

Chloroplast genome of *Sargassum horneri* (Sargassaceae, Phaeophyceae): comparative chloroplast genomics of brown algae

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Abstract *Sargassum horneri* (Turner) C. Agardh is an important canopy-forming seaweed native to the northwestern Pacific coast growing in the upper sublittoral zone. In this study, the chloroplast genome of *S. horneri* was fully sequenced and compared with three other brown algal chloroplast genomes. The *S. horneri* chloroplast genome is 124,068 bp in size with an inverted repeat of 5436 bp and is slightly smaller than the other three brown algal chloroplast DNAs (cpDNAs). It contains 173 genes, including 6 ribosomal RNA (rRNA) genes, 28 transfer RNA (tRNA) genes, and 139 protein-coding genes (PCGs). The coding sequence constitutes 86.32 % of the *S. horneri* cpDNA. The total spacer size was found to be reduced to 16,967 bp with an average length of 101.0 bp, which makes the *S. horneri* cpDNA more compact than the other three brown algal cpDNAs. Sixteen small inverted and nine tandem repeats were detected in *S. horneri* chloroplast genomes. Two introns in *trnL2* and *trnW* genes were identified with the size of 209 and 90 bp, respectively. Its gene content and order are identical to those of *Fucus vesiculosus* cpDNA, indicating that the chloroplast genome organization at the level of the order Fucales is highly conserved.

Keywords *Sargassum horneri* · Chloroplast genome · Brown alga · Phaeophyceae · Sargassaceae

Introduction

The Phaeophyceae (brown algae) belong within the stramenopiles and are a group of multicellular marine eukaryotes, having chloroplasts that originate from secondary endosymbiosis, in which a red alga was engulfed by a nonphotosynthetic protist (Keeling 2010). After their own independent evolution for more than 200 million years (Silberfeld et al. 2010), the current brown algal group consists of a multitude of taxa including 19 orders, 62 families, 473 genera, and more than 2000 species with various morphological and physiological characteristics (Charrier et al. 2012; Liu and Pang 2015b). Brown algae are one of the major photosynthetic producers of organic carbon on rocky intertidal shores of the world and play an important role both as human food and for the habitats that they form (Loureiro et al. 2015).

Compared to rhodophytes, green algae, and land plants (primary endosymbionts), chloroplasts of autotrophic stramenopiles (secondary endosymbionts) display their own characteristics in morphology, pigment composition, genome organization, and gene content (Cattolico et al. 2008; Dorrell and Smith 2011). The interpretation of information implied in chloroplast DNA (cpDNA) of ecologically diverse species could improve our understanding of stramenopile plastid function, phylogeny, and evolution (Keeling 2004). To date, over 40 plastid genomes have been sequenced among stramenopiles. However, only three of them are from brown algae including *Fucus vesiculosus* (order Fucales), *Saccharina japonica* (order Laminariales), and *Ectocarpus siliculosus* (order Ectocarpales) (Le Corguillé et al. 2009; Wang et al. 2013). The brown algal plastid genomes are

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125.0–140.0 kb in size; contain 173–185 genes including 6 ribosomal RNA (rRNA) genes, 28–31 transfer RNA (tRNA) genes, and 139–148 protein-coding genes (PCGs); and appear to be highly rearranged in genome architectures (Table 1). Genome sequence analysis of more brown algal cpDNAs is a promising approach for further understanding the evolutionary history of this eukaryotic lineage.

Sargassaceae, in the order Fucales, is the largest family in Phaeophyceae containing 52 genera and 495 species. *Sargassum* C. Agardh is the most species-rich genus in this family and contains 347 species (including varieties) (Guiry and Guiry 2015). *Sargassum* species usually form dense underwater forests on rocky coastlines of tropical and temperate regions serving as habitats for myriad vertebrate and invertebrate species (Mattio and Payri 2011). *Sargassum horneri* is an important perennial, canopy-forming seaweed native to the northwestern Pacific coast growing in the upper sublittoral zone (Hu et al. 2011). During the past several years, its large-scale biomass has been continuously observed drifting in the Yellow Sea and the East China Sea (Komatsu et al. 2008). In its native distribution areas, e.g., the coastal water of Zhejiang Province of China, its biomass has been observed to decrease

steadily and even disappear in certain regions (Sun et al. 2008). Thus, this seaweed has already been chosen as one of the main algal species to be used to reconstruct seaweed beds in China. An efficient technique for producing young *S. horneri* seedlings has been established (Pang et al. 2009).

Previously, we reported the complete mitochondrial genome sequence of *S. horneri* as well as four other *Sargassum* species in subgenus *Bactrophyucus*, with genome organization highly similar to that of *F. vesiculosus* (Liu and Pang 2015a, b; Liu et al. 2015). To gain further insight into the evolutionary biology of the alga, in this study, the complete sequence of *S. horneri* chloroplast genome has been sequenced and compared with three other brown algal cpDNAs in the aspects of genome features, gene content, and genome organization.

Materials and methods

Sample collection and DNA extraction

Mature plants of *S. horneri* (Turner) C. Agardh were initially collected from the rocky shore at Xiaohuyu, Nanji

Table 1 General properties of chloroplast genomes in four species of brown algae

	<i>Sargassum horneri</i>	<i>Fucus vesiculosus</i> ^a	<i>Saccharina japonica</i> ^a	<i>Ectocarpus siliculosus</i> ^a
Accession number	KP881334	FM957154	JQ405663	FP102296
Genome size (bp)	124,068	124,986	130,584	139,954
Inverted repeat region a (IRa, bp)	5436	5370	5494	8616
Small single-copy region (SSC, bp)	39,885	39,959	42,809	42,711
Inverted repeat region b (IRb, bp)	5436	5370	5492	8616
Large single-copy region (LSC, bp)	73,311	74,287	76,789	80,011
Overall G+C content (%)	30.61	28.94	31.05	30.67
Spacer G+C content (%)	18.05	14.95	19.61	20.48
Predicted coding sequence (%)	86.32	85.84	83.52	80.40
Predicted total gene content	173	173	176	185
rRNA/tRNA/protein-coding gene content	6/28/139	6/28/139	6/31/139	6/31/148
Intron number	2	1	2	0
Total spacer size (bp)	16,967	17,698	21,515	27,434
Average spacer size (bp)	101.0	105.3	126.6	150.7
Pairs of overlapping genes	5	5	6	3
Total overlap size (bp)	75	75	121	65
Average overlap size (bp)	15.0	15.0	20.2	21.7
Start codons: ATG	138	137	137	144
Start codons: GTG	1	2	2	3
Start codons: TTG	0	0	0	1
Stop codons: TAA	111	113	114	129
Stop codons: TAG	25	17	24	14
Stop codons: TGA	3	9	1	5

^a In order to ensure that the comparisons are with accuracy and consistency, the chloroplast genome sequences of *F. vesiculosus*, *S. japonica*, and *E. siliculosus* have been reanalyzed and re-annotated with the same method as that in the *S. horneri* cpDNA

Islands, Wenzhou, Zhejiang Province, China (27° 27' N, 121° 04' E), in April 2007 (Liu et al. 2015). Plants were transported to the laboratory in cool boxes (5–8 °C) within 24 h after collection. Fresh algal tissue was selected and stored in the ultra-low temperature freezer (−80 °C) for DNA extraction. Algal tissue was ground to fine powder in liquid nitrogen. Total DNA was isolated using a Plant Genomic DNA Kit according to the manufacturer’s instructions (Tiangen Biotech, China). The DNA quality and quantity were assessed by electrophoresis on 1.0 % agarose gel.

PCR amplification and sequencing

The whole chloroplast genome of *S. horneri* was amplified using the long PCR and primer walking techniques (Cheng et al. 1994). Primer sets were designed according to three known brown algal cpDNAs (*F. vesiculosus*, *S. japonica*, and *E. siliculosus*) and used to amplify the entire *S. horneri* chloroplast genome in 15 large fragments (Supplementary Table S1). PCR reactions were carried out in 50-μL reaction mixtures containing 32 μL of sterile distilled H₂O, 10 μL of 5× PrimeSTAR GXL buffer (5 mM Mg²⁺ plus, Takara,

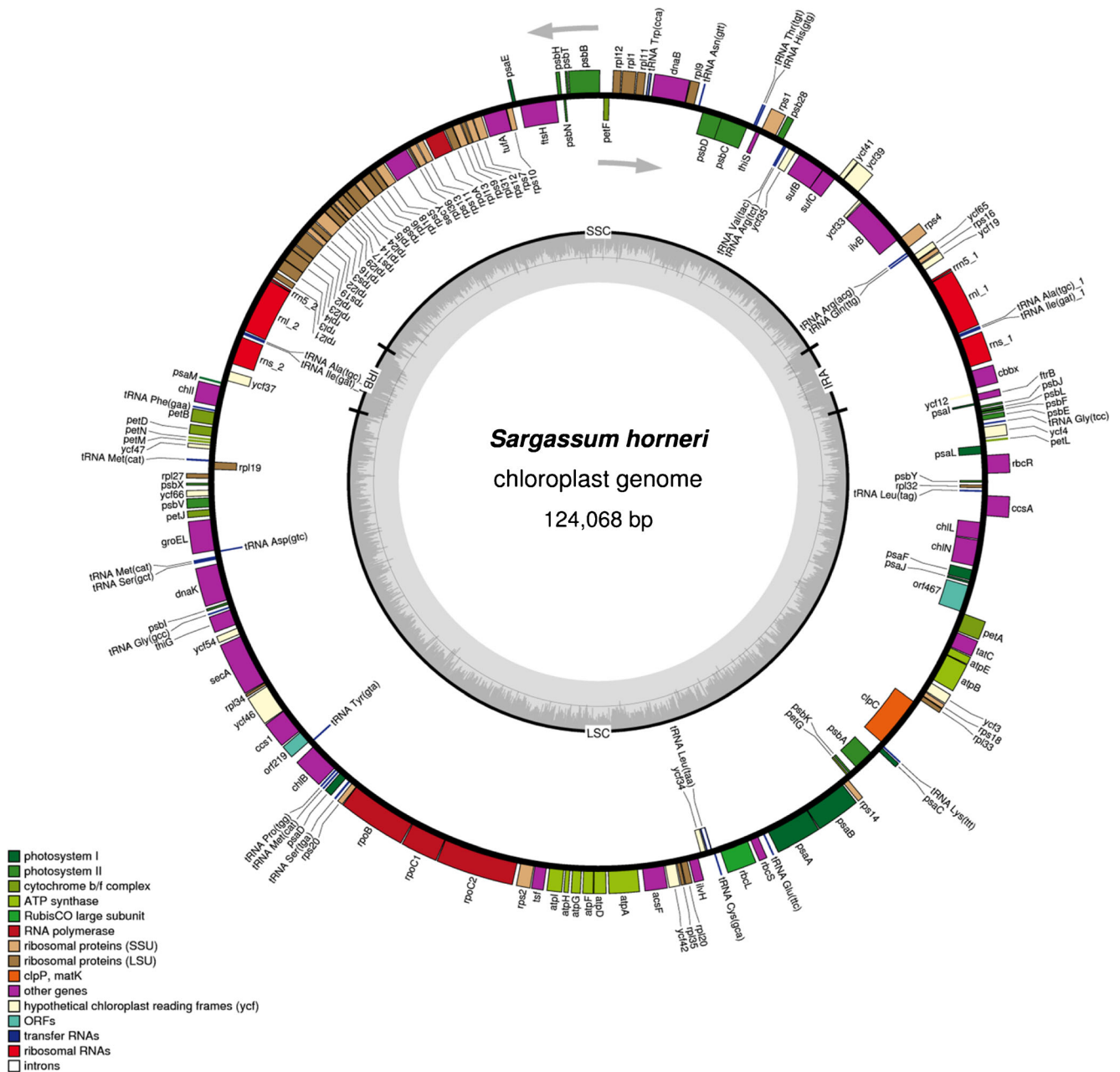


Fig. 1 The chloroplast genome map of *Sargassum horneri*. Annotated genes are colored according to the functional categories. Genes on the outside are transcribed in the *clockwise direction*, whereas genes on the inside are transcribed in the *counterclockwise direction*

Table 2 Properties of small inverted and tandem repeats in chloroplast genomes of brown algae

	<i>Sargassum horneri</i>	<i>Fucus vesiculosus</i>	<i>Saccharina japonica</i>	<i>Ectocarpus siliculosus</i>
Number of small inverted repeats (SIR)	16	15	16	17
Located between genes on opposite coding strands	8	8	6	10
Located between genes on the same coding strand	8	7	10	7
Total length of SIRs (bp)	1264	1326	1441	1429
% of cpDNA	1.02	1.06	1.10	1.02
Stem size range (bp)	23–55	21–60	21–74	25–69
Average stem size (bp)	35.1±9.5	32.7±10.7	44.4±14.8	40.4±12.3
Loop size range (bp)	0–96	0–244	0–6	0–9
Average loop size (bp)	2.9±2.3 ^a	5.4±4.9 ^a	1.9±1.8	3.2±2.5
Number of tandem repeats (TR)	9	3	7	8
Located in genes	4	2	3	5
Located in intergenic spacers	5	1	4	3
Total length of TRs (bp)	394	125	281	348
% of cpDNA	0.32	0.10	0.22	0.25
TR size range (bp)	25–112	37–44	25–91	29–53
Average TR size (bp)	43.8±26.9	41.7±4.0	49.3±29.5	43.5±8.6
Period size range (bp)	8–45	13–22	2–42	14–25
Average period size (bp)	18.8±11.2	18.7±4.9	19.6±16.4	20.3±4.3
Copy number	1.9–5.1	2.0–2.6	1.9–16.0	1.9–2.5

^a The 96-bp loop located between *trnE* and *psaA* was not included in the *S. horneri* column. In the *F. vesiculosus* column, two large loops, the 244 bp *petM-ycf47-trnM* and the 27 bp *rpl19-rpl27* ones, were not included

Table 3 Genes identified in the chloroplast genome of *Sargassum horneri*

Gene classification	Gene designations ^a
Ribosomal RNA genes (6) ^b	<i>rnl-1, rnl-2, rns-1, rns-2, rrm5-1, rrm5-2</i>
Transfer RNA genes (28)	<i>trnA-1(ugc), trnA-2(ugc), trnC(gca), trnD(guc), trnE(uuc), trnF(gaa), trnG-1(gcc), trnG-2(ucc), trnH(gug), trnI-1(gau), trnI-2(gau), trnK(uuu), trnL-1(uag), trnL-2(uaa), trnM-1(cau), trnM-2(cau), trnM-3(cau), trnN(guu), trnP(ugg), trnQ(uug), trnR-1(acg), trnR-2(ucu), trnS-1(gcu), trnS-2(uga), trnT(ugu), trnV(uac), trnW(cca), trnY(gua)</i>
Protein-coding genes (137)	
Transcription and translation (52)	<i>rpl1, rpl2, rpl3, rpl4, rpl5, rpl6, rpl9, rpl11, rpl12, rpl13, rpl14, rpl16, rpl18, rpl19, rpl20, rpl21, rpl22, rpl23, rpl24, rpl27, rpl29, rpl31, rpl32, rpl33, rpl34, rpl35, rpl36, rps1, rps2, rps3, rps4, rps5, rps7, rps8, rps9, rps10, rps11, rps12, rps13, rps14, rps16, rps17, rps18, rps19, rps20, dnaB, rpoA, rpoB, rpoC1, rpoC2, tsf, tufA</i>
Photosystem I (12)	<i>psaA, psbA, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbN, psbT, psbV, psbX, psbY, ycf12</i>
Photosystem II (18)	<i>psb28, psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbN, psbT, psbV, psbX, psbY, ycf12</i>
Electron transport and ATP synthesis (19)	<i>atpA, atpB, atpD, atpE, atpF, atpG, atpH, atpI, ccs1, ccsA, petA, petB, petD, petF, petG, petJ, petL, petM, petN</i>
Carbon assimilation (6)	<i>ilvB, ilvH, rbcL, rbcS, thiG, thiS</i>
Light harvesting and chl biosynthesis (5)	<i>acsF, chlB, chlI, chlL, chlN</i>
Signal transduction (3)	<i>cbbX, ftrB, rbcR</i>
Protein import (3)	<i>secA, secY, tatC</i>
Fe-S assembly (2)	<i>sufB, sufC</i>
Chaperones (2)	<i>dnaK, groEL</i>
Proteolysis (2)	<i>clpC, fisH</i>
Conserved hypothetical genes (15)	<i>orf219, orf467, ycf19, ycf33, ycf34, ycf35, ycf37, ycf39, ycf41, ycf42, ycf46, ycf47, ycf54, ycf65, ycf66</i>

^a Genes are classified according to their function

^b Numbers within parentheses indicate the number of genes in a specific functional group

Japan), 4 μL of dNTP mixture (2.5 mM each), 1 μL of each primer (10 μM), 1 μL of PrimeSTAR GXL DNA polymerase (1.25 units μL^{-1} , Takara, Japan), and 1 μL of DNA template (approximate 50 ng).

PCR amplification was performed on a T-Gradient Thermoblock Thermal Cycler (Whatman Biometra, Germany) with an initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 50–52 °C for 50 s, extension at 68 °C for 1 min kb^{-1} , and a final extension at 68 °C for 10 min. Long PCR products were purified using a Qiaquick Gel Extraction Kit (Qiagen, Germany). Sequencing reactions were performed using ABI 3730 XL automated sequencers (Applied Biosystems, USA).

Genome assembly and annotation

The DNA sequences were manually edited and assembled using the BioEdit v7.1.9 software (Hall 1999). The DNA sequence of the complete chloroplast genome of *S. horneri* was determined by comparison with published sequences for three brown algae (Le Corguillé et al. 2009; Wang et al. 2013). PCGs and putative open reading frames (ORFs) were annotated by NCBI ORF Finder and BLAST similarity searches of the nonredundant databases at NCBI (Altschul et al. 1997). rRNA genes were identified by RNAmmer 1.2 software (Lagesen et al. 2007) and by comparing *S. horneri* cpDNA with rRNA genes from other brown algal cpDNAs. tRNA genes were searched for by reconstructing their cloverleaf structures using the tRNAscan-SE 1.21 software with default parameters (Schattner et al. 2005). The physical map of the circular chloroplast genome was generated using Organellar Genome DRAW (Lohse et al. 2013). The genome sequence has been deposited in GenBank with the accession number KP881334.

Genome analysis

To date, three complete chloroplast genome sequences of brown algae have been reported, but their functional genes, especially tRNA and rRNA genes, have been annotated based on different methods or software, which caused the differences in gene length and number, influencing the accuracy of their comparison. In order to solve this problem, we reanalyzed three reported brown algal cpDNA sequences with the same method, and part of the obtained results were different from those previously reported. A total of 173 genes in the *S. horneri* cpDNA were sorted by their function and divided into basic functional groups. Base composition and pairwise comparison were determined by the MEGA 5.2 software (Tamura et al. 2011). The identity percentages of gene sequences were evaluated using the BioEdit v7.1.9 software (Hall 1999). Small inverted repeats (SIRs) were identified with Inverted Repeats Finder using the default settings, and

the additional constraint that repeats had to be >75 % similar (<http://tandem.bu.edu/cgi-bin/irdb/irdb.exe>). Tandem repeats (TRs) were found with Tandem Repeats Finder using default settings (Benson 1999).

Results and discussion

Genome features

The chloroplast genome of *S. horneri* has a size of 124,068 bp, which is smaller than three other brown algal cpDNAs sequenced to date (Le Corguillé et al. 2009; Wang et al. 2013). The *S. horneri* cpDNA is mapped as a canonical quadripartite structure with two 5436 bp inverted repeat regions (IRs), which divide the circular molecule into a small single-copy region (SSC 39,885 bp) and a large single-copy region (LSC 73,311 bp) (Fig. 1). The *S. horneri* IRs contain five gene loci, including three rRNA and two tRNA genes, which are the same as those of *F. vesiculosus* and *S. japonica*, but different from that of *E. siliculosus* in which the IRs are much longer (8616 bp) containing 11 gene loci.

The overall G+C content for *S. horneri* cpDNA is 30.61 % and in the range of brown algae from 28.94 % for *F. vesiculosus* to 31.05 % for *S. japonica* (Table 1). The coding sequence constitutes 86.32 % of the *S. horneri* cp genome. The total spacer size in *S. horneri* cpDNA is 16,967 bp with an average length of 101.0 bp, and smaller than that in three other brown algal cpDNAs. The decrease of the spacer size makes the *S. horneri* cpDNA more compact than the other three brown algal cpDNAs. The spacer G+C content is only 18.05 % in *S. horneri* cpDNA.

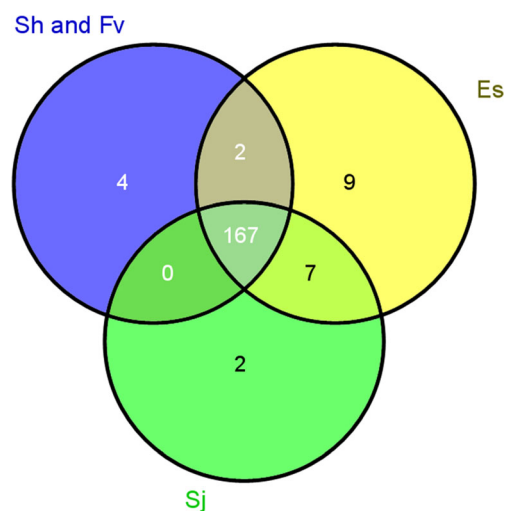


Fig. 2 Venn diagram of gene content of the plastid genomes of *Sargassum horneri* and *Fucus vesiculosus* (Sh and Fv, blue) in order Fucales, *Saccharina japonica* (Sj, green) in order Laminariales, and *Ectocarpus siliculosus* (Es, yellow) in order Ectocarpales

Five pairs of genes are found overlapping by 4 to 53 bp in the *S. horneri* cpDNA; i.e., *ftbB* and *ycf12* overlapped by 6 bp, *sufC* and *sufB* by 4 bp, *rps1* and *thiS* by 4 bp, *psbC* and *psbD* by 53 bp, and *rpl23* and *rpl4* by 8 bp. The overlaps of *psbC-psbD* (53 bp) and *rpl23-rpl4* (8 bp) are highly conserved among the four brown algal cpDNAs. These two overlapping regions are also observed in diatom plastid genomes (e.g., Oudot-Le Secq et al. 2007; Tanaka et al. 2011; Galachyants et al. 2012).

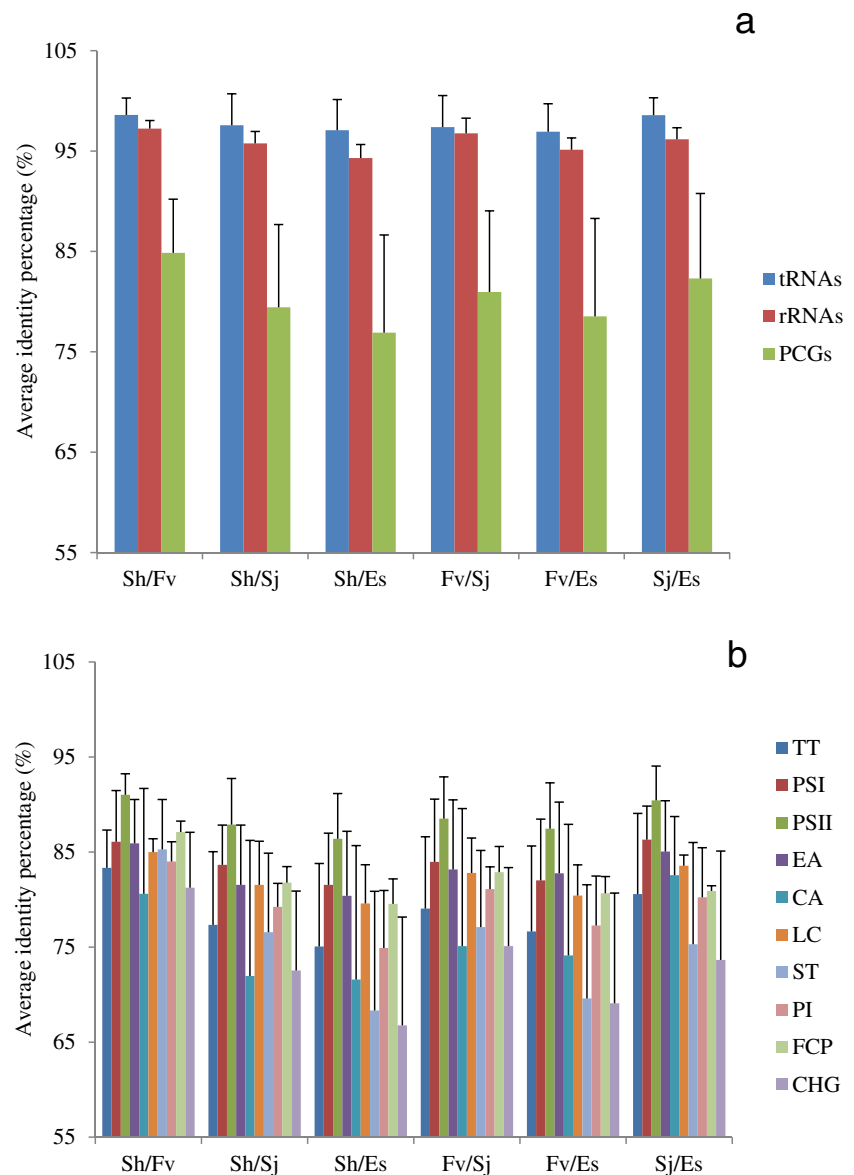
Small inverted and tandem repeats

Brown algal chloroplast genomes harbor multiple small inverted repeats (SIRs) and tandem repeats (TRs). The *S. horneri* cpDNA contains 16 SIRs and 9 TRs (Table 2), representing 1.02 and 0.32 % of the *S. horneri*

genome, respectively, which is similar to those of three other brown algal cpDNAs. The total repeat number found in brown algal cpDNAs (18–25) is similar to that in diatoms (Oudot-Le Secq et al. 2007; Galachyants et al. 2012), more than that in Pelagophyceae (Ong et al. 2010), and less than that in Raphidophyceae (Cattolico et al. 2008).

The SITs of *S. horneri* cpDNA are composed of a stem structure which ranges from 23 to 55 bp in size (average 35.1 ± 9.5 bp) and a small loop domain averaging only 2.9 ± 2.3 bp. Approximately half of SIRs in brown algal cpDNAs are located between two genes transcribed on opposite coding strands, which is similar to bacterial genomes and other reported stramenopile members (e.g., Lillo et al. 2002; Cattolico et al. 2008). Although some SIRs held by four brown algae are located in the same positions of cpDNAs, there is no sequence

Fig. 3 The comparison of average identity percentages **a** of tRNA, rRNA, and protein-coding gene (PCG) sequences and **b** of the specific functional PCG nucleotide sequences from *S. horneri* (Sh), *F. vesiculosus* (Fv), *S. japonica* (Sj), and *E. siliculosus* (Es). *TT* transcription and translation; *PSI* photosystem I; *PSII* photosystem II; *EA* electron transport and ATP synthesis; *CA* carbon assimilation; *LC* light harvesting and chl biosynthesis; *ST* signal transduction; *PI* protein import; *FCP* Fe-S assembly, chaperones, and proteolysis; and *CHG* conserved hypothetical genes.



homology between them. TRs have a period of 18.8 ± 11.2 bp with a copy number ranging from 1.9 to 5.1 in the *S. horneri* cpDNA. The average size of TR element is 43.8 ± 26.9 bp.

Genes in the *S. horneri* cpDNA

The gene set of *S. horneri* cpDNA consists of 173 genes, including 6 rRNA genes located in the IRs, 28 tRNA genes sufficient for messenger RNA translation, and 139 PCGs. The PCG set includes 52 genes responsible for transcription and translation, 12 photosystem I-associated genes, 18 photosystem II-associated genes, 19 for electron transport and ATP synthesis, 6 for carbon assimilation, 5 for light harvesting and chl biosynthesis, 3 for signal transduction, 3 for protein import, 2 for Fe-S assembly, 2 chaperones-associated genes, 2 proteolysis-associated genes, and 15 conserved hypothetical genes (Table 3).

The brown algal plastid genomes show high similarity in terms of gene content and composition. In total, 167 chloroplast genes (6 rRNAs, 28 tRNAs, and 133 PCGs) are shared by these four brown algae (Fig. 2). Two genes (*petL* and *ycf54*) found in *S. horneri* and *F. vesiculosus* cpDNAs were present in *E. siliculosus*, but absent in *S. japonica*. Four PCGs (*psb28*, *sufB*, *sufC*, and *thiG*) were only found in Fucales. Two new tRNA genes (*trnM-4* and *trnF-2*) predicted in the *S. japonica* cpDNA were not detected in three other brown algal cpDNAs. The differences in gene content might be due to gene loss or function transfer to the nucleus.

In *S. horneri* cpDNA, two introns located in *trnL2* and *trnW* genes were identified with the size of 209 and 90 bp, respectively. The former intron in *trnL2* gene was present in *F. vesiculosus* (219 bp) and *S. japonica* (234 bp), but lost in *E. siliculosus*; the latter in *trnW* was only detected in the *S. horneri* cpDNA which might be formed by repeat mutation. In addition, only in the *S. japonica trnF2* gene (tRNA-Phe) located between *ycf35* and *ycf24* was the specific intron (31 bp) found.

All PCGs encoded by *S. horneri* cpDNA start with the ATG codon with the exception of *psbF*, which starts with GTG codon. Unusual start codons have been identified in three other brown algal cpDNAs, e.g., a GTG codon found at the beginning of *psbF* and *rpl3* in *F. vesiculosus*; *psbF* and *rps8* in *S. japonica*; and *rps8*, *rpl3*, and *rbcR* in *E. siliculosus* and a TTG codon at the beginning of *Escp99* in *E. siliculosus*. Three stop codons are employed, and approximately 79.86 % of PCGs (111 of 139 genes) terminate with TAA stop codon, compared with 17.99 % with TAG and 2.16 % with TGA.

Gene order and identity

The gene order of *S. horneri* cpDNA is identical to that of *F. vesiculosus*, indicating that the genome organization of cpDNAs is conservative at the level of the order Fucales.

The comparison by genome-scale alignment shows that chloroplast genomes of *S. horneri* and *F. vesiculosus* display the same gene synteny and have an overall nucleotide sequence identity of 82.1 %. Considering the genome organizations of *S. japonica* and *E. siliculosus*, multiple genome rearrangements occurred during the evolution of the Phaeophyceae (Le Corguillé et al. 2009).

Although gene order of brown algal cpDNAs are different at the order level (Wang et al. 2013), their gene length and identity display high similarity (Supplementary Tables S2 and S3). Gene identities in rRNA and tRNA genes of four brown algae are higher than those in PCGs (Fig. 3a). Among the PCGs, photosystem II-associated genes are most conserved based on both nucleotide and amino acid sequences (Fig. 3b). It is worth noting that the size of *ilvH* gene shows large variation in different brown algal cpDNAs, which is 489 bp in *S. horneri* and much shorter than 651 bp in *F. vesiculosus*, 603 bp in *S. japonica*, and 585 bp in *E. siliculosus*, due to the premature termination codon of the *ilvH* gene.

Conclusion

Sargassum horneri is the fourth brown algal species to have its complete cpDNA sequenced. The new data obtained will provide important information for us to understand plastid evolution as well as phylogeny in brown algae, especially the Sargassaceae. However, limited plastid genomic information severely restricts more detailed investigation on the evolution of the brown algae. Additional sequencing of unsampled taxonomic groups is necessary so that patterns of genome organization in cpDNAs could be further investigated for a better understanding of phylogenomic relationships in brown algae.

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