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IMPLEMENTING THE STRATEGIC ACTION PROGRAMME FOR THE YELLOW SEA LARGE MARINE ECOSYSTEM:
RESTORING ECOSYSTEM GOODS AND SERVICES AND CONSOLIDATION OF A LONG-TERM REGIONAL
ENVIRONMENTAL GOVERNANCE FRAMEWORK
(UNDP/GEF YSLME Phase II Project)

**Interim report on progress of drifting *Sargassum*
horneri in Yellow Sea
(Genetic diversity of benthic and floating populations of
Sargassum in western Yellow Sea)**

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1. Background

Floating *Sargassum* has been increasingly observed in the western Yellow Sea of China, and caused detrimental impacts on the coastal aquaculture in recent years. As we described in the first report, substantial pelagic *Sargassum* was accumulated in the shallow water of Subei Shoal in spring of 2017, affecting about 70% (in average) of the *Pyropia* aquaculture rafts. Besides, large-scale floating rafts of *Sargassum* occurred annually in ECS since early 2000s, especially in the region between the eastern continental shelf and the Kuroshio oceanic front (Komatsu et al., 2007; 2008; Filippi et al., 2010; Mizuno et al., 2014). The satellite remote sensing detected an unusual northward expansion of the pelagic *Sargassum* in 2017, which even intruded into the Yellow Sea (Qi et al., 2017), indicating the close association of the *Sargassum* blooms in ECS and Subei Shoal of YS. Besides, another significant *Sargassum* bloom was reported in YS during October of 2016 to early 2017 (Xing et al., 2017). Unlike that in ECS, the winter bloom was initiated from the southwestern coast of Shandong Peninsula, drifting southward and accumulating in Subei Shoal region (Xing et al., 2017). Other than these, little was known about the relationships or interactions among these *Sargassum* blooms.

The following genetic analyses revealed a low genetic diversity among the floating *Sargassum* samples in Yellow Sea (Su et al., 2017; Liu et al., 2018; Byeon et al., 2019). Whereas, little has been done on the benthic populations, even less was known about the genetic affiliations of the floating *Sargassum* with any benthic populations so far. In this research, we screened the various floating and benthic samples of *Sargassum*, including those from the Subei Shoal, offshore water of Yellow Sea, Bohai Sea and a few benthic populations. This work would provide additional data assisting the investigation on the blooming dynamics of the pelagic *Sargassum* in Yellow Sea.

2. Material and methods

2.1 Sample collections

As we reported in the Report 1 (Distribution of benthic populations of *Sargassum horneri* along the coasts of Shandong and Jiangsu provinces, and seasonal variation of drifting *S. horneri* in western Yellow Sea), only a few discrete benthic populations (four confirmed so far) of *S. horneri* have been identified along the coasts of Liaoning, Shandong and Zhejiang provinces. Two benthic samples were collected from Yantai of Shandong and Zhoushan of Zhejiang, respectively (Fig. 1). The other two haven't

been sampled yet, due to limited funding and time. The floating samples were collected from the Subei Shoal, offshore water of YS and Bohai Sea in 2017, 2016 and 2019, respectively (Table 1). The gross morphology of the samples was recorded by the digital camera and a dissecting microscope (Nikon DS-Ri2).

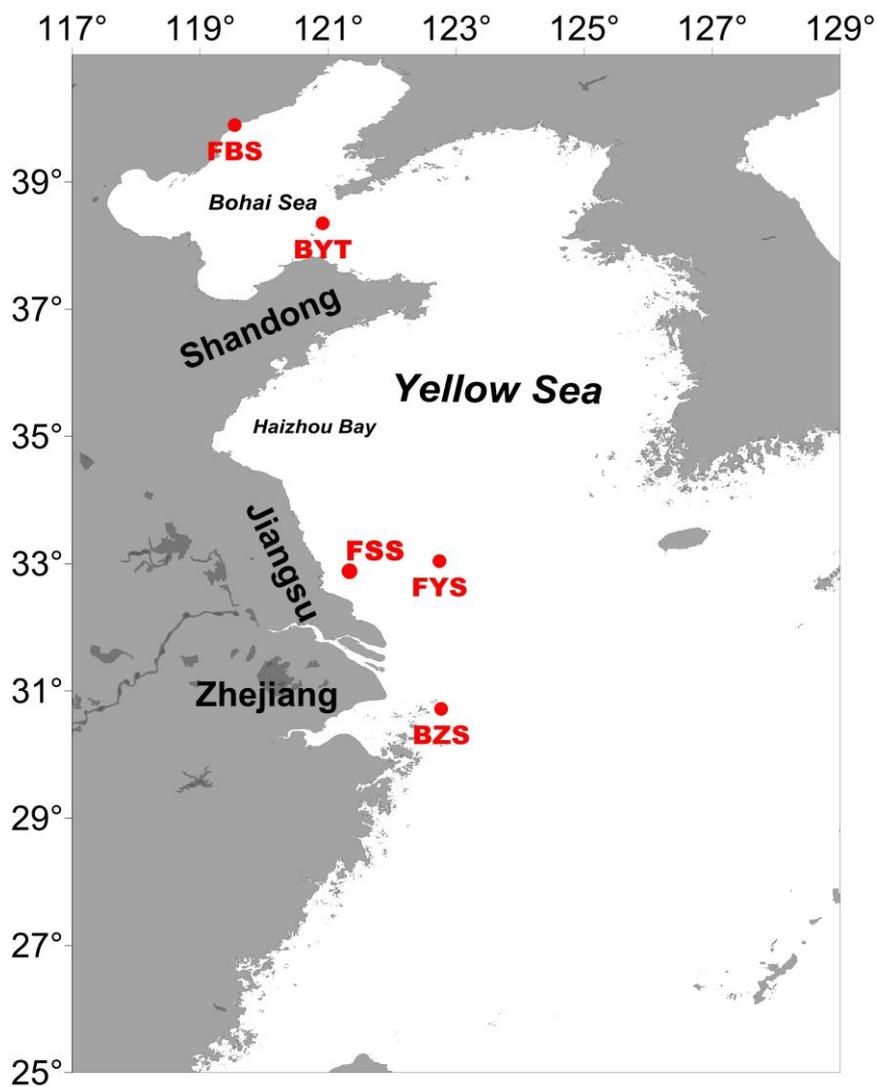


Fig. 1 Geographic locations of the five *S. horneri* samples for this study.

See Table 1 for the detailed sample information.

Table 1 Sample information for the floating and benthic *S. horneri*.

Code	Sampling date	Floating (F) or Benthic (B)	No.	Location	Note
BYT	08/2017	B	4	Yantai, Shandong Province	Provided by Shanghai Ocean University
BZS	03/2018	B	10	Zhoushan, Zhejiang Province	Provided by Marine Biology Institute of Shandong Province
FSS	05/2017	F	15	Subei Shoal, southwestern YS	
FYS	12/2016	F	5	Offshore water in Yellow Sea	
FBS	06/2019	F	8	Coast of Qinhuangdao, Bohai Sea	

2.2 Genetic screening and analysis

The samples were gently rinsed by the filtered seawater and transported back in frozen. In the laboratory, a small piece of tissue (~ 1 cm thallus) was cut by the sterilized blade and homogenized in the lysis buffer using a portable homogenizer. The genomic DNA was then extracted using the E.Z.N.A.[®] Plant DNA DS Kit (Omega Bio-tek, Inc., GA, USA) following the manufacture's protocol. The nuclear rDNA internal transcribed spacer (ITS) fragments (including ITS1, 5.8S and ITS2) were amplified using the primer pairs: F 5'-GGAAGTAAAAGTCGTAACAAG-3', R 5'-TCCTCCGCTTATTGATATGC-3' and F 5'-CGATGAAGAACGCAGCGAAATGCGAT-3', R 5'-TCCTCCGCTTAGTATATGCTTAA-3' (Lv et al., 2018). The PCR cycling was: pre-denature at 94°C for 3 min, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C, extending at 72°C for 10 min. The mitochondrial DNA cytochrome oxidase I (Cox I) fragments were amplified using the primer pair: F 5'-CCAACCAAYAAAGATATWGGTAC-3' and R 5'-GGATGACCAAARAACCAAAA-3' (Lane et al., 2007). The PCR products were visualized on a 1% agarose gel, and sequenced directly by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) at both directions after purification. For the cases that the PCR products were not clean enough, they were ligated with pMD[®]18-T vectors (TaKaRa Co.,

Dalian, China) and transformed into DH5 α competent cells (TaKaRa) following the manufacture's instructions. Two to four clones with inserts were screened and sequenced for the ITS fragments.

The resulted ITS sequences were blasted and aligned with the references retrieved from the Genbank database (<http://www.ncbi.nlm.nih.org>) using ClustalW algorithm (Thompson et al. 1994) in MacVector 15 (Accelrys, CA, U.S.). The phylogenetic relationship between the sequences from this study and the references was evaluated through the Maximum-likelihood (ML) and Bayesian analyses. The robustness of the resulted tree was assessed by bootstrapping for 1000 replicates.

3. Results

3.1 Morphological variations of the floating *S. horneri*

As reported below, the floating *Sargassum* seaweeds collected from SS, YS and BS are comprised exclusively of *S. horneri*. So the following morphology descriptions were all for this species, *S. horneri*.

The thalli of the floating samples were yellow to dark brown. The

individual plant was often large and heavy, < 1 – 2 m in length. The thalli could be longer and larger as observed by the other studies (Zhang et al., 2019). Main axes were elongated, about 2 – 4 mm in diameter, cylindrical with secondary branches or the other accessories (leaves, receptacles and vesicles) spiraling distributed on the axes. The neighboring branches (or accessories) were about 120° and 2 – 4 cm apart. Leaves were petiolate, alternatively pinnatisected, deeply dissected near to the midrib. The gas-filled vesicles were cylindrical, elongated to usually 3 – 5 times of the breadth. Each vesicle was usually crowned with a pinnatifid leaf, a very distinctive characteristic of this species (Fig. 2). There were generally 1 – 2 vesicles along with one leaf and/or 1 receptacle at each leaf node (Fig. 2). The receptacles were stout and elongated and simple. The male receptacles were cylindrical, 5 – 10 cm or more in length, gradually attenuating above and with a short stipe below (Fig. 2). We did not observe female receptacles in our samples.

We compared the floating samples from the central Subei Shoal (FSS) and the offshore water in Yellow Sea (FYS), and observed distinctive morphological differences. The *S. horneri* seaweeds of FSS comprised more dense branches or accessories and in darker brown color compared to those of FYS. Male receptacles were observed in FSS, while not in FYS. Interestingly, significant young sprouts were observed growing on the old

thalli of FSS (Fig. 3), suggesting certain reproductions of this species in Subei Shoal region.

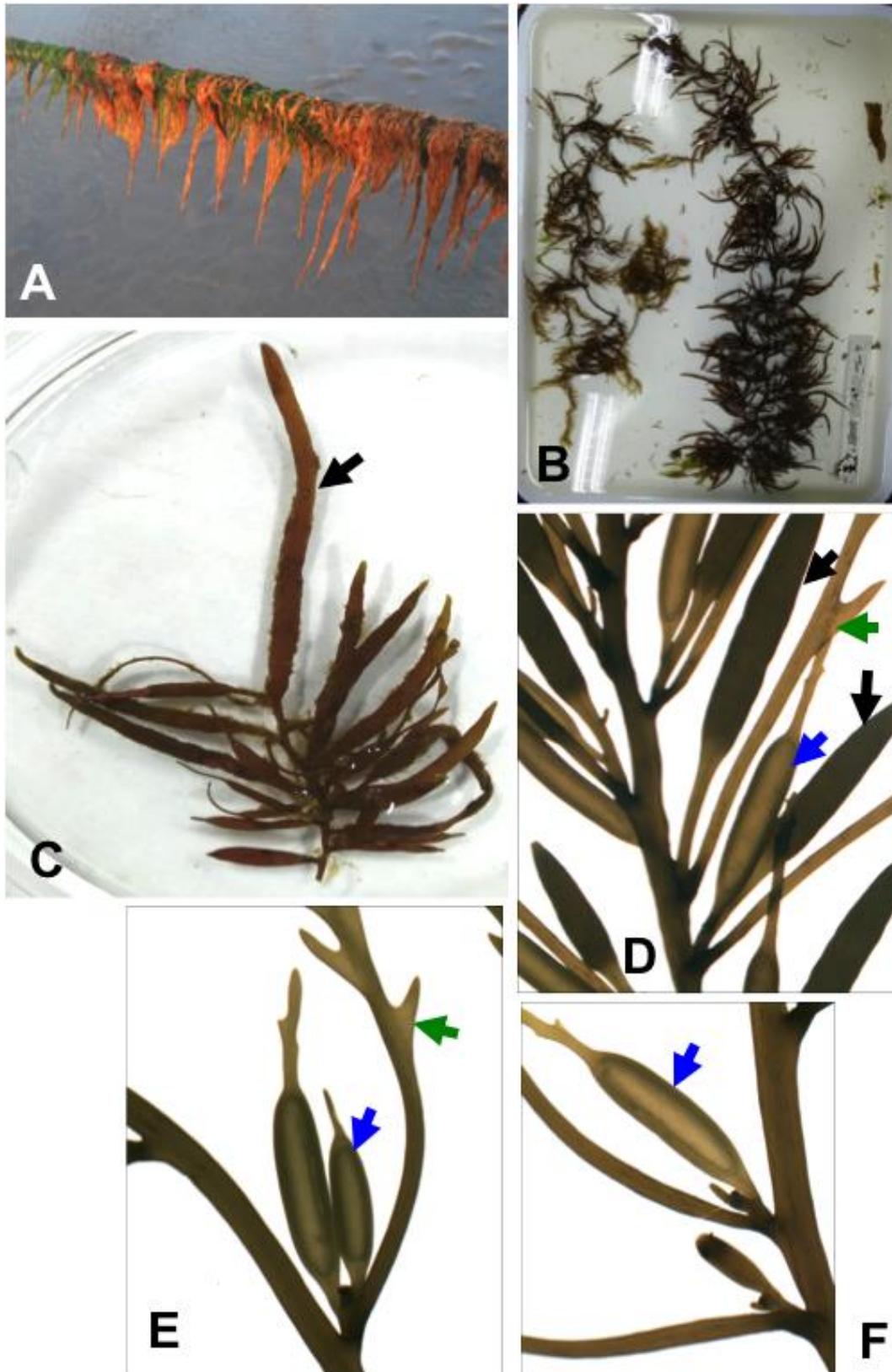


Fig. 2 Gross morphology of the floating *S. horneri*. A, free floating *S. horneri* wrapped on the rafts. B, *S. horneri* thalli with (right) and without

receptacles (left). C, male receptacles (black arrow). D, mature *S. horneri* with receptacles (black arrow), vesicles (blue arrow) and leaves (green arrow) at each node. E, two vesicles at each node. F, one vesicle at each node.

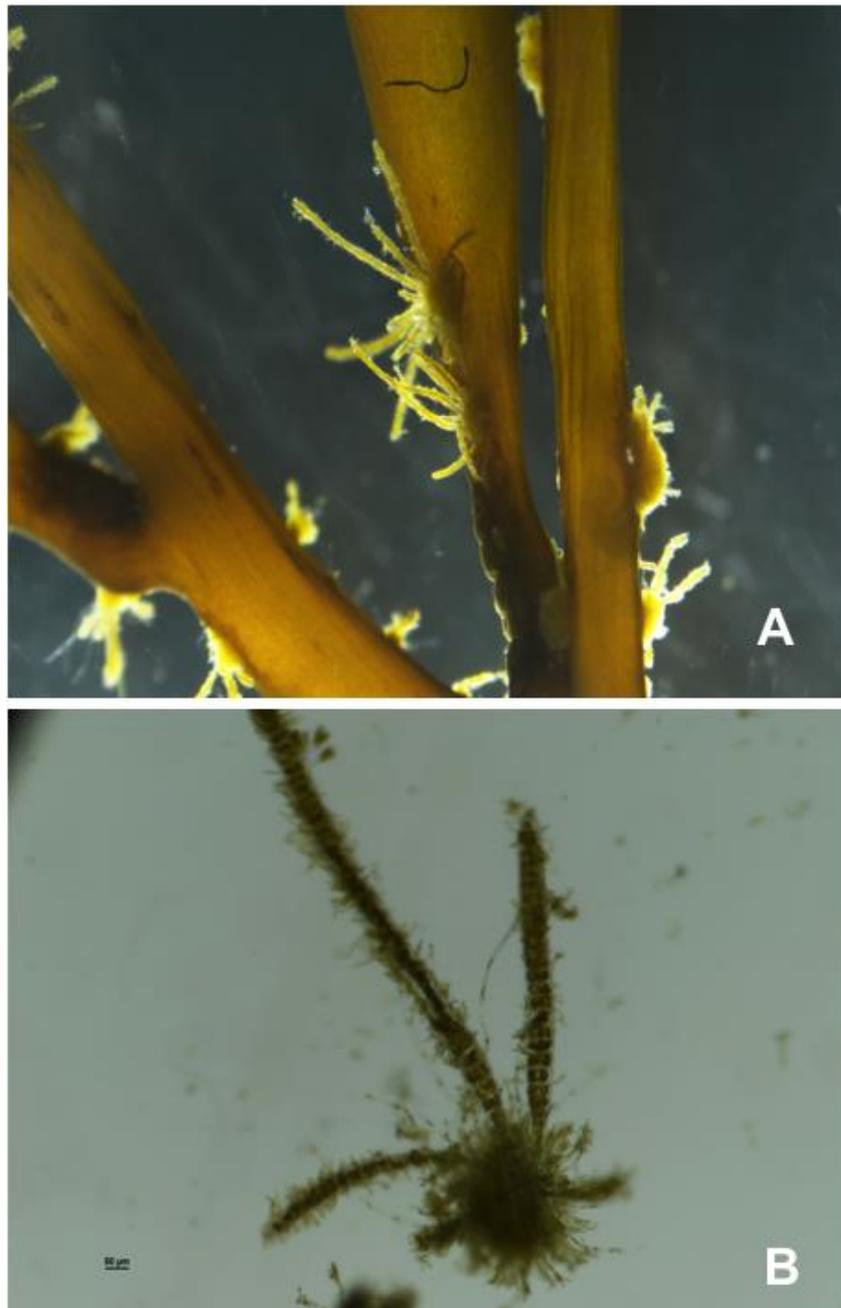


Fig. 3 Young sprouts growing from the old thalli (A) and close image of

the sprouts (B).

3.2 Genetic homogeneity of the benthic and floating samples

Due to the limited time, the genetic analyses haven't been completed yet. So far, we compared the sequence variations of the ITS and CoxI fragments and found little genetic divergence within the benthic and floating populations, and between the benthic and floating samples either.

3.2.1 Sequence variation of ITS

The total length of the ITS sequences was 1506 bp, including partial ITS1 (765 bp), complete 5.8S (159 bp) and ITS2 (582 bp). There was only one polymorphic site observed, locating at 1014 bp from 5' end of the ITS2 region. There were two alleles detected at this locus from the samples screened, C and T. Another heterozygote Y (C or T, Fig. 4) was also observed. The haplotype compositions of the five samples were summarized in Table 2. It was noted that the benthic populations showed relatively lower haplotype diversity than those floating samples. The two benthic populations (BYT and BZS) comprised distinct haplotypes (C or T), respectively. The floating *S. horneri* from Subei Shoal (FSS) comprised mostly C type (86.7%) and small proportions of T and Y types (6.7%).

Floating *S. horneri* from offshore (FYS) were all C type (100%), while those from Bohai Sea (FBS) were mostly T type (75%). Based on the haplotype frequencies (Table 1), the genetic composition of the floating *S. horneri* seems to be closely related to the benthic population nearby. For example, most pelagic *S. horneri* from offshore water of YS and Subei Shoal (SS) were identical to the benthic samples from Zhoushan (BZS). While floating seaweeds from Bohai Sea (FBS) were genetically similar to the benthic ones in Yantai, which was geographically closer to the sampling location of FBS compared with those of FSS and FYS (Fig. 1). Whereas, this conclusion probably needs to be further investigated since limited samples and individuals were included in the analysis and only one polymorphic locus was used in this study. More samples from a larger geographic range and from multiple years will be screened and analyzed to study the representativeness and stability of the haplotype frequencies of the benthic and floating populations of *S. horneri*. In addition, existence of the T type in FSS, Y type (likely a heterozygote, but needs to analyze more genes) in both FSS and FBS indicated multiple sources for the floating *S. horneri* in these regions (esp. for the 2017 spring bloom in Subei Shoal), which was consistent with our field observations (Report 1).

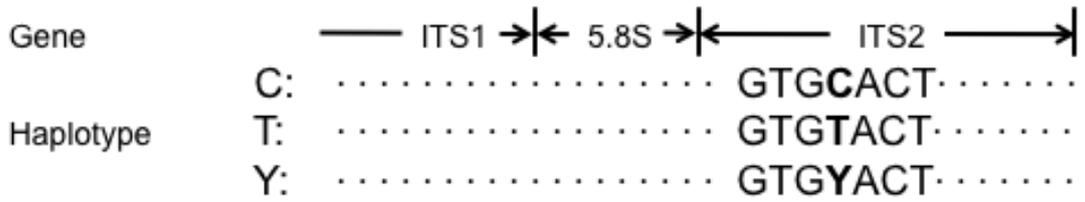


Fig. 4 Polymorphism of the ITS gene detected from the *S. horneri* samples.

Table 2 Haplotype composition of the five *S. horneri* populations screened

Sample code	No. (%) of each haplotype			Total
	C	T	Y	

BYT	0 (0.0)	4 (100.0)	0 (0.0)	4
BZS	10 (100.0)	0 (0.0)	0 (0.0)	10
FSS	13 (86.7)	1 (6.7)	1 (6.7)	15
FYS	5 (100.0)	0 (0.0)	0 (0.0)	5
FBS	0 (0.0)	6 (75.0)	2 (25.0)	8

3.2.2 Sequence variation of Cox I

The resulted Cox I sequences were 655 bp in length, and they were 100% identical throughout all the five samples screened. Blastn search indicated that they were 100% identical to the benthic *S. horneri* from Nanji Island of Zhejiang Province (KJ938300), which was the most dominant haplotype of the floating *S. horneri* in Yellow Sea in 2017 (Liu et al., 2018).

4. Summary and discussion

Following the Report 1 on distribution of benthic populations and seasonal variation of the floating *S. horneri* in YS, detailed morphological and molecular studies were conducted on the five samples, including two benthic samples from Yantai (BYT) and Zhoushan (BZS), respectively, three floating populations from offshore of Yellow Sea (FYS), Subei Shoal (FSS) and Bohai Sea (FBS). Consistent with the previous research, all samples comprised exclusively a single species *S. horneri* and were highly genetically homogeneous. Only one polymorphic site was detected in ITS gene, and there were two alleles and one heterozygote observed for these *S. horneri* samples screened. The two benthic populations comprised reciprocally distinctive haplotypes, and the floating samples showed a relatively higher diversity. The genetic composition of the floating *S. horneri* seems to be closely related to the benthic population nearby, while this conclusion needs further investigation. Existence of alternative haplotypes indicated multiple sources for the floating populations of *S. horneri* in these regions (esp. for the 2017 spring bloom in Subei Shoal), which was congruent with our field observation (Report 1). And evident morphological difference was observed for the various floating *S. horneri*, suggesting probable distinct physiological and reproductive responses to the environmental variation in different regions. Additional research is ongoing to distinguish the benthic populations of *S. horneri* along the

Chinese coasts and to further investigate the detailed structure of the floating populations and their affiliations with any benthic populations.

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