Comparing Complete Mitochondrion Genome of Bloom-forming Macroalgae from the Southern Yellow Sea, China

Jing Xia¹, Peimin He¹, Jinlin Liu^{1,*}, Wei Liu¹, Yichao Tong¹, Yuqing Sun¹, Shuang Zhao¹, Lihua Xia², Yutao Qin², Haoifei Zhang², and Jianheng Zhang^{1,*}

¹College of Marine Ecology and Environment, Shanghai Ocean University, Shanghai, China, 201306

²East China Sea Environmental Monitoring Center, State Oceanic Administration, Shanghai, China, 201206

Abstract. The green tide in the Southern Yellow Sea which has been erupting continuously for 14 years. Dominant species of the free-floating *Ulva* in the early stage of macroalgae bloom were *Ulva compressa*, *Ulva flexuosa*, *Ulva prolifera*, and *Ulva linza* along the coast of Jiangsu Province. In the present study, we carried out comparative studies on complete mitochondrion genomes of four kinds of bloom-forming green algae, and provided standard morphological characteristic pictures of these *Ulva* species. The maximum likelihood phylogenetic analysis showed that *U. linza* is the closest sister species of *U. prolifera*. This study will be helpful in studying the genetic diversity and identification of *Ulva* species.

1 Introduction

Green tides, which occur widely in many coastal areas, are caused primarily by flotation, accumulation, and excessive proliferation of green macroalgae, especially the members of the genus *Ulva* [1-3]. China has the high frequency outbreak of the green tide [4-10]. Especially, the world's largest green tide events, have occurred annually from 2007 to 2020 along the coast of the Yellow Sea, China, seriously affecting marine environments and ecological services functions [11-15].

Figure 1. Green tide outbreak in the Southern Yellow Sea On June 18, 2019.

An interesting phenomenon called *Ulva* species succession were found at the early stage of the blooms in the Southern Yellow Sea. The initial floating species was identified as *Ulva compressa*; *Ulva flexuosa* and *Ulva linza* were the second floating species; and *Ulva prolifera* was the last [16-18]. However, during the drifting process of the blooms, *U. prolifera* became the dominant species and other species disappeared

gradually [19]. Thus, it was meaningful to carry out comparative studies on organelle genomes of these bloom-forming green algae.

2 The specimen and data preparation

In our previous studies, mitochondrion genome of *U. flexuosa* (NC035809; KX455878) [20], *U. linza* (NC029701; KU189740) [21], *U. compressa* (KX595276), and *U. prolifera* (KU161104) were conducted [22]. We downloaded these sequences from US National Center for Biotechnology Information (NCBI: www.ncbi.nlm.nih.gov/) (Table 1). A total of 6 complete mitochondrion genome of *Ulva* and 1 outgroup called *Pseudendoclonium akinetum* (AY359242) were used during this study.

Table 1. Detailed information on the samples used for genetic analysis collected from NCBI database.

Species	GenBank information	Sequence length/bp	Specimen location
<i>U. flexuosa</i>	NC035809K X455878	71,545 71,545	SHOUM, China
<i>U. linza</i>	NC029701K U189740	70,858 70,858	SHOUM, China
<i>U. compressa</i>	KX595276	62,311	SHOUM, China
<i>U. prolifera</i>	KU161104	61,962	SHOUM, China

Also, specimens were stored in the herbarium of Shanghai Ocean University Museum (SHOUM). Based on these four kinds of *Ulva* specimens, we took clear

*Corresponding authors: 15721539745@139.com (Jinlin Liu); jh-zhang@shou.edu.cn (Jianheng Zhang)

morphological photographs by using the camera model GE-X2600.

3 Result and discussion

A Maximum-likelihood (ML) phylogenetic tree with 6 complete mitochondrion genome of *Ulva* and 1 outgroup called *P. akinetum* (AY359242) was constructed by using the MEGA 7 software (Figure 2) [21-22], which showed *U. prolifera* is closely related to *U. linza*. In addition, the standard morphological characteristics of these *Ulva* species were presented in Figure 2.

The formation of the green tide in the Southern Yellow Sea of China is complicated. Many researches demonstrated that the significant amount of attached *Ulva* species on the *Pyropia* aquaculture rafts were considered as the main source of the blooms in the Southern Yellow Sea [23-25]. The attached *Ulva* species consisted of four species which were *U. compressa*, *U. linza*, *U. flexuosa* and *U. prolifera*. During the harvest

season of *Pyropia* aquaculture, as a kind of fouling macroalgae, the attached *Ulva* were removed artificially, resulting in the initial formation of the blooms.

These four kinds of *Ulva* species could be distinct from the complete mitochondrion genomes (Figure 2). Also, the morphological characteristics of these bloom-forming species are different during the initial formation of the bloom process (Figure 2), and in combine with these methods above, we can distinguish the *Ulva* species.

The largest green tide bloom areas in the Southern Yellow Sea of China from 2007 to 2019 was shown in Figure 3. China has the high frequency outbreak of the green tide, in 2009, the cover area of green tide outbreaks in the Southern Yellow Sea of China has reached 2,100 km² (Figure 3) [26-28]. Maybe through the application of biological prevention, strengthening breeding management and other methods, we can fundamentally solve the problem of green tides [29-30].

Figure 2. Maximum likelihood phylogenetic tree for free-floating *Ulva* species based on mitochondrion genomes.

Figure 3. Annual statistics of the largest green tide bloom area in the Southern Yellow Sea of China from 2007 to 2019.

4 Conclusion

In this thesis, we analyzed complete mitochondrion genomes of the free-floating *Ulva* species during the early stage of macroalgae bloom in the Southern Yellow Sea and provided standard morphological pictures of these *Ulva* species, which will be useful for studying the genetic diversity and phylogenetic history of *Ulva* species in the Southern Yellow Sea.

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Disclosure statement

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are openly available in National Center for Biotechnology Information (NCBI) at <https://www.ncbi.nlm.nih.gov>, accession number: NC035809, KX455878, NC029701, KU189740, KX595276, and KU161104.

Author information:

Liu Jinlin (1996–), College of Marine Ecology and Environment, Shanghai Ocean University, male, born in Nantong, Jiangsu Province, Ph.D., majoring in marine science.

References

1. Blomster. J, Back. S, Fewer. D.P, Kiirikki. M, Lehvo. A, Maggs. C.A, Stanhope. M.J. Novel morphology in Enteromorpha (Ulvophyceae) forming green tides. *Am J Bot.* 89(11): p. 1756–1763 (2002).
2. Merceron. M, Antoine. V, Auby. I, Morand. P. In situ growth potential of the subtidal part of green tide forming *Ulva* spp. *Stocks. Sci Total Environ.* 384(1–3): p. 293–305 (2007).
3. Nelson. T.A, Haberland. K, Nelson. A.V, Ribarich. H, Hotchkiss. R, Van-Alstyne. K.L, Buckingham. L, Simunds. D.J, Fredrickson. K. Ecological and physiological controls of species composition in green macroalgal blooms. *Ecology.* 89(5): p. 1287–1298 (2008).
4. Smetacek. V, Zingone. A. Green and golden seaweed tides on the rise. *Nature.* 504: p. 84–88 (2013).
5. Zhang. J.H, Liu. C.C, Yang. L.L, Gao. S, Ji. X, Huo. Y.Z, Yu. K.F, Xu. R, He. P.M. The source of the *Ulva* blooms in the East China Sea by the combination of morphological, molecular and numerical analysis. *Estuar Coast Shelf Sci.* 164: p. 418–424 (2015).
6. Cui. J.J, Zhang. J.H, Monotilla. A.P, Huo. Y.Z, Shi. J.T., Zhao. X.H, Kang. X.Y, He. P.M. Assessment of blooming *Ulva* macroalgae production potential in the Yellow Sea, China. *Phycologia.* 58(5): p. 535–541 (2019).
7. Liu. J.L, Zhao. X.H, Kang. X.Y, Zhuang. M.M, Ding. X.W, Zhao. L.J, Wen. Q.L, Zhu. Y, Gu. K, Bao. Q.J, Yang. X.Q, Zhang. J.H, He. P.M. Good news: we can identify *Ulva* species erupted in the Yellow Sea more easily and cheaply now. *Conserv Genet Resour.* 12(3): p. 447–449 (2020).
8. Zhao. X.H, Cui. J.J, Zhang. J.H, Shi. J.T, Kang. X.Y, Liu. J.L, Wen. Q.L, He. P.M. Reproductive strategy of the floating alga *Ulva prolifera* in blooms in the Yellow Sea based on a combination of zoid and chromosome analysis. *Mar Pollut Bull.* 146: p. 584–590 (2019).

9. Kang. X.Y, Liu. J.L, Yang. X.Q, Cui. J.J, Zhao. L.J, Wen. Q.L, Fu. M.L, Zhang. J.H, He. P.M. The complete mitochondrial genome of a green macroalgae species: *Ulva meridionalis* (Ulvales: Ulvaceae). Mitochondrial DNA Part B. 5(1): p. 760–761 (2020).
10. Xiao. J., Wang. Z.L, Song. H.J, Fan. S.L, Yuan. C, Fu. M.Z, Miao. X.X, Zhang. X.L, Su. R.G, Hu. C.M. An anomalous bi-macroalgal bloom caused by *Ulva* and *Sargassum* seaweeds during spring to summer of 2017 in the western Yellow Sea, China. Harmful Algae. 93: p. 101760 (2020).
11. Liu. D.Y, Keesing. J.K, Xing. Q.G, Shi. P. World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. Mar Pollut Bull. 58: p. 888–895 (2009).
12. Hu. C.M, Li. D.Q, Chen. C.S, Ge. J.Z, Muller-Karger. F.E, Liu. J.P, Yu. F, He. M.X. On the recurrent *Ulva prolifera* blooms in the Yellow Sea and East China Sea. J Geophy Res-Oceans. 115: p. C05017 (2010).
13. Shan. J.Z, Li. J.M, Xu. Z.H. Estimating ecological damage caused by green tides in the Yellow Sea: A choice experiment approach incorporating extended theory of planned behavior. Ocean Coastal Manage. 181: p. 104901 (2019).
14. Wang. H, Wang. G.C, Gu. W.H. Macroalgal blooms caused by marine nutrient changes resulting from human activities. J Appl Ecol. 57(4): p. 766–776 (2020).
15. Zhao. X.H, Yang. X.Q, Zhang. J.H, Wen. Q.L, He. P.M. Karyological observations of *Ulva linza* chromosomes. Journal of Oceanology and Limnology. Early Online, DOI:10.1007/s00343-020-9105-x (2020).
16. Hu. S, Yang. H, Zhang. J.H, Chen. C.S, He. P.M. Small-scale early aggregation of green tide macroalgae observed on the Subei Bank, Yellow Sea. Mar Pollut Bull. 81(1): p. 166–173 (2014).
17. Han. W, Chen. L.P, Zhang. J.H, Tian. X.L, Hua. L, He. Q, Huo. Y.Z, Yu. K.F, Shi. D.J, Ma. J.H, He. P.M. Seasonal variation of dominant free-floating and attached *Ulva* species in Rudong coastal area, China. Harmful Algae. 28: p. 46–54 (2013).
18. Wang. S.Y, Huo. Y.Z, Zhang. J.H, Cui. J.J, Wang. Y, Yang. L.L, Zhao. Q.Y, Lu. Y.W, Yu. K.F, He. P.M. Variations of dominant free-floating *Ulva* species in the source area for the worlds largest macroalgal blooms, China: Differences of ecological tolerance. Harmful Algae. 74: p. 58–66 (2018).
19. Zhang. J.H, Huo. Y.Z, Wu. H.L, Yu. K.F, Kim. J.K, Yarish. C, Qin. Y.T, Liu. C.C, Xu. R, He. P.M. The origin of the *Ulva* macroalgal blooms in the Yellow Sea in 2013. Mar Pollut Bull. 89(1–2): p. 276–283 (2014).
20. Cai. C.E, Wang. L.K, Jiang. T, Zhou. L.J, He. P.M, Jiao. B.H. The complete mitochondrial genomes of green tide algae *Ulva flexuosa* (Ulvophyceae, Chlorophyta). Conserv Genet Resour. 10(3): p. 415–418 (2018).
21. Pombert. J.F, Otis. C, Lemieux. C, Turmel. M. The complete mitochondrial DNA sequence of the green alga *Pseudendoclonium akinetum* (Ulvophyceae) highlights distinctive evolutionary trends in the chlorophyta and suggests a sister-group relationship between the Ulvophyceae and Chlorophyceae. Mol Biol Evol. 21(5): p. 922–935 (2004).
22. Kumar. S, Stecher. G, Tamura. K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 33(7): p. 1870–1874 (2016).
23. Huo. Y.Z, Han. H.B, Shi. H.H, Wu. H.L, Zhang. J.H, Yu. K.F, Xu. R, Liu. C.C, Zhang. Z.L, Liu. K.F, He. P.M, Ding. D.W. Changes to the biomass and species composition of *Ulva* sp on *Porphyra* aquaculture rafts, along the coastal radial sandbank of the Southern Yellow Sea. Mar Pollut Bull. 93(1–2): p. 210–216 (2015).
24. Zhang. J.H, Zhao. P, Huo. Y.Z, Yu. K.F, He. P.M. The fast expansion of *Pyropia* aquaculture in "Sansha" regions should be mainly responsible for the *Ulva* blooms in Yellow Sea. Estuar Coast Shelf Sci. 189: p. 58–65 (2017).
25. Zhang. J.H, Shi. J.T, Gao. S, Huo. Y.Z, Cui. J.J, Shen. H, Liu. G.Y, He. P.M. Annual patterns of macroalgal blooms in the Yellow Sea during 2007–2017. PLOS One. 14(1): p. e0210460 (2019).
26. Liu. J.L, Yang. X.Q, Cui. J.J, Zhuang. M.M, Zhao. L.J, Li. J.Y, Liu. Y.K, Wen. Q.L, Fu. M.L, Zhao. S, Zhang. J.H, He. P.M. Complete chloroplast genome of *Ulva meridionalis* (Ulvales: Ulvaceae): an extremely fast-growing green macroalgae. Mitochondrial DNA Part B-Resources. 5(2): p. 1390–1392 (2020).
27. Liu. J.L, Yang. X.Q, Li. J.Y, Wen. Q.L, Liu. W, Song. W.P, He. P.M, Zhang. J.H. Research progress on settled mature *Ulva prolifera* during the outbreak of green tide in the Yellow Sea. Environmental Pollution & Control. 42(5): p. 614–618 (2020).
28. Shi. J.T, Zhang. J.H, Cui. J.J, Zhao. X.H, Kang. X.Y, Liu. J.L, Wen. Q.L, Yang. X.Q, He. P.M. Artificial hybridization and cross-breeding of *Ulva*. Journal of Shanghai Ocean University. 28(5): p. 755–764 (2019).
29. Zhuang. M.M, Liu. J.L, Ding. X.W, He. J.Z, Zhao. S, Wu. L.J, Gao. S, Zhao. C.Y, Liu. D.Y, Zhang. J.H, He. P.M. *Sargassum* blooms in the East China Sea and Yellow Sea: Formation and management. Marine Pollution Bulletin. p. 111845 (2020).
30. Liu. J.L, Xia. J, Zhao. L.J, Gu. K, Kang. X.Y, Ding. X.W, Zhao. X.H, Zhuang. M.M, Zhang. J.H, He. P.M. Species identification of epizoans from *Ulva Lactuca* in Zhoushan sea area by using the DNA barcode technology. Marine Environmental Science. 39(6): p. 874–879+886 (2020).