

Phylogenomic analysis of the chloroplast genome of the green-tide forming macroalga *Ulva intestinalis* Linnaeus (Ulvophyceae, Chlorophyta)

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ABSTRACT

Ulva intestinalis Linnaeus 1753 (Ulvophyceae, Chlorophyta) is a marine green macroalga that is distributed on coasts of the Yellow Sea and the Bohai Sea in China. Here, the complete chloroplast genome of *U. intestinalis* was constructed and analyzed comparatively. The chloroplast genome of *U. intestinalis* is a 99,041-bp circular molecule that harbors a total of 112 genes including 71 protein-coding genes (PCGs), 26 transfer RNA genes (tRNAs), three ribosomal RNA genes (rRNAs), three free-standing open reading frames (*orfs*) and nine intronic *orfs*, and ten introns in seven genes (*atpA*, *infA*, *psbB*, *psbC*, *petB*, *rmlL*, and *rnnS*). The maximum likelihood (ML) phylogenomic analysis shows that *U. intestinalis* firstly groups with *Ulva compressa*, and then these two species together with the *Ulva australis*–*Ulva fenestrata*–*Ulva rotundata* subclade form a monophyletic clade, *Ulva* lineage II. *U. intestinalis* chloroplast genome is the only one in *Ulva* lineage II where the reversal of a collinear block of two genes (*psbD*–*psbC*) did not occur, and its genome structure is consistent with that of most chloroplast genomes in *Ulva* lineage I, indicating that the similarity of genome structure is not completely related to the genetic relationship of *Ulva* species. Our genomic data will facilitate the development of specific high-resolution chloroplast molecular markers for rapid identification of *U. intestinalis*, and help us understand its population diversity and genetic characteristics on a global scale.

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The macroalgal genus *Ulva* Linnaeus 1753 (Ulvophyceae, Chlorophyta) includes ~85 species of green seaweeds that grow in marine, estuarine and freshwater environments worldwide (Guiry and Guiry 2021). It is noticeable that *Ulva* species are opportunistic algae that tend to form green tides and marine fouling in nutrient-rich waters around the world (Liu et al. 2013; Wang et al. 2015). Thus far, the complete chloroplast genomes (cpDNAs) have been sequenced and constructed for 14 species in genus *Ulva* (e.g. Melton et al. 2015; Cai et al. 2017; Suzuki et al. 2018; Fort et al. 2021), and these *Ulva* cpDNAs share the same set of 100 conserved genes including 71 protein-coding genes (PCGs), 26 transfer RNA genes (tRNAs), three ribosomal RNA genes (rRNAs), and show different genome architectures due to multiple rearrangement events (Liu and Melton 2021).

Ulva intestinalis Linnaeus 1753 is a common marine green seaweed that has a cosmopolitan distribution with cases reported from the coasts of Asia, Australia, Europe, and America (Hayden and Waaland 2004; Heesch et al. 2009; Kirkendale et al. 2013; Steinhagen et al. 2019), and is also well-known as a typical green-tide forming macroalga (Leskinen et al. 2004; Kostamo et al. 2008). In China, this alga

is distributed from temperate coasts (e.g. Yellow Sea and Bohai Sea) to subtropical coasts (e.g. South China Sea) (Titlyanova et al. 2012). In this study, we collected the thalli of *U. intestinalis* from the intertidal zone of Zhifu District (N 37°32'21", E 121°24'29"), Yantai, Shandong Province, China in November 2020. Its specimen was deposited in the collection of marine algae in KLMEES of IOCAS (Feng Liu, liufeng@qdio.ac.cn) under the voucher number 2020-YT-Uin-1. Fresh algal tissue from one individual thallus was used for DNA extraction using a Plant Genome DNA Kit (DP305, Tiangen Biotech, Beijing, China). The extracted DNA was segmented by Covaris S220 ultrasonic crater. The qualified libraries were sequenced on an Illumina NovaSeq platform (Illumina, USA) using paired-end sequencing. The complete chloroplast genome of *U. intestinalis* was constructed by the GetOrganelle software. The annotation of PCGs, tRNAs, rRNAs, open reading frames (*orfs*), and introns was performed using Open Reading Frame Finder (<https://www.ncbi.nlm.nih.gov/orffinder>), tRNAscan-SE 2.0 (Chan et al. 2021), and RNAweasel Tool (<https://megasun.bch.umontreal.ca/cgi-bin/RNAweasel>).

The chloroplast genome of *U. intestinalis* is a 99,041-bp circular molecule (GenBank accession number: MZ158703)

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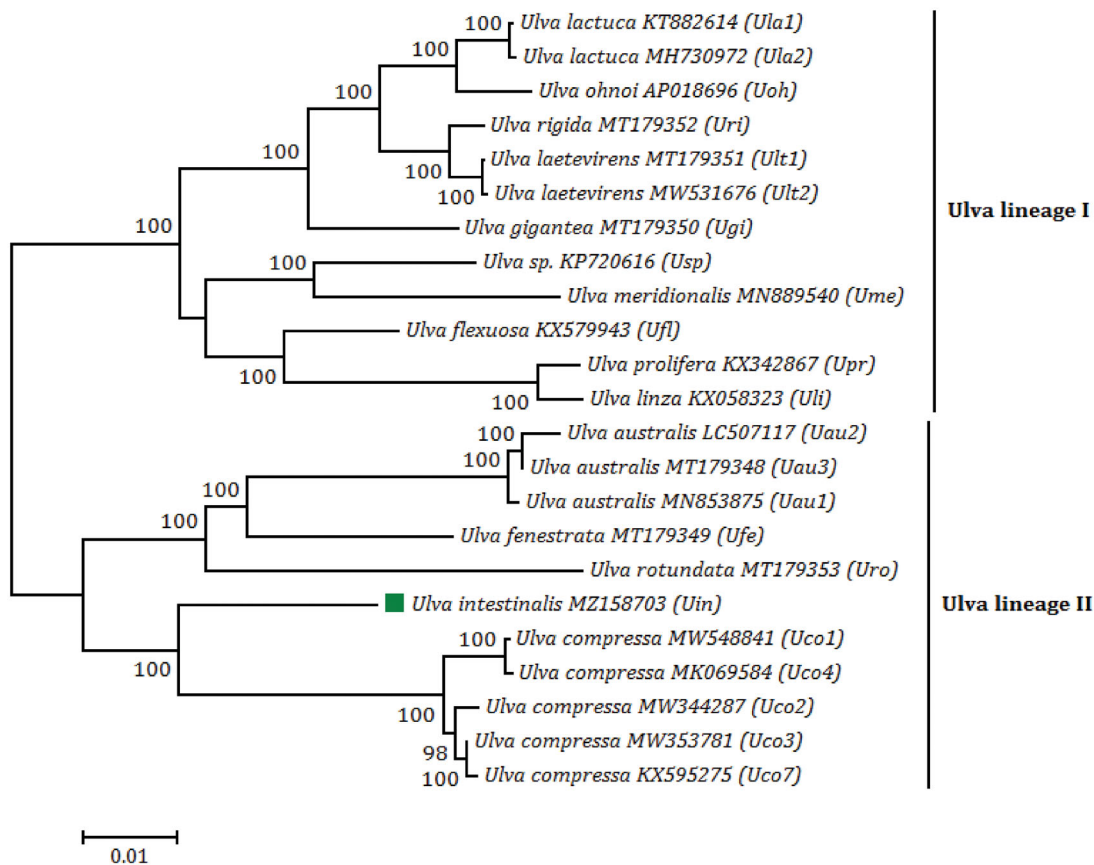


Figure 1. The maximum likelihood (ML) phylogenomic tree was constructed with 1000 bootstrap replicates based on amino acid (aa) sequences of 71 common PCGs from chloroplast genomes of 15 *Ulva* species, using MEGA 7.0. *U. compressa* MK069585 (*Uco5*) and *U. compressa* MT916929 (*Uco6*) were absent in this tree, due to their incomplete chloroplast genomes and faulty assembly of some PCGs (*rpoA*, *rpoB*, *rpoC1*, and *rpoC2*) (Liu and Melton 2021). Branch lengths are proportional to the amount of sequence change, which are indicated by the scale bar below the trees.

with the G + C content of 24.97%. This genome harbored a total of 112 genes including 71 PCGs, 26 tRNAs, three rRNAs, three specific free-standing *orfs*, and nine intronic *orfs* (intron encoded proteins). Ten introns are identified to be present in seven genes including *atpA* (one intron), *infA* (one), *psbB* (three), *psbC* (one), *petB* (one), *rrnL* (two), and *rrnS* (one). Introns embedded in *atpA*, *psbB*, *psbC*, *rrnL*, and *rrnS* belong to group I intron, each of which contained an *orf* encoding a putative LAGLIDADG, or GIY-YIG, or H-N-H homing endonuclease. Introns embedded in *infA* and *petB* belong to group II intron (Liu and Melton 2021). The intron *infA*-62 in *U. intestinalis* is 596 bp in size and its intronic *orf* was completely degenerate. The intron *petB*-69 is 2216 bp in size and harbors an intronic *orf* which encodes a putative reverse transcriptase/maturase (RTM). The three specific *orfs* including *orf103*, *orf136*, and *orf169* had little sequence similarity to any PCGs in the GenBank based on the search of blastp.

The phylogenomic tree is constructed with 1000 bootstrap replicates based on maximum likelihood (ML) analysis of amino acid (aa) sequences of 71 common PCGs from chloroplast genomes of 15 *Ulva* species, using MEGA 7.0. The initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model (Kumar et al. 2016). There were a total of 22,776 positions in the final aa dataset. The

phylogenomic analysis showed that 15 *Ulva* species fell into two clades, *Ulva* lineage I and II, with high bootstrap support values (100%). *U. intestinalis* firstly groups with *Ulva compressa*, and then these two species together with the *Ulva australis*–*Ulva fenestrata*–*Ulva rotundata* subclade forms a monophyletic clade, *Ulva* lineage II (Figure 1). Previously, we found that a locally collinear block of two genes (*psbD*–*psbC*) has been inverted in chloroplast genomes of *Ulva* lineage II including *U. compressa*, *U. australis*, *U. fenestrata*, and *U. rotundata* (Liu and Melton 2021), whereas this reversal event did not occur in *U. intestinalis* cpDNA. The genome structure of *U. intestinalis* cpDNA is consistent with that of most chloroplast genomes in *Ulva* lineage I, excluding *Ulva sp.* and *Ulva meridionalis*, indicating that the similarity of genome structure is not completely related to the genetic relationship of *Ulva* species.

U. intestinalis displays great intraspecific morphological and cytological variation at different growth stages or under different environmental conditions (Leskinen et al. 2004), which makes it difficult to identify species solely based on morphological characteristics. Our genomic data in this study will facilitate the development of specific high-resolution chloroplast molecular markers for rapid identification of the green-tide forming alga, *U. intestinalis*, and help us understand its population diversity and genetic characteristics on a global scale.

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Disclosure statement

The authors are responsible for the content and writing of the paper. No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI under the accession number MZ158703 (<https://www.ncbi.nlm.nih.gov/nuccore/MZ158703.1>). The associated BioProject, SRA, and Bio-Sample numbers are PRJNA728799 (<https://www.ncbi.nlm.nih.gov/bioproject/728799>), SRR14493638 (<https://trace.ncbi.nlm.nih.gov/Traces/sra/?run=SRR14493638>), and SAMN19103542 (<https://www.ncbi.nlm.nih.gov/biosample/SAMN19103542>), respectively.

References

- Cai C, Wang L, Zhou L, He P, Jiao B. 2017. Complete chloroplast genome of green tide algae *Ulva flexuosa* (Ulvophyceae, Chlorophyta) with comparative analysis. *PLOS One*. 12(9):e0184196.
- Chan PP, Lin BY, Mak AJ, Lowe TM. 2021. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucl Acids Res*. gkab688. <https://doi.org/10.1093/nar/gkab688>.
- Fort A, McHale M, Cascella K, Potin P, Usadel B, Guiry MD, Sulpice R. 2021. Foliose *Ulva* species show considerable inter-specific genetic diversity, low intra-specific genetic variation, and the rare occurrence of inter-specific hybrids in the wild. *J Phycol*. 57(1):219–233.
- Guiry MD, Guiry GM. 2021. *AlgaeBase*. World-Wide Electronic Publication. Galway: National University of Ireland [accessed 2021 Aug 24]. <https://www.algaebase.org>.
- Hayden HS, Waaland JR. 2004. A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific. *Phycologia*. 43(4): 364–382.
- Heesch S, Broom JES, Neill KF, Farr TJ, Dalen JL, Nelson WA. 2009. *Ulva*, *Umbraulva* and *Gemina*: genetic survey of New Zealand taxa reveals diversity and introduced species. *Eur J Phycol*. 44(2):143–154.
- Kirkendale L, Saunders GW, Winberg P. 2013. A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism. *J Phycol*. 49(1):69–81.
- Kostamo K, Blomster J, Korpelainen H, Kelly J, Maggs CA, Mineur F. 2008. New microsatellite markers for *Ulva intestinalis* (Chlorophyta) and the transferability of markers across species Ulvaceae. *Phycologia*. 47(6): 580–587.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 33(7): 1870–1874.
- Leskinen E, Alström-Rapaport C, Pamilo P. 2004. Phylogeographical structure, distribution and genetic variation of the green algae *Ulva intestinalis* and *U. compressa* (Chlorophyta) in the Baltic Sea area. *Mol Ecol*. 13(8):2257–2265.
- Liu F, Melton JT. 2021. Chloroplast genomes of the green-tide forming alga *Ulva compressa*: comparative chloroplast genomics in the genus *Ulva* (Ulvophyceae, Chlorophyta). *Front Mar Sci*. 8:668542.
- Liu F, Pang S, Chopin T, Gao S, Shan T, Zhao X, Li J. 2013. Understanding the recurrent large-scale green tide in the Yellow Sea: temporal and spatial correlations between multiple geographical, aquacultural and biological factors. *Mar Environ Res*. 83:38–47.
- Melton JT, Leliaert F, Tronholm A, Lopez-Bautista JM. 2015. The complete chloroplast and mitochondrial genomes of the green macroalga *Ulva* sp. UNA00071828 (Ulvophyceae, Chlorophyta). *PLOS One*. 10(4): e0121020.
- Steinhagen S, Karez R, Weinberger F. 2019. Surveying seaweeds from the Ulvales and Fucales in the world's most frequently used artificial waterway, the Kiel Canal. *Bot Mar*. 62(1):51–61.
- Suzuki S, Yamaguchi H, Hiraoka M, Kawachi M. 2018. Mitochondrial and chloroplast genome sequences of *Ulva ohnoi*, a green-tide-forming macroalga in the Southern coastal regions of Japan. *Mitochondrial DNA B Resour*. 3(2):765–767.
- Titlyanova TV, Titlyanov EA, Xia B, Bartsch I. 2012. New records of benthic marine green algae (Chlorophyta) for the island of Hainan (China). *Nova Hedwigia*. 94(3–4):441–470.
- Wang Z, Xiao J, Fan S, Li Y, Liu X, Liu D. 2015. Who made the world's largest green tide in China?—an integrated study on the initiation and early development of the green tide in Yellow Sea. *Limnol Oceanogr*. 60(4):1105–1117.