

The green tide in Yingkou, China in summer 2021 was caused by a subtropical alga—*Ulva meridionalis* (Ulvophyceae, Chlorophyta)*

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Abstract The large-scale green tide caused by *Ulva* has occurred successively in the Yellow Sea since 2007, and new events of green tide also continued to appear in nearby sea areas, indicating an undergoing rapid development of occurrence patterns for harmful macroalgal blooms (HMBs) along coastal China. In August 2021, a green tide occurred for the first time in Bayuquan sea area of Yingkou city, Liaoning Province in the Bohai Sea. In this study, morphological and molecular approaches were used to identify the causative species as *U. meridionalis*, an alien subtropical alga previously found to dominate green tides in the South China Sea. According to the hydrological data of Bayuquan in summer 2021, combined with morphological and developmental observations for this alga, we hypothesized that the disturbance caused by the typhoon In-Fa might have detached the local *U. meridionalis* from substrates, especially for those thalli with poorly developed holdfasts, and the ensuing wave-free period with unusually high temperature, which fell in the reported optimum growth temperature for *U. meridionalis*, might have provided the favorable conditions for the final bloom of the floating seaweeds. This is the first report on the bloom of subtropical *U. meridionalis* in the north temperate sea zone, indicating that the ecological risk of causing green tides in the future by this rapidly spreading species deserves high attention.

Keyword: Bohai Sea; green tide; holdfast; alien species; *Ulva meridionalis*; Yingkou

1 INTRODUCTION

Harmful macroalgal blooms (HMBs) refer to the overgrowth and accumulation of floating macrophytes, which has occurred more and more frequently worldwide in recent decades (Smetacek and Zingone, 2013). The green tide is one type of HMBs, mainly caused by green seaweeds from *Ulva* (Ulvophyceae, Chlorophyta) (Fletcher, 1996). These green algae are typical opportunistic seaweeds that can grow rapidly especially when nutrients and water temperature conditions are favourable. In addition to damage to

the marine environment (Valiela et al., 1997), once the green tide occurs on the urban seashore, it will have a serious impact on aquaculture (Wang et al., 2011),

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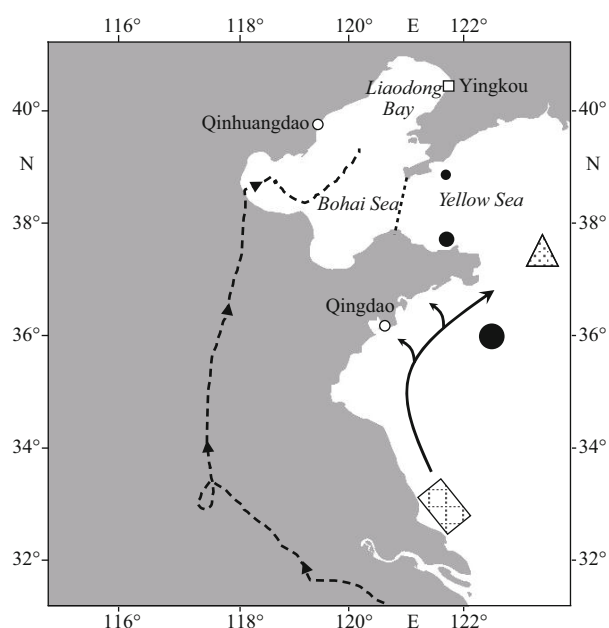


Fig.1 Sampling site in the coastline of Yingkou, North China

The white square represents the sampling site. Slash-filled rectangle represents the source area of the Yellow Sea green tide. Black circles indicate the potential settlement regions of massive drifting *Ulva*. Dotted triangle represents the northernmost position of drifting *U. prolifera*. Solid line with branched arrows mark the drifting route of the green tide. Dashed line with arrows mark the path of typhoon In-Fa. Dotted line separates the Yellow Sea from the Bohai Sea.

tourism economy, and even daily life.

In August 2021, a green tide event occurred in the coastal waters of Bayuquan, Yingkou City, Liaoning Province, located in Liaodong Bay, Bohai Sea. According to the reports from the website of the local government management department, on August 9, it was first noticed that there was only a small amount of green seaweed gathering on the shore of Shanhai Square beach. However, from August 10 to 12, a large amount of algae suddenly appeared in the same area, floating in the seawater or stranded on the sand beach. Based on the on-site aerial survey with unmanned aerial vehicle (UAV), this green tide was distributed along the coastal zone. The sea area covered by floating green seaweeds was about 1 000 m long and 50 m wide, with a maximum coverage area of about 0.05 km², mainly covering the intertidal zone. The local government management department urgently organized environmental investigation and sample analysis, and tentatively identified the causative species as *U. prolifera* O. F. Müller informally (Department of Natural Resources of Liaoning Provincial, 2021a). This green tide event lasted 15 days in total. On August 23, it was reported that this green tide had basically disappeared and the sea area returned to normal after timely cleaning the floating algae in the seawater and

those stranded on the beach (Department of Natural Resources of Liaoning Provincial, 2021b).

In recent years, coastal China has been widely threatened by green tides. The first report came from the Yellow Sea green tide in 2007 (Jiang et al., 2008), which occurred in successive years and ranked first on scale in the world (Liu et al., 2009). Genetic analysis showed that this event was dominated by a unique “floating ecotype” of *U. prolifera*, which is genetically different from attached populations widespread in China (Zhao et al., 2013, 2015; Jiang and Zhao, 2018), and later was identified as *U. prolifera* subsp. *qingdaoensis* (Cui et al., 2018). Moreover, it was suggested that the drifting *U. prolifera* from the Yellow Sea may expand to the East China Sea, resulting in the green tides in the Gouqi Island since 2011 (Zhang et al., 2015). In the Bohai Sea, *U. prolifera* has also been reported to cause summer green tides since 2015 (Song et al., 2019a), but whether it belonged to the same “floating ecotype” was unclear. For green tides in the South China Sea, three alien species, *U. meridionalis*, *U. tepida*, and *U. chaugulii*, were identified as causative species in 2018 (Xie et al., 2020). Since the floating algae in Yingkou has been tentatively identified as *U. prolifera*, in addition to the local origin, there are at least two possible origins that need special attention: one is from the blooming *U. prolifera* in Qinhuangdao which might be driven east by a near typhoon In-Fa (National Meteorological Center, 2021); second, considering the north limit of drifts (Li et al., 2020), and possible main settlement region in previous years (Geng et al., 2019; Zhao et al., 2022), the drifting *U. prolifera* from the Yellow Sea may also move further northward to the Bohai Sea as shown in Fig.1.

To determine the origin of this event, accurate identification on both species and intraspecies level is very necessary and the most priority. In this study, the blooming seaweeds in Yingkou were collected for molecular investigation with multi genetic markers, and detailed morphological characterization was also combined. These results provide basic data to resolve the process and mechanism for this event.

2 MATERIAL AND METHOD

2.1 Seaweeds collection and culture

During the blooming period of green tide in August 2021, all samples of floating green seaweeds were collected in the coastal waters near Bayuquan of Yingkou city, Liaodong Bay, Bohai

Sea (40°13'13"N, 122°4'37"E) (Fig.1), where there were large concentrations of blooming macrophytes. After algal samples were transported back to the laboratory in a container with ice packs, they were washed with sterilized seawater to eliminate any epiphytes and contaminants. Then, some intact, clean, and healthy individuals were selected for culture in separate Petri dishes with Von Stosch's Enriched Medium (VSE medium) which was renewed once a week. All seaweeds samples were cultured at 16 °C with a 12-h:12-h light (L):dark (D) photoperiod and illumination intensity of 75 $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$.

2.2 Molecular identification

To prepare genomic DNA templates for polymerase chain reaction (PCR) detections, approximately 5 mm² of thallus for each individual was cut off with a sterilized blade for DNA extraction, using a Plant Genomic DNA Extraction Kit (Tiangen Biotech Co. Ltd., Beijing, China) following the provided instruction. The PCR mixtures contained 10 μL of 2 \times Taq polymerase (Novoprotein Scientific Inc., Suzhou, China), 0.4 μL of each reverse and forward primer (10 $\mu\text{mol/L}$), 2 μL of genomic DNA, and 7.2 μL of ultrapure water, for a total volume of 20 μL . A total of five pairs of primers and their corresponding PCR profiles, including ITS-a and ITS-d for internal transcribed spacer (ITS, including ITS1, 5.8S and ITS2) (Leskinen and Pamilo, 1997), *rbcL*-RH1 and *rbcL*-1385r for *rbcL* (Manhart, 1994), *tufGF4* and *tufAR* for *tufA* (Saunders and Kucera, 2010), 5S-F and 5S-R for 5S rDNA spacer (Shimada et al., 2008), and YSF-F and YSF-R for sequence characterized amplified region (SCAR) marker which was specific to the floating ecotype of *U. prolifera* dominating the green tides in the Yellow Sea (Zhao et al., 2015), were used in this study.

All PCR products were visualized by gel electrophoresis in a 1.5% agarose gel stained with the dye Super GelRed (US Everbright Inc., Suzhou, China), and purified by a Gel Extraction Kit (200) (OMEGA Bio-Tek, Georgia, USA) following the manufacturer's instructions. After commercial sequencing (Ruibio BioTech Co. Ltd., Qingdao, China), partial obtained representative sequences were uploaded to the GenBank database. The phylogenetic analysis was performed with the DNA sequences including those of samples and references from the GenBank, with those from *Ulvaria* or *Blidingia* as outgroups. The maximum likelihood (ML) phylogenetic trees was constructed using MEGA 6.0 (Tamura et al., 2013), with 1 000 bootstrap replicates.

2.3 Morphological characterization

Intact, clean, and healthy samples were selected to record the gross morphology of the specimens, including the shape, size, branches, and holdfast of the thalli. Photographs of the morphological features of the specimens were taken by a Canon PC2152 digital camera (Canon Inc., Tokyo, Japan). Morphologically typical and well-formed samples were selected and pressed on herbarium sheets for making specimens, and deposited in the Marine Biological Museum of Chinese Academy of Sciences (MBMCAS) at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China. Random sampling was used for statistical works to analyze the branching characteristics and the proportion of individual plants with holdfasts ($n=100$). According to the protocols described previously (Ma et al., 2020), two indexes of branching morphology, i.e., the top branching order and the intensity of primary branches, were measured for statistical analysis ($n=100$).

A BH2 light microscope (Olympus Corp., Tokyo, Japan) was employed for examination of microscopic characters of samples, such as the chloroplasts number and location, as well as the shape and arrangement of surface cells. The thalli were crosscut to observe the cell location in transverse view, or stained with Lugol's iodine solution to observe the number of pyrenoids. All microscopic photographs of the specimens were taken by a CCD camera (Scope Tek MDC200, Mingshi, Ningbo, China) mounted on the microscope. In order to compare the differences or stability of some morphological features between the thalli from field collection and those from laboratory culture, some samples were selected to induce reproduction with a described method (Hiraoka and Enomoto, 1998). After the formation, release, and germination of germ cells, new plantlets were cultured in Petri dishes under the same conditions mentioned above.

2.4 Analysis of hydrological data from Bayuquan

The daily sea surface temperature (SST) and wave height data of Bayuquan sea area from July to September in 2020 and 2021 were collected from the website of the Municipal Government of Yingkou City (<http://www.yingkou.gov.cn>), and Microsoft Excel software (Microsoft Excel 2019) was used for data statistics and analysis.

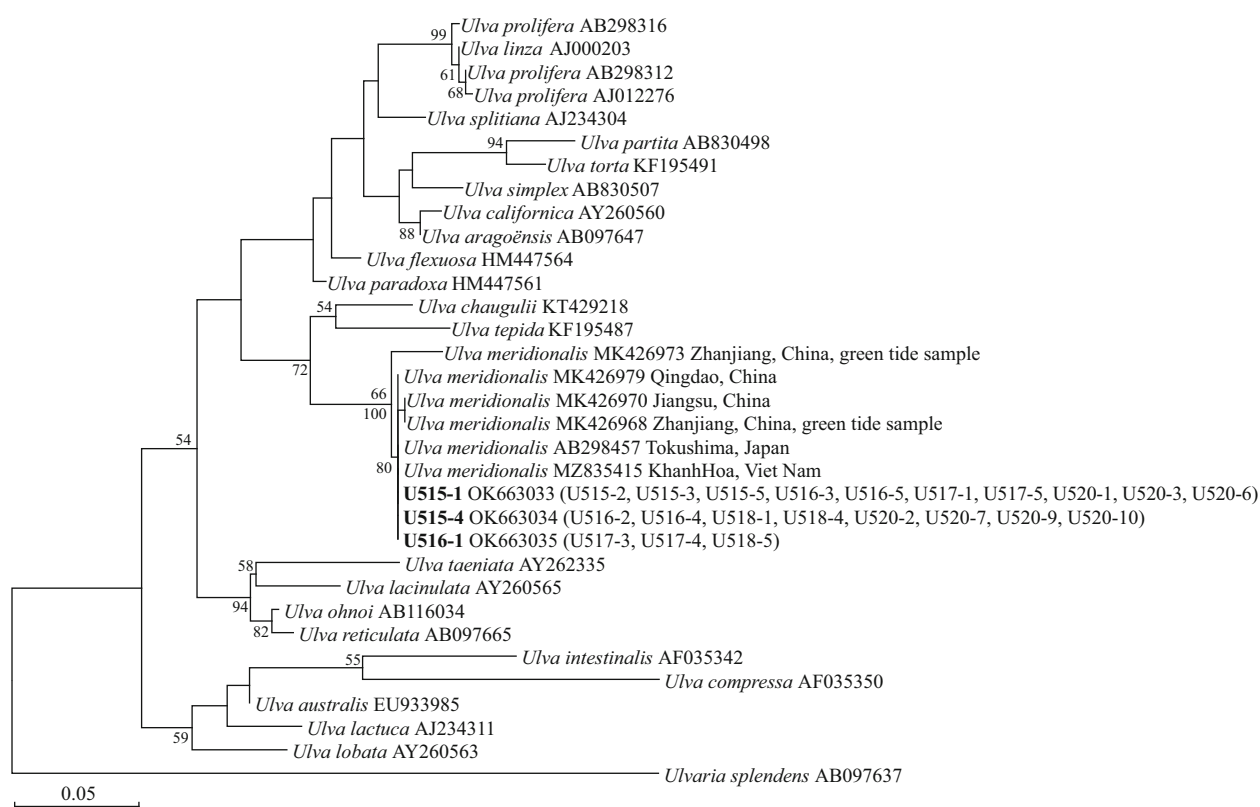


Fig.2 Maximum likelihood (ML) phylogenetic tree based on DNA sequences of ITS

Numbers at the nodes indicate bootstrap values. GenBank accession numbers for each reference sequence and representative sample sequences were provided. Samples in brackets shared identical sequences with the sample in bold outside the brackets.

3 RESULT

3.1 Molecular identification

A total of 54 nucleotide sequences of ITS, including 24 samples from this investigation and 30 reference sequences downloaded from GenBank, were used to construct the ML tree for species identification and phylogenetic analysis. As shown in Fig.2, all sequences from samples were highly identical and gathered into a cluster which consisted of all reference sequences from *U. meridionalis*, including all available sequences from samples outside China, and those from specimens that have caused green tides in the South China Sea in 2018 (Xie et al., 2020). Besides, it was found that all sample ITS sequences were highly identical (99.21%) to the short ITS2 sequence (AB598807) from the holotype of *U. meridionalis* (Horimoto et al., 2011). On the contrary, all sample sequences were clearly in a different clade from that of *U. prolifera*, and the genetic distances between them was approximately 8.6%–9.0%. Similar results were shown in the *tufA*-ML tree which was constructed with 19 sample sequences and 20 references (Fig.3). Besides, an ML tree based on *rbcL* sequences was

also constructed and similar topological structure was shown, in which there was a high identity between sequences from our sample and the holotype of *U. meridionalis* from Japan (Supplementary Fig.S1). Since this causative species had been tentatively identified as *U. prolifera*, the PCR detection with 5S or SCAR were also performed, but no amplified products were obtained. Combined with all data of molecular identification with multiple markers, it can be determined that the single dominant species causing this green tide event was *U. meridionalis* R. Horimoto & S. Shimada, rather than *U. prolifera*.

3.2 Morphological characterization

In order to provide additional evidence for species identification and detect the adaptive characteristics under the specific life mode of floating, morphological and anatomical observation was carried out on samples. All samples appeared to be *Enteromorpha*-like species that were tubular and monostromatic thalli. The length of thalli was 5–15 cm, and the breadth of main axis was approximately 1 mm. Two voucher specimens, MBM287241 (Fig.4a) and MBM287242, have been made from typical individuals, and

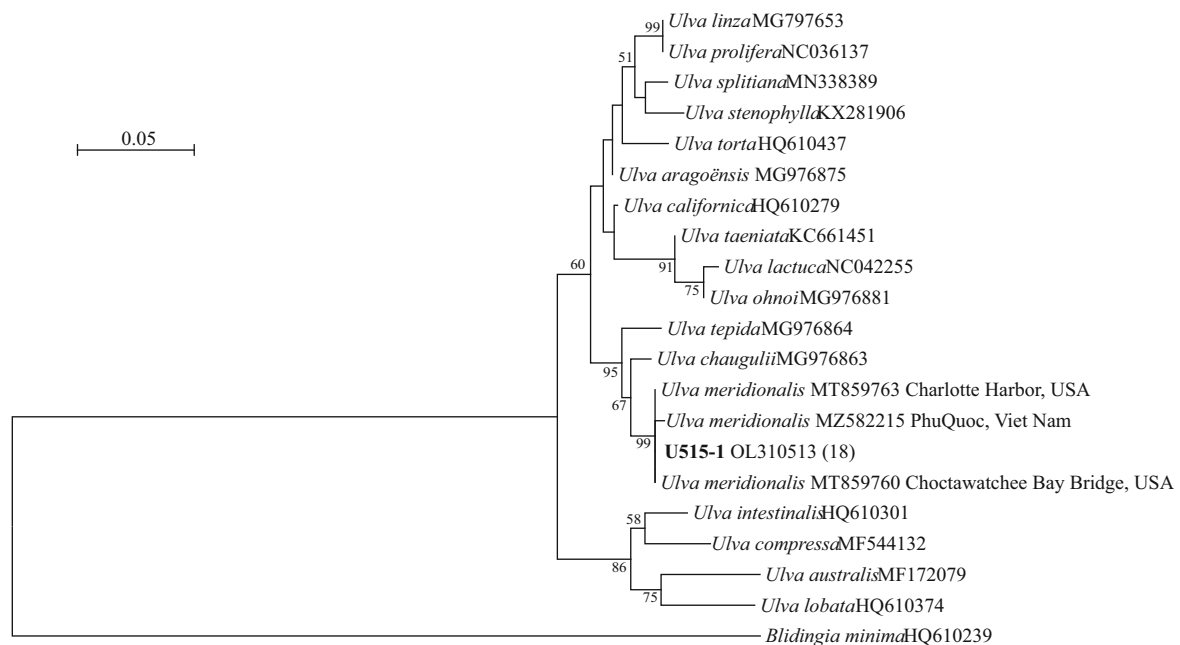


Fig.3 Maximum likelihood (ML) phylogenetic tree based on DNA sequences of *tufA*

Numbers at the nodes indicate bootstrap values. GenBank accession numbers for each sequence were provided. Numbers in brackets following the sample ID represent the amount of the identical sequences. Sample number in bold were from this study.



Fig.4 Morphological and anatomical characteristics of samples

a. voucher specimen (MBM287241); b-c. air bubbles inside tubular algae; d-e. holdfasts in field-collected samples; f-g. holdfasts in germinated seedlings; h. germinated seedlings attaching to the bottom of Petri dish; i. transverse constriction; j. single-tubular type in transverse view; k. double-tubular structure in surface view; l. bow-shaped double-tubular type in transverse view; m. chloroplast location in surface view from a field-collected sample; n. chloroplast location in surface view from a germinated seedling; o. cell location in transverse view; p. pyrenoids. Triangular arrows in (a, d, f) indicate holdfasts; triangular arrow in (i) indicates a transverse constriction; long-tail arrows in (h) indicate floating bigger individuals. Scale bars: 1 cm in a, h; 3 mm in b, d, f, i, k; 100 μ m in c, e; 50 μ m in g, j, l; 10 μ m in m-o; 5 μ m in p.

deposited at the MBMCAS. Each thallus had an obvious branched main axis, but only primary branches were detected. The primary branches were roughly evenly distributed along the main axis, with a density of 0.47–2.90/cm ($n=100$). In freshly collected samples, the tubular algae were common with air bubbles inside, which may have made it easier to float during the blooming period in seawater (Fig.4b & c). It should be noted that according to observation and statistics, about 49% of the field-collected samples were found to have holdfasts ($n=100$), but they were generally poorly developed and very weak (Fig.4d & e). This distinct feature was observed repeatedly in the next generation propagated in the laboratory. It was shown that the released and germinated seedlings can develop holdfasts to grow on the bottom of Petri dishes (Fig.4f & g), but the development of holdfasts was so weak that even gently disturbs like renew of media would detach seedlings in bigger sizes from the bottom of Petri dishes (Fig.4h). On the surface view, transverse constrictions were obvious and common on the thalli (Fig.4i). For freshly collected samples, each thallus showed a single-tubular type in transverse view (Fig.4j). However, after a period of culture in the laboratory, along the main axis of thalli, the two opposite layers of cells near the central axis began to approach and bond with each other to form a vesicular structure on both sides (Fig.4k). Therefore, the cross section exhibited a bowknot-shaped double-tubular type (Fig.4l), which was reported highly specific to *U. meridionalis* (Xie et al., 2020).

The anatomical observation of the surface view showed that cells were polygonal with rounded corners, and irregularly arranged throughout the thalli. It is worth noting that for the field-collected samples, each cell contained a single chloroplast, and most of the chloroplasts were adherent to one side of the cell (Fig.4m). On the contrary, the chloroplasts were found to fill the whole cells from the samples of generated seedlings (Fig.4n). The cross section of samples showed that the cell was located in the middle of thalli (Fig.4o). After staining with Lugol's iodine, each cell was shown to possess 1–4 pyrenoids, and most cells only had 1–2 pyrenoid (one, 30.0%; two, 51.4%; three, 17.2%; four, 1.4%; $n=70$) (Fig.4p).

3.3 Analysis of hydrological data from Bayuquan

As shown in Fig.5 that based on the data of Bayuquan sea area in 2020 and 2021, the SST generally increased first and then decreased from July to September, and obviously higher in 2021 than that

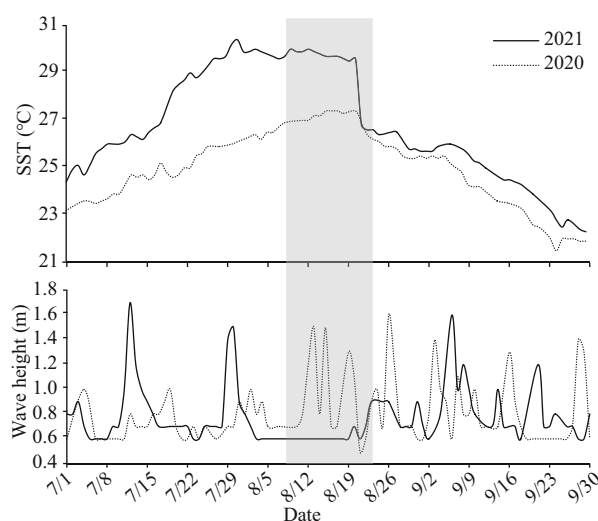


Fig.5 SST and wave height in Bayuquan from July to September in 2020 and 2021

The shaded area represents the duration of the green tide in Yingkou.

in 2020. Every year, there was a high temperature period between about July 15 and August 25, during which the average SST in 2020 was 26.1 °C and the highest temperature was 27.4 °C, while the average SST in 2021 was 29.0 °C and the highest temperature was 30.4 °C, indicating that the warming range and the speed of SST increase and decrease in 2021 were significantly higher than that in 2020. During July–September 2021, the dates with higher waves were mainly in July and September, while in August there was a 16-day long period of low waves, accompanied by high temperatures. It should be noted that there were two peaks of wave heights in July, which were formed on July 12 by the Bohai low pressure and July 30 by the typhoon In-Fa respectively (<http://www.yingkou.gov.cn>). On the contrary, in 2020 the dates with higher waves were mostly in August and September, almost affecting the whole high-temperature period.

4 DISCUSSION

Based on results of molecular identification with multiple markers, it was shown that the sequences of samples from the green tide of Yingkou were highly identical to that of the holotype of *U. meridionalis*, and other widespread *U. meridionalis* specimens. Moreover, the 5S rDNA spacer and SCAR signal that should be present in the floating ecotype of *U. prolifera* in the Yellow Sea, were not detected. Therefore, it can be determined that the single dominant species causing this green tide event was *U. meridionalis* rather than *U. prolifera*. Additional evidence supporting this

conclusion was also provided from the morphological and anatomical observation. In addition to a previous brief morphological description with the holotype of *U. meridionalis* that collected from brackish water habitat (Horimoto et al., 2011), Xie et al. (2020) supplemented more details to the conspecific samples that were from marine habitats. It was shown that for samples in this study, first, the transverse constrictions were common on the thalli and only primary branches were detected, which were consistent with the previous description. It was found that two features, i.e., the chloroplasts location in surface view and the number of pyrenoids, were very similar to the description from those in brackish water habitats, but different from those in marine habitats, suggesting that it may result from the lower salinity in Bayuquan area (Song et al., 2017). Secondly, some characteristics, such as the typical bow-shaped double-tubular structure in marine *U. meridionalis*, were found to be absent in field samples but reproducible after subsequent laboratory culture. We speculate that this may be related to the development stages for this species as previously shown (Xie et al., 2020). In addition, some distinct features of our samples were also noticed. It has been reported that the primary branches always occurred in the basal region of the thalli and rarely in the mid or upper regions (Horimoto et al., 2011; Xie et al., 2020). However, they were roughly evenly distributed along the main axis in this study. Based on the identification results, it was indicated that the origin of Yingkou green tide was not from the floating ecotype of *U. prolifera* in the Yellow Sea, nor from the transport of blooming algae in Qinhuangdao, but probably from the native algae, since nearly half of samples were found to have holdfasts.

Bohai Sea is a semi-closed sea with a low degree of seawater exchange. Due to the increasing impact of human activities, the Bohai Sea receives about one billion tons of wastewater every year (Duan et al., 2010), resulting in increased eutrophication levels. In addition to frequent red tides (Zhao et al., 2004), brown tides (Zhang et al., 2012), jellyfish blooms (Dong et al., 2010), green tides (Xing et al., 2016; Song et al., 2019a), and even overgrowth of seagrass (Xu et al., 2019) have occurred in recent decades. It indicates that the marine ecosystem in the offshore area has been seriously out of balance. Bayuquan is located at the estuary of Liaohe River in Liaodong Bay, with the highest DIN value in the Bohai Sea due to the main impact of Liaohe River runoff (Wang et al., 2009). It was known that Bayuquan was a sea area

with frequent red tides and serious impacts (Yu et al., 2018), but as far as we know, this event is the first green tide in this area. Based on the hydrological data of Bayuquan in summer 2021, here we speculated about the possible occurrence process of this event. During the high temperature period, typhoon In-Fa entered the Bohai Sea around July 30 (National Meteorological Center, 2021), which brought greater waves to Bayuquan about twice as high as usual. Due to the weak holdfasts observed in the field samples, it was speculated that some *U. meridionalis* could be detached from substrates by the wave disturbance. After the typhoon, there was a period of high temperatures and calm for about half a month, which was conducive to the rapid growth of floating *U. meridionalis*. In particular, the average SST during the high temperature period in 2021 is significantly increased by 2 °C compared with that in 2020, and the highest SST is up to 30.4 °C. Since it was reported that *U. meridionalis* grown explosively in a narrow range of temperatures of 25–30 °C (Hiraoka et al., 2020a), the unusually high temperature might be a critical condition for the rapid proliferation of this subtropical alga in eutrophication waters. Finally, the SST began to drop rapidly from August 20, and with the emergency cleanup, the green tide event quickly ended.

Ulva meridionalis was initially identified and described in the brackish water habitats from Okinawa, Japan (Horimoto et al., 2011). According to its global distribution pattern, Xie et al. (2020) suggested it a subtropical species, and reported that it had caused green tides at low latitudes along the coast of the South China Sea in 2018. Because a unique bowknot-shaped structure found only in the transverse view of this species has never been described in any previous records from China, the current distribution of *U. meridionalis* in China was considered a result of recent introduction which was followed by rapid local spreads. In the Bohai Sea, this species was only detected occasionally in 2014 (Xie et al., 2020), and was usually absent from most seaweed surveys (Wang and Wang, 2009; Liu et al., 2011; Yu et al., 2017; Song et al. 2019b). In this study, we reported for the first time that *U. meridionalis* could also cause green tide in the north temperate sea zone, suggesting that the recent colonization of this species in the Bohai Sea might have reached a certain scale. Recent invasions to China with rapid local spreads of *Ulva* species have been noticed, and it was considered to be driven by the global warming

(Xie et al., 2020; Wei et al., 2022). It was predicted that with ocean warming, tropical *Ulva* species would spread to temperate regions and cause green tides (Hiraoka, 2021), with *U. ohnoi* and *U. reticulata* as typical examples (Hiraoka et al., 2020b). This study demonstrates that subtropical *U. meridionalis* also spreads to the temperate zone to cause green tides, supporting the predictions of the latest studies.

In addition to the known facts that *U. meridionalis* has an extremely prominent ability to grow rapidly (Hiraoka, 2012; Hiraoka et al., 2020a; Liu et al., 2020; Tsubaki et al., 2020) and cause blooms (Tsubaki et al., 2017; Xie et al., 2020). Here we suggested that there were at least two other possible reasons for the rapid northward extension of the sea area where this species bloomed along coastal China. First, although *U. meridionalis* grew at an optimum of 25–30 °C (Hiraoka et al., 2020a), it has been reported to distribute in all four sea areas of China, from 20°01'N to 39°55'N (Xie et al., 2020), indicating that it was likely an eurythermal species that could tolerate and grow over a wide temperature range. This might help expand its distribution and give it the opportunity to bloom in summer at higher latitudes when temperature and nutrient conditions are favorable. Similar phenomena have been found with the Bohai Sea red tide events which occurred mainly in August or September and usually dominant by eurythermal species or those adapted to high temperature (Zhao et al., 2004; Dou et al., 2020). Second, it seems that the spread of *U. meridionalis* was likely to rely on settlement, i.e., living with holdfasts attached to substrates, to achieve colonization of populations and continuous invasion into new habitats (Xie et al., 2020). Thus, in new habitats, when a certain number of individuals detached from substrates under severe disturbances, they would be possible to bloom in new locations. A negative example came from the Yellow Sea green tide. It was found that the thalli of the floating ecotype of *U. prolifera* can only be detected on nori rafts from the Subei radial sand ridges, but they have been almost completely missing in all intertidal zones over years (Zhao et al., 2015; Zhang et al., 2018). It has been proved that after the blooming stage with long-distance drifting, the wide-spread micro-propagules of *U. prolifera* were difficult to survive in new habitats (Miao et al., 2018; Zhao et al., 2018, 2022), and this might partly explain why the source of the Yellow Sea green tide has never spread northward. This indicated that although the unattached forms have the ability to build up a large biomass, forming massive green tides

(Smetacek and Zingone, 2013; Hiraoka, 2021), the extremely weak fixation ability reduces the expansion of new habitats for resident populations. Therefore, the current distribution and holdfast development of attached populations of *U. meridionalis* in the Bohai Sea need to be further investigated, and the ecological risk of this species causing green tides in the future also deserves high attention.

5 CONCLUSION

In August 2021, a green tide occurred for the first time in Bayuquan sea area of Yingkou, Liaoning Province in the Bohai Sea. In this study, morphological and molecular approaches were used to identify the causative species as *U. meridionalis*, a subtropical alga previously found to dominate green tides in the South China Sea. According to the hydrological data of Bayuquan in summer 2021, combined with morphological and developmental observations for this alga, the possible occurrence process of this event was speculated. This is the first report on the bloom of subtropical *U. meridionalis* in the north temperate sea zone, indicating that the ecological risk of causing green tides in the future by this rapidly spreading species deserves high attention.

6 DATA AVAILABILITY STATEMENT

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

7 ACKNOWLEDGMENT

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Electronic supplementary material

Supplementary material (Supplementary Fig.S1) is available in the online version of this article at <https://doi.org/10.1007/s00343-022-2014-4>.