frontiers in Marine Science

ORIGINAL RESEARCH
published: 16 March 2022
doi: 10.3389/fmars.2022.850710

Understanding the Evolution of Mitochondrial Genomes in the Green Macroalgal Genus *Ulva* (Ulvophyceae, Chlorophyta)

Feng Liu[1,2,3], James T. Melton III[4], Hongshu Wang[1,2,3], Jing Wang[1,2,3] and Juan M. Lopez-Bautista[5]*

[1] CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China, [2] Marine Ecology and Environmental Science Laboratory, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, China, [3] Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, China, [4] Spelman College, Atlanta, GA, United States, [5] Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, United States

OPEN ACCESS

Edited by:
Jin Liu,
Peking University, China

Reviewed by:
Guang Gao,
Xiamen University, China
Sonia Andrade,
University of São Paulo, Brazil

***Correspondence:**
Feng Liu
liufeng@qdio.ac.cn;
prcliufeng@sina.cn

Specialty section:
This article was submitted to Marine Molecular Biology and Ecology, a section of the journal Frontiers in Marine Science

Received: *08 January 2022*
Accepted: *09 February 2022*
Published: *16 March 2022*

Citation:
Liu F, Melton JT III, Wang H, Wang J and Lopez-Bautista JM (2022) Understanding the Evolution of Mitochondrial Genomes in the Green Macroalgal Genus Ulva (Ulvophyceae, Chlorophyta). Front. Mar. Sci. 9:850710. doi: 10.3389/fmars.2022.850710

To gain more insights into the evolution of mitochondrial genomes (mitogenomes or mtDNAs) in the green macroalgal genus *Ulva* (Ulvophyceae, Chlorophyta), we sequenced seven *Ulva* mitogenomes from six species as well as one *Percursaria* mitogenome as outgroup, and compared them with the available *Ulva* mtDNA data. Our comparative analyses unveiled many novel findings. First, the *Ulva* mitogenomes shared a total of 62 core genes including 29 protein-coding genes (PCGs), three ribosomal RNA genes (rRNAs), 26 transfer RNA genes (tRNAs), three conserved free-standing open reading frames (*orf*s), and one putative RNA subunit of RNase P (*rnpB*). The *rrn5* gene previously unrecognized is present in all sequenced ulvalean mitogenomes, which is situated between *trnG(ucc)* and *trnW(cca)*. Second, the evolution of tRNAs in *Ulva* mitogenomes is related to different processes, including duplication, transposition, remolding, degeneration, loss and recruitment of tRNAs. The duplication of three tRNAs, i.e., *trnT1(ugu)*, *trnI1(gau),* and *trnM2(cau)*, was observed in *Ulva* mitogenomes. Third, the DNA-directed RNA polymerases (*rpo*s), belonging to single-subunit DNA-dependent RNA polymerase (ssRNAP) family, are common in ulvalean mitogenomes. A total of three full-length and 55 split *rpo*s have been detected in these 33 ulvalean mitogenomes. Fourth, six types of group I/II introns are detected at 29 insertion sites which are related to seven host genes (*atp1*, *cox1*, *cox2*, *nad3*, *nad5*, *rnl*, and *rns*) in these ulvalean mitogenomes. One group IB intron, i.e., intron *cox1*-214 which carried a GIY-YIG homing endonuclease (GHE), was observed for the first time in *Ulva* organelle genomes. Finally, phylogenomic analyses based on mitogenome dataset showed that the *Ulva* was split into two sister clades, representing *Ulva* lineage I and II, which was consistent to the results based on plastid genome dataset. Our study provides more important findings to better understand the evolution of mitochondrial genome in green algae.

Keywords: mitochondrial genome, Ulvophyceae, green algae, group I/II intron, DNA-directed RNA polymerase, mitochondrial 5S rRNA

INTRODUCTION

The green macroalgal genus *Ulva* Linnaeus 1753 (Ulvophyceae, Chlorophyta) is the most speciose genus in the order Ulvales, and harbors at least 86 species accepted taxonomically all over the world (Guiry and Guiry, 2021). *Ulva* species are widely distributed in marine and estuarine environments, and some species could live in fresh water (Mareš et al., 2011; Rybak, 2016). *Ulva* species are known for their rapid, proliferous growth in eutrophic conditions, forming harmful macroalgal blooms known as green tides (Wang et al., 2019). For example, the green tides caused by *Ulva prolifera* have broken out continuously in the Yellow Sea of China for 15 years from 2007 to 2021 (Liu et al., 2013; Wang et al., 2021), which had serious negative impacts on the local economy and ecosystem (Ye et al., 2011; Wang et al., 2015). Morphological characteristics of *Ulva* species are highly variable at the intraspecific level, due to different environmental conditions (Blomster et al., 2002; Gao et al., 2016; Liu F. et al., 2020). Phylogenetic methods based on common DNA markers, e.g., the nuclear internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) and the chloroplast RUBISCO LSU (*rbc*L) gene, are more reliable for species identifications in *Ulva* (Hayden and Waaland, 2004; Hughey et al., 2019).

Recently, organelle genomes (mtDNAs and cpDNAs) as potential molecular markers have been proved to be a valuable tool as for phylogenetic and evolutionary studies to understand the molecular species concepts and species boundaries in the genus *Ulva* (Fort et al., 2020; Liu F. et al., 2020; Liu and Melton, 2021). Mitochondria, which evolved from a single endosymbiotic event involving an α-proteobacterium-like ancestor (Gray et al., 2001; Martin et al., 2015), apparently remained more faithful to their eukaryotic host compared with plastids which were acquired via primary or secondary or higher-order symbiotic events (Keeling, 2010). Compared with the common DNA markers, the mitochondrial genome (mitogenome or mtDNA) with rich genetic information can accurately reflect the evolutionary history of nuclear genome (Burger and Nedelcu, 2012), and systematically describe intraspecific and interspecific evolutionary relationships at various taxonomic levels (e.g., Joardar et al., 2012; Park et al., 2015).

Mitogenome data are currently growing at an accelerated pace using faster high-throughput sequencing technologies. Thus far, a total of 25 mitogenomes from 15 *Ulva* species have been sequenced and deposited in the GenBank database (**Table 1**). On the whole, the sequenced *Ulva* mitogenomes were highly conserved in content of core genes, gene order, and genome architecture at the intragenus level (e.g., Liu and Pang, 2016; Melton and Lopez-Bautista, 2016; Zhou et al., 2016a,b; Liu M. et al., 2020). Some important findings have been unveiled previously in the sequenced *Ulva* mitogenomes. The content of group I/II introns varied greatly at the intraspecific level (e.g., *Ulva australis* and *Ulva compressa*), due to the inconsistent dispersal or invasion of introns as self-splicing and mobile genetic elements (Liu et al., 2017; Liu F. et al., 2020). The frequent integration and rapid turnover of foreign DNA fragments which usually contain specific open reading frames (*orf*s) and transfer RNA genes (tRNAs) caused the variations in gene content at both intraspecific and interspecific levels (Liu F. et al., 2020). Many repeat sequences are scattered in *Ulva* mitogenomes and change rapidly even at the intraspecific level (Hanyuda et al., 2016; Cai et al., 2018a,b). Genome rearrangement causes changes in the distribution of core genes, from coding on one strand to two strands (Liu F. et al., 2020).

With the accumulation of *Ulva* mitochondrial genome data, intraspecific and interspecific comparisons could be carried out more systematically. In the present study, we sequenced seven *Ulva* mitogenomes from six distinct species including *Ulva flexuosa* (*Ufl1* and *Ufl2*), *Ulva torta* (*Uto*), *Ulva prolifera* (*Upr1*), *Ulva intestinalis* (*Uin*), *Ulva* sp. TM637 (*Usp1*), and *Ulva* sp. TM708 (*Usp2*), as well as one *Percursaria* mitogenome as an outgroup. These newly sequenced mitogenomes as well as the available *Ulva* mtDNA data from the GenBank database were comparatively analyzed to reveal more details of mitochondrial genome evolution.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Three algal samples including *Ulva flexuosa* (*Ufl1*), *Ulva prolifera* (*Upr1*), and *Ulva intestinalis* (*Uin*) collected in China were transported to laboratory in coolers (5–8°C) after sampling (**Supplementary Table 1**). Fresh tissue from one individual thallus was used for DNA extraction using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Four algal samples including *Ulva torta* (*Uto*), *U. flexuosa* (*Ufl2*), *Ulva* sp. TM637 (*Usp1*), and *Ulva* sp. TM708 (*Usp2*) from the United States were preserved in silica gel after collection and as herbarium vouchers, which were submitted to the University of Alabama Herbarium (UNA) (**Supplementary Table 1**). The algal thallus (UTEX LB 1423) of *Percursaria percursa* (*Ppe*) was from the UTEX Culture Collection of Algae[1]. Dried tissue from one individual thallus for United States samples was used to extract DNA with a Qiagen Plant DNA Extraction Kit (QIAGEN, Valencia, CA, United States). Species identification was performed based on phylogenetic analyses of two common marker datasets (the nuclear ITS region and the chloroplast *rbc*L gene) (Liu et al., 2013).

DNA Sequencing and Mitogenome Assembly

The concentration and quality of isolated DNA were measured with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States). For the samples from China, the purified DNA was fragmented into 350 bp and used to construct short-insert libraries. The short fragments were sequenced using an Illumina HiSeq 4000 sequencing platform. For the samples from the United States, paired end reads (150 bp) were sequenced at Cold Spring Harbor Laboratory on an Illumina MiSeq platform. Poor quality sequences and sequencing adapters were removed using Trim Galore! v0.3.7. Eight ulvalean

[1]https://utex.org/products/utex-lb-1423

TABLE 1 | General features of 33 ulvalean mitochondrial genomes from 19 *Ulva* species and one *Percursaria* species for comparative analysis.

Lineage	Species	Abbr.	Accession number	Size (bp)	A + T (%)	Core genes	Introns	Specific genes	References
						PCGs/rRNAs/tRNAs/*orf*s/*rnpB*	(I/II)	*rpo*s/tRNAs/*orf*s	
Ulva I	*Ulva torta*	*Uto*	MH013471	65,772	65.86	29/3/28/3/1	6 (4/2)	3/0/4	This study
	Ulva flexuosa	*Ufl1*	KY626326	71,527	65.84	29/3/28/3/1	11 (6/5)	2/1/5	This study
	Ulva flexuosa	*Ufl2*	MH013470	63,526	65.31	29/3/28/3/1	7 (3/4)	0/0/1	This study
	Ulva flexuosa	*Ufl3*	KX455878	71,545	65.84	29/3/28/3/1	11 (6/5)	2/1/4	Cai et al., 2018b
	Ulva meridionalis	*Ume*	MN861072	>62,887	65.80	29/3/28/3/1	7 (3/4)	2/1/4	Kang et al., 2020
	Ulva prolifera	*Upr1*	MZ438677	63,843	66.04	29/3/28/3/1	9 (6/3)	0/2/2	This study
	Ulva prolifera	*Upr2*	KT428794	63,845	66.04	29/3/28/3/1	9 (6/3)	0/2/2	Liu and Pang, 2016
	Ulva prolifera	*Upr3*	KU161104	61,962	66.14	29/3/28/3/1	9 (6/3)	0/1/0	Zhou et al., 2016a
	Ulva linza	*Uli*	KU189740	70,858	65.39	29/3/28/3/1	13 (7/6)	0/2/1	Zhou et al., 2016b
	Ulva lactuca	*Ula1*	KU182748	62,021	67.77	29/3/29/3/1	4 (3/1)	0/0/3	Liu M. et al., 2020
	Ulva lactuca	*Ula2*	KT364296	61,614	67.51	29/3/29/3/1	4 (3/1)	1/0/3	Melton and Lopez-Bautista, 2016
	Ulva lactuca	*Ula3*	MH763013	>61,125	67.27	29/3/29/3/1	4 (3/1)	0/0/2	Hughey et al., 2019
	Ulva ohnoi	*Uoh*	AP018695	65,326	65.89	29/3/30/3/1	7 (3/4)	0/0/2	Suzuki et al., 2018
	*Ulva lacinulata**	*Ulc*	MT179357	79,723	67.44	29/3/30/3/1	13 (9/4)	3/2/5	Fort et al., 2020
	Ulva sp. A AF-2021*	*Usp4*	MT179358	88,318	66.61	29/3/30/3/1	14 (8/6)	5/2/9	Fort et al., 2020
	Ulva gigantea	*Ugi*	MT179356	66,743	67.04	29/3/28/3/1	11 (7/4)	0/0/2	Fort et al., 2020
	Ulva sp. TM637	*Usp1*	MH013467	67,506	67.54	29/3/28/3/1	7 (6/1)	3/1/1	This study
	Ulva sp. UNA00071828	*Usp3*	KP720617	73,493	67.83	29/3/28/3/1	10 (6/4)	5/0/2	Melton et al., 2015
Ulva II	*Ulva intestinalis*	*Uin*	MZ571476	68,139	65.14	29/3/28/3/1	10 (7/3)	3/0/4	This study
	Ulva compressa	*Uco1*	MH013469	61,700	61.96	29/3/28/3/1	7 (4/3)	0/0/0	Liu F. et al., 2020
	Ulva compressa	*Uco2*	MH093740	62,791	63.52	29/3/28/3/1	5 (4/1)	2/1/3	Liu F. et al., 2020
	Ulva compressa	*Uco3*	KY626327	62,477	63.03	29/3/28/3/1	4 (2/2)	4/1/2	Liu F. et al., 2020
	Ulva compressa	*Uco4*	KX595276	62,311	63.08	29/3/27/3/1	4 (2/2)	4/1/2	Cai et al., 2018a
	Ulva compressa	*Uco5*	MK069586	>66,587	62.27	29/3/28/3/1	6 (2/4)	4/3/1	GenBank
	Ulva compressa	*Uco6*	MK069587	67,021	61.16	29/3/28/3/1	9 (4/5)	0/0/0	GenBank
	Ulva australis	*Uau1*	KX530816	69,333	64.14	29/3/27/3/1	8 (3/5)	1/1/1	Liu et al., 2017
	Ulva australis	*Uau2*	KX530817	64,602	65.11	29/3/27/3/1	6 (3/3)	1/1/1	Liu et al., 2017
	Ulva australis	*Uau3*	MT179354	64,466	65.07	29/3/27/3/1	6 (3/3)	1/1/1	Fort et al., 2020
	Ulva sp. TM708	*Usp2*	MH013468	55,814	66.78	29/3/28/3/1	4 (4/0)	0/0/1	This study
	Ulva fenestrata	*Ufe*	MT179355	59,026	64.63	29/3/29/3/1	4 (1/3)	0/1/0	Fort et al., 2020
	Ulva expansa	*Uex*	MH730971	64,143	65.87	29/3/28/3/1	10 (5/5)	2/0/0	Hughey et al., 2018
	*Ulva rigida**	*Uri*	MT179359	88,416	63.58	29/3/28/4/1	9 (2/7)	9/0/6	Fort et al., 2020
Outgroup	*Percursaria percursa*	*Ppe*	MZ911851	>59,664	66.80	29/3/28/3/1	7 (5/2)	1/0/0	This study

**Ulva laetevirens (MT179357), Ulva rigida (MT179358), and Ulva rotundata (MT179359) were corrected to Ulva lacinulata (MT179357), Ulva sp. (MT179358), and Ulva rigida (MT179359), respectively (Fort et al., 2020).*

mitogenomes were constructed using a combination of *de novo* and reference-guided assemblies. The mitogenome of *U. prolifera* (KT428794) was used as the reference genome for assembly. Mitogenome assembly was done with both A5 (Tritt et al., 2012) and Geneious R7 (Kearse et al., 2012). The mtDNA assembly was examined using the MEM algorithm of BWA v0.7.17 (Li and Durbin, 2010), and the mutation sites were verified using VarScan v2.3.9 (Koboldt et al., 2009). Incomplete genomes were closed by iteratively mapping the trimmed reads on to the contig with the Geneious 7.1 software (Biomatters[2]).

Genome Annotation

Protein-coding genes (PCGs) were annotated by Open Reading Frame (ORF) Finder at the National Center for Biotechnology Information (NCBI) website[3], DOGMA (Wyman et al., 2004) and ORF finder in the Geneious 7.1 software. Transfer RNA genes (tRNAs) were searched for by reconstructing their cloverleaf structures using the tRNA scan-SE 1.21 software with default parameters (Chan et al., 2021). The large and small subunit ribosomal RNA genes (rRNAs) were identified by comparing newly sequenced ulvalean mtDNAs with homologous rRNA genes from the known *Ulva* mtDNAs deposited in the GenBank database, respectively (**Table 1**). The 5S rRNA gene (*rrn5*) was found by the RNAweasel Tool[4] and RNA Folding (Zuker, 2003). Intron insertion-sites were identified manually based on the alignments of nucleotide (nt) sequences for homologous genes with or without introns from these 33 ulvalean mitogenomes. The corresponding genes (*atp1*, *cox1*, *cox2*, *nad3*, *nad5*, *rnl,* and *rns*)

[2]http://www.geneious.com

[3]https://www.ncbi.nlm.nih.gov/orffinder

[4]https://megasun.bch.umontreal.ca/cgi-bin/RNAweasel/RNAweaselInterface.pl

in the *U. compressa* (KY626327) (*Uco3*) mitogenome were used as a reference (Liu F. et al., 2020). Intron name was defined as host gene plus insertion site. The free-standing and intronic *orf*s greater than 300 bp were found by Open Reading Frame Finder at the NCBI website. The class and core structure of all these introns were determined using the software RNAweasel and RNA Folding. We observed that some annotations were incomplete or incorrect in the *Ulva* mitogenome data deposited in the GenBank database. In order to ensure the accuracy of our comparative analysis, we re-annotated all of the deposited *Ulva* mitogenomes with the same method.

Phylogenetic Analysis of the Core RNA Polymerase Domains

The aa sequences of DNA-dependent RNA polymerase genes (*rpo*s) were searched based on three full-length *rpo* genes found in *Ulva* mitogenomes as a query dataset through the BLAST program at the NCBI website[5]. A total of 45 closest Rpo proteins were obtained from the GenBank database for phylogenetic analysis. The core Rpo domains in these 48 Rpo proteins were determined by significant Pfam-A matches (Punta et al., 2012). Multiple sequence alignments of core Rpo domains were conducted using ClustalX 1.83 with the default settings (Thompson et al., 1997). To avoid phylogenetic artifacts caused by convergent base composition induced by synonymous substitutions (Cox et al., 2014), the ML phylogenetic tree was constructed based on aa sequences of core Rpo domains with 1,000 bootstrap replicates using MEGA 7.0 (Kumar et al., 2016). The phylogenetic relationships were inferred based on the Jones et al. w/freq. model (Jones et al., 1992). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. There was a total of 902 positions in the final dataset of Rpos.

Phylogenetic Analysis of the Reverse Transcriptase Domains in Group IIA/IIB Introns

In group IIA/IIB introns of *Ulva* organelle genomes, the intron-encoded protein (IEP or intronic *orf*) was one reverse transcriptase/maturase (RTM). Three novel group IIA introns including intron *atp1*-1095, *cox1*-312, and *nad5*-1057 have been found for the first time in *Ulva* mitogenomes. To further understand the relationships between the new RTMs and those previously reported (Liu F. et al., 2020; Liu and Melton, 2021), we performed the phylogenetic analysis of the conserved reverse transcriptase (RT) domains which were determined by significant Pfam-A matches (Punta et al., 2012). The amino acid (aa) sequences of 94 RT domains (54 in mtDNAs and 40 in cpDNAs) from group IIA/IIB introns were subjected to concatenated alignments using ClustalX 1.83 with the default settings (Thompson et al., 1997). Maximum Likelihood (ML) phylogenetic tree was constructed for the RT dataset based on the Jones et al. w/freq. model (Jones et al., 1992) with 1,000 bootstrap replicates using MEGA 7.0 (Kumar et al., 2016). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. There was a total of 330 positions in the final dataset of RT domains.

Comparative Genomic and Phylogenomic Analyses

Base composition of these 33 ulvalean mtDNAs was determined using MEGA 7.0 (Kumar et al., 2016). The nt sequences of 61 genes (including 29 PCGs, three rRNAs, 26 tRNAs, and three conserved *orf*s) and the aa sequences of 32 genes (including 29 PCGs and three *orf*s) were subjected to concatenated alignments using ClustalX 1.83 with the default settings, respectively (Thompson et al., 1997). For the nt sequence dataset of the 61 genes, the evolutionary history was inferred by using the ML method based on the Tamura-Nei model (Tamura and Nei, 1993). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. For the aa sequence dataset of the 32 genes, the evolutionary history was inferred by using the ML method based on the Jones et al. w/freq. model (Jones et al., 1992). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using a JTT model. There was a total of 39,070 and 10,491 positions in the final nt and aa datasets, respectively. Phylogenomic analysis was conducted with 1,000 bootstrap replicates using MEGA 7.0 (Kumar et al., 2016).

RESULTS AND DISCUSSION

Mitogenome Features and Gene Content

Eight newly sequenced ulvalean mitochondrial genomes (seven *Ulva* mtDNAs and one *Percursaria* mtDNA) were acquired in this study and compared with 25 known *Ulva* mitogenomes to understand the evolution of *Ulva* mtDNAs. The *Ulva* mitogenomes ranged in size from the smallest one, 55,814 bp in *Ulva* sp. TM708 (*Usp2*), to the largest one, 88,416 bp in *U. rigida* (*Uri*) (**Table 1**). The *Usp2* mtDNA is the second smallest mitochondrial genome in Ulvophyceae to date, which lies between the 45,971-bp mitogenome of *Codium fragile* (Bryopsidales) and the 56,761-bp mitogenome of *Oltmannsiellopsis viridis* (Oltmansiellopsidales) (Pombert et al., 2004, 2006). All ulvalean mitogenomes were biased toward A + T nucleotides and the A + T content ranged from 61.16% in *U. compressa* (*Uco6*) to 67.83% in *Ulva* sp. (*Usp3*).

These 33 ulvalean mitogenomes shared a total of 62 core genes including 29 PCGs, three rRNAs (*rnl*, *rns* and *rrn5*), 26 tRNAs, three conserved free-standing *orf*s, and one putative RNA subunit of RNase P (*rnpB*) (**Table 2**). The homologs of these three conserved *orf*s share identical positions in all sequenced ulvalean mtDNAs, but based on blastp and Pfam, we can not determine their function. In mitogenome of *U. rigida* (*Uri*), the conserved *orf* located between *rps2* and *trnL1(uaa)* was split into two *orf*s, *orf326* and *orf199* (**Figure 1**). All these core genes

[5]http://www.ncbi.nlm.nih.gov/blast

TABLE 2 | Functional classification of genes (including *orf*s) identified among these 33 ulvalean mitochondrial genomes.

Functional classification	Genes
rRNAs (3)*	*rnl*, *rns*, *rrn5*
Core tRNAs (28)	
Conserved tRNAs (26)	*trnA1(ugc)*, *trnC(gca)*, *trnD(guc)*, *trnE(uuc)*, *trnF(gaa)*, *trnG(ucc)*, *trnH(gug)*, *trnI1-1,2(gau)*, *trnK1(uuu)*, *trnL1(uaa)*, *trnL2(uag)*, *trnM1(cau)*, *trnM2-1,2(cau)*, *trnM3(cau)*, *trnN1(guu)*, *trnP1(ugg)*, *trnQ(uug)*, *trnR1(ucu)*, *trnR2(gcg)*, *trnR3(ucg)*, *trnS1(gcu)*, *trnS2(uga)*, *trnT1-1,2(ugu)*, *trnV1(uac)*, *trnW(cca)*, *trnY1(gua)*
Degraded or functionally altered tRNAs (2)**	*trnS3(cga)*, *trnX1/I2(uau)/K2(uuu)/P2(ugg)/V2(uac)*
Specific tRNAs (10)	*trnA2(agc)*, *trnY2(gua)*, *trnL3(caa)*, *trnK3(uuu)*, *trnL4(uag)*, *trnL5(caa)*, *trnX2*, *trnX3*, *trnX4*, *trnX5*
Miscellaneous RNAs (1)	*rnpB*
Core PCGs and *orf*s (32)	
Complex I (8)	*nad1, nad2, nad3, nad4, nad4L, nad5, nad6, nad7*
Complex III (1)	*cob*
Complex IV (3)	*cox1, cox2, cox3*
Complex V (5)	*atp1, atp4, atp6, atp8, atp9*
Ribosomal proteins (12)	*rpl5, rpl14, rpl16, rps2, rps3, rps4, rps10, rps11, rps12, rps13, rps14, rps19*
Conserved *orf*s with unknown function (3)***	*orf500*, *orf312*, *orf225*
Specific PCG (1)	
DNA-dependent RNA polymerase (1)	*rpo*
Specific *orf*s	(see **Supplementary Table 3**)

**Numbers within parentheses indicate the number of genes in a specific functional group. **Among these 33 ulvalean mitogenomes, the countparts of trnX1/I2/K2/P2/V2 were lost only in the mtDNAs of U. compressa (Uco4) and U. australis (Uau1-3), and the trnS3(cga) gene has undergone more severe degradation or even loss in the mtDNA of U. rigida (Uri). ***The orf500, orf312, and orf225 from the Ulva torta mtDNA were used to represent three conserved orfs detected in ulvalean mtDNAs.*

are coded on the same strand, and have the identical gene order in these ulvalean mitogenomes with the exception of one *U. compressa* mitogenome (*Uco1*), in which a collinear block of eight genes (*rps11-rps19-rps4-rpl16-trnR3-trnQ-trnE-trnS3*) has been inverted (Liu F. et al., 2020).

It is worth noting that the mitochondrial 5S rRNA gene (*rrn5*) previously unrecognized in *Ulva* mitogenomes was situated between *trnG(ucc)* and *trnW(cca)* in all sequenced ulvalean mitogenomes, while it was flanked by *rnl* and *trnI(gau)* in ulotrichalean mtDNAs. The sequences of domain β were conserved among the mitochondrial 5S rRNAs in Ulvales, but were different from the counterparts in Ulotrichales. Similar to that in mtDNAs of brown algae and some Ochrophyta lineages, the mitochondrial *rrn5* gene in Ulvales was folded into a secondary structure by adopting a permuted triskelion shape (Valach et al., 2014; **Figure 2A**). We observed that the 5S rRNAs in ulvalean mtDNAs displayed a much larger structural variability than these in other lineages. The interior loop B in stem C is very asymmetric (**Figure 2B**), which makes it difficult to recognize the *rrn5* genes. The sequences (approximately 35 bp) of domain γ including helix V, loop E, helix IV and loop D were highly conserved among *rrns* genes in not only Ulvales but also Ulotrichales. However, the homologous sequence was not detected in the mitogenomes of Bryopsidales and Oltmannsiellopsidales, as might be due to the sequence divergence, compositional bias and/or structural deviation of mitochondrial 5S rRNA genes (Valach et al., 2014).

The putative RNA subunit of mitochondrial RNase P (*rnpB*) could be detected in ulvalean mitogenomes using RNAweasel, which was situated in the latter part of the conserved *orf* (e.g., *orf500* in *U. torta*) between *rps2* and *trnL1(uaa)*. The *rnpB* gene is encoding the RNA component of RNase P which participates in the generation of mature 5′-ends of tRNAs by cleaving the 5′-leader elements of tRNA precursors (Daoud et al., 2012; Shaukat et al., 2021). The highly variable sequence of *rnpB* makes it difficult to be found in mtDNAs. Thus far, the mitochondrial *rnpB* gene was found only in mtDNAs of the prasinophyte green alga *Nephroselmis olivacea* (Turmel et al., 1999), some ascomycete fungi (Seif et al., 2005) and jakobid protists (Lang et al., 1997; Burger et al., 2013).

Evolution of Transfer RNA Genes in *Ulva* Mitogenomes

The number of tRNA genes in *Ulva* mitogenomes showed slight differences at the interspecific level, ranging from 27 to 30 (**Table 2**). A total of 26 core tRNA genes are highly conserved in terms of both composition and structure, and shared by all sequenced ulvalean mitogenomes. The secondary structure of most tRNAs showed typical clover structures with the exception of the *trnS3(cga)* gene located between *trnE(uuc)* and *nad5*. It seems that *trnS3(cga)* has undergone insertion or deletion mutations in *Ulva* mtDNAs, causing the change of their secondary structures, or even severe degradation in the DHU arm and DHU loop, which led to the loss of this gene in the mtDNA of *U. rigida* (*Uri*). The *trnX1* flanked by *cox3* and *rpl14* displayed variation in the anticodon loop sequences, which caused a marked change in function among different *Ulva* mtDNAs (Liu F. et al., 2020). This tRNA gene has been remolded into *trnK2(uuu)* in *U. flexuosa* (*Ufl1-3*) and *U. meridionalis* (*Ume*); or *trnP2(ugg)* in *U. prolifera* (*Upr1-3*) and *U. linza* (*Uli*); or *trnI2(uau)* in *U. compressa* (*Uco1-3*, *5,* and *6*); or *trnV2(uac)* in *Ulva* sp. TM708 (*Usp2*), *U. fenestrata* (*Ufe*), *U. expansa* (*Uex*), and *U. rigida* (*Uri*). This gene was lost in the mtDNAs of *U. compressa*

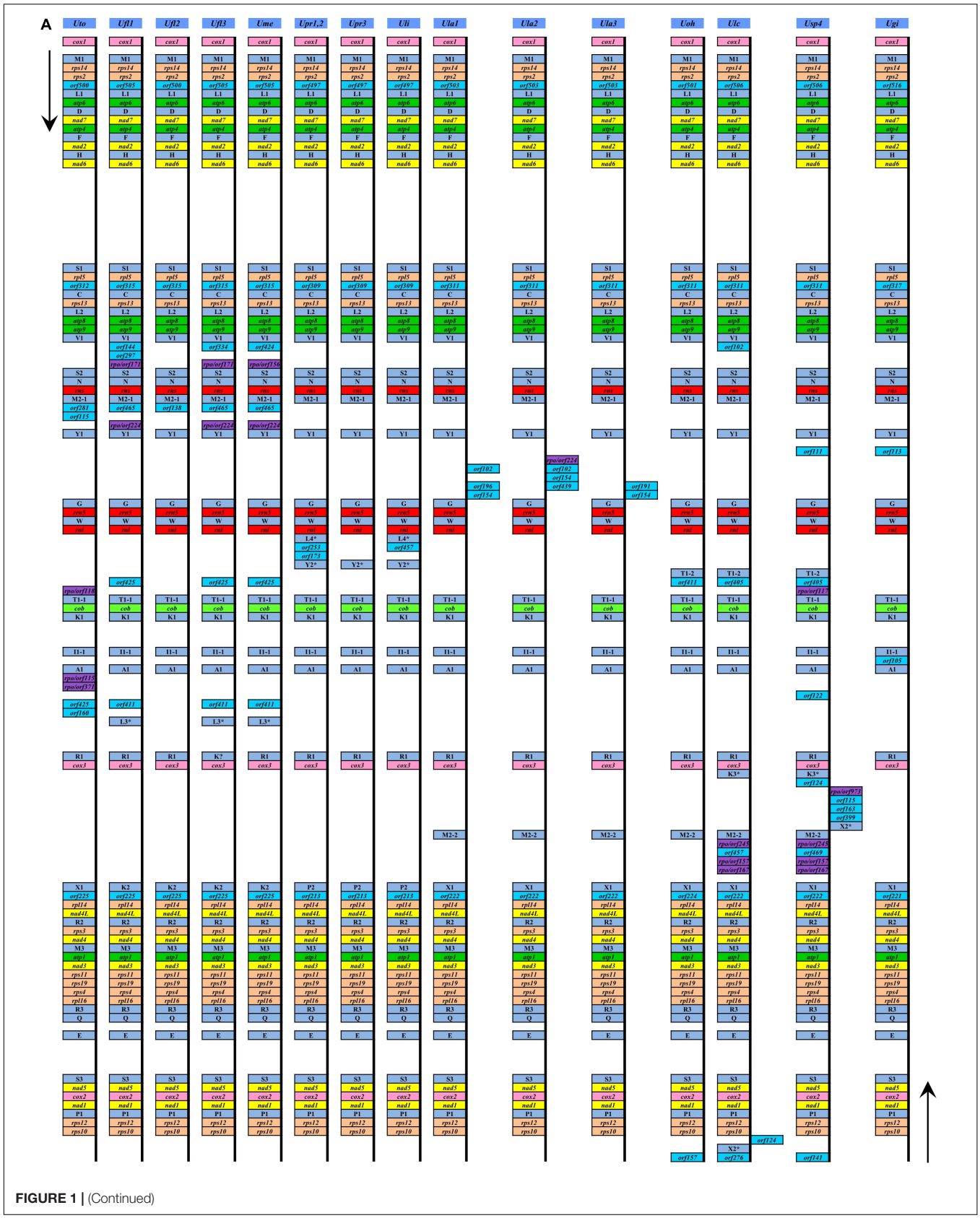


FIGURE 1 | (Continued)

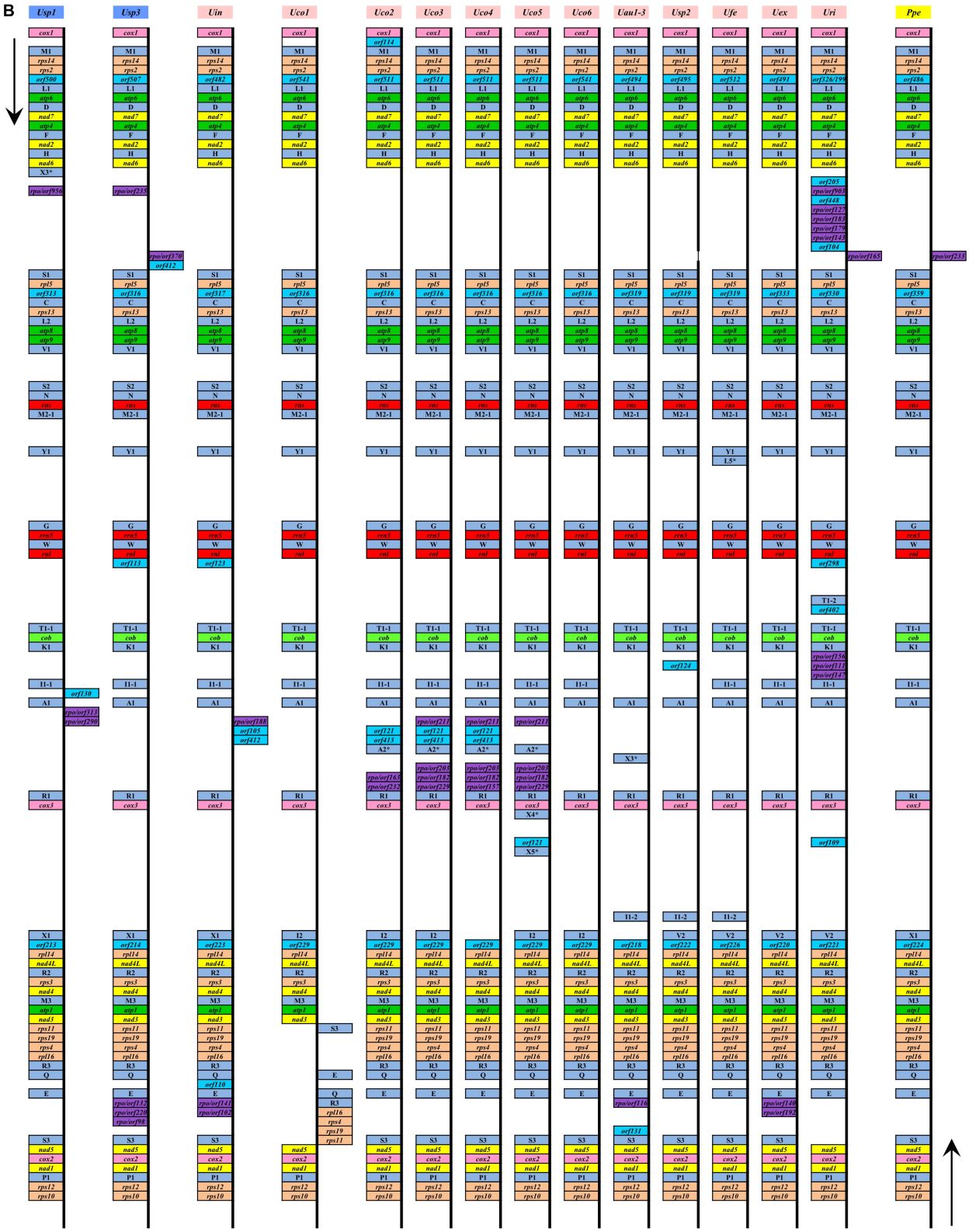
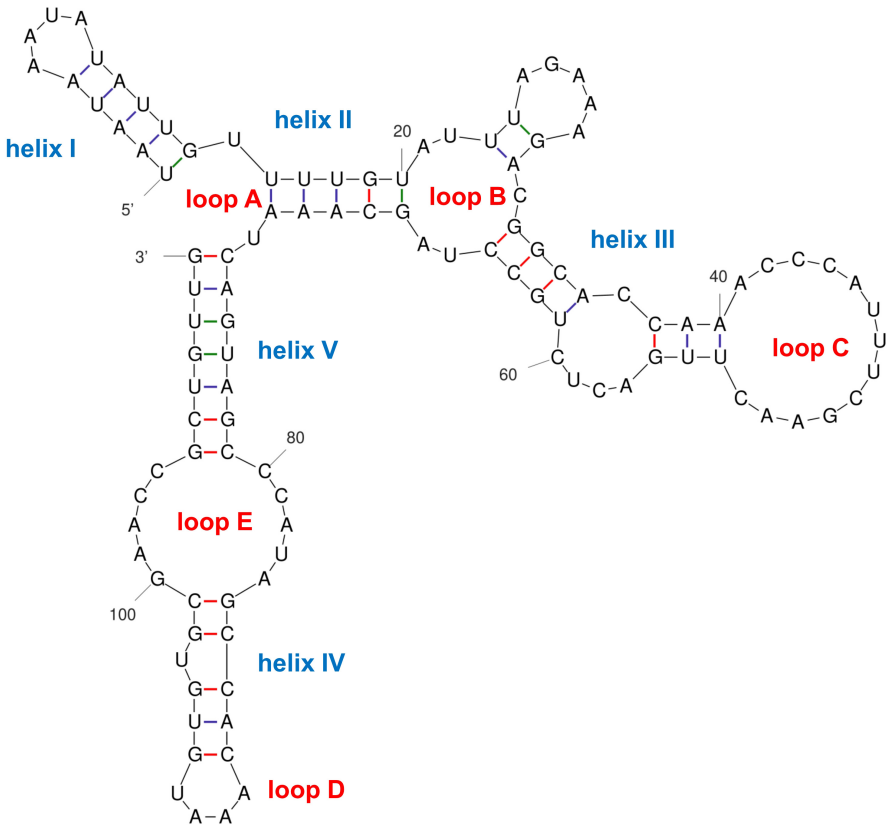


FIGURE 1 | Comparison of genome organization and gene order of these 33 sequenced ulvalean mitochondrial genomes **(A,B)**. The arrows indicated the direction of gene transcription. Different gene types were shown as filled boxes in different colors.

A



B

rrn5	Domain α (5'-)			Domain β									Domain γ (-3')						
	H-I		H-I	L-A	H-II	L-B	H-III	L-C	H-III	L-B	H-II	L-A	H-V	L-E	H-IV	L-D	H-IV	L-E	H-V
Uto	UAAU	UAAUGU	AUUG	U	UUUGU	AUUGAAAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACCCUGCC	UA	GCAAA	U	CAGUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUUG
Ufl1-3	AAU	AAAUGC	AUU	GU	UUUGU	AUUGAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	UA	GCAAA	U	CAGUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUUG
Ume	AAU	AAAUGC	AUU	GU	UUUGU	AUUGAGAAAGA	AGGCACCAA	ACCCAUUUCGAAC	UUGACUUUGCCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCAC	AAAG	GUGUGC	GAACC	GCUGUUG
Upr1-3	UAAUA	AAUA	UAUUG	U	UUUGU	AUUUAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	UA	GCAAA	U	CAGUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUUG
Uli	UAAUA	AAUA	UAUUG	U	UUUGU	AUUUAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	UA	GCAAA	U	CAGUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUUG
Ula1-3	AAUG	AAUA	CAUU	GU	UUUGU	AUUGAGAAAAAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	UA	GCAAA	UC	AAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUU
Uoh	AAUG	AAUA	CAUU	GU	UUUGU	AUUGAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	UA	GCAAA	UC	AAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUU
Ulc	AAUG	AAUA	CAUU	GU	UUUGU	AUUUAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	CA	GCAAA	UC	AAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUU
Usp4	AAUG	AAUA	CAUU	GU	UUUGU	AUUUAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCC	CA	GCAAA	UC	AAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUU
Ugi	AAU	AAAUAC	AUU	GU	UUUGU	AUGGAGAAAGAC	GGCACCAA	ACCCAUUUCGAAC	UUGACUUUGCC	UA	GCGAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUUG
Usp1	AAUA	AACA	UAUU	GU	UUUGU	AUUGAGA	AGGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCCU	A	GCAAA	UC	AAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUU
Usp3	AAUA	AAUA	UAUU	GU	UUUGU	AUAAAAUAAAA	AAGCACCAA	ACCCAUUUAGAAC	UUGACUUUGCUU	A	ACAAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACC	GCUGUUG
Uin	AAUA	AACG	UAUU	GU	UUUGU	AUUGAUAAAAAUA	AGGCACCAA	ACCCAUUUCGAAC	UUGACCCUGCCU	A	GCAAA	UCA	AUAGC	CCAUA	GCCGC	AAAU	GCGUGC	GAACC	GCUGU
Uco1-6	AAUA	AACA	UAUU	GU	UUUGU	AUAUAAAAAAGCA	AGGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCCU	A	ACAAA	UC	AAUAGC	CCAUA	GCCGC	AAAU	GCGUGC	GAACC	GCUGUU
Uau1-3	AAUA	AACA	UAUU	GU	UUUGU	AUUGACGAAAAAGA	AGGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCGC	AAAU	GUGUGC	GAACU	GCUGUUG
Usp2	AAUA	AACA	UAUU	GU	UUUGU	AUUGACGAAAAAUA	AGGCACCAAA	CCCAUUUCGAA	UUUGACUCUGCCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACU	GCUGUUG
Ufe	AAUA	AACA	UAUU	GU	UUUGU	AUUGACGAAAAUA	AGGCACCAA	ACCCAUUUCGAAC	UUGACUCUGCCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACU	GCUGUUG
Uex	AAUA	AACA	UAUU	GU	UUUGU	AUUGACAAAAAUA	AGGCACCAA	ACCCAUUUCGAAC	UUGACCUUGCCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACU	GCUGUUG
Uri	AAUA	AACA	UAUU	GU	UUUGU	AUAUCAAAAAA	AGGCACCAA	ACCCAUUUCGAAC	UUGACCCUGCCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAACU	GCUGUUG
Ppe	UGAU	ACA	AUUG	U	UUUGU	CUUUUAA	AGACACCAA	ACCCAUUCCGAAC	UUGACACUGUCU	A	GCAAA	U	CAAUAGC	CCAUA	GCCAC	AAAU	GUGUGC	GAAUC	GCUGUUG

FIGURE 2 | The permuted 5S rRNA genes (*rrn5*) detected in ulvalean mitochondrial genomes **(A,B)**. **(A)** Potential secondary structure of the mitochondrial 5S rRNA gene in *Ulva prolifera* (*Upr1-3*). **(B)** Alignment of the mitochondrial 5S rRNA sequences in Ulvales. Shaded nucleotides indicated that bases could be paired.

(*Uco4*) and *U. australis* (*Uau1-3*) (Liu et al., 2017; Liu F. et al., 2020). Loss and structural changes of tRNAs at interspecific and intraspecific level reflected their rapid evolution in *Ulva* mtDNAs (Noutahi et al., 2019). The functions of the lost tRNA genes could be replaced by those from the nucleus or other organelles (Adams and Palmer, 2003; Pino et al., 2010).

A

Species	*trnT1-1,2 (ugu)*	Acceptor stem		DHU stem	DHU loop	DHU stem		Anticodon stem	Anticodon loop	Anticodon stem		TΨC stem	TΨC loop	TΨC stem	Acceptor stem
Uto	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAAU	GCCAC	UUAUCGC
Ufl1,3	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAG	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAAU	GCCAC	UUAUCGC
Ufl2	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAG	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Ume	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Upr1-3	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uli	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAG	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Ula1-3	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uoh	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	GAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uoh	T1-2	GCGAUAA	UA	GUUU	AAUGGUA	AAAG	U	UUUGA	UUUGUAA	UCAAA	GAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Ulc	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAAU	GCCAC	UUAUCGC
Ulc	T1-2	GUGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAAU	GCCAC	UUAUCGC
Usp4	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Usp4	T1-2	GUGAUAA	UA	GUUU	AAUGGUA	AAAG	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Ugi	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAU	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Usp1	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Usp3	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uin	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uco1-6	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uau1-3	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Usp2	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Ufe	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CAUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uex	T1-1	GCGAUAA	UA	GUUU	AACGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uri	T1-1	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGU	GCCAC	UUAUCGC
Uri	T1-2	GCGAUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAGG	GCCAC	UUAUCGC
Ppe	T1-1	GCGGUAA	UA	GUUU	AAUGGUA	AAAC	U	UUUGA	UUUGUAA	UCAAA	CGUU	GUGAG	UUCAAAU	GCCAC	UUACGGC

B

Species	*trnI1-1,2 (gau)*	Acceptor stem		DHU stem	DHU loop	DHU stem		Anticodon stem	Anticodon loop	Anticodon stem		TΨC stem	TΨC loop	TΨC stem	Acceptor stem
Uto	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGU	CGCGU	AGUAGUG
Ufl1-3	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGU	CGCGU	AGUAGUG
Ume	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGU	CGUGU	AGUAGUG
Upr1-3	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Uli	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Ula1-3	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Uoh	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Ulc	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGACU	ACUGG	UUCGAGU	CGCGU	AGUAGUG
Usp4	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGACU	ACUGG	UUCGAGU	CGCGU	AGUAGUG
Ugi	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGACU	ACUGG	UUCGAGU	CGCGU	AGUAGUG
Usp1	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Usp3	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGUGU	AGUAGUG
Uin	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAUU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Uco1-6	I1-1	CACUACU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGCGU	AGUAGUG
Uau1-3	I1-2	CACUGCU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACAGG	UUCAAGC	CGUGU	AGUAGUG
Usp2	I1-2	CACUGCU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACAGG	UUCAAGC	CGUGU	AGUAGUG
Ufe	I1-1	CACUGCU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGCC	UUGAUAA	GGCCG	AGGGU	UCAGG	UUCAAGU	CGUGU	AGUAGUG
Ufe	I1-2	CACUGCU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACAGG	UUCAAGU	CGUGU	AGUAGUG
Uex	I1-1	CACUGCU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACAGG	UUCAAGC	CGUGU	AGUAGUG
Uri	I1-1	CGCUACU	UA	GCUU	AAUUGGUCA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACAGG	UUCAAGU	CUUGU	AGUAGCG
Ppe	I1-1	CACUGCU	UA	GCUU	AAUUGGUUA	AAGC	G	CGGUC	UUGAUAA	GGCCG	AGAGU	ACUGG	UUCGAGC	CGAGU	AGUAGUG

C

Species	*trnM2-1,2 (cau)*	Acceptor stem		DHU stem	DHU loop	DHU stem		Anticodon stem	Anticodon loop	Anticodon stem		TΨC stem	TΨC loop	TΨC stem	Acceptor stem
Uto	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Ufl1-3	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Ume	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Upr1-3	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Uli	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Ula1-3	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Ula1-3	M2-2	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCU
Uoh	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Uoh	M2-2	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCU
Ulc	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Ulc	M2-2	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCU
Usp4	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Usp4	M2-2	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCU
Ugi	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAAU	CCUAA	GCCUGCG
Usp1	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Usp3	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCG
Uin	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Uco1-6	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Uau1-3	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Usp2	M2-1	UGCAGGU	UA	GAGU	AAUCGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Ufe	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Uex	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Uri	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA
Ppe	M2-1	UGCAGGU	UA	GAGU	AAUUGGUA	ACUU	G	CUAGG	CUCAUGC	CCUAG	AGAU	UUAGG	UUCAAGU	CCUAA	GCCUGCA

FIGURE 3 | The aligned sequences of three tRNA genes with duplication mutation in *Ulva* mitogenomes. **(A)** *trnT1(ugu)*. **(B)** *trnI1(gau)*. **(C)** *trnM2(cau)*. Shaded nucleotides indicated that bases could be paired.

We found that the tRNA duplication events occurred frequently in the evolution of *Ulva* mitogenomes. The mitogenomes of *U. ohnoi* (*Uoh*), *U. lacinulata* (*Ulc*), *Ulva* sp. (*Usp4*), and *U. rigida* (*Uri*) have two perfect copies of *trnT1(ugu)*, both of which are located between *rnl* and *cob* (**Figure 3A**). Considering that *Uoh*, *Ulc*, and *Usp4* have a relatively distant

relationship with *Uri*, it seems that the duplication of *trnT1(ugu)* happened at least twice independently in *Ulva* mitogenomes. The *trnI1-1(gau)* located between *trnK1(uuu)* and *trnA1(ugc)* was duplicated in *U. fenestrata* (*Ufe*), and the *trnI1-2(gau)* has been translocated at the upstream of *trnV2* (**Figure 3B**). Our previous study found that *trnI1(gau)* in *U. australis* (*Uau1-3*) was translocated from the intergenic region of *trnK1-trnA1* to that of *cox3-rpl14* (Liu et al., 2017), which was similar to that in *Ulva* sp. TM708 (*Usp2*). Based on their phylogenetic relationships, it is more reasonable that the transposition of *trnI1(gau)* occurred in common ancestor of the *U. australis-Ulva* sp.-*U. fenestrata* clade after its duplication, and then the previous *trnI1-1(gau)* was subsequently lost in mtDNAs of *Uau1-3* and *Usp2*. In the *U. lactuca-U. ohnoi-U. lacinulata-Ulva* sp. clade, there are two copies of *trnM2(cau)* situated in different locations (**Figure 3C**), i.e., the intergenic regions of *rns-trnY1* and *cox3-trnX1*, respectively, indicating that *trnM2(cau)* experienced the process of duplication and transposition in this clade.

The frequency of some specific tRNAs differed significantly at the interspecific level (**Table 2**). For example, *trnY2(gua)* was observed to exist only in the mtDNAs of *U. prolifera* (*Upr1-3*) and *U. linza* (*Uli*), and *trnX2* was present only in mtDNAs of *U. lacinulata* (*Ulc*) and *Ulva* sp. (*Usp4*). These specific tRNAs are most likely recruited into the mitogenomes through the integration of exogenous DNA fragments (e.g., mitochondrial plasmids) (Handa, 2008). Overall, the evolution of tRNAs in *Ulva* mitogenomes is similar to that in other eukaryotic lineages (e.g., insects, fungi and higher plants), which is related to different evolutionary processes, including duplication, transposition, remolding, degeneration, loss and recruitment of tRNAs (e.g., Lang, 2014; Zhang et al., 2018; Li et al., 2021).

DNA-Dependent RNA Polymerase Genes and Specific Free-Standing Open Reading Frames

Among these specific free-standing *orf*s, a total of 58 *orf*s were annotated as the full-length or split DNA-dependent RNA polymerase genes (*rpo*s) in these 33 ulvalean mitogenomes. Only three *rpo*s were complete, including *orf903* in *U. rigida* (*Uri*), *orf956* in *Ulva* sp. TM637 (*Usp1*) and *orf973* in *Ulva* sp. (*Usp4*), and the left 55 *orf*s ranging in size from 98 to 371 aa seem to be the different remnants after the degradation of the intact *rpo*s (**Figure 4**). Obviously, the rapid mutation accumulation (e.g., insertion and deletion) led to the frameshift and destruction of *rpo*s in these ulvalean mtDNAs. These *rpo*s were detected to be located in nine specific intergenic regions including *nad6-trnS1*, *trnV1-trnS2*, *trnM2-1-trnY1*, *trnY1-trnG*, *rnl-trnT1-1*, *trnK1-trnI1-1*, *trnA1-trnR1*, *cox3-trnX1,* and *trnE-trnS3*, either in a forward or reverse order (**Figure 4**).

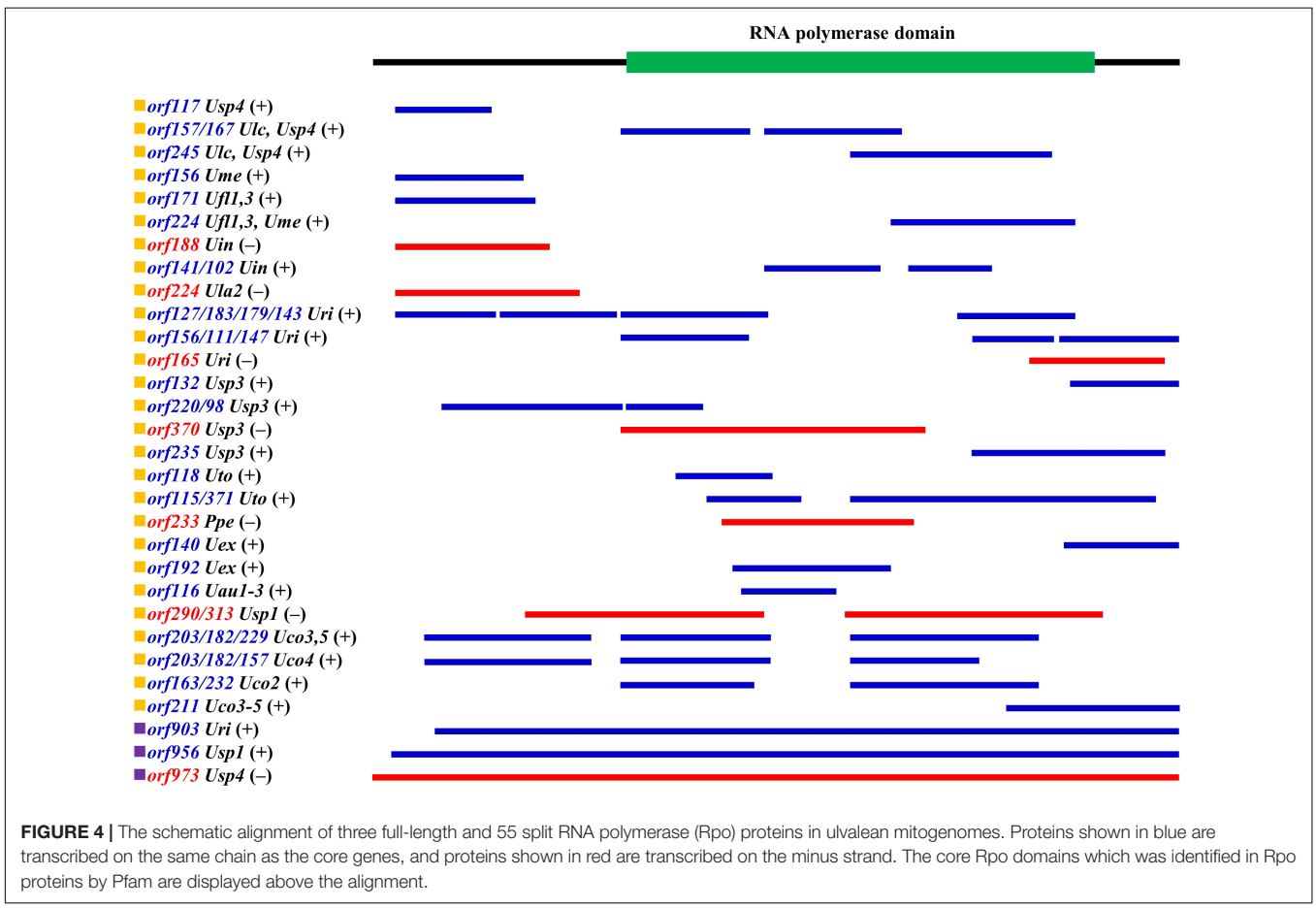


FIGURE 4 | The schematic alignment of three full-length and 55 split RNA polymerase (Rpo) proteins in ulvalean mitogenomes. Proteins shown in blue are transcribed on the same chain as the core genes, and proteins shown in red are transcribed on the minus strand. The core Rpo domains which was identified in Rpo proteins by Pfam are displayed above the alignment.

The *Ulva* mitochondrial Rpos belong to T7-phage-type RNA polymerase which was a group of single-subunit Rpo (ss-Rpo) family. The ss-Rpo family is structurally and evolutionarily distinct from the multi-subunit family of Rpos (including bacterial and eukaryotic sub-families) (Cermakian et al., 1997). *Ulva* mitogenomes lack the ancestral a-proteobacterial polymerase genes, as was the same as almost all eukaryotic mitogenomes except jacobids (Yin et al., 2010; Peralta-Castro et al., 2020). Transcription of mitochondrial proteins involves nuclear or mitochondrial encoded single subunit T7-phage-type Rpos which probably has replaced the a-proteobacterial polymerase. In this regard, the mitogenomes in *Ulva* are completely different from chloroplast genomes which still retain the core subunits (*rpoA*, *rpoB*, *rpoC1,* and *rpoC2*) of the plastid-encoded Rpo derived from their cyanobacterial ancestor (Liu and Melton, 2021).

Based on the blastp searches, the entire T7-phage-type *rpo* genes closely related to those in *Ulva* mtDNAs could be found in the nuclear or mitochondrial genomes of eukaryotes (higher plants, brown algae and fungi), as well as genomes of prokaryotes (bacteria and viruses). A total of 45 Rpo proteins from the GenBank database were finally used for phylogenetic analysis. The ML tree inferring from the analysis of core Rpo domains in the 48 *rpo* genes showed that three *Ulva* full-length Rpos clustered together, representing a Chlorophyta-specific Rpo lineage (**Figure 5**). As found in higher plants and fungi (Clark-Walker, 1992; Handa, 2008; Warren et al., 2016), the observed Rpo genes in *Ulva* mtDNAs most likely come from mitochondrial linear plasmids which carry genes usually coding for an RNA/DNA polymerase or more often for both, as well as other ORFs (Cermakian et al., 1996). These linear plasmids were observed to be sometimes entirely integrated into the mitogenome. The mitogenome of brown alga *Pylaiella littoralis* contains a putative completely integrated linear plasmid which harbors an entire T7-phage-type Rpo gene (Rousvoal et al., 1998; Oudot-Le Secq et al., 2001).

At the interspecific level, Ulvalean mitogenomes sometimes shared some specific homologous *orf*s which were located in different positions in these genomes (**Supplementary Table 3**). For example, among the 17 homologous *orf*s in group 1, which were from four intergenic regions (**Supplementary Table 3**), 13 *orf*s showed sequence similarity to the putative bacterial transposase with alignment scores of approximately 50%, and the left four *orf*s are more like remnants of their degradation. The *orf124* which was only found in mtDNA of *Ulva* sp. TM708 (*Usp2*) contained the transferase hexapeptide (six repeats) of putative bacterial origin. The left specific *orf*s had little sequence similarity to any PCGs in the GenBank database based on the search of blastp.

All sequenced *Ulva* mitogenomes shared the same set of core genes, but they showed great variations in the content of specific genes including *rpo* genes, specific *orf*s (more than 100 codons) and recruited tRNAs (**Tables 1**, **2**). These variations are mainly caused by different acquisitions of foreign DNA fragments from diverse sources including nucleus, plastids, bacteria, viruses, and mitochondrial plasmids (e.g., Wang et al., 2007; Gandini and Sanchez-Puerta, 2017; Liu F. et al., 2020). The integrations of foreign fragments in *Ulva* mtDNAs lead to the generation of large specific intergenic regions. Nine intergenic regions flanked by core genes vary greatly in size and sequence even at intraspecific levels, indicating that these hot spot regions are undergoing drastic dynamic changes which involve the recent capture of exogenous DNA fragments (Liu and Melton, 2021) and the frequent mutation or loss of intergenic regions which may occur through recombination-excision processes or by slipped-strand mispairing for small regions (Clark-Walker, 1992). Based on these hot spot regions, specific DNA markers could be designed and developed to specifically identify species or populations in the genus *Ulva*.

Diversity and Evolution of *Ulva* Mitochondrial Introns

Obviously, these sequenced ulvalean mitogenomes show great changes in intron content from four introns in *Ulva* sp. TM708 (*Usp2*), *U. lactuca* (*Ula1-3*), *U. compressa* (*Uco3* and *4*) and *U. fenestrata* (*Ufe*) to 14 in *Ulva* sp. (*Usp4*) (**Table 3**). Introns account for 8.77% of mitogenome in *Usp2* to 27.80% in *U. linza* (*Uli*) in size. The reported ulvophycean mitogenomes contain a certain number of introns (Pombert et al., 2004, 2006; Melton et al., 2015; Turmel et al., 2016), which are not only ribozymes that catalyze their own splicing, but also retroelements that usually harbor an intron-encoded protein (IEP) and can insert themselves into new locations. Most of introns encoded a homing endonuclease or a reverse transcriptase/maturase (RTM) in *Ulva* mtDNAs, while IEPs in some introns degenerated and even completely lost (Liu et al., 2017; Liu F. et al., 2020).

From a comparative analysis of these intron locations, a total of 29 intron insertion sites were detected at seven mitochondrial genes (*atp1*, *cox1*, *cox2*, *nad3*, *nad5*, *rnl,* and *rns*) (**Table 3**). Three types of group I introns including group IB (complete, LHE), group IB (complete, GHE) and group ID, and three of group II introns including group IIA, group IIB and group II (LHE) were detected in these ulvalean mtDNAs. Five intron insertion sites were found for the first time in *Ulva* mitogenomes, two introns (intron *cox1*-214 and *cox1*-900) belonging to group IB and three (intron *atp1*-1095, *cox1*-312, and *nad5*-1057) belonging to group IIA. Intron DNA sequences at the same insertion site were homologous among these ulvalean mtDNAs, and these cognate introns shared the highly conserved RNA secondary structures of ribozyme components. Similar to that in chloroplast genomes, group IB intron was the most prevalent in ulvalean mitogenomes, and was found at ten insertion sites. The LHEs from different families of group IB introns displayed great genetic diversity (Liu and Melton, 2021), but their ribozyme components showed similarity in RNA secondary structures. One group ID intron (intron *cox1*-709) formerly detected to be present only in the *U. prolifera*-*U. linza*-*U. flexuosa*-*Ulva* sp. clade (Liu F. et al., 2020), were found to be scattered in mitogenomes of two *Ulva* lineages (I and II), indicating this group ID intron might be frequently homing or jumping.

In *Ulva* chloroplast genomes, intron-encoded homing endonucleases from three distinct families (LAGLIDADG, GIY-YIG, and H-N-H) have been found in group I introns

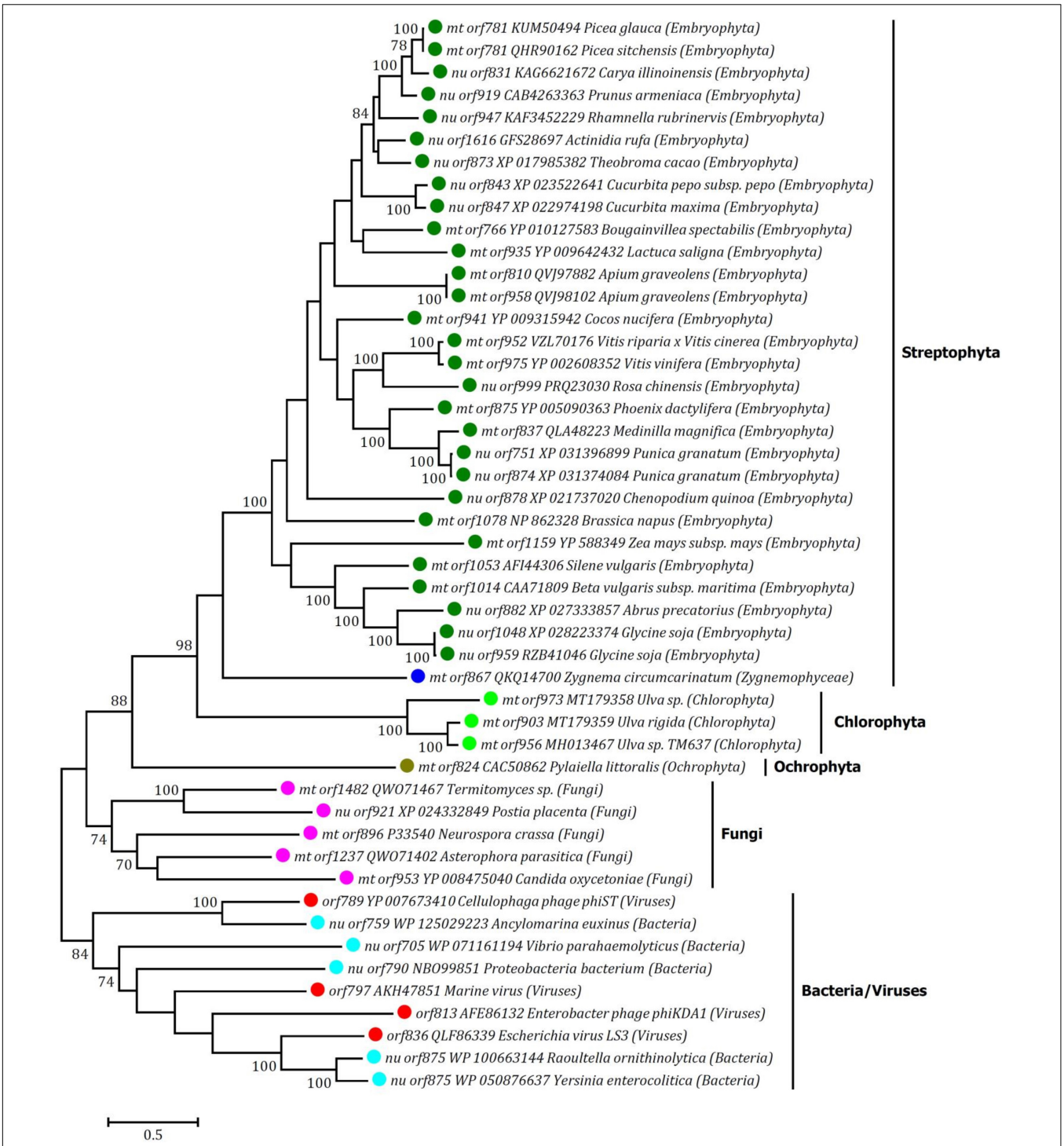


FIGURE 5 | Phylogenetic tree based on Maximum Likelihood (ML) analysis of amino acid (aa) sequences of 48 RNA polymerase (Rpo) domains from Rpo proteins. The ML analysis was conducted with 1,000 bootstrap replicates using MEGA 7.0. The bootstrap support values greater than 70% were displayed at branches. Branch lengths were proportional to the amount of sequence change, which were indicated by the scale bar below the trees. The tree was rooted with Rpo proteins from bacteria and viruses as outgroups.

(Wang et al., 2021), while all ORF-containing group I introns previously known in *Ulva* mitogenomes encode a LAGLIDADG homing endonuclease (LHE). In this study, we found that the IEPs from nine group IB (complete) introns were LAGLIDADG homing endonucleases (LHEs), while intron *cox1*-214 carried a GIY-YIG homing endonuclease (GHE). Group IB (complete,

TABLE 3 | The updated information on insertion site, size and group of introns detected in these 33 ulvalean mitogenomes.

Introns*	*Uto*	*Ufl1*	*Ufl2*	*Ufl3*	*Ume*	*Upr1,2*	*Upr3*	*Uli*	*Ula1,3*	*Ula2*	*Uoh*	*Ulc*	*Usp4*	*Ugi*	*Usp1*	*Usp3*
atp1-540																1,345
atp1-990											2,589					
atp1-1095																
cox1-199			2,495		un			2,525								
cox1-214					un							1,352	1,357			
cox1-281			1,419		un	1,408	1,408	1,406	1,401	1,401		1,408	1,398	1,427	1,428	
cox1-312					un											
cox1-386	1,247	1,291	1,281	1,293	un	1,277	1,276	1,277	1,249	1,248	1,269	1,229	1,229		1,258	1,287
cox1-643					un											
cox1-709		1,594		1,594	un	1,434	1,433	1,434				1,478				1,590
cox1-731**	1,097	1,125	1,125	1,125	un							1,120	1,114	1,119		
cox1-760			2,692		un											
cox1-874		1,591	1,506	1,591	un	1,509	1,509	1,509				1,667		1,424		
cox1-900					un							1,114	1,114	1,129		
cox1-1107		1,128		1,128	>735			1,156	1,109	1,109	1,127	1,165	1,156	1,221	1,129	1,162
cox1-1125		1,139		1,139	1,135	1,117	1,117	1,118			1,134	1,137		1,140	1,120	1,240
cox2-424	2,460										2,539		2,463	2,480		
cox2-751	2,428															
nad3-215																2,727
nad3-216						2,503	2,503	2,516	2,481	2,481	2,498		2,456	1,634		
nad5-800	1,185					1,227	1,227	1,232					1,169	696	1,175	1,309
nad5-1057																
rnl-1963														1,250		
rnl-2080		1,550		1,550	1,545							1,554	1,551			
rnl-2698		1,380		1,380	1,375			1,393				1,359	1,359			
rnl-2747	578	1,197		1,198	1,194	1,202	1,202	1,250				1,138	1,142	797	1,098	
rns-420		1,368		1,368	1,368			1,402				686	1,387			1,319
rns-670		1,004	1,490	1,004	1,049	1,098	1,098	1,481					830		1,424	1,534
rns-780											1,028					2,449

Introns*	*Uin*	*Uco1*	*Uco2*	*Uco3,4*	*Uco5*	*Uco6*	*Uau1*	*Uau2,3*	*Usp2*	*Ufe*	*Uex*	*Uri*	*Ppe*
atp1-540							1,157	1,157	1,338		1,303	1,256	
atp1-990													
atp1-1095												2,547	
cox1-199					2,501								
cox1-214													
cox1-281	1,310												
cox1-312													2,331
cox1-386	1,293	1,446	1,370			1,549	1,270	1,269	1,285	1,268		1,380	1,346
cox1-643		2,508				2,508				2,517			
cox1-709	1,539												
cox1-731**	1,320												1,097
cox1-760							2,604					2,627	2,622
cox1-874	1,510										1,157	1,621	
cox1-900	1,111								1,127				1,105
cox1-1107		1,142	1,195	1,188	1,212	1,142	1,129	1,129	1,145		1,157		1,209
cox1-1125	1,125	1,147	1,148	1,156	1,156	1,147					1,170		
cox2-424		2,561		2,581	2,581	2,560	2,463			2,434		2,541	
cox2-751			2,459	2,460	2,460	2,626	2,380	2,380				2,411	
nad3-215													
nad3-216		2,542			2,507	2,560						2,513	
nad5-800	1,219										1,180		1,247
nad5-1057										2,649		2,631	

(Continued)

TABLE 3 | (Continued)

Introns*	*Uin*	*Uco1*	*Uco2*	*Uco3,4*	*Uco5*	*Uco6*	*Uau1*	*Uau2,3*	*Usp2*	*Ufe*	*Uex*	*Uri*	*Ppe*
rnl-1963							2,364	2,364			1,681		
rnl-2080											1,546		
rnl-2698	1,359												
rnl-2747		1,139	1,149			1,139					566		
rns-420	1,338										619		
rns-670											623		
rns-780						2,438	2,415	2,415					

**Intron insertion-sites were determined by comparing homologous genes relative to the mitogenome of U. compressa (Uco3). Intron name was defined as host gene plus insertion site. Different colored boxes denoted different groups of introns: group IB (complete, LHE), blue; group IB (complete, GHE), yellow; group ID, green; group IIA, red; group IIB, orange; and group II (LHE), pink. **The intron cox1-734 (Liu F. et al., 2020) was corrected to intron cox1-731 in this table.*

GHE) intron was observed to be present only in the mtDNAs of two closely related species, *U. lacinulata* (*Ulc*) and *Ulva* sp. (*Usp4*), indicating that it is most probably the result of an independent insertion event occurring in their common progenitor. In addition, the GHE was also observed to be encoded in group I (derived, A) intron (e.g., intron *psbB*-489, *psbB*-772, *psbC*-882, and *psbD*-740) in *Ulva* chloroplast genomes.

Group IIA/IIB introns were present at eight and five insertion sites in these ulvalean mitogenomes, respectively, and both of them usually encoded an RTM (Dai et al., 2003; Liu F. et al., 2020). All of three newly discovered group II intron, i.e., intron *atp1*-1095, *cox1*-312, and *nad5*-1057, belonged to group IIA intron, indicating that group IIA intron is frequently involved in recent invasion or homing in *Ulva* mitogenomes. Based on phylogenetic analysis of reverse transcriptase (RT) domains in RTMs from *Ulva* mtDNAs and cpDNAs, RTs can be clearly clustered in two clades, representing group IIA and IIB lineages, respectively (**Figure 6**). RT family encoded in intron *nad5*-1057 had a close relationship with that in intron *cox1*-760, while both of RT families encoded in intron *atp1*-1095 and *cox1*-312 showed novel sequence characteristics, respectively, and represented two new intron families. The conserved YXRYADDXXXGXXG catalytic motif in RT segment 5 was shared by RT domains from group IIA introns (**Supplementary Figure 1**), which was much longer than those (RYADD) from group IIB introns (**Supplementary Figure 2**; Bonen and Vogel, 2001). All of group IIA introns (100%) contained an intact tripartite RTM gene in these ulvalean mitogenomes, while the RTMs in some group IIB introns have obviously degenerated or been completely lost, e.g., intron *nad3*-216 and *rnl*-1963 in *U. gigantea* (*Ugi*), and intron *rns*-780 in *U. ohnoi* (*Uoh*). Similar phenomena have been found in *P. littoralis* (Ikuta et al., 2008), but the mechanism by which the integrity of RTMs in group IIA introns is maintained successfully remains an open question. Group IIB introns with incomplete or missing RTMs should retain splicing competence to ensure that housekeeping genes function properly.

Interestingly, some group II introns in *Ulva* mitogenomes did not encode an RTM, but instead encoded an LHE. These mitochondrial LHEs in group II introns have close relationships with that in group IB introns (Liu and Melton, 2021). For a long time, it is considered that there is no genetic evolution relationship between group I and group II introns (Kelchner, 2002; Haugen et al., 2005). These findings indicated that there was a certain genetic relationship between group I and group II introns, at least between group IB (LHE) and group II (LHE) introns. Considering the great differences in ribozyme structure and splicing mechanism between group IB (LHE) and group II (LHE) introns (Seetharaman et al., 2006; Stoddard, 2011), the close genetic relationships of their IEPs are likely related to the evolution processes for both of introns. It appears that group I and II introns might recruit or employ the same IEP components (e.g., LHE) in their evolution, but these processes are still unclear.

Phylogenomic Analysis

Single DNA markers (e.g., ITS, *rbc*L, and 18S rDNA) or combined marker sequences contain limited phylogenetic signals to understand genetic relationships in *Ulva* species. Trees inferred from phylogenetic analysis based on these datasets have important implications for the phylogeny and classification of *Ulva* species (e.g., Hayden et al., 2003; Hayden and Waaland, 2004), but it is still restricted by the number of genetic difference signals in these DNA markers, especially in the differentiation of closely related species (e.g., *U. linza* vs. *U. prolifera*) or intraspecific relationships (Shimada et al., 2010; Liu et al., 2013). Phylogenomic trees based on mitogenome data and/or plastid genome data as well as organelle genome structure information could provide a more comprehensive understanding of evolutionary systematics and molecular species concepts in this morphologically simple group of macroalgae, due to their rich genetic information at the genome level (Liu F. et al., 2020; Liu and Melton, 2021).

The *Ulva* mitogenomes sequenced thus far represent a large portion of the genetic diversity at the intragenus level as they were sampled from each of the major clades in this genus. Phylogenomic analysis using maximum likelihood (ML) method based on the mitochondrial nt and aa sequence datasets showed that the 19 *Ulva* species were divided into two sister clades with strong support values (100%), representing *Ulva* lineage I and II, respectively (**Figure 7**). Many mutation sites in mtDNAs are completely consistent within each of these two lineages, but there are significant differences between these two lineages. For example, a three-base insertion occurred at the 5′ end of *rps14* in all mtDNAs of *Ulva* lineage II, not in the mtDNAs of lineage I and *P. percursa* (*Ppe*) (**Supplementary Figure 3**), and a 15-base insertion was detected in *rps2* of *Ulva* lineage II, not in that of lineage I (**Supplementary Figure 4**). In addition, some genomic features such as gene content, tRNA duplication, distribution of some introns, gene order, and genome rearrangement could

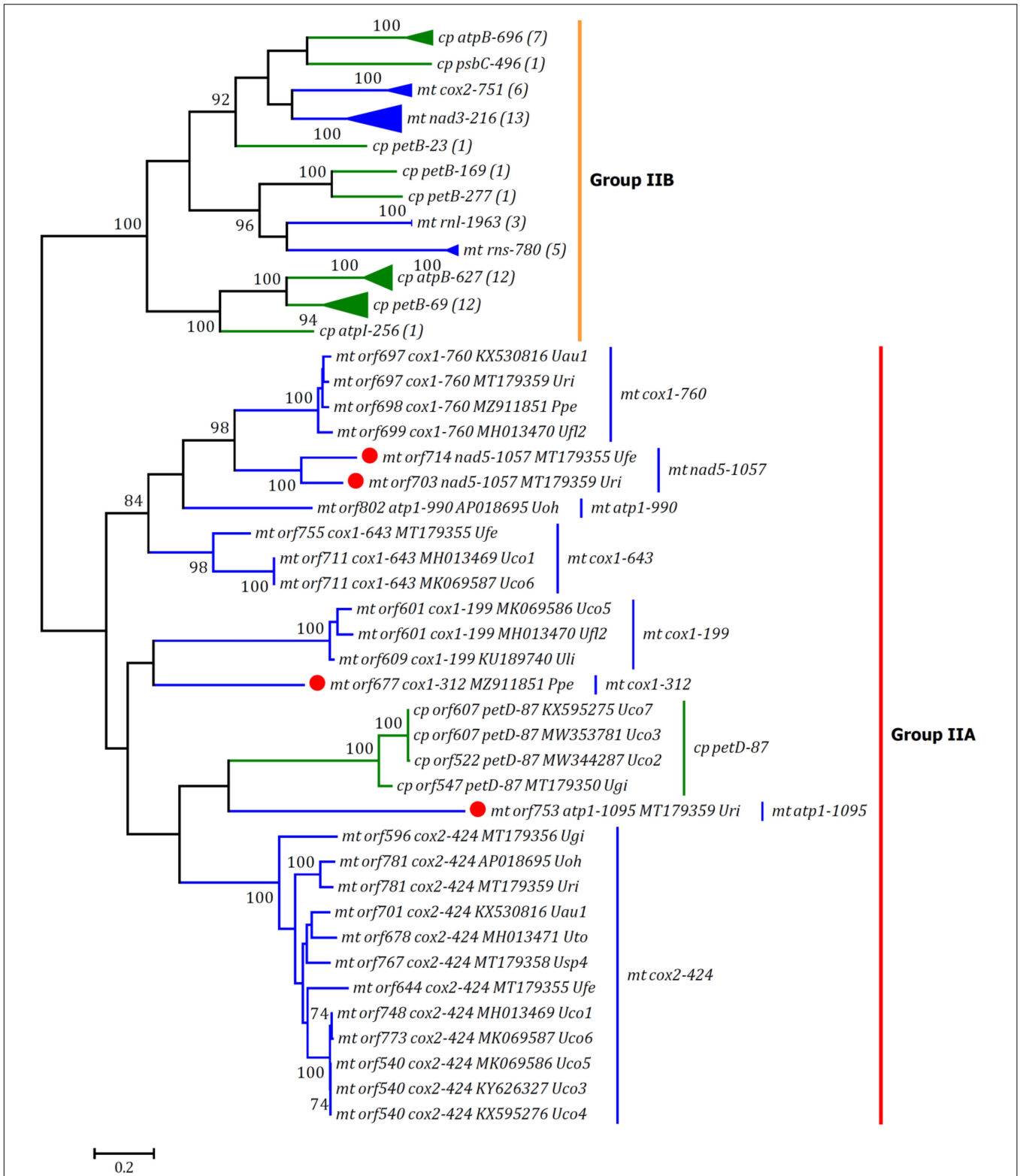


FIGURE 6 | Unrooted phylogenetic tree based on Maximum Likelihood (ML) analysis of amino acid (aa) sequences of 94 reverse transcriptase (RT) domains (54 in mtDNAs and 40 in cpDNAs) from group IIA/IIB introns. RT domains from mtDNAs were shown in blue and RT domain from cpDNAs in green. Numbers within parentheses in group IIB clade indicated the number of RT domains found in each cognate intron family. The ML analysis was conducted with 1,000 bootstrap replicates using MEGA 7.0. The bootstrap support values greater than 70% were displayed at branches. Branch lengths were proportional to the amount of sequence change, which were indicated by the scale bar below the trees.

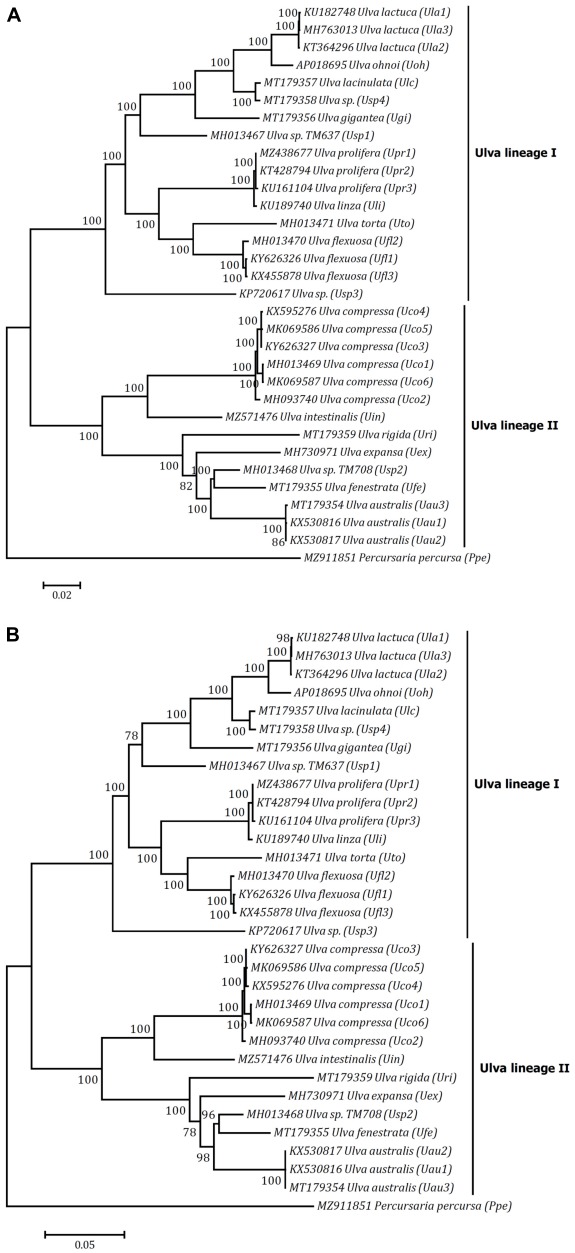


FIGURE 7 | Phylogenomic trees based on Maximum Likelihood (ML) analysis of the nucleotide (nt) sequences of 61 genes **(A)** and the amino acid (aa) sequences of 32 genes **(B)** in 32 ulvalean mitogenomes. The mitogenome of *Ulva meridionalis* (*Ume*) was not included due to its incomplete *cox1* gene. The ML analysis was conducted with 1,000 bootstrap replicates using MEGA 7.0. The bootstrap support values greater than 70% were displayed at branches. Branch lengths were proportional to the amount of sequence change, which were indicated by the scale bar below the trees. The tree was rooted with *Percursaria percursa* (*Ppe*) as an outgroup.

partially support the current evolutionary relationships in lineage I and II. The *trnY2(gua)* was present only in the *U. linza-U. prolifera* (LP) clade, two copies of *trnM2(cau)* were present only in the *U. lactuca-U. ohnoi-U. lacinulata-Ulva* sp. clade, and the GHE-containing group IB introns were found only in the *U. lacinulata-Ulva* sp. clade.

Relationships of taxa in the present tree are generally congruent with those based on plastid genome dataset (Liu and Melton, 2021; Wang et al., 2021). Minor topological differences between trees are most likely due to the different evolutionary rates between two gene datasets from mitogenomes and plastid genomes. Although two monophyletic clades in *Ulva*, namely lineage I and II, were well supported by genomic data (**Figure 7**), few morphological synapomorphies for these two clades were identified in this group of green macroalgae, due to their high degree of phenotypic plasticity caused by environment conditions (Hayden et al., 2003). Both lineage I and II consist of green seaweeds with multiple morphotypes including tubular and blade morphologies. Considering that the genus *Ulva* contains more than 80 known species worldwide as well as many cryptic species, further phylogenetics studies including more taxa is needed to clarify the infrageneric taxonomy and to understand the evolutionary relationships in *Ulva*.

CONCLUSION

Size variations of *Ulva* mitogenomes caused by integration of foreign DNA fragments, gain or loss of group I/II introns, and abundance of repetitive sequences have been well shown at the interspecific and intraspecific level in our previous studies (Liu et al., 2017; Liu F. et al., 2020). This study uncovered many novel important findings in the evolution of *Ulva* mitogenomes. These ulvalean mitogenomes shared a total of 62 core genes including 29 PCGs, three rRNAs, 26 tRNAs, three conserved *orf*s, and one putative *rnpB*. The *rrn5* gene previously unrecognized is present in all ulvalean mitogenomes, and this gene is folded into a secondary structure by adopting a permuted triskelion shape. The evolution of tRNAs in *Ulva* mitogenomes is related to duplication, transposition, remolding, degeneration, loss or recruitment. The DNA-directed RNA polymerases (*rpo*s) are common in ulvalean mitogenomes and a total of three full-length and 55 split *rpo*s have been detected in these 33 ulvalean mitogenomes. The GHE-containing group IB introns were found for the first time in *Ulva* mtDNAs, which expand our understanding of intron diversity in *Ulva* mitogenomes. All of three newly discovered group II intron belonged to group IIA intron, indicating that group IIA intron is frequently involved in recent invasion or homing in *Ulva* mitogenomes. Phylogenomic analyses based on mitogenome dataset showed that the *Ulva* was split into two sister clades, representing *Ulva* lineage I and II. This study provides new insights on the genetics, systematics, and evolution of *Ulva* species. The comparative analysis of these ulvalean mitogenomes enriches our understanding of the mitogenome evolution in Ulvophyceae.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and

accession number(s) can be found below: NCBI (accessions: MH013471, KY626326, MH013470, MZ438677, KP720617, MZ571476, MH013468, and MZ911851).

AUTHOR CONTRIBUTIONS

FL and JM designed the study and contributed equally to this work. FL, JM, HW, JW, and JL-B performed the experiments. FL performed the analysis and wrote the manuscript. All authors have read and approved the final version of the manuscript.

FUNDING

This work was financially supported by the Strategic Priority Research Program of Chinese Academy of Sciences (No. XDA23050302/XDA23050403), the Science and Technology Basic Resources Investigation Program of China (No. 2018FY100200), the Key Research Program of Frontier Sciences, Chinese Academy of Sciences (No. QYZDB-SSW-DQC023), the Major Scientific and Technological Innovation Project of Shandong Province (No. 2019JZZY020706), the National Natural Science Foundation of China (No. 41876165), the National Science Foundation (NSF) HBCU-UP (No. 1436759) for postdoctoral fellowship, and NSF through Assembling the Tree of Life for Green Algae GRAToL (DEB 1036495).

ACKNOWLEDGMENTS

The authors wish to thank Wei Luan, Manman Liu, Yu Wang, and Nansheng Chen for their assistance in algal collection and data analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.850710/full#supplementary-material

REFERENCES

Adams, K. L., and Palmer, J. D. (2003). Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol. Phylogenet. Evol.* 29, 380–395. doi: 10.1016/s1055-7903(03)00194-5

Blomster, J., Bäck, S., Fewer, D. P., Kiirikki, M., Lehvo, A., Maggs, C. A., et al. (2002). Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides. *Am. J. Bot.* 89, 1756–1763. doi: 10.3732/ajb.89.11.1756

Bonen, L., and Vogel, J. (2001). The ins and outs of group II introns. *Trends Genet.* 17, 322–331. doi: 10.1016/s0168-9525(01)02324-1

Burger, G., Gray, M. W., Forget, L., and Lang, B. F. (2013). Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists. *Genome Biol. Evol.* 5, 418–438. doi: 10.1093/gbe/evt008

Burger, G., and Nedelcu, A. M. (2012). "Mitochondrial genomes of algae," in *Advances in Photosynthesis and Respiration Including Bioenergy and Related Processes: Genomics of Chloroplasts and Mitochondria*, eds R. Bock and V. Knoop (Dordrecht: Springer), 125–157. doi: 10.1089/omi.2011.0074

Cai, C., Liu, F., Jiang, T., Wang, L., Jia, R., Zhou, L., et al. (2018a). Comparative study on mitogenomes of green tide algae. *Genetica* 146, 529–540. doi: 10.1007/s10709-018-0046-7

Cai, C., Wang, L., Jiang, T., Zhou, L., He, P., and Jiao, B. (2018b). The complete mitochondrial genomes of green tide algae *Ulva flexuosa* (Ulvophyceae, Chlorophyta). *Conserv. Genet. Resour.* 10, 415–418. doi: 10.1007/s12686-017-0838-6

Cermakian, N., Ikeda, T. M., Cedergren, R., and Gray, M. W. (1996). Sequences homologous to yeast mitochondrial and bacteriophage T3 and T7 RNA polymerases are widespread throughout the eukaryotic lineage. *Nucleic Acids Res.* 24, 648–654. doi: 10.1093/nar/24.4.648

Cermakian, N., Ikeda, T. M., Miramontes, P., Lang, B. F., Gray, M. W., and Cedergren, R. (1997). On the evolution of the single-subunit RNA polymerases. *J. Mol. Evol.* 45, 671–681. doi: 10.1007/pl00006271

Chan, P. P., Lin, B. Y., Mak, A. J., and Lowe, T. M. (2021). tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res.* 49, 9077–9096. doi: 10.1093/nar/gkab688

Clark-Walker, G. D. (1992). Evolution of mitochondrial genomes in fungi. *Int. Rev. Cytol.* 141, 89–127. doi: 10.1016/s0074-7696(08)62064-1

Cox, C. J., Li, B., Foster, P. G., Embley, T. M., and Civán, P. (2014). Conflicting phylogenies for early land plants are caused by composition biases among synonymous substitutions. *Syst. Biol.* 63, 272–279. doi: 10.1093/sysbio/syt109

Dai, L., Toor, N., Olson, R., Keeping, A., and Zimmerly, S. (2003). Database for mobile group II introns. *Nucleic Acids Res.* 31, 424–426. doi: 10.1093/nar/gkg049

Daoud, R., Forget, L., and Lang, B. F. (2012). Yeast mitochondrial RNase P, RNase Z and the RNA degradosome are part of a stable supercomplex. *Nucleic Acids Res.* 40, 1728–1736. doi: 10.1093/nar/gkr941

Fort, A., McHale, M., Cascella, K., Potin, P., Usadel, B., Guiry, M. D., et al. (2020). 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, 219–233. doi: 10.1111/jpy.13079

Gandini, C. L., and Sanchez-Puerta, M. V. (2017). Foreign plastid sequences in plant mitochondria are frequently acquired via mitochondrion-to-mitochondrion horizontal transfer. *Sci. Rep.* 7:43402. doi: 10.1038/srep43402

Gao, G., Zhong, Z. H., Zhou, X. H., and Xu, J. T. (2016). Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: a laboratory experiment. *Harmful Algae* 59, 51–58. doi: 10.1016/j.hal.2016.09.004

Gray, M. W., Burger, G., and Lang, B. F. (2001). The origin and early evolution of mitochondria. *Genome Biol.* 2:reviews1018.1–reviews1018.5. doi: 10.1186/gb-2001-2-6-reviews1018

Guiry, M. D., and Guiry, G. M. (2021). *AlgaeBase*. Available online at: http://www.algaebase.org (Accessed September 25, 2021)

Handa, H. (2008). Linear plasmids in plant mitochondria: peaceful coexistences or malicious invasions?. *Mitochondrion* 8, 15–25. doi: 10.1016/j.mito.2007.10.002

Hanyuda, T., Heesch, S., Nelson, W., Sutherland, J., Arai, S., Boo, S. M., et al. (2016). Genetic diversity and biogeography of native and introduced populations of *Ulva pertusa* (Ulvales. Chlorophyta). *Phycol. Res.* 64, 102–109. doi: 10.1111/pre.12123

Haugen, P., Simon, D. M., and Bhattacharya, D. (2005). The natural history of group I introns. *Trends Genet.* 21, 111–119. doi: 10.1016/j.tig.2004.12.007

Hayden, H. S., Blomster, J., Maggs, C. A., Silva, P. C., Stanhope, M. J., and Waaland, J. R. (2003). Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur. J. Phycol.* 38, 277–294.

Hayden, H. S., and Waaland, J. R. (2004). A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific. *Phycologia* 43, 364–382.

Hughey, J. R., Maggs, C. A., Mineur, F., Jarvis, C., Miller, K. A., Shabaka, S. H., et al. (2019). Genetic analysis of the Linnaean *Ulva lactuca* (Ulvales, Chlorophyta) holotype and related type specimens reveals name misapplications, unexpected origins, and new synonymies. *J. Phycol.* 55, 503–508. doi: 10.1111/jpy.12860

Hughey, J. R., Miller, K. A., and Gabrielson, P. W. (2018). Mitogenome analysis of a green tide forming *Ulva* from California, USA confirms its identity as *Ulva expansa* (Ulvaceae, Chlorophyta). *Mitochondrial DNA B Resour.* 3, 1302–1303. doi: 10.1080/23802359.2018.1535859

Ikuta, K., Kawai, H., Müller, D. G., and Ohama, T. (2008). Recurrent invasion of mitochondrial group II introns in specimens of *Pylaiella littoralis* (brown alga),

collected worldwide. *Curr. Genet.* 53, 207–216. doi: 10.1007/s00294-008-0178-x

Joardar, V., Abrams, N. F., Hostetler, J., Paukstelis, P. J., Pakala, S., Pakala, S. B., et al. (2012). Sequencing of mitochondrial genomes of nine *Aspergillus* and *Penicillium* species identifies mobile introns and accessory genes as main sources of genome size variability. *BMC Genomics* 13:698. doi: 10.1186/1471-2164-13-698

Jones, D. T., Taylor, W. R., and Thornton, J. M. (1992). The rapid generation of mutation data matrices from protein sequences. *Comput. Appl. Biosci.* 8, 275–282. doi: 10.1093/bioinformatics/8.3.275

Kang, X., Liu, J., Yang, X., Cui, J., Zhao, L., Wen, Q., et al. (2020). The complete mitochondrial genome of a green macroalgae species: *Ulva meridionalis* (Ulvales: Ulvaceae). *Mitochondrial DNA B Resour.* 5, 760–761. doi: 10.1080/23802359.2020.1715856

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649. doi: 10.1093/bioinformatics/bts199

Keeling, P. J. (2010). The endosymbiotic origin, diversification and fate of plastids. *Philos. Trans. R. Soc. B.* 365, 729–748. doi: 10.1098/rstb.2009.0103

Kelchner, S. A. (2002). Group II introns as phylogenetic tools: structure, function, and evolutionary constraints. *Am. J. Bot.* 89, 1651–1669. doi: 10.3732/ajb.89.10.1651

Koboldt, D. C., Chen, K., Wylie, T., Larson, D. E., McLellan, M. D., Mardis, E. R., et al. (2009). VarScan: variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics* 25, 2283–2285. doi: 10.1093/bioinformatics/btp373

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054

Lang, B. F. (2014). "Mitochondrial genomes in fungi," in *Molecular Life Sciences*, eds R. D. Wells, J. S. Bond, J. Klinman, B. S. S. Masters, and E. Bell (New York: Springer), 1–7.

Lang, B. F., Burger, G., O'Kelly, C. J., Cedergren, R., Golding, G. B., Lemieux, C., et al. (1997). An ancestral mitochondrial DNA resembling a eubacterial genome in miniature. *Nature* 387, 493–497. doi: 10.1038/387493a0

Li, H., and Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 26, 589–595. doi: 10.1093/bioinformatics/btp698

Li, R., Lei, Z., Li, W., Zhang, W., and Zhou, C. (2021). Comparative mitogenomic analysis of *Heptageniid mayflies* (Insecta: Ephemeroptera): conserved intergenic spacer and tRNA gene duplication. *Insects* 12:170. doi: 10.3390/insects12020170

Liu, F., and Melton, J. T. (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. doi: 10.3389/fmars.2021.668542

Liu, F., Melton, J. T., and Bi, Y. (2017). Mitochondrial genomes of the green macroalga *Ulva pertusa* (Ulvophyceae, Chlorophyta): novel insights into the evolution of mitogenomes in the Ulvophyceae. *J. Phycol.* 53, 1010–1019. doi: 10.1111/jpy.12561

Liu, F., Melton, J. T., Lopez-Bautista, J. M., and Chen, N. (2020). Multiple intraspecific variations of mitochondrial genomes in the green-tide forming alga, *Ulva compressa* Linnaeus (Ulvophyceae, Chlorophyta). *Front. Mar. Sci.* 7:714. doi: 10.3389/fmars.2020.00714

Liu, F., and Pang, S. (2016). The mitochondrial genome of the bloom-forming green alga *Ulva prolifera*. *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 27, 4530–4531. doi: 10.3109/19401736.2015.1101548

Liu, F., Pang, S., Chopin, T., Gao, S., Shan, T., Zhao, X., et al. (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. doi: 10.1016/j.marenvres.2012.10.007

Liu, M., Liu, F., Chen, N., Melton, J. T., and Luo, M. (2020). Mitochondrial genomes and phylogenomic analysis of *Ulva lactuca* Linnaeus (Ulvophyceae, Chlorophyta). *Mitochondrial DNA B.* 5, 1638–1639. doi: 10.1080/23802359.2020.1745712

Mareš, J., Leskinen, E., Sitkowska, M., Skácelová, O., and Blomster, J. (2011). True identity of the European freshwater *Ulva* (Chlorophyta, Ulvophyceae) revealed by a combined molecular and morphological approach. *J. Phycol.* 47, 1177–1192. doi: 10.1111/j.1529-8817.2011.01048.x

Martin, W. F., Garg, S., and Zimorski, V. (2015). Endosymbiotic theories for eukaryote origin. *Philos. Trans. R. Soc. B.* 370:20140330. doi: 10.1098/rstb.2014.0330

Melton, J. T., Leliaert, F., Tronholm, A., and Lopez-Bautista, J. M. (2015). The complete chloroplast and mitochondrial genomes of the green macroalga *Ulva* sp. UNA00071828 (Ulvophyceae, Chlorophyta). *PLoS One* 10:e0121020. doi: 10.1371/journal.pone.0121020

Melton, J. T., and Lopez-Bautista, J. M. (2016). *De novo* assembly of the mitochondrial genome of *Ulva fasciata* Delile (Ulvophyceae, Chlorophyta), a distromatic blade-forming green macroalga. *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 27, 3817–3819. doi: 10.3109/19401736.2015.1082095

Noutahi, E., Calderon, V., Blanchette, M., El-Mabrouk, N., and Lang, B. F. (2019). Rapid Genetic Code Evolution in Green Algal Mitochondrial Genomes. *Mol. Biol. Evol.* 36, 766–783. doi: 10.1093/molbev/msz016

Oudot-Le Secq, M. P., Fontaine, J. M., Rousvoal, S., Kloareg, B., and Loiseaux-De Goër, S. (2001). The complete sequence of a brown algal mitochondrial genome, the Ectocarpale *Pylaiella littoralis* (L.) Kjellm. *J. Mol. Evol.* 53, 80–88. doi: 10.1007/s002390010196

Park, S., Grewe, F., Zhu, A., Ruhlman, T. A., Sabir, J., Mower, J. P., et al. (2015). Dynamic evolution of *Geranium* mitochondrial genomes through multiple horizontal and intracellular gene transfers. *New Phytol.* 208, 570–583. doi: 10.1111/nph.13467

Peralta-Castro, A., García-Medel, P. L., Baruch-Torres, N., Trasviña-Arenas, C. H., Juarez-Quintero, V., Morales-Vazquez, C. M., et al. (2020). Plant Organellar DNA Polymerases Evolved Multifunctionality through the Acquisition of Novel Amino Acid Insertions. *Genes* 11:1370. doi: 10.3390/genes11111370

Pino, P., Aeby, E., Foth, B. J., Sheiner, L., Soldati, T., Schneider, A., et al. (2010). Mitochondrial translation in absence of local tRNA aminoacylation and methionyl tRNA Met formylation in Apicomplexa. *Mol. Microbiol.* 76, 706–718. doi: 10.1111/j.1365-2958.2010.07128.x

Pombert, J.-F., Beauchamp, P., Otis, C., Lemieux, C., and Turmel, M. (2006). The complete mitochondrial DNA sequence of the green alga *Oltmannsiellopsis viridis*: evolutionary trends of the mitochondrial genome in the Ulvophyceae. *Curr. Genet.* 50, 137–147. doi: 10.1007/s00294-006-0076-z

Pombert, J.-F., Otis, C., Lemieux, C., and Turmel, M. (2004). 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, 922–935. doi: 10.1093/molbev/msh099

Punta, M., Coggill, P. C., and Eberhardt, R. Y. (2012). The Pfam protein families database. *Nucleic Acids Res.* 40, D290–D301.

Rousvoal, S., Oudot, M.-P., Fontaine, J.-M., Kloareg, B., and Loiseaux-de Goër, S. (1998). Witnessing the evolution of transcription in mitochondria: the mitochondrial genome of the primitive brown alga *Pylaiella littoralis* (L.) Kjellm. encodes a T7-like RNA polymerase. *J. Mol. Biol.* 277, 1047–1057. doi: 10.1006/jmbi.1998.1679

Rybak, A. S. (2016). Ecological preferences of freshwater *Ulva flexuosa* (Ulvales; Ulvophyceae): development of macroalgal mats in a Tulce fishpond (Wielkopolska Region, Poland). *Oceanol. Hydrobiol. Stud.* 45, 100–111. doi: 10.1515/ohs-2016-0010

Seetharaman, M., Eldho, N. V., Padgett, R. A., and Dayie, K. T. (2006). Structure of a self-splicing group II intron catalytic effect or domain 5: parallels with spliceosomal U6 RNA. *RNA* 12, 235–247. doi: 10.1261/rna.2237806

Seif, E., Leigh, J., Liu, Y., Roewer, I., Forget, L., and Lang, B. F. (2005). Comparative mitochondrial genomics in zygomycetes: bacteria-like RNase P RNAs, mobile elements and a close source of the group I intron invasion in angiosperms. *Nucleic Acids Res.* 33, 734–744. doi: 10.1093/nar/gki199

Shaukat, A.-N., Kaliatsi, E. G., Skeparnias, I., and Stathopoulos, C. (2021). The Dynamic Network of RNP RNase P Subunits. *Int. J. Mol. Sci.* 22:10307. doi: 10.3390/ijms221910307

Shimada, S., Nagano, M., Hiraoka, M., Ichihara, K., Mineur, F., and Zhu, W. (2010). Phylogeographic analysis of the genus *Ulva* (Ulvales, Chlorophyta), including bloom sample in Qingdao, China. *Coast. Mar. Sci.* 34, 117–122.

Stoddard, B. L. (2011). Homing endonucleases: from microbial genetic invaders to reagents for targeted DNA modification. *Structure* 19, 7–15. doi: 10.1016/j.str.2010.12.003

Suzuki, S., Yamaguchi, H., Hiraoka, M., and 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, 765–767. doi: 10.1080/23802359.2018.1483778

Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol*. 10, 512–526. doi: 10.1093/oxfordjournals.molbev.a040023

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The ClustalX windows interface flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, 4876–4882. doi: 10.1093/nar/25.24.4876

Tritt, A., Eisen, J. A., Facciotti, M. T., and Darling, A. E. (2012). An integrated pipeline for *de novo* assembly of microbial genomes. *PLoS One* 7:e42304. doi: 10.1371/journal.pone.0042304

Turmel, M., Lemieux, C., Burger, G., Lang, B. F., Otis, C., Plante, I., et al. (1999). The complete mitochondrial DNAsequences of *Nephroselmis olivacea* and *Pedinomonas minor*: two radically different evolutionary patterns within green algae. *Plant Cell* 11, 1717–1730. doi: 10.1105/tpc.11.9.1717

Turmel, M., Otis, C., and Lemieux, C. (2016). Mitochondrion-to-chloroplast DNA transfers and intragenomic proliferation of chloroplast group II introns in *Gloeotilopsis* green algae (Ulotrichales, Ulvophyceae). *Genome Biol. Evol.* 8, 2789–2805. doi: 10.1093/gbe/evw190

Valach, M., Burger, G., Gray, M. W., and Lang, B. F. (2014). Widespread occurrence of organelle genome-encoded 5S rRNAs including permuted molecules. *Nucleic Acids Res.* 42, 13764–13777. doi: 10.1093/nar/gku1266

Wang, D., Wu, Y. W., Shih, A. C. C., Wu, C. S., Wang, Y. N., and Chaw, S. M. (2007). Transfer of chloroplast genomic DNA to mitochondrial genome occurred at least 300 MYA. *Mol. Biol. Evol.* 24, 2040–2048. doi: 10.1093/molbev/msm133

Wang, H., Liu, F., Wang, J., and Chen, N. (2021). Phylogenomic analysis of the chloroplast genome of the green-tide forming macroalga *Ulva intestinalis* Linnaeus (Ulvophyceae, Chlorophyta). *Mitochondrial DNA B* 6, 3052–3054. doi: 10.1080/23802359.2021.1978889

Wang, Y., Liu, F., Liu, X., Shi, S., Bi, Y., and Moejes, F. W. (2019). Comparative transcriptome analysis of four co-occurring *Ulva* species for understanding the dominance of *Ulva prolifera* in the Yellow Sea green tides. *J. Appl. Phycol.* 31, 3303–3316.

Wang, Z., Xiao, J., Fan, S., Li, Y., Liu, X., and 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, 1105–1117.

Warren, J. M., Simmons, M. P., Wu, Z., and Sloan, D. B. (2016). Linear Plasmids and the Rate of Sequence Evolution in Plant Mitochondrial Genomes. *Genome Biol. Evol.* 8, 364–374. doi: 10.1093/gbe/evw003

Wyman, S. K., Jansen, R. K., and Boore, J. L. (2004). Automatic annotation of organellar genomes with DOGMA. *Bioinformatics* 20, 3252–3255. doi: 10.1093/bioinformatics/bth352

Ye, N., Zhang, X., Mao, Y., Liang, C., Xu, D., Zou, J., et al. (2011). 'Green tides' are overwhelming the coastline of our blue planet: taking the world's largest example. *Ecol. Res.* 26, 477–485. doi: 10.1007/s11284-011-0821-8

Yin, C., Richter, U., Börner, T., and Weihe, A. (2010). Evolution of plant phage-type RNA polymerases: the genome of the basal angiosperm Nuphar advena encodes two mitochondrial and one plastid phage-type RNA polymerases. *BMC Evol. Biol.* 10:379. doi: 10.1186/1471-2148-10-379

Zhang, L., Yu, D., Storey, K. B., Cheng, H., and Zhang, J. (2018). Higher tRNA gene duplication in mitogenomes of praying mantises (Dictyoptera, Mantodea) and the phylogeny within Mantodea. *Int. J. Biol. Macromol.* 111, 787–795. doi: 10.1016/j.ijbiomac.2018.01.016

Zhou, L., Wang, L., Zhang, J., Cai, C., and He, P. (2016a). Complete mitochondrial genome of *Ulva linza*, one of the causal species of green macroalgal blooms in Yellow Sea, China. *Mitochondrial DNA B* 1, 31–33. doi: 10.1080/23802359.2015.1137806

Zhou, L., Wang, L., Zhang, J., Cai, C., and He, P. (2016b). Complete mitochondrial genome of *Ulva prolifera*, the dominant species of green macroalgal blooms in Yellow Sea, China. *Mitochondrial DNA B.* 1, 76–78. doi: 10.1080/23802359.2015.1137831

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31, 3406–3415. doi: 10.1093/nar/gkg595

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.