



# Understanding the Evolution of Mitochondrial Genomes in Phaeophyceae Inferred from Mitogenomes of *Ishige okamurae* (Ishigeales) and *Dictyopteris divaricata* (Dictyotales)

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## Abstract

To gain further insight into the evolution of mitochondrial genomes (mtDNAs) in Phaeophyceae, the first recorded characterization of an Ishigeophycidae mtDNA from *Ishige okamurae* (Yendo), and only the second recorded characterization of a Dictyotophycidae mtDNA from *Dictyopteris divaricata* (Okamura) Okamura are presented in this study. The 35,485 bp *I. okamurae* mtDNA contained 36 protein-coding genes (PCGs), 22 tRNAs, three rRNAs, and four open reading frames (*orfs*), and the 32,021 bp *D. divaricata* mtDNA harbored 35 PCGs, 25 tRNAs, three rRNAs, and three *orfs*. The A + T content in *D. divaricata* (61.69%) was the lowest recorded in sequenced brown algal mtDNAs. The *I. okamurae* mtDNA displayed unique genome features including an elevated start-codon usage bias for GTG, while the organization of *D. divaricata* mtDNA was identical to that of *Dictyota dichotoma*. Phylogenetic analysis based on the amino acid sequence dataset of 35 PCGs indicated that *I. okamurae* (Ishigeophycidae) diverged early from the Fucophycidae–Dictyotophycidae complex, which was confirmed by the comparative analysis of the mitogenome structure. The novel mitogenome data made available by this study have improved our understanding of the evolution, phylogenetics, and genomics of brown algae.

**Keywords** Mitochondrial genome · Brown algae · Ishigeales · Dictyotales · Evolution · Phaeophyceae

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## Introduction

Phaeophyceae, or brown algae, are a group of multicellular photosynthetic organisms in the Heterokontophyta (Stramenopiles) (Yang et al. 2012; Charrier et al. 2012). Most

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brown algae species are found predominantly in marine environments, with only a few freshwater species identified thus far (e.g., *Pleurocladia lacustris*) (Wehr et al. 2013). Phylogenetic studies have shown that Discosporangiales and Ishigeales were the monophyletic early-diverging lineages, while the other brown algae were clustered into two superclades representing two subclasses, Dictyotophycidae and Fucophycidae (Guiry and Guiry 2018; Liu et al. 2017a). Brown algae are important primary producers in our oceans, forming essential ecological structures known as marine forests and seaweed beds that provide food and shelter to a diverse range of invertebrates, fishes, sea turtles, birds, and mammals (Laffoley et al. 2011, Witherington et al. 2012; Wikipedia 2018). Large-scale cultivation of commercial kelps (e.g., *Saccharina* and *Undaria*) using artificial floating raft systems is commonplace in several East Asian countries (Tseng 2001). On the other hand, some *Sargassum* species (e.g., *Sargassum horneri* and *Sargassum natans*) can maintain high growth rates while in a floating state within the pelagic zone, forming harmful macroalgal blooms known as “golden tides,” which can cause considerable damage to the local environment and economy (Smetacek and Zingone 2013; Liu et al. 2017b).

The launch of several genome sequencing projects to understand the evolutionary history of brown algae led to the complete genome sequences of two species, *Ectocarpus siliculosus* and *Saccharina japonica*, to date (Cock et al. 2010; Ye et al. 2015). Considering that more than 2000 brown algal species have been identified worldwide (Guiry and Guiry 2018), our understanding of the evolutionary history of brown algae still remains very limited. Organelle genomes (mtDNAs and cpDNAs) carry important genetic information widely utilized in phylogenetics and comparative genomics implemented to broaden our understanding of Phaeophyceae evolutionary history. The advent of molecular systematics has brought additional insights, reshaping evolutionary concepts associated with brown algae (Silberfeld et al. 2014).

Previous work has unveiled the organelle genome sequences in several brown algal species (e.g., Oudot-Le Secq et al. 2001, 2002, 2006; Le Corguillé et al. 2009; Yotsukura et al. 2010; Zhang et al. 2013; Wang et al. 2013; Liu et al. 2015, 2017a, b; Liu and Pang 2016b). So far, the complete mitochondrial genomes (mtDNAs) of 41 brown algal species have been sequenced (Table 1), which has provided considerable insight into evolutionary and phylogenetic concepts of brown algae. The sequenced mtDNAs are from several orders belonging to two subclasses including the order Dictyotales in subclass Dictyotophycidae (1 species), and four orders in Fucophycidae including Ectocarpales (7 species), Laminariales (15 species), Desmarestiales (1 species), and Fucales (17 species) (i.e., the ELDF complex). There are some general observations of the complete mtDNAs reported thus far including that they

are single circular molecules of 31.6–58.5 kb in size; harbor 35–36 protein-coding genes (PCGs), three ribosomal RNA genes (rRNAs), 24–26 transfer RNA genes (tRNAs), and 2–16 open reading frames (*orfs*); and display highly conserved genome architecture (Liu and Pang 2015a).

*Ishige okamurae* Yendo grows in the upper intertidal zones of the coastlines of South China, Japan, and Korea (Lee et al. 2009), and is the holotype of the order Ishigeales, subclass Ishigeophycidae (Guiry and Guiry 2018). Previous studies have shown Ishigeales to be a monophyletic early-diverging lineage in Phaeophyceae, yet very little is known about the genome information within this order (Liu et al. 2017a). *Dictyopteris divaricata* (Okamura) Okamura inhabits littoral and sublittoral rock zones and belongs to the order Dictyotales, subclass Dictyotophycidae (Guiry and Guiry 2018). To date, only one species, *Dictyota dichotoma*, from Dictyotales has had its mitogenome completely sequenced (Oudot-Le Secq et al. 2006).

In this study, we present the first recorded characterization of an Ishigeophycidae mtDNA from *Ishige okamurae* (Yendo), and only the second recorded characterization of a Dictyotophycidae mtDNA from *Dictyopteris divaricata* (Okamura) Okamura. The two novel mtDNA sequences are then compared to the mtDNA sequences reported in Dictyotales, Ectocarpales, Laminariales, Fucales, and Desmarestiales, providing further insights into the evolution of mitogenomes in the class Phaeophyceae.

## Materials and Methods

### Algal Sampling and DNA Extraction

Adult plants of *Ishige okamurae* Yendo were collected from the rocky shore of Houjiang, Zhangpu, Fujian Province, China (23°54'N, 117°46'E) on January 2, 2018. Algal thalli of *Dictyopteris divaricata* (Okamura) Okamura were sampled from the rocky shore of No. 3 bathing beach of Qingdao, Shandong Province, China (36°03'N, 120°22'E) on July 16, 2016 (Liu et al. 2017a). Seaweed samples were transported to the laboratory in coolers (5–8 °C) after collection. Clean thalli without epiphytic brown algae were examined by microscopy and selected for storage in –80 °C freezers. Frozen tissue from the original algal samples was used for DNA extraction. The algal tissue was ground to fine powder in liquid nitrogen using a Scientz-48 Tissue Grinder (Scientz Biotech, Ningbo, China). Total DNA was extracted using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The concentration and quality of isolated DNA was evaluated by electrophoresis on 1.0% agarose gel.

**Table 1** The known mitochondrial genome sequences (mtDNAs) in the class Phaeophyceae

| Subclass                     | Order                  | Genus                      | Species                        | Accession number             | References                  |
|------------------------------|------------------------|----------------------------|--------------------------------|------------------------------|-----------------------------|
| Ishigeophycidae (1)          | Ishigeales (1)         | <i>Ishige</i> (1)          | <i>Ishige okamurae</i>         | MG940857                     | This study                  |
| Dictyotophycidae (2)         | Dictyotales (2)        | <i>Dictyopteris</i> (1)    | <i>Dictyopteris divaricata</i> | MG940856                     | This study                  |
|                              |                        | <i>Dictyota</i> (1)        | <i>Dictyota dichotoma</i>      | AY500368                     | Oudot-Le Secq et al. (2006) |
| Fucophycidae (≈ 40)          | Ectocarpales (7)       | <i>Pylaiella</i> (1)       | <i>Pylaiella littoralis</i>    | AJ277126                     | Oudot-Le Secq et al. (2001) |
|                              |                        | <i>Pleurocladia</i> (1)    | <i>Pleurocladia lacustris</i>  | KU164873                     | Wang (2014)                 |
|                              |                        | <i>Ectocarpus</i> (1)      | <i>Ectocarpus siliculosus</i>  | FP885846                     | GenBank                     |
|                              |                        | <i>Endarachne</i> (1)      | <i>Endarachne binghamiae</i>   | MF374731                     | GenBank                     |
|                              |                        | <i>Petalonia</i> (1)       | <i>Petalonia fascia</i>        | KJ957769                     | Liu and Pang (2015b)        |
|                              |                        | <i>Scytosiphon</i> (1)     | <i>Scytosiphon lomentaria</i>  | KJ995702                     | Liu and Pang (2016a)        |
|                              |                        | <i>Colpomenia</i> (1)      | <i>Colpomenia peregrina</i>    | KM244739                     | Liu et al. (2016b)          |
|                              | Laminariales (≈ 15)    | <i>Undaria</i> (1)         | <i>Undaria pinnatifida</i>     | KF319031                     | Li et al. (2015)            |
|                              |                        | <i>Costaria</i> (1)        | <i>Costaria costata</i>        | KF384641                     | Qu et al. (2015)            |
|                              |                        | <i>Saccharina</i> (≈ 11)   | <i>Saccharina japonica</i>     | AP011493                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina longissima</i>   | JN099684                     | Zhang et al. (2013)         |
|                              |                        |                            | <i>Saccharina angustata</i>    | AP011498                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina coriacea</i>     | AP011499                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina ochotensis</i>   | AP011495                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina longipedalis</i> | AP011497                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina diabolica</i>    | AP011496                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina religiosa</i>    | AP011494                     | Yotsukura et al. (2010)     |
|                              |                        |                            | <i>Saccharina sculpera</i>     | KR350664                     | Zhang et al. (2016)         |
|                              |                        |                            | <i>Saccharina latissima</i>    | KM675818                     | GenBank                     |
|                              |                        |                            | <i>Saccharina</i> sp.          | KT315643                     | GenBank                     |
|                              |                        | <i>Laminaria</i> (2)       | <i>Laminaria digitata</i>      | AJ344328                     | Oudot-Le Secq et al. (2002) |
|                              |                        |                            | <i>Laminaria hyperborea</i>    | JN099683                     | Zhang et al. (2013)         |
|                              | Fucales (17)           | <i>Coccophora</i> (1)      | <i>Coccophora langsdorfi</i>   | KU255794                     | Graf et al. (2017)          |
|                              |                        | <i>Turbinaria</i> (1)      | <i>Turbinaria ornata</i>       | KM501562                     | Liu and Pang (2015a)        |
|                              |                        | <i>Sargassum</i> (13)      | <i>Sargassum confusum</i>      | MG459430                     | Liu et al. (2018)           |
|                              |                        |                            | <i>Sargassum horneri</i>       | KJ938300                     | Liu et al. (2015)           |
|                              |                        |                            | <i>Sargassum thunbergii</i>    | KP280065                     | Liu and Pang (2016d)        |
|                              |                        |                            | <i>Sargassum vachellianum</i>  | KR132242                     | Bi and Zhou (2016)          |
|                              |                        |                            | <i>Sargassum muticum</i>       | KJ938301                     | Liu and Pang (2016c)        |
|                              |                        |                            | <i>Sargassum hemiphyllum</i>   | KM210510                     | Liu et al. (2016a)          |
|                              |                        |                            | <i>Sargassum fusiforme</i>     | KJ946428                     | Liu et al. (2016c)          |
|                              |                        |                            | <i>Sargassum polycystum</i>    | KT280278                     | Liu et al. (2017b)          |
|                              |                        |                            | <i>Sargassum spinuligerum</i>  | KT276514                     | Liu et al. (2017b)          |
| <i>Sargassum ilicifolium</i> |                        |                            | KT272403                       | Liu et al. (2017b)           |                             |
| <i>Sargassum aquifolium</i>  |                        |                            | KT266809                       | Liu et al. (2017b)           |                             |
| <i>Sargassum natans</i>      |                        |                            | KY084911                       | Amaral-Zettler et al. (2017) |                             |
| <i>Sargassum fluitans</i>    |                        |                            | KY084909                       | Amaral-Zettler et al. (2017) |                             |
| <i>Fucus</i> (2)             |                        | <i>Fucus vesiculosus</i>   | AY494079                       | Oudot-Le Secq et al. (2006)  |                             |
|                              |                        | <i>Fucus distichus</i>     | KY678904                       | Hughey and Gabrielson (2017) |                             |
| Desmarestiales (1)           | <i>Desmarestia</i> (1) | <i>Desmarestia viridis</i> | AY500367                       | Oudot-Le Secq et al. (2006)  |                             |

## PCR Amplification, Sequencing, and Assembly

Both species have previously been identified by morphological and molecular methods (data not given; Liu et al. 2017a). The mitochondrial genome sequences of *I. okamurae* and *D.*

*divaricata* were amplified using the long PCR and primer walking techniques (Cheng et al. 1994). The novel primer sets were designed and used to amplify the entire *I. okamurae* and *D. divaricata* mitochondrial genomes into four and five long fragments, respectively (Supplementary data:

**Fig. 1** The mitochondrial genome maps of **a** *Ishige okamurae* and **b** *Dictyopteris divaricata*. Annotated genes are colored according to the functional categories. Genes on the inside are transcribed in the clockwise direction, whereas genes on the outside are transcribed in the counterclockwise direction. The ring of bar graphs on the inner circle shows the GC content in dark gray

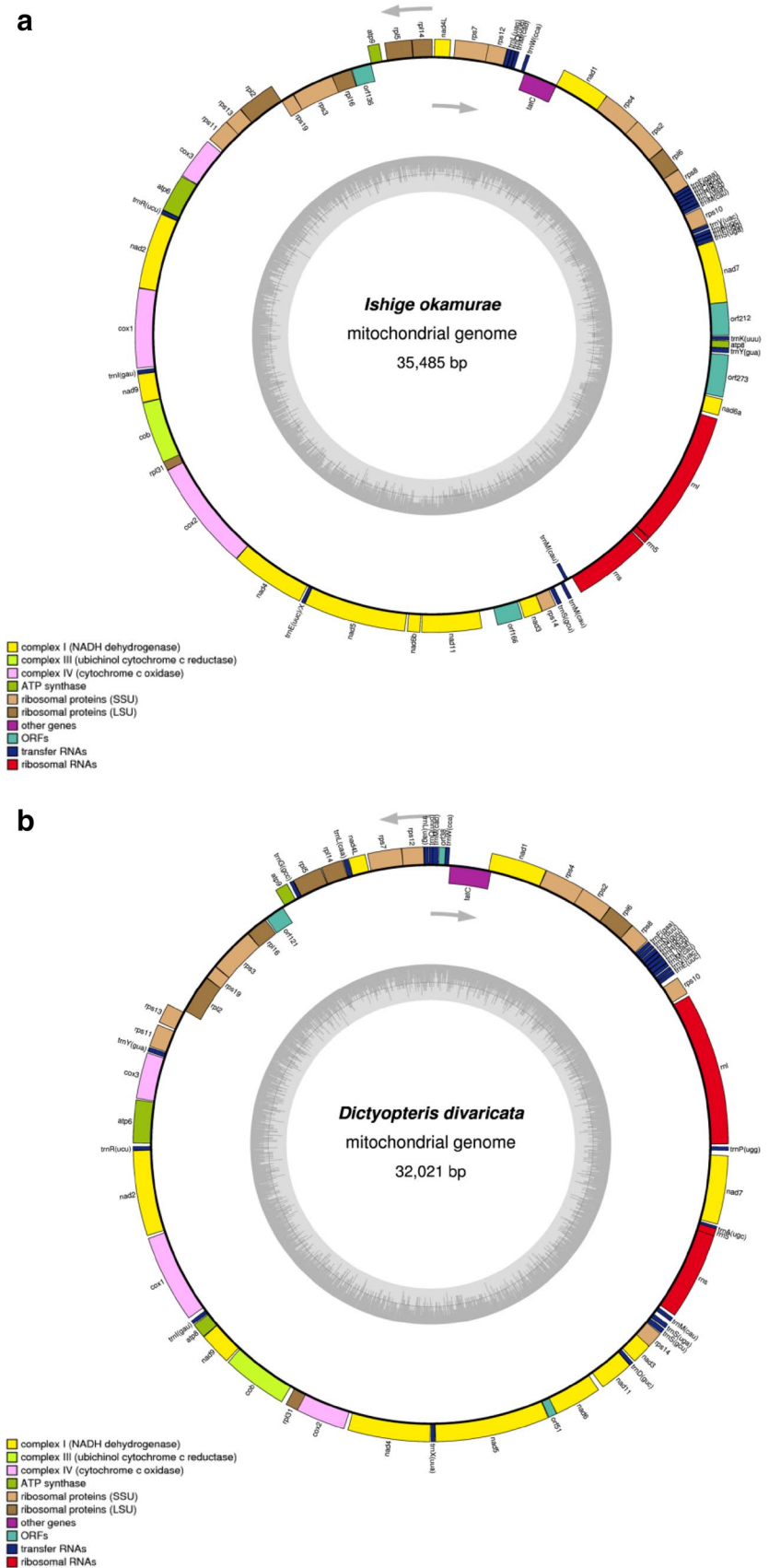


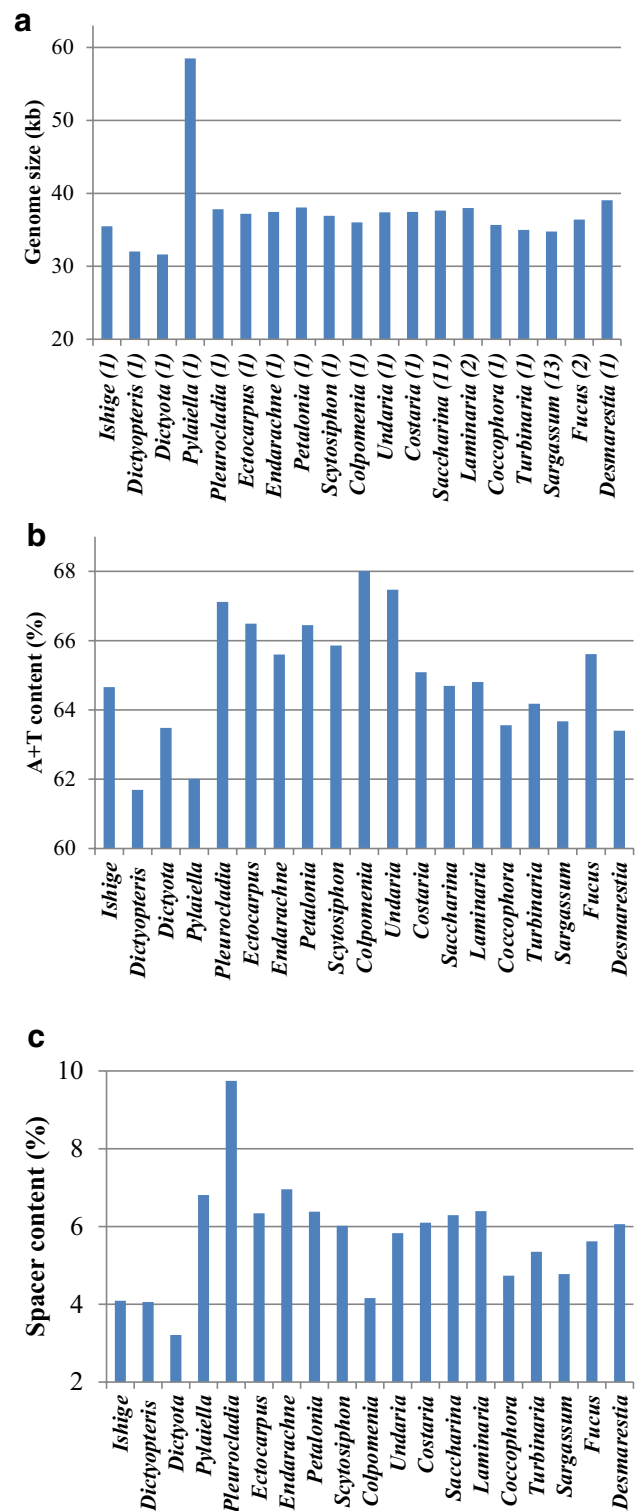
Table S1). PCR reactions were carried out in 50 L reaction mixtures containing 32 L of sterile distilled H<sub>2</sub>O, 10 L of 5 × PrimeSTAR GXL buffer (5 mM Mg<sup>2+</sup> plus, Takara, Japan), 4 L of dNTP mixture (2.5 mM each), 1 L of each primer (10 μM), 1 L of PrimeSTAR GXL DNA polymerase (1.25 units/L, Takara, Japan), and 1 L of DNA template (approximately 50 ng). PCR amplification was performed on a TC1000-G Thermal Cycler (Sciogex, USA) with an initial denaturation at 94 °C for 3 min, followed by 30–35 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, and a final extension at 68 °C for 10 min (Liu et al. 2015). Long PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Germany) and Sanger sequencing from both forward and reverse reactions was performed for all fragments, causing the whole mitogenome sequences to be covered twice. In addition, some specific gene sequences for the *I. okamurae* mtDNA including *cox2*, *nad6*, *nad11*, were amplified and sequenced to confirm their unique characteristics. Sequencing reactions were performed using ABI 3730 XL automated sequencers (Applied Biosystems, USA). The DNA sequences were assembled using Geneious 7.1 software (Biomatters, <http://www.geneious.com>). This resulted in one scaffold of 35,485 bp for *I. okamurae* and 32,021 bp for *D. divaricata*.

## Genome Annotation and Analysis

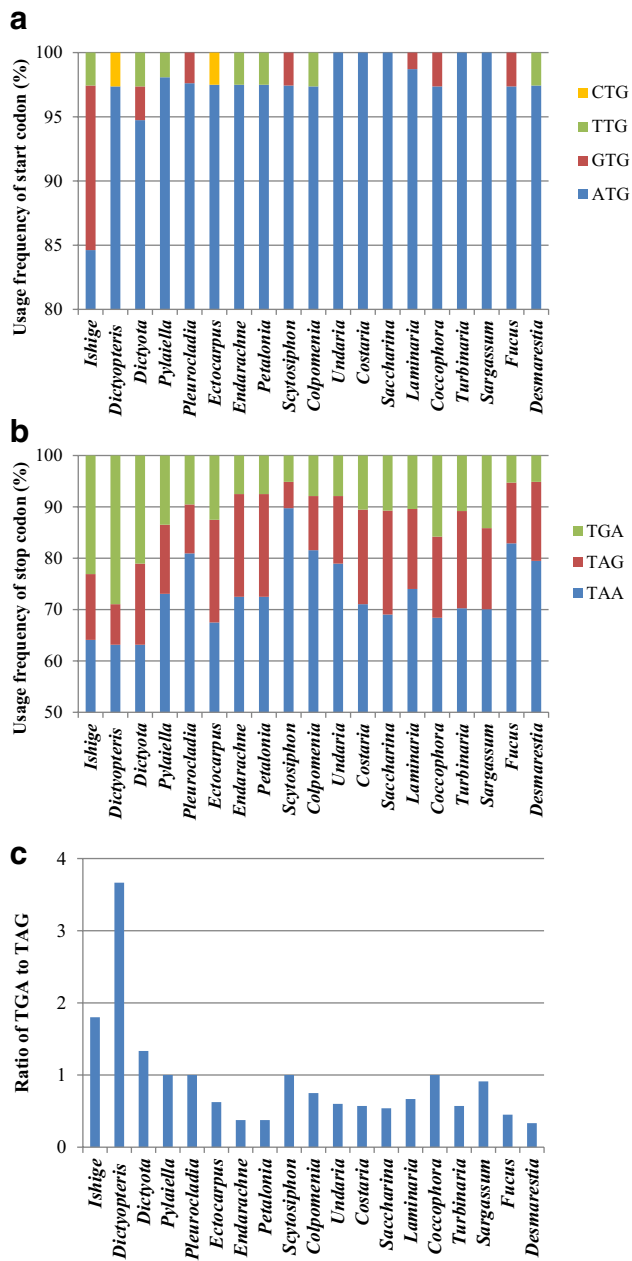
Protein-coding genes (PCGs) and open reading frames (*orfs*) were annotated using NCBI ORF Finder, Dual Organellar Genome Annotator (DOGMA) (Wyman et al. 2004), and BLAST similarity searches of the non-redundant databases at NCBI (Altschul et al. 1997). Ribosomal RNA genes were delimited by direct comparison to sequenced brown algal orthologues using MEGA v.7.0 (Kumar et al. 2016). Transfer RNA genes were identified by reconstructing their cloverleaf structures using the tRNAscan-SE 1.21 software with default parameters (Schattner et al. 2005; Lowe and Chan 2016). The physical maps of the circular mitochondrial genomes were constructed with Organellar Genome DRAW (OGDraw) (Lohse et al. 2013). Base composition was determined by MEGA v.7.0. The mitochondrial genome sequences of *I. okamurae* and *D. divaricata* have been deposited in GenBank with the Accession Numbers MG940857 and MG940856, respectively.

## Phylogenetic Analysis

Phylogenetic relationships within Phaeophyceae were analyzed based on the amino acid (aa) sequence dataset of 35 PCGs including 17 ribosomal proteins (*rps2–4*, 7, 8, 10–14, and 19; *rpl2*, 5, 6, 14, 16, and 31), 10 NADH dehydrogenase complex subunits (*nad1–7*, 4L, 9, and 11), three cytochrome



**Fig. 2** The comparison of **a** genome size, **b** A+T content, and **c** spacer content among the mtDNAs of 19 genera in brown algae. The mean values were used for *Saccharia* (11 species), *Laminaria* (2), *Sargassum* (13), and *Fucus* (2)



**Fig. 3** The comparison of usage frequency of **a** start codon and **b** stop codon, and **c** ratio of TGA to TAG among the mtDNAs of 19 genera in brown algae. The mean values were used for *Saccharia* (11 species), *Laminaria* (2), *Sargassum* (13), and *Fucus* (2)

oxidase subunits (*cox1–3*), three ATPase subunits (*atp6*, *8*, and *9*), apocytochrome b (*cob*), and a secY-independent transporter protein (*tatC*). *Heterosigma akashiwo* (GQ222228) was selected as an out-group taxon for analysis of the aa dataset. We re-annotated the mitochondrial genome of *H. akashiwo* and found that it shared the same 35 PCGs as Phaeophyceae. The aa sequences were subjected to concatenated alignments using ClustalX 1.83 with the default settings (Thompson et al. 1997). Bayesian Inference (BI)

**Fig. 4** Comparison of mitogenome organization of the 19 genera in brown algae. The genome-specific tRNAs and PCGs were indicated by an asterisk (\*). The black brackets showed the two regions experienced more frequent rearrangements. The genome-specific *orfs* were not shown

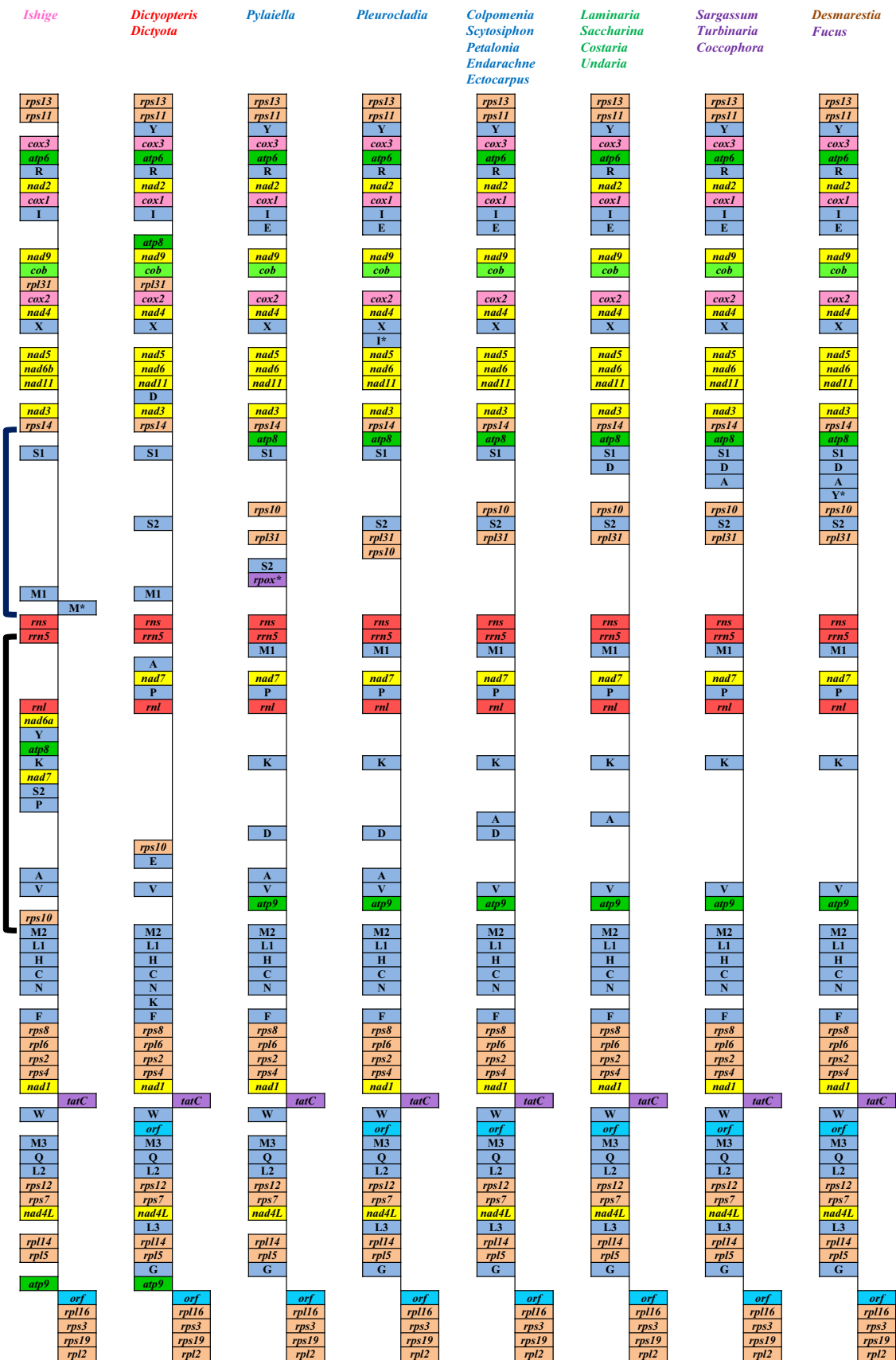
analysis was performed based on the best scoring alternative model of MtREV + G + I using MrBayes v.3.2 (Huelsenbeck and Ronquist 2001). One million generations were run and posterior probabilities determined using the Markov chain Monte Carlo (MCMC) method for tree reconstructions. Every 1000th generation was saved, and the first 100 generations discarded as burn-in. Posterior probability values for the majority-rule consensus trees constructed were calculated. Maximum likelihood (ML) analysis of the aa dataset was run based on the JTT matrix-based model (Jones et al. 1992) with 1000 bootstrap replicates using MEGA v.7.0 (Kumar et al. 2016). Multiple sequence alignment of the brown algal mitochondrial genome sequences was performed using the Mauve Genome Alignment v.2.3.1 (Darling et al. 2004) using the progressive Mauve algorithm (Darling et al. 2010).

## Results and Discussion

### Genome Features and Codon Usage

The mitochondrial genomes of *I. okamurae* and *D. divaricata* were 35,485 bp and 32,021 bp, respectively (Fig. 1a, b). Comparative analysis of the mtDNA size of 19 brown algal genera indicated that the Dictyotales mtDNAs tend to be smaller (Fig. 2a). The A + T content of *I. okamurae* and *D. divaricata* were 64.66% and 61.69%, respectively. To date, the *D. divaricata* mtDNA displayed the lowest A + T content of all sequenced brown algal mtDNAs (Fig. 2b). The spacer contents were 4.09% and 4.06% in the *I. okamurae* and *D. divaricata* mtDNAs, respectively, both of which were lower than all sequenced mtDNAs in Fucophycidae (4.16–6.81%) but higher than *D. dichotoma* (3.21%) (Fig. 2c). The sequenced mtDNAs in Ishigeophycidae and Dictyotophycidae had an elevated number of reduced spacer regions, suggesting that their mitogenomes evolved to be more compact than Fucophycidae. The *I. okamurae* genes overlapped by a total of 104 bp in 15 different locations ranging from 1 to 23 bp, while the *D. divaricata* genes overlapped by 160 bp in 12 locations from 1 to 60 bp. Two overlapping regions, *rps8-rpl6* (4 bp) and *rpl6-rps2* (1 bp), previously observed to be highly conserved in Fucophycidae and Dictyotophycidae mtDNAs (Liu and Pang 2015a), were also conserved in *I. okamurae* and *D. divaricata*.

Most of the PCGs in *I. okamurae* and *D. divaricata* mtDNAs had a methionine (ATG) start codon and a TAA



stop codon (Fig. 3a, b), which was similar to that in other brown algal mtDNAs (Graf et al. 2017). However, compared with the mtDNAs in Fucophycidae, some novel features in codon usage were observed in mtDNAs of *I. okamurae* and *D. divaricata*. An increase in a start-codon usage bias for GTG was observed in the *I. okamurae* mtDNA where five genes (*nad5*, *nad9*, *rps2*, *rps8*, and *orf136*) started with the GTG codon (Fig. 3a). The frequency of stop-codon usage bias for TGA was higher than TAG in *I. okamurae* and *D. divaricata*, which was similar to what was reported for *D. dichotoma* (Fig. 3b, c). However, the usage frequency of TGA was lower than TAG in nearly all mtDNAs of Fucophycidae. The variance of codon usage in brown algal mtDNAs suggests a diverse evolutionary pattern in different lineages.

### Novel Features in Genes

The mtDNA of *I. okamurae* contained a total of 65 genes including 36 protein-coding genes (PCGs), 22 tRNA genes, three rRNA genes, and four *orfs* (Fig. 1a). The *D. divaricata* mtDNA harbored 66 genes including 35 protein-coding genes (PCGs), 25 tRNA genes, three rRNA genes, and three *orfs* (Fig. 1b). The gene content of *D. divaricata* was identical to that previously reported in *D. dichotoma*. Unlike the other brown algal mitogenomes, the *nad6* gene in the *I. okamurae* mtDNA was split into two genes, *nad6a* and *nad6b*, which were 7.5 kb apart from each other (Fig. 1a). The two *nad6* genes can be independently transcribed into two mRNAs and translated into separate polypeptides (Edqvist et al. 2000). The observed *nad6* gene in the *I. okamurae* mtDNA might be associated with the posttranslational fusion of the two polypeptides (Odintsova and Yurina 2005).

The *nad11* in *I. okamurae* was approximately 180 aa longer than its homologues in Fucophycidae and Dictyotophycidae. Based on protein BLAST, the extra 180 aa was predicted to be an Actinobacteria sulfotransferase. The size of *atp8* in *D. divaricata* was identical to that in *D. dichotoma* but was approximately 30 aa longer than those found in *I. okamurae* and Fucophycidae. Previous studies showed that the *cox2* gene in Fucophycidae mtDNAs contained a large in-frame insertion which introduced an extra region about 761–1010 aa long in the middle of this gene (Oudot-Le Secqet et al. 2006, Liu et al. 2015; Graf et al. 2017). However, this insertion was absent in the *cox2* of *D. divaricata* and *D. dichotoma*. In *I. okamurae*, however, we observed that a much smaller insertion of approximately 330 aa was present in the *cox2* gene.

Two conserved *orfs*, located in *trnW(cca)-trnM3(cau)* and *atp9-rpl16* spacers, were shared by nearly all known brown algal mtDNAs, with the exception of *I. okamurae* and *Pylaiella littoralis* where the conserved *orfs* located in *trnW(cca)-trnM3(cau)* are lost. Unique species-specific *orfs* identified include one *orf* (*orf51*) located between *nad5* and

*nad6* in two Dictyotales mtDNA sequences, and three *orfs* (*orf166*, *orf273*, and *orf212*) with unknown functions only detected in *I. okamurae*.

Four tRNA genes, *trnG(gcc)*, *trnL3(caa)*, *trnD(guc)*, and *trnE(uuc)*, common in the mtDNAs of Fucophycidae and Dictyotophycidae, were lost from *I. okamurae*. An extra tRNA gene, *trnM(cau)*, located between *rns* and *trnM1(cau)* that transcribed onto different strands was found in *I. okamurae* only (Fig. 3).

### Genome Rearrangement

The structures of brown algal mtDNAs were analyzed and compared by combining the brown algal basal lineages of *I. okamurae* and *D. divaricata* with that of Fucophycidae and Dictyotophycidae to understand the evolution of mtDNA genome architecture. The genome organization of *I. okamurae* mtDNA was different from that of Fucophycidae and Dictyotophycidae, while the architecture of *D. divaricata* mtDNA was identical to that of *D. dichotoma*. The brown algal mtDNA demonstrated a highly conserved genome organization at the order level (Liu and Pang 2015a), akin to cpDNAs in Phaeophyceae (Liu et al. 2017a).

Two ribosomal gene blocks, *rps8-rpl6-rps2-rps4* and *rpl16-rps3-rps9-rpl2-rps13-rps11*, and two tRNA gene blocks, MLHCNF and MQL, were observed in all the sequenced mtDNAs of the three subclasses revealing that brown algal mitogenomes are highly conserved (Liu and Pang 2015a). The *rps8-rpl6-rps2-rps4* ribosomal gene block was also conserved in the mtDNA of *H. akashiwo* (Masuda et al. 2011), which belonged to the class Raphidophyceae and has a sister relationship with Phaeophyceae (Guiry and Guiry 2018). Furthermore, the second ribosomal gene block, *rpl16-rps3-rps9-rpl2-rps13-rps11*, was reduced to the *rpl16-rps3-rps9-rpl2* block in *H. akashiwo*.

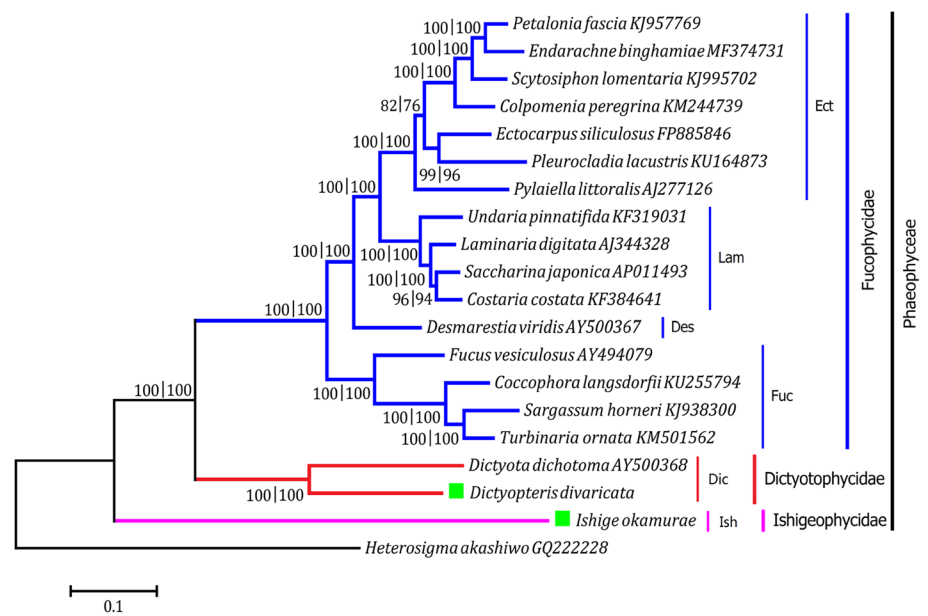
Despite generally maintaining a highly conserved mitogenomic architecture, the study observed that two regions, *rps14-rns* and *rrn5-trnM2(cau)*, experienced greater variability in brown algal mtDNAs, exhibiting structural diversity not only between the three subclasses but also at the inter and intra-order level in Fucophycidae. The SPAV tRNA gene block was present in *I. okamurae* mtDNA, but absent in Fucophycidae and Dictyotophycidae. The *trnM1(cau)-rns-rrn5-rnl* rRNA gene block was observed in *I. okamurae* and *H. akashiwo* mtDNAs, but was interrupted in the mtDNAs of Fucophycidae and Dictyotophycidae as a result of genome rearrangement events (Fig. 4).

### Basal Lineage of Brown Algae

Phylogenetic relationship analyses based on the aa sequence dataset of 35 PCGs showed that the 19 species representing 19 genera from six orders were clustered



**Fig. 5** Phylogenetic tree constructed from analysis of amino acid (aa) sequences of 35 PCGs in brown algal mtDNAs. The tree was rooted with *Heterosigma akashiwo* (Raphidophyceae). The numbers at internal nodes (BI/ML) indicated Bayesian Inference (BI) posterior probability values and maximum likelihood (ML) bootstrap values, respectively. Branch lengths are proportional to the amount of amino acid substitutions per site, which are indicated by the scale bar below the tree



into three clades, Fucophycidae, Dictyotophycidae, and Ishigeophycidae, with high support values (BI/ML = 100%) (Fig. 5). Fucophycidae and Dictyotophycidae were sister taxa that evolved from a common ancestor forming the Fucophycidae–Dictyotophycidae (FD) complex. The early divergence of *I. okamurae* (Ishigeophycidae) from the FD complex, which includes Ectocarpales, Laminariales, Desmarestiales, Fucales, and Dictyotales, suggests its basal lineage in brown algae. The polytomy of Fucophycidae, also known as the brown algal crown radiation (BACR) (Rousseau et al. 2001), likely represents a gradual diversification spanning most of the Lower Cretaceous rather than a sudden radiation (Silberfeld et al. 2010).

The phylogenetic relationships uncovered in our study were consistent with the result of phylogeny inferred from the combined dataset of seven genes (Silberfeld et al. 2010, 2014), and was further confirmed by comparative analysis of the mitogenome structure of brown algae. The genome organization of Dictyotophycidae showed greater similarity to that of Fucophycidae when compared to *I. okamurae* (Supplementary data: Fig. S1). The *I. okamurae* mtDNA exhibited a novel genome architecture, and more genes appeared in new positions (Fig. 4), suggesting a distant relationship with the FD complex.

The mitogenomic data uncovered in this study emphasize that the Phaeophyceae basal lineage mtDNAs exhibit novel features, suggested an even greater diversity of mitogenome evolution in brown algae than previously thought, providing further insights into the evolutionary history, phylogenetics, and genomics of brown algae.

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## Compliance with Ethical Standards

**Conflicts of Interest** The authors declare that they have no conflict of interest

**Ethics Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

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