Complete mitochondrial genome of the brown alga *Sargassum horneri* (Sargassaceae, Phaeophyceae): genome organization and phylogenetic analyses

Feng Liu · Shaojun Pang · Xia Li · Jing Li

Abstract The genetics and molecular biology of the ecologically important brown alga, *Sargassum horneri* (Turner) C. Agardh, are poorly known. In this investigation, the complete nucleotide sequence of the mitochondrial (mt) genome of *S. horneri* was determined using long PCR and primer walking techniques. The mt genome is 34,606 bp in length and contains 3 ribosomal RNA genes, 25 transfer RNA genes, 35 protein-coding genes, and 2 open reading frames (ORFs). The overall AT content of the genome is 63.84 %, and the intergenic spacers constitute only 4.29 %. The genome organization of *S. horneri* mitochondrial DNA (mtDNA) is very similar to *Fucus vesiculosus* except that the counterparts of one putative tRNA^{Tyr} gene and ORF379 in *Fucus* were missing from *S. horneri* mtDNA. Phylogenetic analyses based on 3 ribosomal RNA genes and 35 protein-coding genes suggest that *S. horneri* has a closer relationship with *F. vesiculosus* than other analyzed brown algae. *Sargassum horneri* is the first species of Sargassaceae to have its mitochondrial genome sequenced. This will provide useful information on both population genetics and molecular evolution of the related species.

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

Introduction

The Phaeophyceae (brown algae) are multicellular photosynthetic marine organisms and display great morphological and physiological diversity (Yang et al. 2012). There are approximately 2,000 species of brown algae all over the world (Charrier et al. 2012). However, the data on their mitochondrial genomes are limited until now. The first complete mitochondrial DNA (mtDNA) sequence from *Pylaiella littoralis* (Ectocarpales) was reported in 2001 (Oudot-Le Secq et al. 2001). To date, the mitochondrial genomes of brown algae have been completed in 16 species/subspecies belonging to seven genera (Table 1). The information of complete mitochondrial genomes provides important insights into our understanding of the evolution of the Phaeophyceae and their organellar genomes (Oudot-Le Secq et al. 2006; Yotsukura et al. 2009; Zhang et al. 2013).

Mitochondria arose from a single endosymbiotic event of a α-proteobacterial ancestor and prokaryotic nature across the eukaryotic domain (Gray et al. 2001). The well-conserved genetic function of mtDNA involved several biological processes, e.g., electron-transport and oxidative phosphorylation, a distinctive protein-synthesizing system, RNA maturation, and protein import (Lang et al. 1999). More evidence highlights the divergent trends in mtDNA structure and gene expression mechanisms of many eukaryotic lineages (Burger et al. 2003). In the past decades, specific, short mitochondrial fragments were used in investigation of molecular evolution and reconstruction of phylogeny among Phaeophyceae (e.g., Engel et al. 2008; Draisma et al. 2010; Silberfeld et al. 2010). However, short fragments can only be used to a limited extent to resolve phylogenetic relationships at higher taxonomic levels. In recent years, the phylogeny of higher-level taxa have often been reconstructed based on the complete mtDNA sequences, which provide adequate information especially for the reliable resolution of the phylogenetic relationships of closely related species (Yotsukura et al. 2009; Zhang et al. 2013).

Sargassum is a widely distributed genus on rocky intertidal shores worldwide and represents one of the most species-rich

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Table 1 Completely sequenced mitochondrial genomes from brown algae

Taxa	Order	Accession number	Size of mitochondrial genome (bp)	Reference
Pylaiella littoralis	Ectocarpales	AJ277126	58,507	Oudot-Le Secq et al. (2001)
Ectocarpus_siliculosus	Ectocarpales	FP885846	37,189	GenBank
Dictyota dichotoma	Dictyotales	AY500368	31,617	Oudot-Le Secq et al. (2006)
Fucus vesiculosus	Fucales	AY494079	36,392	Oudot-Le Secq et al. (2006)
Desmarestia viridis	Desmarestiales	AY500367	39,049	Oudot-Le Secq et al. (2006)
Laminaria digitata	Laminariales	AJ344328	38,007	Oudot-Le Secq et al. (2002)
Laminaria hyperborea	Laminariales	JN099683	37,976	Zhang et al. (2013)
Saccharina japonica	Laminariales	AP011493	37,657	Yotsukura et al. (2009)
Saccharina japonica var. religiosa	Laminariales	AP011494	37,657	Yotsukura et al. (2009)
Saccharina japonica var. diabolica	Laminariales	AP011496	37,657	Yotsukura et al. (2009)
Saccharina japonica var. ochotensis	Laminariales	AP011495	37,656	Yotsukura et al. (2009)
Saccharina longipedalis	Laminariales	AP011497	37,657	Yotsukura et al. (2009)
Saccharina angustata	Laminariales	AP011498	37,604	Yotsukura et al. (2009)
Saccharina coriacea	Laminariales	AP011499	37,500	Yotsukura et al. (2009)
Saccharina longissima	Laminariales	JN099684	37,628	Zhang et al. (2013)
Saccharina japonica x latissima	Laminariales	JF937591	37,638	GenBank

genera of the marine macrophytes (Mattio and Payri 2011). *Sargassum horneri* (Turner) C. Agardh is a dioecious macroalga specially distributed in the Pacific Northwest coasts (Hu et al. 2011). This alga forms forests or meadows in sublittoral regions, serving as nursery habitats and spawning grounds for marine invertebrates and fish (Yatsuya 2008). Because of its ecological and environmental importance, *S. horneri* has received much attention over the past 30 years thus much of its basic physiological processes involving growth, development, reproduction, and taxonomy (e.g., Choi et al. 2008; Komatsu et al. 2008; Uwai et al. 2009). However, basic understanding of its genome is unknown.

In this investigation, the complete sequence of mitochondria genome was determined for *S. horneri*. Comparative genomic analyses and its structure and sequence were completed. The new information should provide a better understanding of mitochondrial genome diversity in the Phaeophyceae and insights into molecular evolution of Sargassaceae.

Materials and methods

Sample collection and DNA extraction

Mature plants of *Sargassum horneri* (Turner) C. Agardh were collected from the rocky shore at Xiaohuyu, Nanji Islands, Wenzhou, Zhejiang Province, China (27°27′N, 121°04′E) in April 2007 (Pang et al. 2009). Plants were transported to the laboratory in coolers (5–8 °C) within 24 h after collection. Algal culture was performed in 80-L polypropylene (PP) tanks under solar irradiance. Fresh algal tissue was selected and stored in the ultra-low temperature freezer (−80 °C). Frozen tissue was used for DNA extraction. Algal tissue was ground to fine powder in liquid nitrogen. Total DNA was extracted using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The concentration and the quality of isolated DNA were assessed by electrophoresis on 1.0 % agarose gel (Liu et al. 2013).

PCR amplification and sequencing

The whole mitochondrial genome of *S. horneri* was amplified using the long PCR technique (Cheng et al. 1994) and a primer walking method. Primer sets for the long PCR were as follows: mt23S-FB (Draisma et al. 2010)/trnW-trnI-R (Voisin et al. 2005), trnW-trnI-F (Voisin et al. 2005)/CAR4A (Kogame et al. 2005), CAF4A (Kogame et al. 2005)/cox1-1378R (Silberfeld et al. 2010), cox1-117 F (Bittner et al. 2008)/nad5-R (5′-AGCATAACGACAGTTAAACT-3′, this study), cox2-F (5′-TTGGWAAAAGATTATGCTCTTAA-3′, this study)/trnS-R (5′-TTGATTTAGCAAACCAAGGCTT-3′, this study), and trnS-F (5′-AAGCCTTGGTTTGCTAAA TCAA-3′, this study)/mt23S-RB (Draisma et al. 2010). Primer

sets were used to amplify the entire *S. horneri* mitochondrial genome in six large fragments. 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, 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, Goettingen, 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).

Sequence alignment and genome analysis

The DNA sequences were manually edited and assembled using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). DNA sequences of the complete mitochondrial genome of *S. horneri* were determined by comparison with published sequences for several brown algae (Oudot-Le Secq et al. 2001, 2002, 2006; Yotsukura et al. 2009; Zhang et al. 2013). Protein-coding genes were annotated by Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi) and DOGMA (Wyman et al. 2004). Ribosomal RNA genes were identified by BLAST searches (Altschul et al. 1997) of the nonredundant databases at the National Center for Biotechnology Information. Transfer RNA genes were searched for by reconstructing their cloverleaf structures using the tRNAscan-SE 1.21 software with default parameters (http://lowelab.ucsc.edu/tRNAscan-SE/. Schattner et al. 2005). Gene map of mitochondrial genome was generated using OGDRAW (Lohse et al. 2013).

Phylogenetic analysis

Seven brown algal complete mitochondrial sequences were used along with *S. horneri* in phylogenetic analysis. *Dictyota dichotoma* (AY500368) was used as outgroup based on Oudot-Le Secq et al. (2006). Phylogenetic relationships within Phaeophyceae were evaluated based on two datasets from mtDNA. One dataset included three ribosomal RNA genes (*rnl*, *rns*, and *rrn5*), and another contained 35 functionally known protein-coding genes as well as their concatenated protein sequences (*rps2-4*, *rps7*, *rps8*, *rps10-14*, *rps19*; *rpl2*, *rpl5*, *rpl6*, *rpl14*, *rpl16*, *rpl31*; *nad1-7*, *nad9*, *nad11*; *cob*; *cox1-3*; *atp6*, *atp8*, *atp9*; and *tatC*). Both datasets were subjected to concatenated alignments using ClustalX 1.83 with the default settings (Thompson et al. 1997). Base composition and pairwise comparison were examined by the MEGA 5.2 software (Tamura et al. 2011). Neighbor-joining (NJ) and maximum likelihood (ML) trees were conducted with 1,000 bootstrap replicates using MEGA5.2. The evolutionary distances were computed based on the Kimura two-parameter model for DNA sequences (Kimura 1980) and on the Whelan and Goldman + Freq. model for protein sequences (Whelan and Goldman 2001). Final results are presented using gaps as fifth character states for bases in DNA sequences.

Results and discussion

Genome organization

The complete mtDNA of *S. horneri* is a circular molecule of 34,606 bp in length (Fig. 1). It is shorter than other available Phaeophyceae mt genomes except for *D. dichotoma* (31,617 bp. Table 1). The *S. horneri* mitochondrium is gene dense, with 95.71 % of the sequence specifying genes and open reading frames (ORFs), and only 4.29 % noncoding (Table 2). The percentage of the intergenic spacer (4.29 %) in *S. horneri* is higher than that in *D. dichotoma* (3.21 %) but lower than those in other brown algae (5.62–6.81 %), which are closely related to the mt genome size and gene content. The overall AT content of *S. horneri* mtDNA is 63.84 %, similar to 62.01 % in *P. littoralis* to 66.49 % in *Ectocarpus siliculosus*. Intergenic spacers of *S. horneri* mtDNA are significantly rich in AT content (74.28 %). This pattern is similar to other brown algal mtDNA with the exception of *P. littoralis*, in which the AT content of protein-encoding region was 70.00 %, higher than that of intergenic spacers (67.41 %).

The *S. horneri* mt genome contains three ribosomal RNA genes (rRNA), 25 transfer RNA genes (tRNA), 35 protein-coding genes identified by sequence homology, as well as two ORFs (Table 3). Its total gene number is 65 and lower than other brown algae (66−79 genes). Genes in *S. horneri* mtDNA are encoded on both strands and are not interrupted by intron. The heavy strand (H) and the light strand (L) encode 59 and 6 genes, respectively.

In *S. horneri* mtDNA, intergenic spacers average only 28.0 nucleotides with a range of 0 to 172 nucleotides, which is significantly shorter than those of other Phaeophyceae mtDNA except for *D. dichotoma* (18.8 nucleotides with a range of 0 to 74 nucleotides). Twelve pairs of genes overlap by 1 to 66 nucleotides, with the average size of 13.7 nucleotides, which are in the same range as those of other brown algae. Two conserved overlapping regions, ATGA overlapped
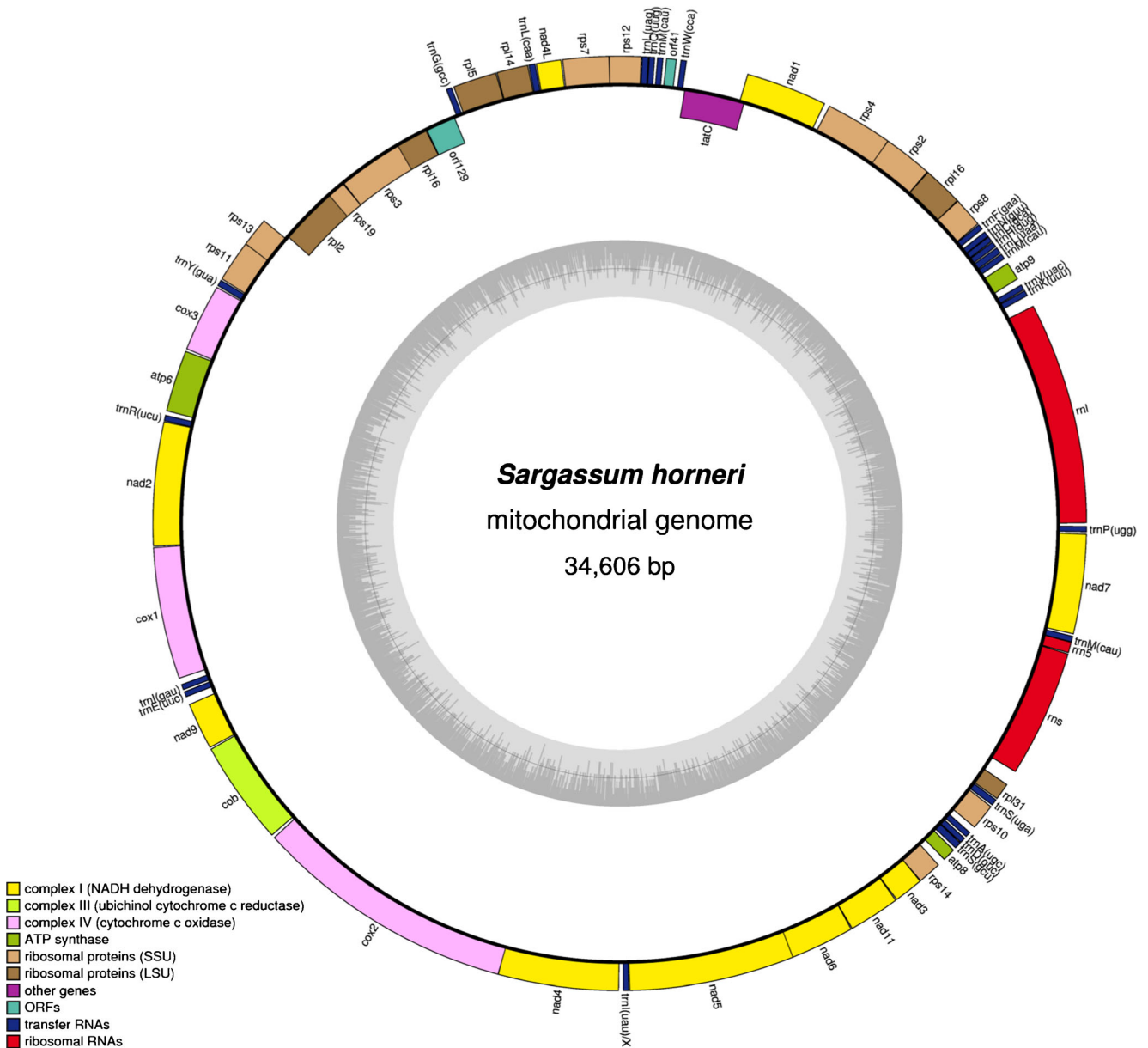
Fig. 1 Gene map of *Sargassum horneri* mitochondrial DNA. Genes (*filled boxes*) shown on the outside of the map are transcribed in a clockwise direction, whereas those on the inside of the map are transcribed counterclockwise

by *rps8* and *rpl6*, and A overlapped by *rpl6* and *rps2* reported by Oudot-Le Secq et al. (2006), are found in *S. horneri* mtDNA.

All protein-coding genes encoded by *S. horneri* mtDNA have a methionine (ATG) start codon. Other start codons have been found in other brown algal mtDNA, e.g., a GTG codon found at the beginning of *rps14* genes in *D. dichotoma*, ORF379 in *Fucus vesiculosus*, and ORF157 gene in *Laminaria digitata*; a TTG codon at the beginning of ORF211 gene in *Desmarestia viridis*, ORF37 gene in *D. dichotoma*, and *nad11* gene in *P. littoralis*, a CTG codon at the beginning of

nad11 gene in *E. siliculosus*. Three stop codons are used, with a preference of 64.86 % for TAA (16.22 % for TAG and 18.92 % for TGA). This feature was shared in all of reported brown algal mtDNA, with proportional changing to a certain extent.

The mitochondrial genome of *S. horneri* shows remarkable similarity to other brown algae in terms of gene content, gene organization, AT composition, and gene sequences. In terms of all this attributes, *S. horneri* mtDNA is more similar to *F. vesiculosus* mtDNA as expected. This suggests that *S. horneri* has a close evolutionary relationship with

Table 2 General features of mitochondrial genomes in eight species of brown algae

Genome features	Sargassum horneri	Fucus vesiculosus AY494079	Laminaria digitata AJ344328	Saccharina japonica AP011493	Dictyota dichotoma AY500368	Desmarestia viridis AY500367	Ectocarpus siliculosus FP885846	Pylaiella littoralis AJ277126
Overall AT content (%)	63.84	65.55	64.86	64.70	63.48	63.40	66.49	62.01
Protein AT content (%)[a]	64.85	66.72	66.00	65.82	64.55	64.34	67.75	70.00
rRNA AT content (%)	58.15	57.81	55.81	56.20	58.88	55.72	57.28	56.59
tRNA AT content (%)	54.40	53.38	52.40	52.89	52.96	52.86	52.79	52.25
Spacer AT content (%)[b]	74.28	77.35	76.84	75.87	76.53	74.06	79.31	67.41
Protein-encoding content (%)	77.73	77.19	77.16	76.79	77.38	77.88	76.83	75.79
Spacer content (%)	4.29	5.62	6.42	6.49	3.21	6.06	6.34	6.81
Spacer size (bp)	0–172	0–422	0–310	0–361	0–74	0–385	0–356	0–632
Average spacer size (bp)	28.0	35.9	45.2	46.1	18.8	43.0	43.7	69.9
Pairs of overlapping genes	12	10	13	13	12	13	14	17
Overlap size (bp)	1–66	1–66	1–54	1–16	1–30	1–60	1–59	1–24
Average overlap size (bp)	13.7	12.4	12.2	7.3	8.3	15.2	14.2	7.9
Stop codons for TAA (%)[c]	64.86	81.58	74.36	68.42	63.16	79.49	67.50	73.08
Stop codons for TAG (%)	16.22	13.16	15.38	21.05	15.79	15.38	20.00	13.46
Stop codons for TGA (%)	18.92	5.26	10.26	10.53	21.05	5.13	12.50	13.46

[a] Unidentified ORFs are thought as protein-encoding genes. Introns in the *P. littoralis* mtDNA are excluded

[b] Introns in the *P. littoralis* mtDNA are not taken as the spacers

[c] The stop codons of unidentified ORFs are included

Table 3 Genes identified in mitochondrial genomes of eight species of brown algae

Genes	Sargassum horneri	Fucus vesiculosus	Laminaria digitata	Saccharina japonica	Dictyota dichotoma	Desmarestia viridis	Ectocarpus siliculosus	Pylaiella littoralis
A. Ribosomal RNA genes (rnl, rns, rrn5)	+	+	+	+	+	+	+	+
B. Transfer RNA genes	25	26	25	25	25	26	25	24
C. Ribosomal protein genes (rps2-4, 7, 8, 10–14, 19; rpl2, 5, 6, 14, 16, 31)[a]	+	+	+	+	+	+	+	+
D. Complex I (nad1-7, 9, 11)	+	+	+	+	+	+	+	+
E. Complex III (cob)	+	+	+	+	+	+	+	+
F. Complex IV (cox1-3)	+	+	+	+	+	+	+	+
G. Complex V (atp6, 8, 9)	+	+	+	+	+	+	+	+
H. tatC	+	+	+	+	+	+	+	+
I. rpo	−	−	−	−	−	−	−	+
J. Unidentified conserved ORFs	ORF41, ORF129	ORF43, ORF131, ORF379	ORF40, ORF129, ORF384	ORF41, ORF130, ORF377	ORF37, ORF111	ORF39, ORF143, ORF622	ORF42, ORF128	ORF127
K. Unidentified unique ORFs	−	−	ORF157	−	ORF54	ORF211	ORF75, ORF183, ORF80	ORF31, ORF97, ORF132, ORF237, ORF39, ORF121, ORF224, ORF261, ORF64, ORF233, ORF557, ORF568, ORF757, ORF795, ORF748
Total	65	67	67	66	66	68	68	79

[a] Genes are classified according to their function. Numbers within parentheses indicate the gene terms in specific functional groups

F. vesiculosus as compared to other brown algae supporting current taxonomic systems. This was confirmed by recent morphological, phylogenic, and classification data (Guiry and Guiry 2014).

The mtDNA genomes from different brown algae display a highly conservative architecture (Oudot-Le Secq et al. 2006; Yotsukura et al. 2009; Zhang et al. 2013), but differ in terms of different genome size. Size of mtDNA genomes are reduced by several mechanisms including but not exclusive of gene transfer to the nucleus, intron loss, elimination of intergenic spacers, and overlap of adjacent coding genes (Burger et al. 2003). Comparisons among eight brown algae have indicated that the reduction of mtDNA size in *S. horneri* are closely associated with the above aspects, e.g., the loss of one putative tRNA^{Tyr} gene and ORF379 found in *F. vesiculosus*, no intron found in genes, the average spacer size of 28.0 bp, and the average overlap size of 13.7 bp.

Ribosomal and transfer RNA genes

The predicted sizes of *rnl* and *rns* genes in *S. horneri* are 2,667 and 1,536 bp, respectively, consistent with currently reported Phaeophyceae rRNAs (Oudot-Le Secq et al. 2006; Yotsukura et al. 2009). Both can fold in accordance with conserved, eubacteria-like model. The *rrn5* gene of the *S. horneri* mtDNA is separated from the *rns* by six nucleotides, TTAA GA and overlaps the tRNA^{Met-3} gene with three nucleotides, TGC.

The *S. horneri* mt genome contains 25 tRNA genes ranging from 72 to 88 nucleotides in size (Table 4). All of the tRNA genes could fold into a typical cloverleaf secondary structure as determined by tRNAscan-SE. The anticodon usage is identical to that found in other brown algae. Among them, six tRNA genes (Leu-1, Leu-2, Leu-3, Tyr, Ser-1, and Ser-2) contain the extra arms. The tRNA^{Met-2} gene located between tRNA^{Gln} and ORF41genes was labeled as tRNA^{Ile} in *F. vesiculosus*, *L. digitata*, *Saccharina japonica*, *D. dichotoma*, *D. viridis*, and *P. littoralis*, but also tRNA^{Met-2} in *E. siliculosus*. The counterparts of the tRNA^{Ile-2} gene were different in other brown algae, e.g., X (uncertain amino acid) in *F. vesiculosus*, *L. digitata* and *S. japonica*, Lys in *D. dichotoma* and *D. viridis*, Ser in *E. siliculosus*, and Arg in *P. littloralis*. However, one putative tRNA gene (the tRNA^{Tyr} gene) shared by *F. vesiculosus* and *D. viridis* mt genomes were not detected in the *S. horneri* mt genome.

Protein-encoding genes and ORFs

The identified mitochondrial protein-encoding gene set is the same as those in currently reported Phaeophyceae mt genomes (Oudot-Le Secq et al. 2006). These 35 genes encoded 17 ribosomal proteins (*rps2-4*, *7*, *8*, *10–14*, *19*; *rpl2*, *5*, *6*, *14*, *16*, *31*), 10 subunits of the NADH dehydrogenase (*nad1-7*, *4 L*, *9* and *11*), apocytochrome b (*cob*), 3 first subunits of the cytochrome oxidase (*cox1-3*), 3 subunits of the ATPase (*atp6*, *8*, and *9*), and a protein transporter component of the *secY*-independent pathway (*tatC*). No notable reduction or extension of gene length was observed compared to other Phaeophyceae except the *cox2* gene. The *cox2* gene in *S. horneri* contains an in-frame insertion of 2,277 bp. Such insertion also exists in the *cox2* genes of *F. vesiculosus* (2,283 bp), *E. siliculosus* (2,946 bp), *P. littoralis* (2,973 bp), *L. digitata* (2,979 bp), *S. japonica* (2,982 bp), and *D. viridis* (2,994 bp), but is absent in the *D. dichotoma cox2* gene. The insertion with a phage-type RNA polymerase gene found in *P. littoralis* mtDNA is not detected in the *S. horneri* mt genome.

Two conserved ORFs (ORF129 and ORF41) are found in *S. horneri* mtDNA, whereas another conserved ORF, ORF379 in *F. vesiculosus*, is missing from *S. horneri* mtDNA. All currently reported Phaeophyceae mt genomes contain only one specific ORF, ORF129 (*S. horneri*), ORF131 (*F. vesiculosus*), ORF129 (*L. digitata*), ORF130 (*S. japonica*), ORF111 (*D. dichotoma*), ORF143 (*D. viridis*), ORF128 (*E. siliculosus*), and ORF127 (*P. littoralis*). The homologous genes of ORF41 were lost in *P. littoralis*, but are present in other brown algae, ORF43 (*F. vesiculosus*), ORF42 (*E. siliculosus*), ORF40 (*L. digitata*), ORF41 (*S. japonica*), ORF39 (*D. viridis*), and ORF37 (*D. dichotoma*). No unique ORF was found in the *S. horneri* mtDNA.

Phylogenetic relationships within Phaeophyceae

In order to carry out a basic phylogenetic analysis, 3 ribosomal RNA genes and 35 protein-coding genes and the derived amino acid sequences identified in *S. horneri* were aligned with other brown algal species. Both maximum likelihood and neighbor-joining analyses were run, with the analyses generating slightly different topologies between two datasets and high bootstrap support values. Phylogenetic trees showed that eight species of brown algae well fell into five clades standing for five different orders: Laminariales (two species), Desmarestiales (one), Ectocarpales (two), Fucales (two), and Dictyotales (one). *Sargassum horneri* was tightly combined with *F. vesiculosus*, forming the Fucales clade in all the phylogenetic trees with high bootstrap support values (99–100 %) (Figs. 2 and 3).

The topology of the trees obtained in this study was similar to that from the previous results based on few mitochondrial, plastid, and nuclear markers (e.g., Silberfeld et al. 2010; Charrier et al. 2012). However, based on the rRNA dataset, Laminariales firstly combined with Desmarestiales with bootstrap values of

Table 4 The aligned sequences of the 25 tRNA genes detected in the *Sargassum horneri* mtDNA

tRNA	Stem	Stem	D loop	Stem	Stem	Stem	Anticodon loop[a]	Stem	Stem	Variable loop	Stem	TΨC loop	Stem	Stem
Lys	GGGTGTG	TA ACTC	AGAT	GGT TA	GAGT AG	TGGA	CT TTT AA	TCCG	AGGG		TC TTGGG	TTCAAAT	CCCAA	CACACCTA
Val	GGGAGCA	TA ACTC	AGT	GGT A	GAGT A	TGTGC	CT TAC AA	GCATG	AAG		TC GTGAG	TTCGATC	CTCTC	TGGTCCCA
Met-1	AACGGTG	TA GTTC	AGT	GGT TA	GAAC A	TTGGA	AT CAT AT	CCCAA	ATGG		C GGGGG	TTCGAAT	CCCTT	TTCCGTTA
Leu-1	GCGACTT	TG GTG	AAATT	GGT ATA	CAC GT	CAGA	CT TAA AA	TCTG	TTT	CTGTTTTAAGAG	T ATCGG	TTCAAGT	CCGAT	AAGTCGTA
His	GCGGATA	TA ACTC	AGAT	GGT TA	GAGT A	CCAGA	TT GTG GC	TTTGG	ACGA		C GGGGG	TTCAAGT	CCCCT	TATTCGCC
Cys	GGCTTGA	TA GTAT	AATG	GGT TA	ATGC G	GCGGG	TT GCA AA	CCCAC	AAA		T GAGGG	TTCAAAT	CCCTC	TCAAGCTT
Asn	TCTTCAA	TA GCTC	AATT	GGT A	GAGC A	TATGG	CT GTT AA	CCATA	GCG		TT GTTGG	TTCGAGT	CCAAT	TTGGGGAG
Phe	GTTTGAG	TA GCTC	AGTT	GGT A	GAAC T	AAGGA	CT GAA AA	TCCTT	AGG		TC GTTGG	TTCAATC	CCAGT	CTCAAACA
Trp	AGGAAGA	TA GTTT	AATT	GGT A	AAAC A	TTGGT	TT CCA AA	GCCAA	CGG		CT GGGGG	TTCAAAT	CCCTC	TCTTTCTG
Met-2/f?	CGGTTTG	TA GTTT	AATA	GGT A	GGC A	TAGTT	CT CAT GA	AGCTA	GTA		TT GCAGG	TTCAACT	CCTGC	CGGACTGA
Gln	TGGAGTC	TA GCC	AAGTT	GGT AA	CAC G	CCCGT	TT TTG GT	GCGGG	GAT		C GAAGG	TTCGAGT	CCTCC	GACTCCAC
Leu-2	GCCAGGG	TG GTG	GAAGT	GGT AGA	CAC G	CTGGG	TT TAG GT	TCCAG	T	CTTTTGTAGG	GTA GGGG	TTCAAAT	CCCT	CTCTGGTA
Leu-3	GCTCCCA	TG GTG	AAATT	GGT AGA	CAC G	GCAGA	TT CAA AA	TCTTT	T	GTCTTTTGAC	AT GCTGG	TTCAAAT	CCGGC	TGGGAGCA
Gly	GCGGAAG	TA ACTC	AATC	GGT A	GAGT G	TAAGC	TT GCC AA	GTTTA	AAG		TT GAGGG	TTCAAAT	CCCTT	CTTTCGCT
Tyr	GAAGGGG	TG ACTG	AGG	GGT TGA	TAGT GT	CAGA	TT GTA GT	TCTG	TT	GGTTTTACC	ATC GTAGG	TTCGAAT	CCTAT	CCCCTTCT
Arg	GCATTTT	TA GCTC	AGT	GGA TA	GAGC A	ACGGT	CT TCT AA	ATCGT	GGG		TC ATAGG	TTCAAAT	CCTAT	AAGATGTA
Ile-1	GGGCCTA	TA ACTC	AGT	GGT A	GAGT A	TACGC	CT GAT AA	GCGTA	AAG		TC GATCG	TTCAATC	CGATC	TAGGCCCA
Glu	GTCCCTT	TG GTCT	AGT	GGT TA	GGAC G	TTGCC	TT TTC AC	GGCAG	AGA		T ATGGG	TTCAATT	CCCAT	AAGGGATT
Ile-2/X?	GTTCTTT	TC GTCT	AAT	GGT TA	GGAT A	TTATC	TT TAT GA	GATAA	AGG		T GCGGG	TTCAAGG	CCCGT	AAAGAACT
Ser-1	GGAGATG	TG GCTG	AGT	GGC TGA	TAGC C	TTGGT	TT GCT AA	ATCAA	T	CTATATTTTTAATGTAG	C GTGGG	TTCGAAT	CCCAC	CATCTCTT
Asp	GGAAAAG	TA ACTC	AGAT	GGT A	GAGT G	CTTGC	TT GTC AT	GCCAG	TGG		TC GCGGG	TTCGAAT	CCCGT	CTTTTTCG
Ala	GGGGATA	TA ACTC	AATT	GGT A	GAGT A	TATGC	TT TGC AA	GCATA	AAG		TT GAGAG	TTCAAAT	CTCTT	TATCTCCA
Ser-2	GGATAAG	TG ACCG	AGT	GGT TTA	TGGT A	TCAGT	TT TGA AA	ACTGA	C	GCAGACAAACTGC	C GTGGG	TTCGAAT	CCTAC	CTTATCCT
Met-3	TGCGTTA	TA GAGC	AGTTA	GGT TA	GCTC A	CCAGG	CT CAT GC	CCTGG	AGG		TC ATAGG	TTCAAAT	CCTGT	TGACGCTA
Pro	CGAGAGA	TA ACGT	AGT	GGT A	GCGT G	TTCGC	TT TGG GA	GTGAA	AAG		TC GTAGG	TTCGAAT	CCTAC	TCTCTTGA

[a] Anticodons are indicated under grey background

Fig. 2 Phylogenetic tree of brown algal relationships derived from three rRNA genes (*rnl*, *rns*, and *rrn5*) in the mt genome. *Dictyota dichotoma* served as outgroups. Numbers in each branch indicated maximum likelihood (ML, *above*) and neighbor-joining (NJ, *below*) bootstrap values
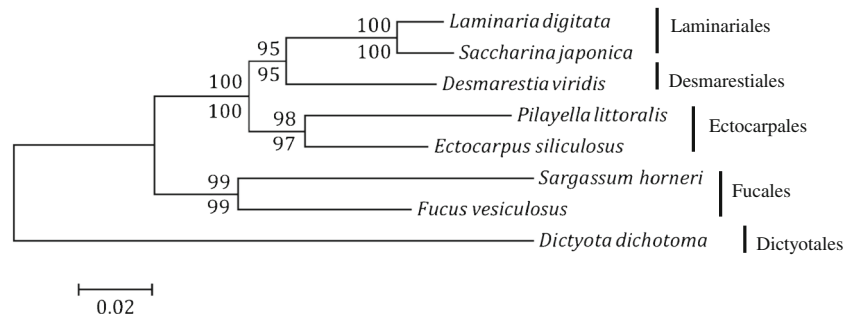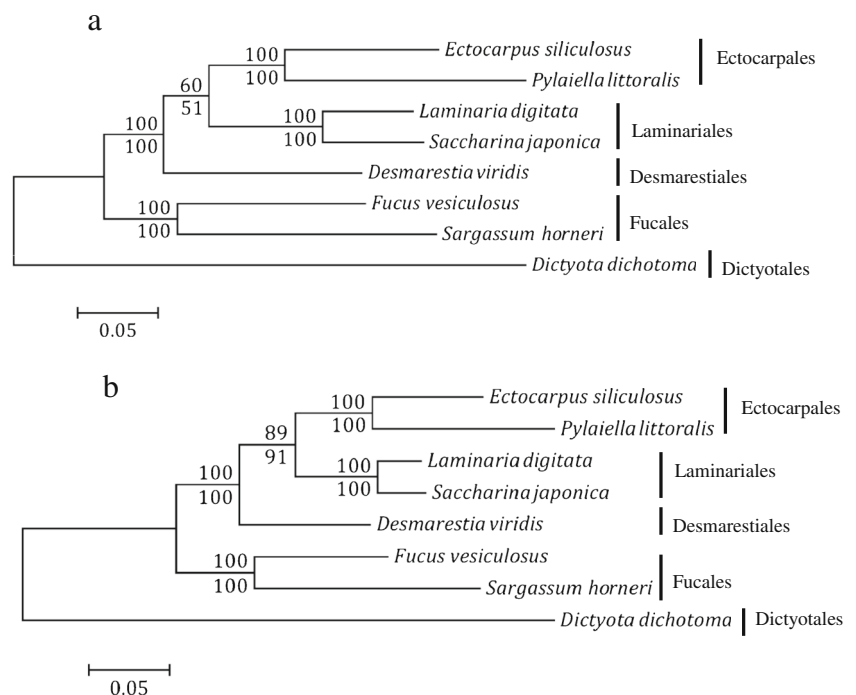

95 % and then Ectocarpales with bootstrap values of 100 %, while, in the latter dataset, Laminariales firstly combined with Ectocarpales with low bootstrap values (ML/NJ=60/51 %) based on DNA sequences but high bootstrap values (ML/NJ=89/91 %) based on protein sequences. These different topologies revealed that the extent of sequence variation or evolution was different between rRNA region and the total protein-coding gene region.

The completely sequenced and annotated mtDNA of *S. horneri* represents an important source of information for understanding not only evolution of the mitochondrial genome in the Phaeophyceae but also provides insights into the evolutionary history of the class. Mitochondrial DNA markers designed for phylogenetics were scarce due to the limited data but very useful to understand phaeophycean phylogenies. Lane et al. (2007) and Uwai et al. (2007) used *cox1* and *cox3* to define species limits and genetic relationships in the orders Laminariales, respectively. The mtDNA 23S sequence (*rnl*) and the variable mt23S-tRNA^{Val} intergenic spacer were employed to infer interspecific and intergeneric relationships and delineate genera within the order Fucales (Coyer et al. 2006; Draisma et al. 2010). Based on ten mitochondrial (*cox1*, *cox3*, *nad1*, *nad4*, and *atp9*), plastid, and nuclear loci, Silberfeld et al. (2010) hypothesized that the brown algal crown radiation (BACR) likely represents a gradual diversification during the Lower Cretaceous instead of a sudden radiation as proposed earlier. Based on the complete mtDNA sequences of *S. horneri*, new mtDNA markers could be selected and employed to study the diversity of *S. horneri* populations and Sargassaceae phylogenetics in the future.

Fig. 3 Phylogenetic tree of brown algal relationships derived from the nucleotide (a) and amino acid (b) datasets of 35 functionally known protein-coding genes (*rps2-4*, *rps7*, *rps8*, *rps10-14*, *rps19*; *rpl2*, *rpl5*, *rpl6*, *rpl14*, *rpl16*, *rpl31*; *nad1-7*, *nad9*, *nad11*; *cob*; *cox1-3*; *atp6*, *atp8*, *atp9*; and *tatC*) in the mt genome. *Dictyota dichotoma* served as outgroups. *Numbers* in each branch indicated maximum likelihood (ML, *above*) and Neighbor-joining (NJ, *below*) bootstrap values

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