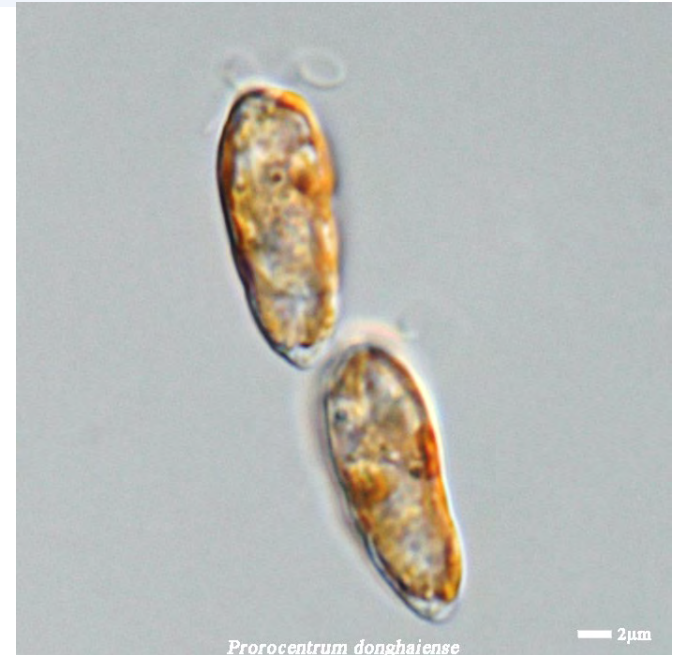
□ Occurred every year since 2000.

□ Mainly concentrated in the inshore area

of the Yangtze River Estuary (Zhejiang and Fujian).

□ High incidences occurred in April-September per year, mainly in

April-June.





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## Morphology, molecular phylogeny and okadaic acid production of epibenthic *Prorocentrum* (Dinophyceae) species from the northern South China Sea

Zhaohe Luo<sup>a,b,1</sup>, Hua Zhang<sup>a,1</sup>, Bernd Krock<sup>c</sup>, Songhui Lu<sup>a,\*</sup>, Weidong Yang<sup>a</sup>, Haifeng Gu<sup>b,\*</sup>

<sup>a</sup> College of Life Science and Technology, Jinan University, Guangzhou 510632, China

<sup>b</sup> Third Institute of Oceanography, SOA, Xiamen 361005, China

<sup>c</sup> Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

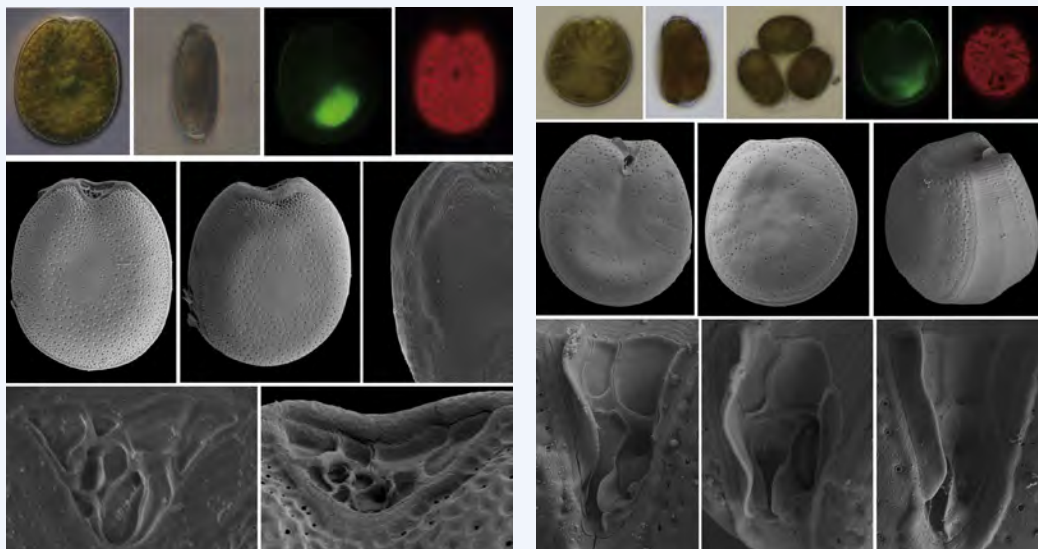


Table 1  
These strains of *Prorocentrum* examined in the present study, including okadaic acid concentrations, collection data and locations.

Species	Strains	Okadaic acid (lg cell <sup>-1</sup> )	DTX-1 (lg cell <sup>-1</sup> )	Collection date	Latitude (N)	Longitude (E)	Station	Location
<i>P. concavum</i>	AS47	–	None	2013.06.08	18°18.63'	109°58.62'	4	Lingshui, Hainan
<i>P. concavum</i>	D56C18	+0.031	None	2013.06.08	18°18.63'	109°58.62'	4	Lingshui, Hainan
<i>P. concavum</i>	D54E11	–0.015	None	2013.06.08	18°18.63'	109°58.62'	4	Lingshui, Hainan
<i>P. concavum</i>	D54F8	–	–	2013.06.08	18°18.63'	109°58.62'	4	Lingshui, Hainan
<i>P. cf. emarginatum</i>	NG21	–	–	2014.08.19	18°14.47'	109°21.50'	8	Sanya, Hainan
<i>P. akayayi</i>	NG2	–	–	2012.11.23	18°18.63'	109°58.62'	4	Lingshui, Hainan
<i>P. akayayi</i>	T0309	–	–	2015.12.03	21°25.24'	109°02.89'	10	Beihai, Guangxi
<i>P. akayayi</i>	T0310	–	–	2015.12.03	21°25.24'	109°02.89'	10	Beihai, Guangxi
<i>P. akayayi</i>	T0312	–	–	2015.12.03	21°25.24'	109°02.89'	10	Beihai, Guangxi
<i>P. akayayi</i>	T0313	–	–	2015.12.03	21°25.24'	109°02.89'	10	Beihai, Guangxi
<i>P. akayayi</i>	T0314	–	–	2015.12.03	21°25.24'	109°02.89'	10	Beihai, Guangxi
<i>P. lima</i>	T0124	2834	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0157b	2886	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0162	5110	910	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0155a	4213	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0159b	2886	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0164	3001	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0302	10,260	1810	2015.08.26	21°54.29'	120°43.55'	2	Kemuning, Taiwan
<i>P. lima</i>	T0163	1275	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. lima</i>	T0175c	551	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. cf. maculatum</i>	T011	5205	None	2014.11.16	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. cf. maculatum</i>	T0102	2117	None	2015.04.16	18°12.48'	109°38.95'	6	Sanya, Hainan
<i>P. cf. maculatum</i>	T0139	3002	None	2015.04.16	18°16.26'	109°44.99'	5	Sanya, Hainan
<i>P. cf. maculatum</i>	T0138	4209	None	2015.04.16	18°16.26'	109°44.99'	5	Sanya, Hainan
<i>P. cf. maculatum</i>	T0179	6879	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. cf. maculatum</i>	T0180	7610	None	2015.06.06	21°01.89'	109°08.48'	9	Beihai, Guangxi
<i>P. japonense</i>	T007	+0.341	None	2015.04.16	18°12.48'	109°38.95'	6	Sanya, Hainan
<i>P. thalysimum</i>	T020	–0.024	None	2015.02.05	18°14.26'	109°44.99'	5	Sanya, Hainan
<i>P. thalysimum</i>	T0100	–0.015	None	2015.04.16	18°12.48'	109°38.95'	6	Sanya, Hainan
<i>P. thalysimum</i>	T0307	–	–	2015.10.18	22°41.59'	117°29.67'	1	Dongshih, Fujian
<i>P. thalysimum</i>	T0401	–	–	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T005	–	–	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T005	–0.017	None	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T0114	–	–	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T0075	–	–	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T0080	–	–	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T0082	–	–	2015.02.04	18°09.61'	109°33.77'	7	Sanya, Hainan
<i>P. thalysimum</i>	T0136	–	–	2015.04.16	18°16.26'	109°44.99'	5	Sanya, Hainan
<i>P. thalysimum</i>	T0146	–	–	2015.04.16	18°16.26'	109°44.99'	5	Sanya, Hainan
<i>P. thalysimum</i>	T0101	–0.014	None	2015.04.16	18°12.48'	109°38.95'	6	Sanya, Hainan
<i>P. thalysimum</i>	T0085	+0.014	None	2015.04.16	18°16.26'	109°44.99'	5	Sanya, Hainan
<i>P. thalysimum</i>	T0084	–	–	2015.04.16	18°16.26'	109°44.99'	5	Sanya, Hainan
<i>P. thalysimum</i>	T0093	–0.038	None	2015.04.17	18°21.63'	110°00.19'	3	Sanya, Hainan
<i>P. thalysimum</i>	T0052	–	–	2015.04.16	18°12.48'	109°38.95'	6	Sanya, Hainan
<i>P. thalysimum</i>	T0064	–	–	2015.04.17	18°21.63'	110°00.19'	3	Sanya, Hainan
<i>P. thalysimum</i>	T0109	–	–	2015.04.17	18°21.63'	110°00.19'	3	Sanya, Hainan
<i>P. thalysimum</i>	T0112	–	–	2015.04.17	18°21.63'	110°00.19'	3	Sanya, Hainan

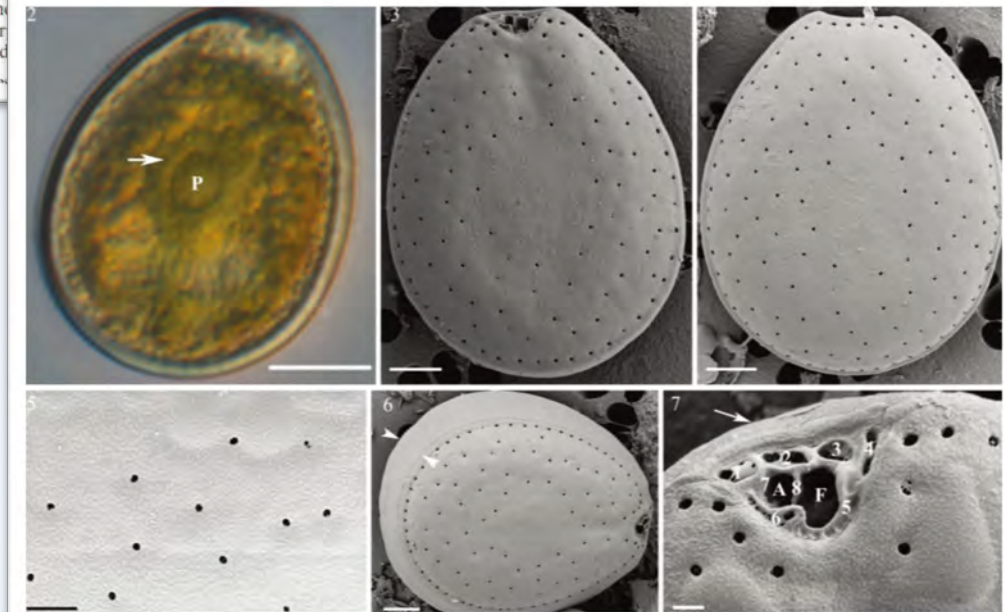
## Morphotypes of *Prorocentrum lima* (Dinophyceae) from Hainan Island, South China Sea: morphological and molecular characterization

HUA ZHANG<sup>1</sup>, YANG LI<sup>2</sup>, JINGYI CEN<sup>1</sup>, HUALONG WANG<sup>1</sup>, LEI CUI<sup>1</sup>, YUELEI DONG<sup>1</sup> AND SONGHUI LU<sup>1\*</sup>

<sup>1</sup>Research Center for Harmful Algae and Marine Biology, Jinan University, Key Laboratory of Eutrophication and Red Tide Prevention of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, China

<sup>2</sup>College of Life Science, South China Normal University, Guangzhou 510631, China

**ABSTRACT:** The benthic dinoflagellate *Prorocentrum lima* has a cosmopolitan distribution that extends from temperate to tropical oceans. The identification of *P. lima* needs detailed morphological observation and molecular characterization because of its similarity to congeners and its morphological variation. In this study, we characterized different morphotypes of *P. lima* found in six sampling sites from Hainan Island, South China Sea, associated with macroalgae and seagrass. Twenty strains were isolated and cultivated, and *P. lima* was characterized using light and scanning electron microscopy in combination with large-subunit (LSU) and internal transcribed spacer (ITS) rDNA phylogenetic analysis. High morphological variability and genetic divergence was observed. Molecular analysis based on LSU and ITS rDNA indicated that the different morphotypes of *P. lima* were genetically based. The morphotypes determined here suggest that all five morphotypes of *P. lima* examined from Hainan Island are distinct. **Key Words:** Genetic divergence, Hainan Island, Morphological variation, Morphotypes





# Introduction

R-1

**Coastline of China : 14,500 Km!**

**Still few detected~!!!**





## Identification Problems????!!!

Planktonic *Prorocentrum*  
can rapidly form HABs:

*P. balticum*  
*P. donghaiense*  
*P. micans*\*  
*P. minimum/cordatum*  
*P. sigmoides*  
*P. triestinum*



### *Prorocentrum* blooms



- In March 1994, St. Helena Bay on South Africa's West Coast experienced massive marine mortality caused by *Prorocentrum micans* and *Ceratium furca*
- About 60 tons of rock lobster and 1500 tons of fish were killed
- $O_2$  concentrations were near zero and  $H_2S$  concentrations were in excess of 50 micromols per liter!

Slide by J. Larsen

for a review see  
Glibert et al. (2012) HA

## Benthic *Prorocentrum* spp.

> 30 species

Toxic species ? ? ?



photo N. Chomérat

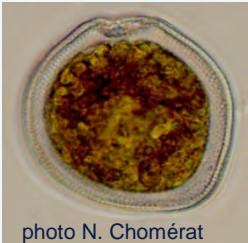


photo N. Chomérat

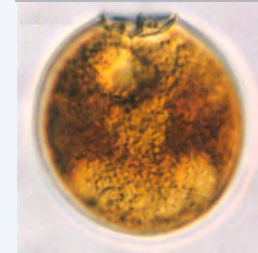
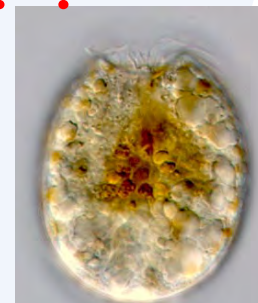
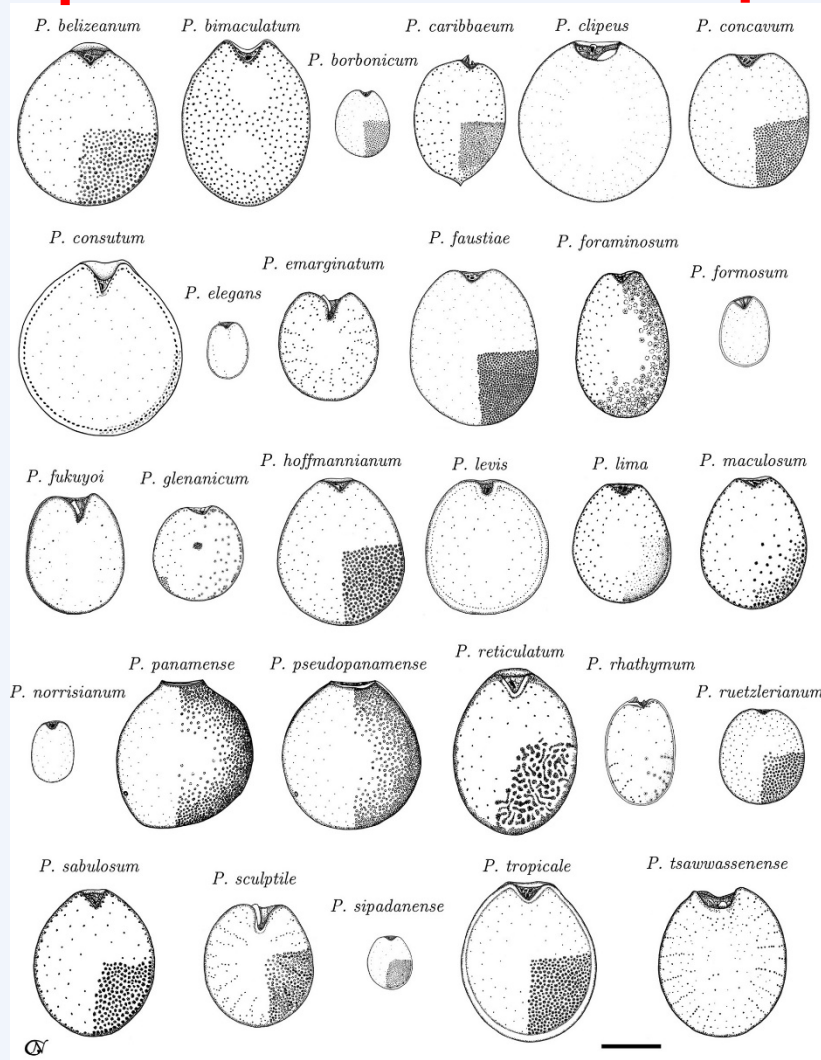
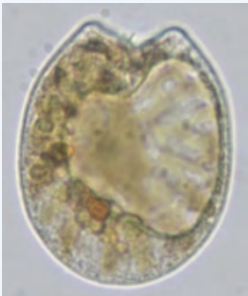
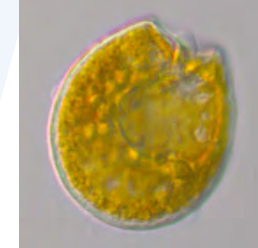


photo N. Chomérat

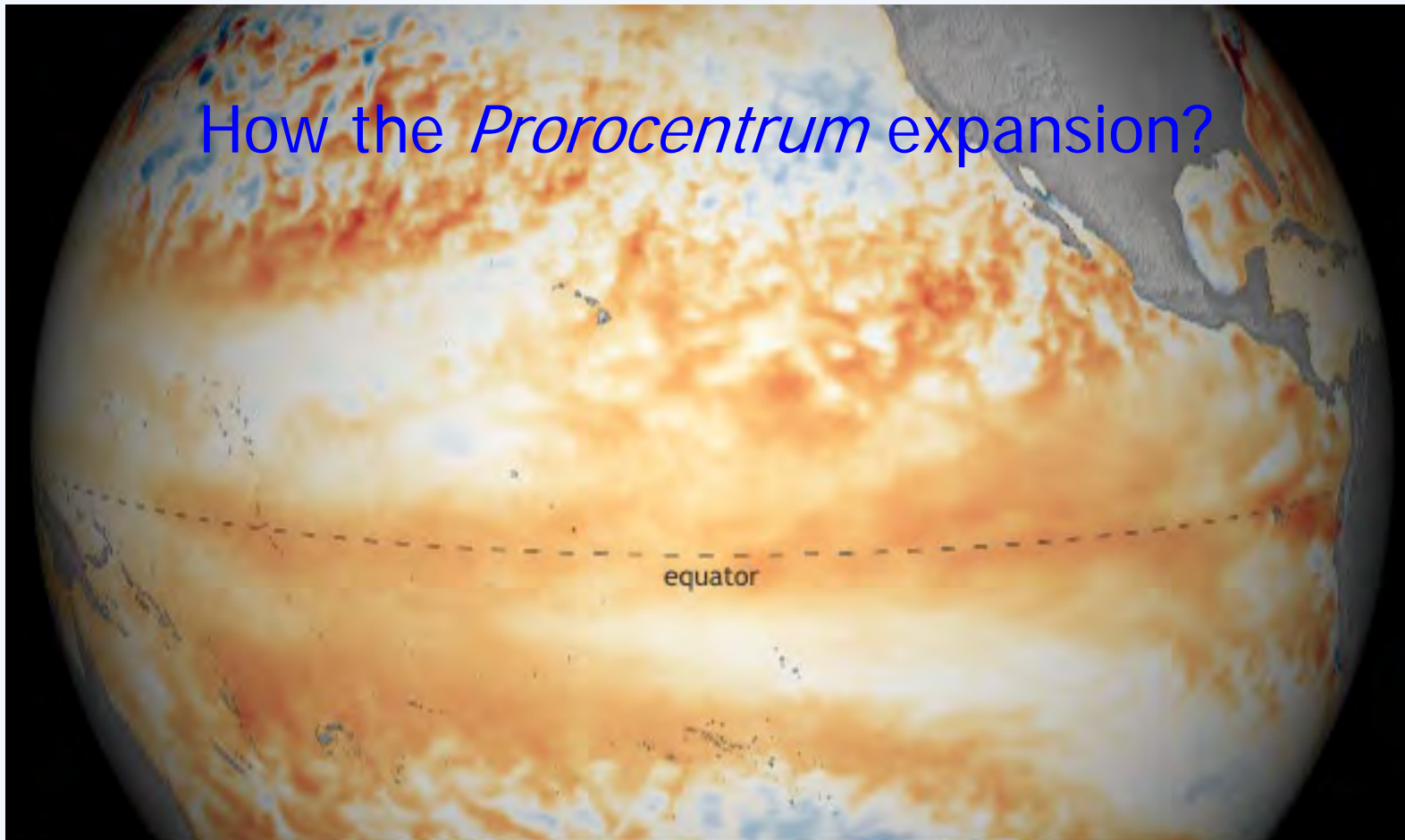




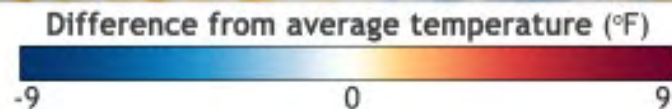
R-3

How the *Prorocentrum* expansion?

ENSO



April 2019  
compared to 1981-2010



Climate.gov/NNVL  
Data: Geo-Polar SST



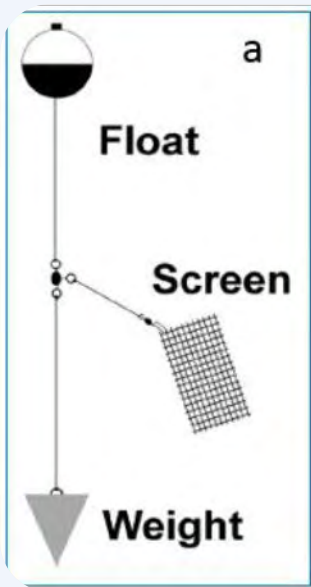
# Method: Sampling sites



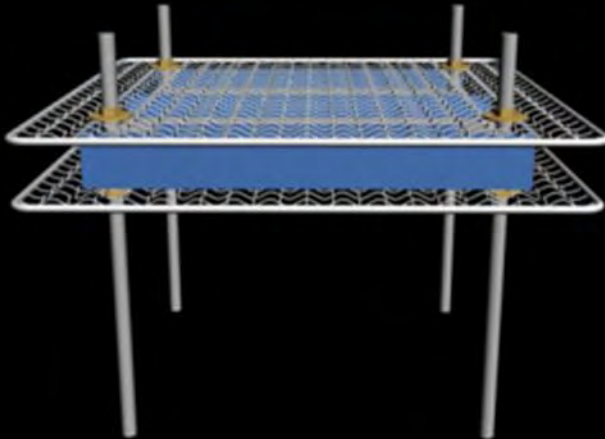
【NSFC】 -Composition, phylogeny and geographical distribution pattern of pelagic and benthic *Prorocentrum* in typical coastal habitats of East China Sea

【Scientific Research Fund of Second Institute of Oceanography】 - Composition, phylogeny, geographical distribution pattern and toxicity of genus *Prorocentrum* in typical coastal habitats of Chinese coastal water

# Methods



Designed by Pengbin WANG



## Benthic sample collections

### 1. Tools

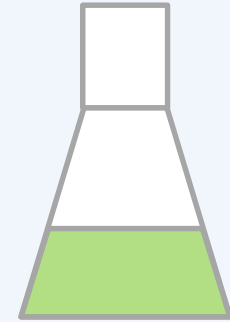
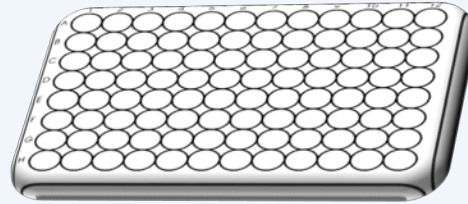
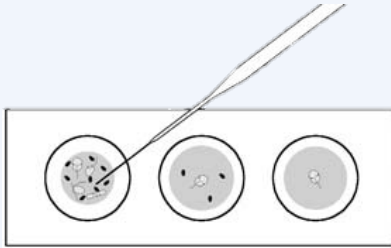




# Methods

## Isolation and culture

f/2 medium; 12L:12D cycle; 20°C



~20 Species have isolated and cultured





# Methods

## Morphological study and identification



Experimental equipment using in this study:

- A. Light microscopy;
- B. Scanning electron microscopy.

# Methods

5'

SSU

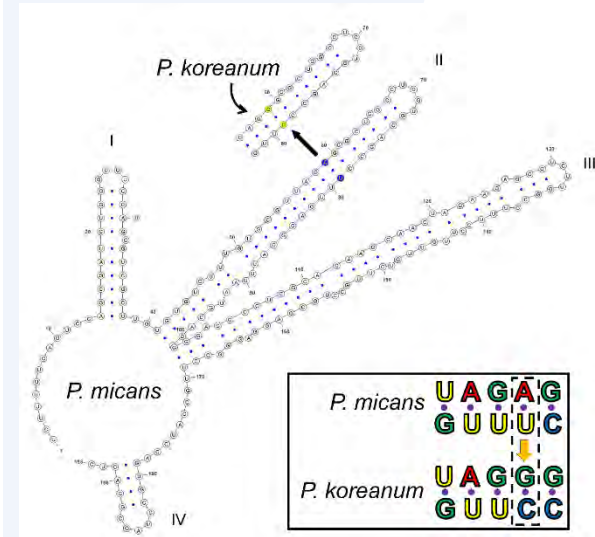
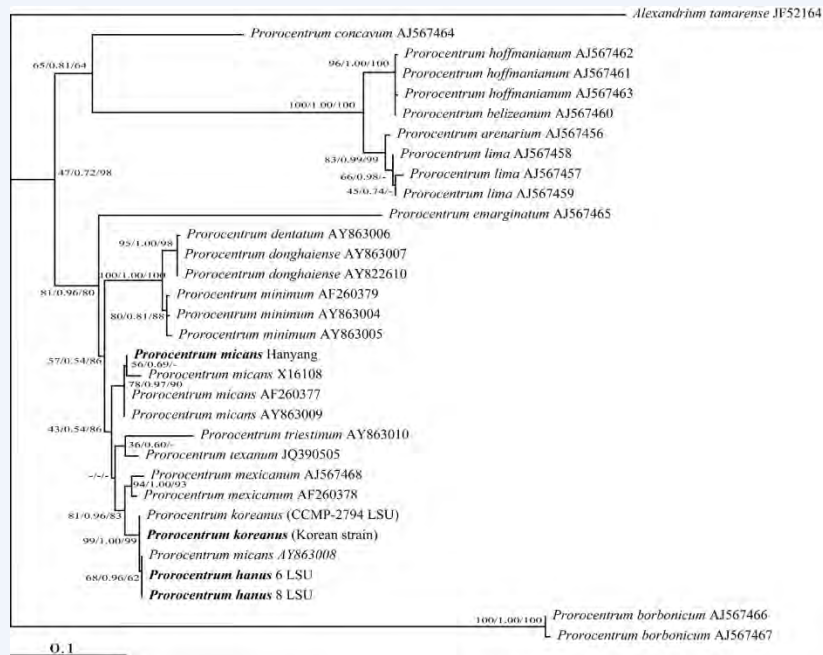
ITS1

5.8S

ITS2

LSU

3'



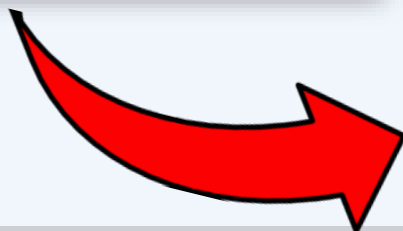
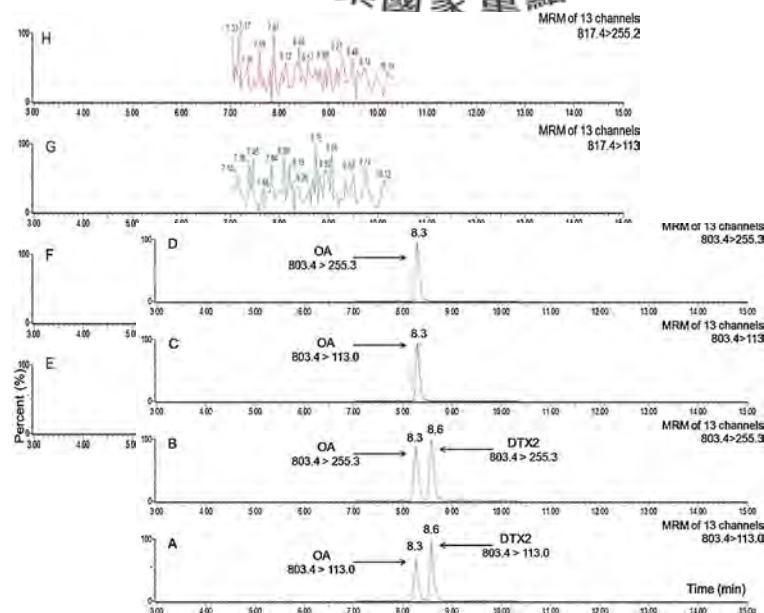
# Methods

## Cooperated with CityU



香港城市大學

City University of Hong Kong



UHPLC-MS/MS



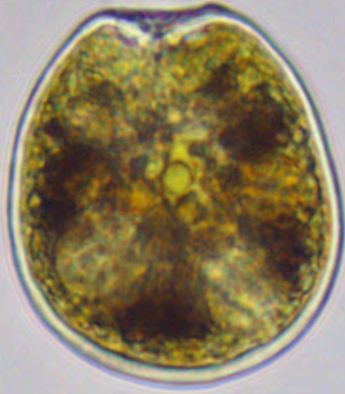
# Results

Strain No.	Name	Strain No.	Name
PR-1	<i>Prorocentrum cf. sculptile</i>	PR-41	<i>Prorocentrum minimum</i>
PR-2	<i>Prorocentrum cf. sculptile</i>	PR-42	<i>Prorocentrum rathymum</i>
PR-3	<i>Prorocentrum cf. sculptile</i>	PR-43	<i>Prorocentrum rathymum</i>
PR-4	<i>Prorocentrum cf. sculptile</i>	PR-44	<i>Prorocentrum triestinum</i>
PR-5	<i>Prorocentrum cf. sculptile</i>	PR-45	<i>Prorocentrum triestinum</i>
PR-6	<i>Prorocentrum cf. sculptile</i>	PR-46	<i>Prorocentrum sp.</i>
PR-7	<i>Prorocentrum cf. sculptile</i>	PR-47	<i>Prorocentrum sp.</i>
PR-8	<i>Prorocentrum cf. sculptile</i>	PR-48	<i>Prorocentrum sp.</i>
PR-9	<i>Prorocentrum clipeus</i>	PR-49	<i>Prorocentrum sp.</i>
PR-10	<i>Prorocentrum concavum</i>	PR-50	<i>Prorocentrum sp.</i>
PR-11	<i>Prorocentrum concavum</i>	PR-51	<i>Prorocentrum sp.</i>
PR-12	<i>Prorocentrum concavum</i>	PR-52	<i>Prorocentrum sp.</i>
PR-13	<i>Prorocentrum concavum</i>	PR-53	<i>Prorocentrum sp.</i>
PR-14	<i>Prorocentrum concavum</i>	PR-54	<i>Prorocentrum sp.</i>
PR-15	<i>Prorocentrum concavum</i>	PR-55	<i>Prorocentrum sp.</i>
PR-16	<i>Prorocentrum concavum</i>	PR-56	<i>Prorocentrum sp.</i>
PR-17	<i>Prorocentrum concavum</i>	PR-57	<i>Prorocentrum sp.</i>
PR-18	<i>Prorocentrum concavum</i>	PR-58	<i>Prorocentrum sp.</i>
PR-19	<i>Prorocentrum concavum</i>	PR-59	<i>Prorocentrum sp.</i>
PR-20	<i>Prorocentrum concavum</i>	PR-60	<i>Prorocentrum sp.</i>
PR-21	<i>Prorocentrum donghaiense</i>	PR-61	<i>Prorocentrum sp.</i>
PR-22	<i>Prorocentrum donghaiense</i>	PR-62	<i>Prorocentrum sp.</i>
PR-23	<i>Prorocentrum donghaiense</i>	PR-63	<i>Prorocentrum sp.</i>
PR-24	<i>Prorocentrum donghaiense</i>	PR-64	<i>Prorocentrum sp.</i>
PR-25	<i>Prorocentrum elegans</i>	PR-65	<i>Prorocentrum sp.</i>
PR-26	<i>Prorocentrum elegans</i>	PR-66	<i>Prorocentrum sp.</i>
PR-27	<i>Prorocentrum fukuyoi</i>	PR-67	<i>Prorocentrum sp.</i>
PR-28	<i>Prorocentrum hongkongum</i>	PR-68	<i>Prorocentrum sp.</i>
PR-29	<i>Prorocentrum lima</i>	PR-69	<i>Prorocentrum sp.</i>
PR-30	<i>Prorocentrum lima</i>	PR-70	<i>Prorocentrum sp.</i>
PR-31	<i>Prorocentrum lima</i>	PR-71	<i>Prorocentrum sp.</i>
PR-32	<i>Prorocentrum lima</i>	PR-72	<i>Prorocentrum sp.</i>
PR-33	<i>Prorocentrum lima</i>	PR-73	<i>Prorocentrum sp.</i>
PR-34	<i>Prorocentrum lima</i>	PR-74	<i>Prorocentrum sp.</i>
PR-35	<i>Prorocentrum lima</i>	PR-75	<i>Prorocentrum sp.</i>
PR-36	<i>Prorocentrum lima</i>	PR-76	<i>Prorocentrum sp.</i>
PR-37	<i>Prorocentrum maculosum</i>	PR-77	<i>Prorocentrum sp.</i>
PR-38	<i>Prorocentrum micans</i>	PR-78	<i>Prorocentrum sp.</i>
PR-39	<i>Prorocentrum micans</i>	PR-79	<i>Prorocentrum sp.</i>
PR-40	<i>Prorocentrum minimum</i>	PR-80	<i>Prorocentrum sp.</i>

More than 80 strains were isolated and cultivated!!!

# Results

*P.concavum*



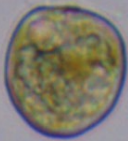
*P.elegans*



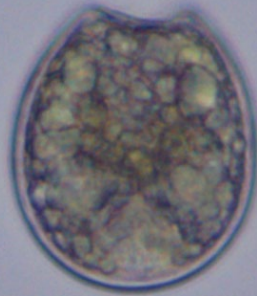
*P. lima*



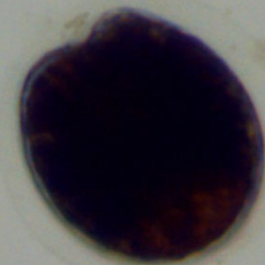
*P.minimum*



*P.cf. sculptile*



*P.cf. sculptile*



Scale bar : 20um



# Results

*P. clipeus*



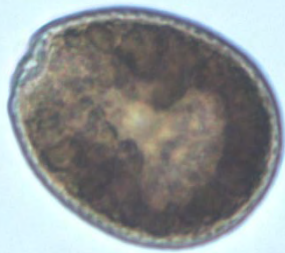
*P. triestinum*



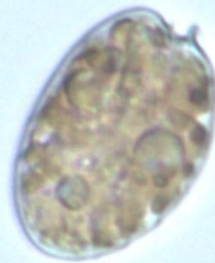
*P. fukuyoi*



*P. maculosum*



*P. rhathymum*



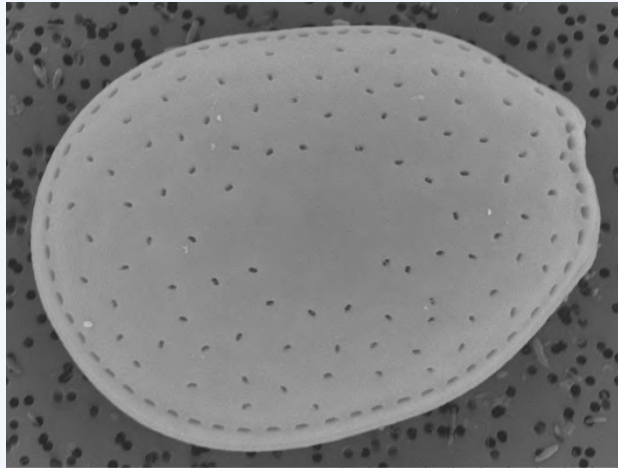
*Prorocentrum* sp.



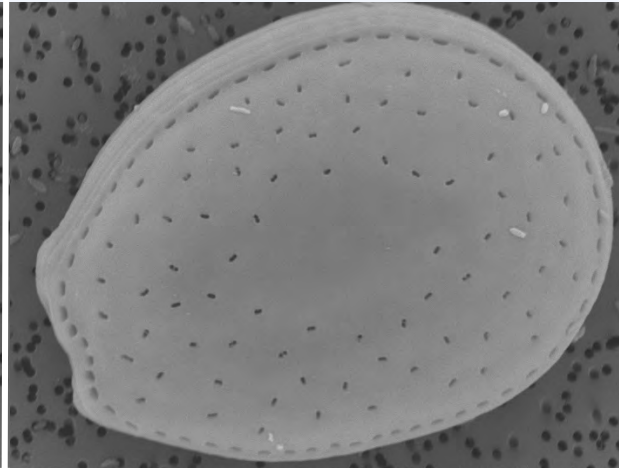


# Results

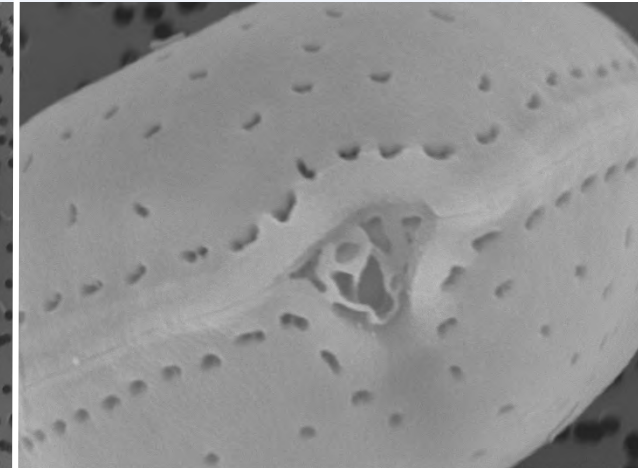
## *Prorocentrum lima*



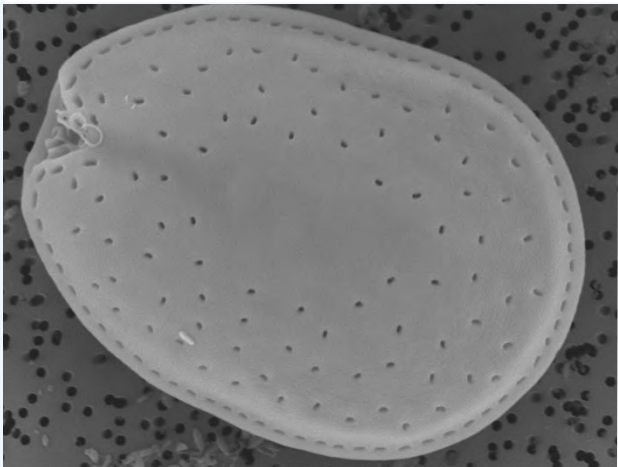
TM-1000\_9806 2018-07-09 D2.7 x4.0k 20 um



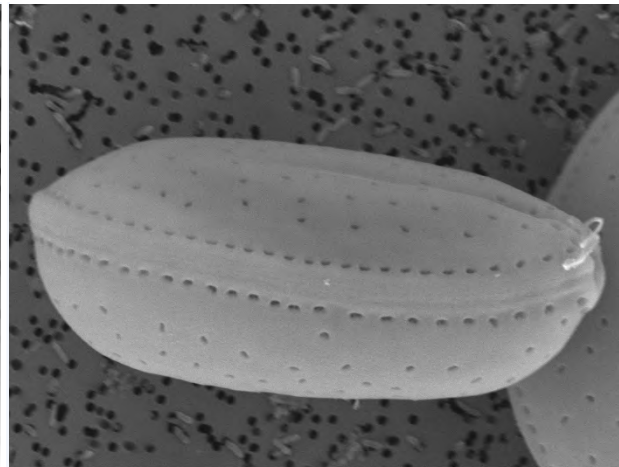
TM-1000\_9801 2018-07-09 D2.7 x4.0k 20 um



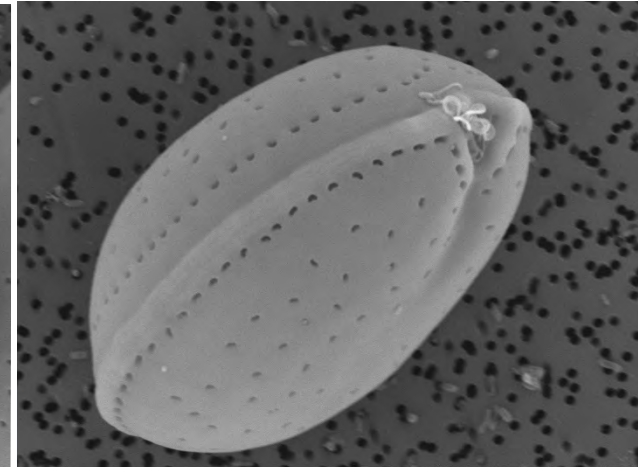
TM-1000\_9839 2018-07-10 D2.7 x8.0k 10 um



TM-1000\_9837 2018-07-10 D2.7 x4.0k 20 um



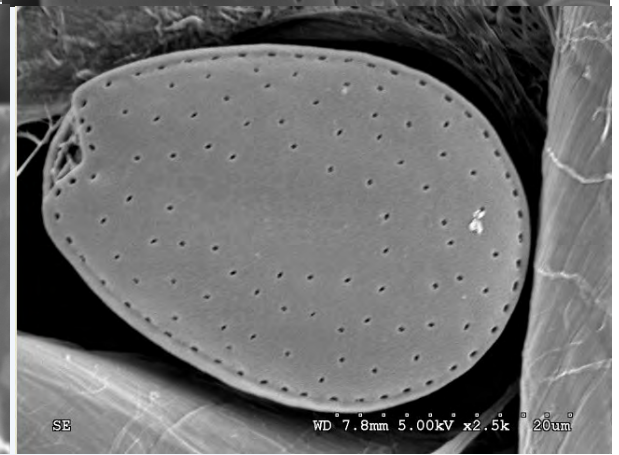
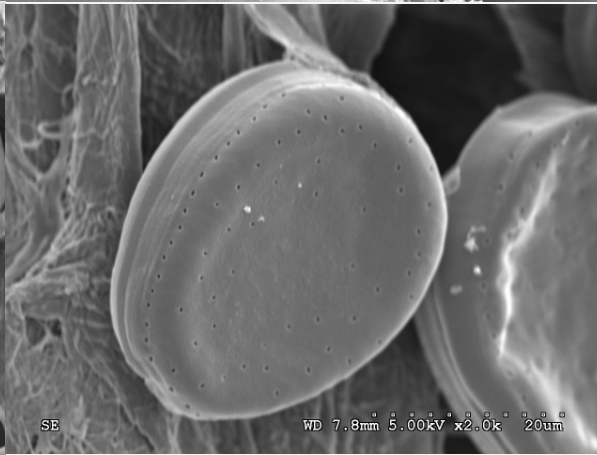
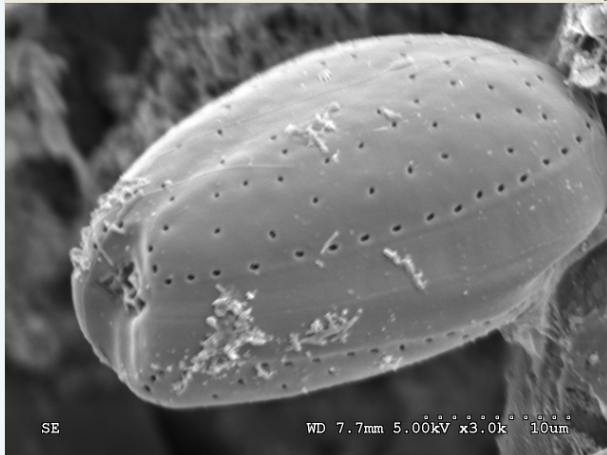
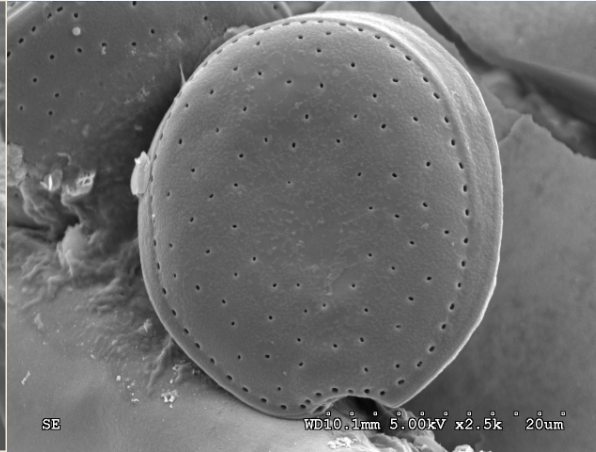
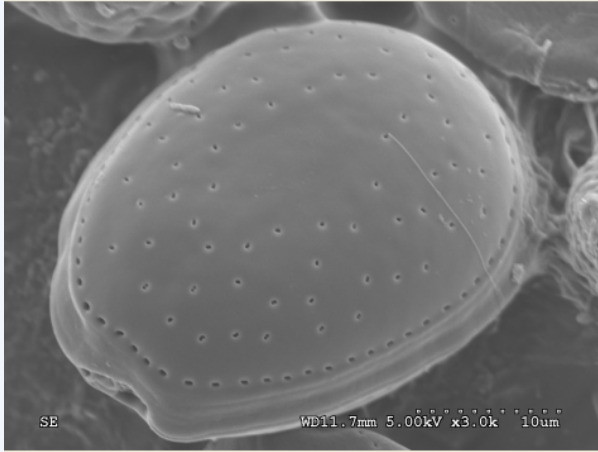
TM-1000\_0208 2018-07-17 D2.8 x4.0k 20 um



TM-1000\_0088 2018-07-15 D2.7 x4.0k 20 um

# Results

## *Prorocentrum* spp.

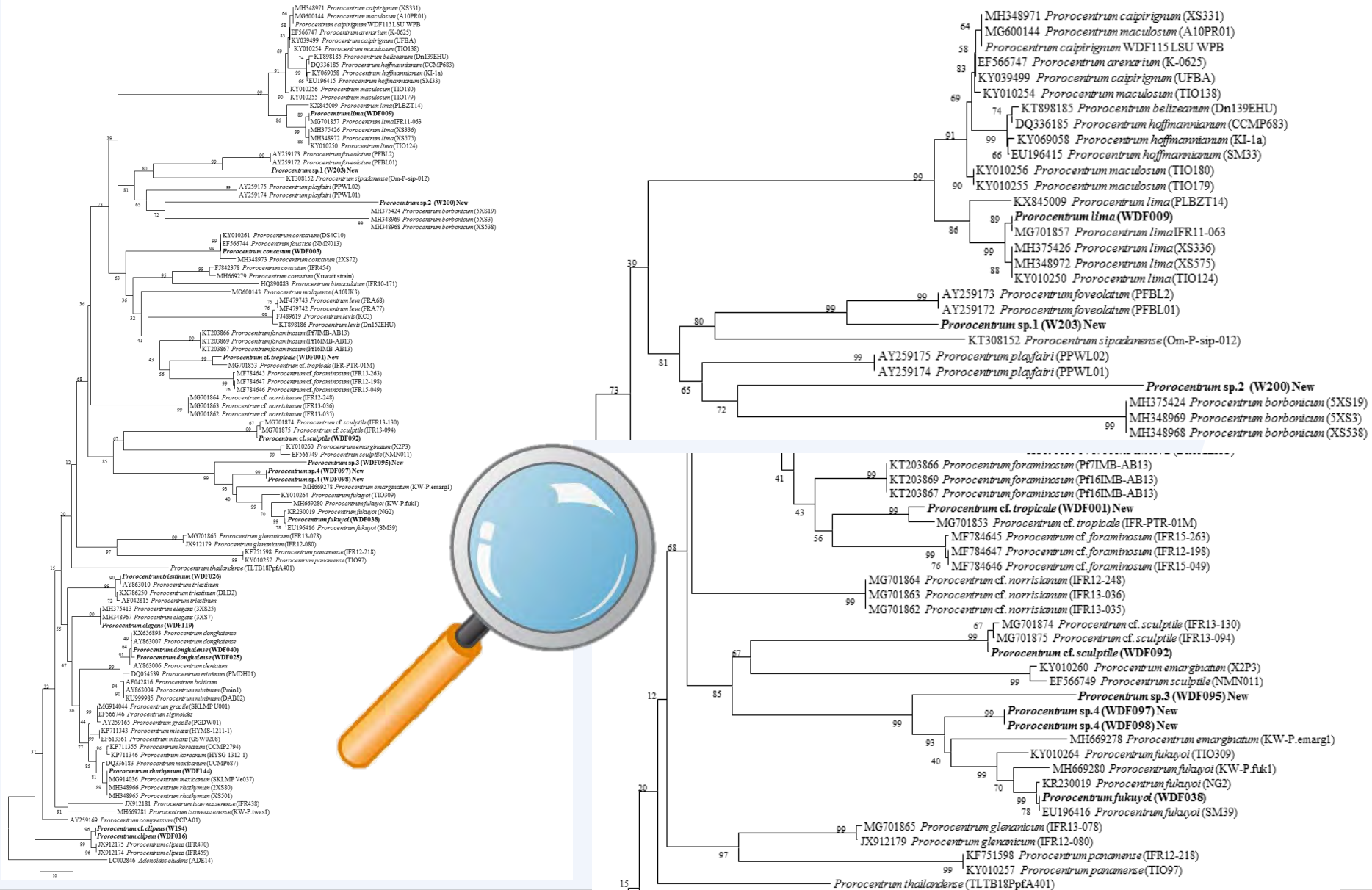








# Results



# Results

## A New Potential Toxic Dinoflagellate from Hong Kong Coastal Waters

### *Prorocentrum hongkongense*

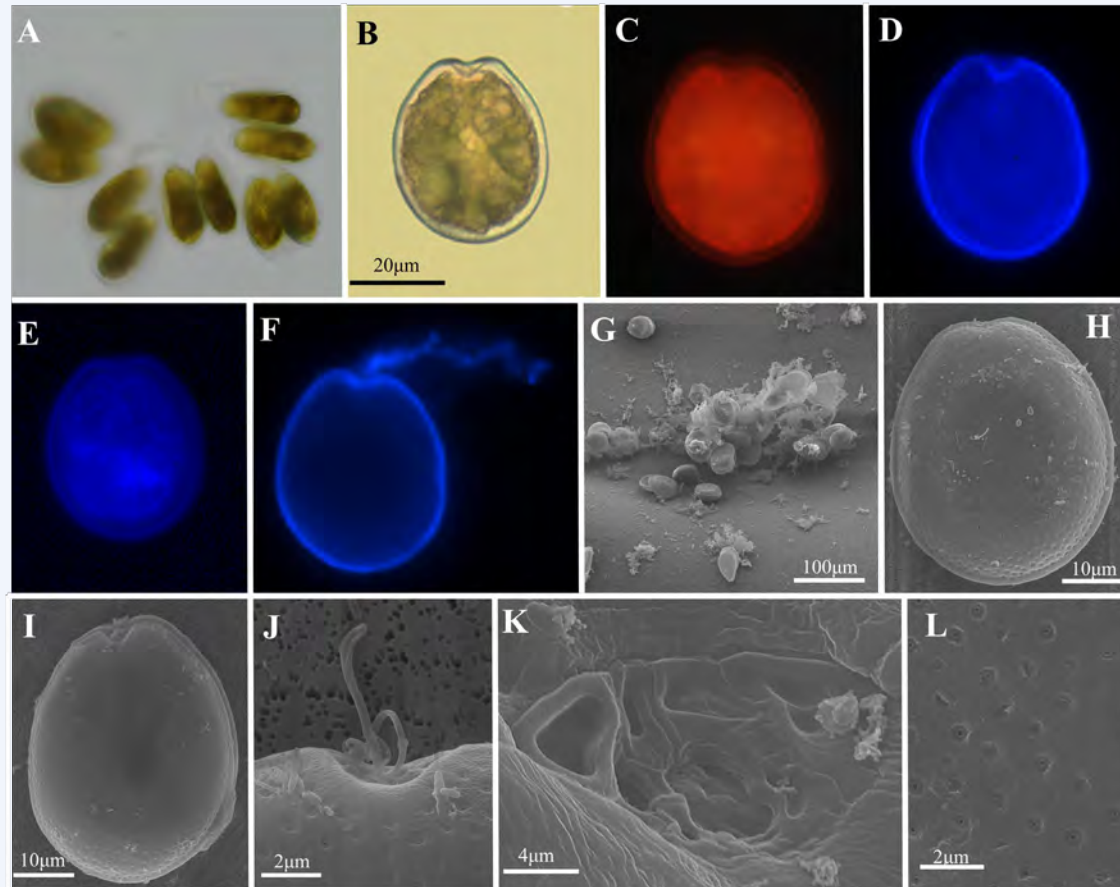


Fig. *Prorocentrum hongkongense*. (A) Colony under view of light microscope. (B) General view. (C) Chloroplasts view under fluorescence microscope. (D) Calcofluor White -stained valve (blue) under fluorescence microscope. (E) DAPI-stained nucleus (blue) under fluorescence microscope. (F) Calcofluor White -stained valve with flagella (blue) under fluorescence microscope. (G) Colony under view of SEM. (H) Left valve. (I) Right valve. (J) Flagella. (K) Periflagellar area. (L) Trichocyst pore fields.

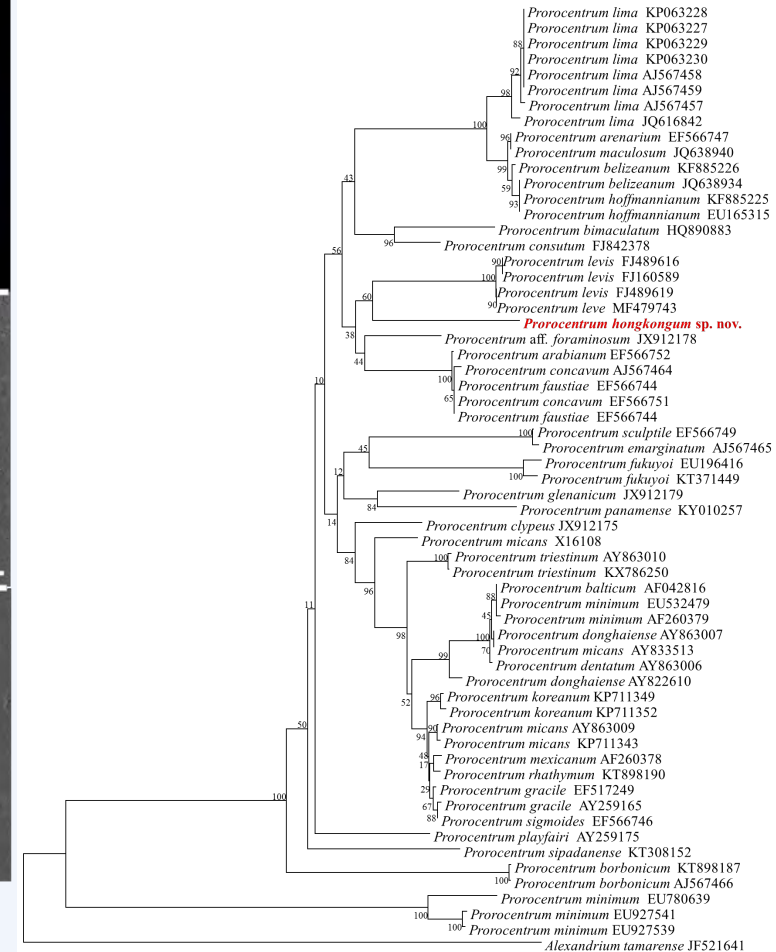


Fig. . Neighbor-joining tree of the LSU region. *Alexandrium tamarense* was included as an out-group.

# NGS on the *Prorocentrum* species detection

M101B



Prorocentrum

Total: 1092

5% of  
Dinophyceae

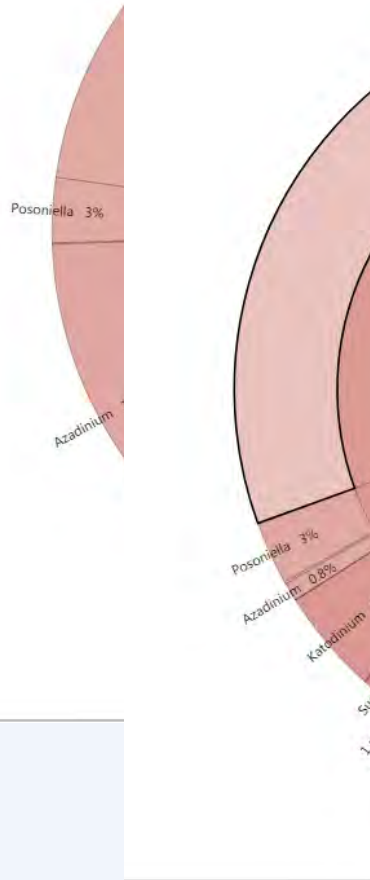
A14



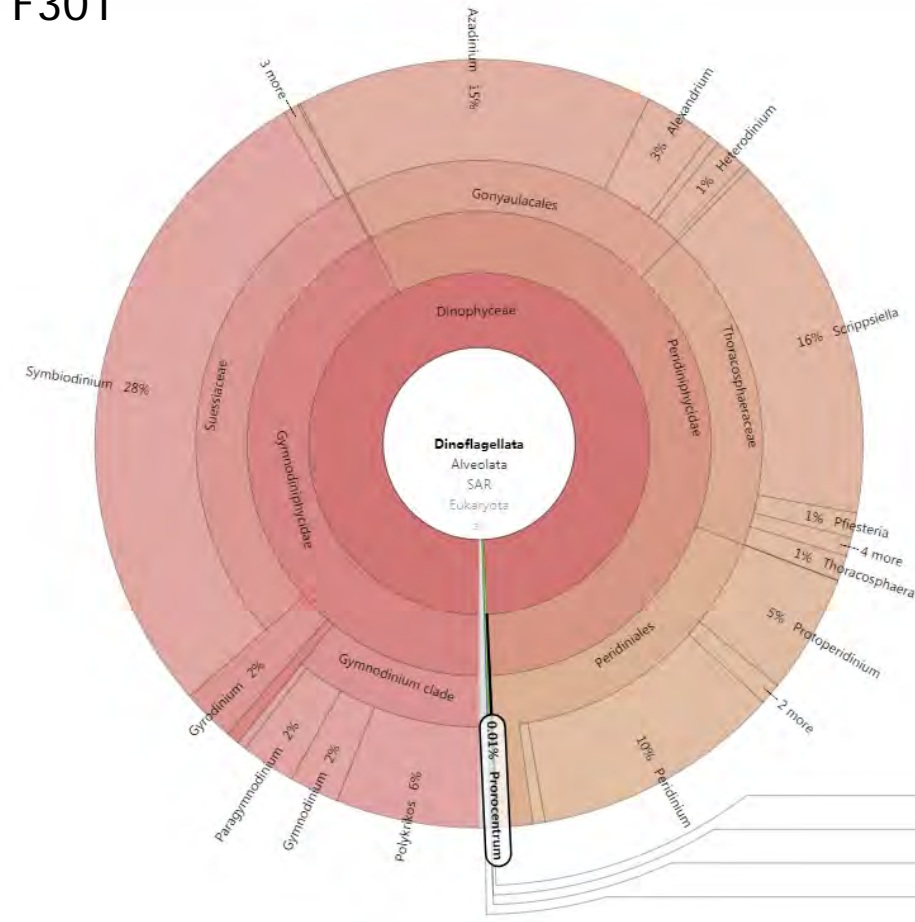
Prorocentrum

Total: 227

1% of  
Dinophyceae



F301



Prorocentrum

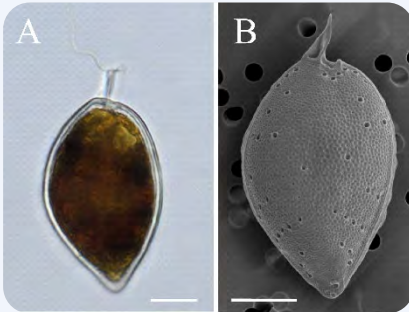
Total: 1

- 0.01% of Dinophyceae
- 0.01% of Dinoflagellata
- 0.004% of Alveolata
- 0.002% of SAR
- 0.001% of Eukaryota
- 0.001% of all

- Incertae Sedis 0.2%
- Noctilucales 0%
- uncultured 0%
- [unassigned Dinoflagellata] 0.5%



# Summary



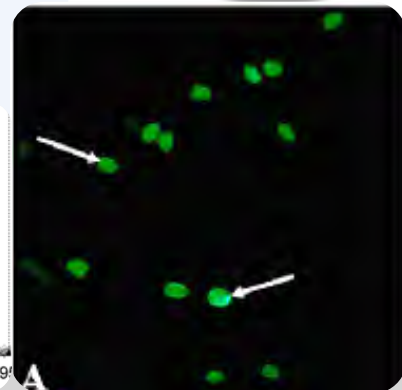
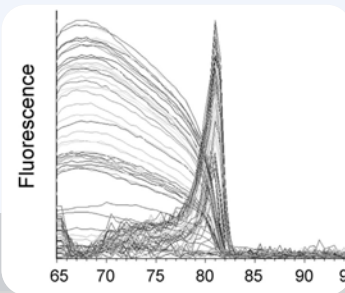
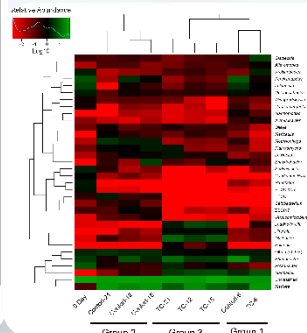
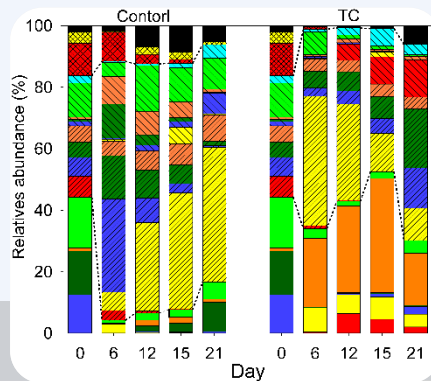
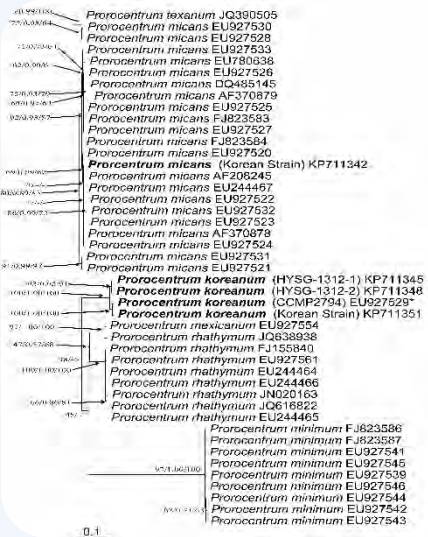
## Taxonomy

## Studies on *Prorocentrum* (Dinophyceae) in the coastal water of China

qPCR/  
FISH

## Phylogeny

## NGS



# Thanks

