MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

# QUALIFICATION THESIS

on the topic **Mitochondrial genomes reveal the differential mechanism between Saccharina and Laminaria**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
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**ASSIGNMENTS**
**FOR THE QUALIFICATION THESIS**
**Bu Pu**

1. Thesis topic **Mitochondrial genomes reveal the differential mechanism between *Saccharina* and *Laminaria***

Scientific supervisor Dr. Sc., Prof. Tetiana Shcherbatiuk
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2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

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WORK CALENDAR

| № | The name of the stages of the qualification thesis | Terms of performance of stage | Note on performance |
|---|---|---|---|
| 1 | Introduction | until 11 April 2025 | |
| 2 | Chapter 1. Literature review | until 20 April 2025 | |
| 3 | Chapter 2. Object, purpose, and methods of the study | until 30 April 2025 | |
| 4 | Chapter 3. Experimental part | until 11 May 2025 | |
| 5 | Conclusions | until 15 May 2025 | |
| 6 | Draw up a bachelor's thesis (final version) | until 25 May 2025 | |
| 7 | Submission of qualification work to the supervisor for feedback | until 27 May 2025 | |
| 8 | Submission of bachelor's thesis to the department for review (14 days before the defense) | 28 May 2025 | |
| 9 | Checking the bachelor's thesis for signs of plagiarism (10 days before the defense) | 01 June 2025 | Similarity coefficient ____% Citation rate ____% |
| 10 | Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense) | 04 June 2025 | |

I am familiar with the task:

Student _____ Bu Pu

Scientific supervisor _____ Tetiana SHCHERBATIUK

## Abstract

**Bu Pu. Mitochondrial genomes reveal the differential mechanism between *Saccharina* and *Laminaria*. Manuscript**.

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In this study, the genera *Saccharina* and *Laminaria*, which are economically important in the Phaeophyceae phylum, were used to obtain the mitochondrial genome data of a total of 10 species of the two genera from the NCBI database, and bioinformatic analysis software such as OGDRAW, Mauve, and CodonW were utilized to execute the genome mapping, covariate analysis, and codon preference analysis, and to reconstruct the phylogenetic tree using the ML (maximum likelihood method) to reconstruct the phylogenetic tree.

The results of the study showed that the genome length of the genus *Saccharina* (37,500-37,657 bp) was shorter than that of the genus *Laminaria* (37,862-38,047 bp), the GC content of the genus *Saccharina* (35.20- 35.30%) was more stable than that of *Laminaria* (34.20-35.20%); five species in *Laminaria* encoded 36-39 protein-coding genes, whereas the number of protein-coding genes in *Saccharina* was highly conserved with 38 genes in all of them; covariance analysis showed that there was a difference in the gene arrangement of the two genera, with gene duplications occurring in the *trn*C to *trn*S regions. Covariance analysis showed that there were differences in gene arrangement between the two genera, with gene duplications and deletions in the *trn*C to *trn*S regions: duplications of the *trn*M gene in *Laminaria ephemera*, deletions of the *trn*K and *trn*S genes in five species of the genus *Saccharina*, and in *Laminaria solidungula*; and codon preferences were found to be weaker in the two genera. The codon preference study revealed that the genomic codon preferences of both genera were weak, and the *rps*10 termination codon was TAG in *Saccharina* and TAA in *Laminaria*, which could be used as molecular characters for intergeneric classification; the evolutionary tree supported the independent classification of *Saccharina* and *Laminaria*, and the species within the genera were closely related, and the intergeneric

differentiation was significant, which verified the revision of the traditional classification by molecular phylogeny.

This study is the first to systematically compare the characteristics of the mitochondrial genomes of the genera *Saccharina* and *Laminaria*. By comparing the structural features, covariance and phylogenetic relationships of the mitochondrial genomes, the structural differences between the two genera and their evolutionary history are revealed, which provides theoretical basis for the taxonomy of Phaeophyceae, the development of germplasm resources, and molecular-assisted breeding.

*Key words: Mitochondrial genome; Structural characterization; Covariance analysis; Brown algae classification; systematic evolution*

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## Introduction

This study systematically investigated the genomic architecture and evolutionary relationships of *Saccharina* and *Laminaria* species through mitochondrial genome sequencing, structural comparative analysis, and codon usage profiling. The mitochondrial genomes of *Saccharina* species (37,500–37,657 bp) were found to be shorter and more GC-stable (35.20–35.30%) compared to those of *Laminaria*species (37,862–38,047 bp; GC 34.20–35.20%). While *Saccharina* exhibited conserved protein-coding gene numbers (38 genes), *Laminaria* displayed significant variability (36–39 genes). Syntenic rearrangements were identified in the *trnC–trnS* regions, with *Laminaria ephemera* showing *trnM*duplications and *Saccharina* species lacking *trnK* and *trnS*. Notably, the termination codon of the *rps10*gene (TAG in *Saccharina* vs. TAA in *Laminaria*) emerged as a genus-specific molecular marker. Phylogenetic reconstruction strongly supported the independent taxonomic status of these genera, resolving long-standing classification ambiguities. Additionally, codon usage bias was weaker in *Saccharina*, primarily driven by mutational pressure rather than natural selection.

**Relevance of the Topic :** As keystone species in marine ecosystems and vital resources for industrial applications, *Saccharina* and *Laminaria* contribute significantly to carbon sequestration, aquaculture, and alginate production. However, unresolved taxonomic controversies hinder the optimization of resource utilization and conservation strategies. Clarifying their evolutionary boundaries through mitochondrial genomics not only advances phycological systematics but also supports germplasm innovation and disease control, offering dual benefits for ecological preservation and bioeconomic development.

**Purpose of the Study :** This research aims to elucidate the structural divergence and evolutionary dynamics of mitochondrial genomes between *Saccharina* and *Laminaria*, delineate their taxonomic boundaries, and provide theoretical foundations for molecular breeding and resource management.

**Object and Methodology**：The study focused on the mitochondrial genomes of 10 species (5 *Saccharina* and 5 *Laminaria*). Genome annotation and visualization were performed using Geneious and OGDRAW, synteny analysis was conducted via the Mauve plugin, codon usage indices (ENc, CAI) were calculated with CodonW, and phylogenetic trees were reconstructed using RAxML and MrBayes.

**Scientific Novelty**：This work represents the first systematic comparison of mitochondrial genomes between *Saccharina* and *Laminaria*, revealing novel features such as genus-specific termination codons, tRNA rearrangements, and weak codon bias. The robust phylogenetic evidence resolves traditional classification disputes and establishes a foundation for developing molecular markers and evolutionary studies in brown algae.

**Practical Significance**：The findings directly inform taxonomic revisions, optimize hybrid breeding strategies, and enhance industrial strain selection. Furthermore, they contribute to evaluating carbon sequestration potential in marine ecosystems and safeguarding genetic resources, demonstrating both academic value and practical applicability in biotechnology and environmental management.

# Chapter I
# LITERATURE REVIEW

## 1.1 Overview of Phaeophyceae

Algae are a group of lower plants with chlorophyll, autotrophic life, without rhizomatous differentiation, reproducing through nutritive cell division or by means of unicellular spores and conidia, and are an important component of the biological resources of the aquatic ecosystems[1].There are a wide variety of species of macroalgae, with different morphologies, which include four *phaeophyceae*: *Phaeophyceae*, *Chlorophyceae*, *Rhodophyceae*, and *Cyanophyceae*[2]. *Phaeophyceae* (brown algae) are a multicellular group of algae with abundant morphological variations. The morphology varies from tiny filamentous structures to chloroplasts that can reach tens of meters in length. Their photosynthetic pigments include chlorophyll a, c1, c2, beta-carotene, and abundant fucoxanthin. Most *Phaeophyceae* grow in marine environments, with a few species inhabiting freshwater. Many *Phaeophyceae* species have economically important applications, such as kelp and wakame. Currently, the *Phaeophyceae* are known to include more than 1800 species 3. In China, marine *Phaeophyceae* are mainly distributed in two regions, namely the Yellow Bohai Sea and the South China Sea. Most of the species of this *phaeophyceae* are distributed in the intertidal zone 4, but some of the marine *Phaeophyceae* have a discontinuous distribution along the coast of China 5. In *Phaeophyceae* plants, a polysaccharide substance, fucoidan, is widely present, and it has been widely used in many fields such as food additives 6, biomedicine 7, and animal feeding 8.

## 1.2 Ecological role and value of Phaeophyceae

*Phaeophyceae*, as one of the macroalgae, has the highest organic carbon content of 32.94% compared to the red algae phylum and the green algae phylum 9. Therefore when all other conditions are equal, the *Phaeophyceae* phylum has the highest amount

of carbon sequestration. *Phaeophyceae* as one of the primary producers in the ocean also remove N, Pi nutrients, absorb heavy metals and maintain marine biodiversity 10. Macroalgae can improve the environment of marine areas and conserve fishery resources by relying on a wide cultivable area and efficient carbon fixation capacity per unit area, while macroalgae ecosystems can synergize to achieve multi-pathway carbon sequestration through biological carbon pumps and microbial carbon pumps 11. *Phaeophyceae* can also be used for animal feeding, which has high nutritional value due to its richness in sugars, lipids, proteins, vitamins, minerals, trace elements and a variety of bioactive substances. It can have a positive effect on the growth performance, immune function, antioxidant function and stress resistance of aquatic animals 12. In addition, the size and biomass of *Phaeophyceae* provide a unique and important habitat for hundreds of species 13.

### 1.3 Taxonomic status and controversy between the genera *Saccharina* and *Laminaria*

The taxonomic controversy between the genera *Saccharina* and *Laminaria* in the Phaeophyceae has been one of the focal points of systematic studies of algae. When Lamouroux established the genus *Laminaria* in 1813, he included in the genus the species now attributed to *Saccharina* (i.e., the original genus *Laminaria* contains both taxa in the modern classification) [16]. However, recent studies have shown that the two genera should be considered as separate species genera [14]. Traditional taxonomy relies heavily on morphological characters, such as leaf morphology, fixator structure and reproductive organ arrangement. For example, typical features of the genus *Laminaria* include smooth or lobed leaf blade margins and cylindrical stipe, while the genus *Saccharina* is classified independently for its broad leaf blades and conspicuous marginal undulations [13]. However, with the development of algal breeding technology, more and more hybrid kelp has been bred, and artificial hybridization can be realized among different closely related species [15]. The limitations of

morphological classification have gradually appeared, and some species have been classified ambiguously due to morphological transition and phenotypic plasticity.

In conclusion, the classification of the algae of the genera *Saccharina* and *Laminaria* is currently facing many problems, and the classification of both is controversial. Species identification is difficult using previous studies, mainly due to different taxonomic sampling, molecular markers, and analytical methods [16].

## 1.4 Research value of the mitochondrial genome

### 1.4.1 Overview of mitochondria

Mitochondria are organelles responsible for energy synthesis in eukaryotic cells, whose origin can be traced back to the endosymbiotic events of α-amoebae about 2 billion years ago [17], and are commonly found in almost all eukaryotic cells, with their numbers usually ranging from a few hundred to several thousand. As an important part of the cell, mitochondria are responsible for carrying out oxidative phosphorylation reactions and synthesizing ATP, which in turn provides the energy needed for various cellular life activities, and is therefore known as the energy factory of the cell [3].

### 1.4.2 Mitochondrial Structure

Mitochondria are composed of two membranes, inner and outer, forming a closed vesicle-like structure. From the inside to the outside, it can be divided into four functional regions: the mitochondrial matrix, the inner mitochondrial membrane, the mitochondrial membrane gap and the outer mitochondrial membrane. The outer membrane of mitochondria has high permeability and is rich in pore proteins, which can facilitate the passage of small molecules and has certain signaling functions involved in intracellular information transmission. The membrane gap between the outer and inner membranes contains a certain amount of fluid and contains a variety of key molecules and enzymes that play an important role in cellular metabolism and

other physiological activities. The inner membrane of mitochondria is a unit membrane that is less permeable and more compact than the outer membrane. The inner membrane usually forms a cristae structure by folding inward, which increases the surface area of the inner membrane, and this special structure helps to increase the efficiency of ATP synthesis. The mitochondrial matrix contains a large number of enzymes involved in respiration, which not only play an important role in cellular energy conversion, but also regulate cellular metabolic activities, which are essential for maintaining normal cellular functions [18].

### 1.4.3 Algal mtDNA structural composition and characterization

Mitochondrial DNA (*mt*DNA) is a type of genetic material outside the nucleus and is usually a covalently closed double-stranded circular molecule [20] Algal mitochondrial genomes show a remarkable diversity in structural features. Their molecular weight usually ranges between 15-70 kb, and most species exhibit a covalently closed loop conformation, but some green algal species such as *Chlamydomonas reinhardtii* and *Polytomella capuana* exist in a unique linear molecular conformation. It is noteworthy that algal mitochondrial DNA generally showed obvious GC base-poor characteristics, and the available data showed that its GC content fluctuated in the range of 13.30%-53.20%, with an overall average value of 38.00%; the GC values of most species were distributed in the range of 20.00%-40.00%, with an average of 33.30%. Comparative analysis among taxonomic orders revealed that the GC contents of species in the Phaeophyceae and Red Algae phylum remained relatively stable, while the GC contents of different species within the Green Algae phylum varied greatly. Particularly special was *Polytomella capuana*, whose mitochondrial genome GC content reached 57.00%, showing a clear GC preference, a value nearly 19.00% higher than the average GC content of algal mitochondrial genomes, indicating the evolutionary specialization of this species in terms of base composition [19].

### 1.4.4 Evolution of algal mitochondria

The evolution of algal mitochondria is a complex and diverse process involving multiple biological mechanisms and ecological adaptations. Mitochondria evolved from an α-amoeboid ancestor through endosymbiotic events, a process that played a key role in the origin of eukaryotes [21]. In algae, the function and structure of mitochondria may change significantly depending on different ecological niches and metabolic demands. For example, in some parasitic algae, mitochondrial function may be readjusted to adapt to the nutrient-rich environment provided by the host [22].

The evolution of algal mitochondria involves not only changes in function, but also genome remodeling and protein relocalization. It has been shown that the mitochondrial genome has undergone significant gene loss and transfer during evolution, and these changes are closely related to the transition of mitochondria from endosymbiotic bacteria to permanent organelles [21]. In addition, the metabolic pathways of algal mitochondria may also be adapted to meet specific energy demands in response to different environmental conditions [22].

Interactions between mitochondria and other organelles have also played an important role in the evolution of algae. It was found that algal mitochondria and plastids underwent significant remodeling during the evolutionary process, and this remodeling was closely related to the transition of algae from aquatic to terrestrial environments [23]. This transition involves not only functional changes in mitochondria and plastids, but also changes in their intracellular localization and mode of interaction.

In summary, the evolution of algal mitochondria is a multilevel process involving genome remodeling, adaptation of metabolic pathways, and interactions with other organelles. These changes have enabled algae to adapt to diverse ecological environments and diversify in the course of evolution.

## 1.5 Methods of mitochondrial genome determination

### 1.5.1 Traditional mitochondrial genome sequencing

This method uses a physical separation technique to enrich intact mitochondria by density gradient centrifugation (e.g., cesium chloride or sucrose media) combined with a differential centrifugation strategy, followed by purification of mtDNA preparation [24]. The isolation efficiency of this method is significantly affected by the degree of tissue fragmentation and organelle integrity, making it more difficult to operate in species with complex organelle membrane structures, such as plants [25].

### 1.5.2 Long fragment PCR-based amplification technique

This method targets the amplification of contiguous large fragments of mtDNA ranging from a few Kb to several tens of Kb from total DNA through the systematic design of multiple sets of specific primers. This method has been widely used in mitochondrial genome studies in insects, mainly due to the fact that their genome sequences are smaller than those of plants [24].

### 1.5.3 High-throughput sequencing technology

In recent years, with the emergence of second-generation sequencing technology, high-throughput sequencing has gradually become an important research technology, promoting the rapid development of mitochondrial genome research. This technology sequences the total genome by large-scale synthesis or splice-joining, and then uses high-performance computers to perform end-pair splicing on the large number of reads obtained to generate contigs, which can effectively identify mitochondrial-associated contigs by comparing the mitochondrial genomes with those of their relatives, which are characterized by a lower base content and a relatively simple structure than the nuclear genome. Compared with the nuclear genome, the mitochondrial genome has a lower base content, a simpler structure, and a non-Mendelian inheritance, which enables high-throughput sequencing of the mitochondrial genome to avoid many of the

problems commonly found in the nuclear genome, such as the interference of repetitive sequences in the sequencing results [30].

## 1.6 Purpose and significance of the present study

Phaeophyceae of the genera *Saccharina* and *Laminaria* are important groups of macroalgae, taxonomically belonging to Heterokontophyta, Phaeophyceae, Laminariales, Laminariaceae [26], and are mainly distributed in the North Pacific and Atlantic coasts, rich in Phaeophyceae, mannitol, iodine and other components, which are applied in the fields of medicine, chemical industry, agriculture and other fields, providing a large number of jobs and creating a huge economic value [27] [19]. However, with the development of molecular biology technology, it has been found that the boundaries between the genera *Saccharina* and *Laminaria* in traditional taxonomy are somewhat blurred and controversial, so it is necessary to study the mitochondrial genes of algae in the genera *Saccharina* and *Laminaria*. In this paper, we will analyze the size, CG content, structural characteristics, and phylogenetic relationships of the mitochondrial genomes of *Saccharina* and *Laminaria* in the light of relevant literature and theories [26]. Meanwhile, understanding the structural characteristics of the mitochondrial genomes and the differences in functional genes is of great significance to the in-depth understanding of the adaptive evolutionary mechanism of Phaeophyceae, as well as guiding its application in breeding for aquaculture.

## Summary of the chapter I

1.Brown algae, as primary producers in marine ecosystems, exhibit remarkable carbon sequestration capacity with organic carbon content up to 32.94%. They play vital roles in heavy metal adsorption, nutrient regulation, and biodiversity maintenance. Economically, components like alginate and mannitol are widely used in food, medicine, and aquaculture, making them crucial marine resources.

2.Traditional classification of *Saccharina* and *Laminaria* relies on morphological traits (e.g., blade morphology, holdfast structure). However, phenotypic plasticity and artificial hybridization have led to taxonomic ambiguities. Molecular markers (e.g., mitochondrial genomes) are proposed as more reliable tools, yet their application requires systematic validation.

3.Complementary use of traditional sequencing (density gradient centrifugation) and high-throughput technologies (e.g., Illumina) has advanced mitochondrial genome research. Bioinformatics tools (e.g., Geneious, Mauve) support genome annotation, synteny analysis, and phylogenetic reconstruction.

# Chapter II

# OBJECT, PURPOSE, AND METHODS OF THE STUDY

## 2.1 Test Methods

### 2.1.1 Data sources

Five species each of the genera *Saccharina* and *Laminaria*, *Saccharina japonica*, *Saccharina angustata*, *Saccharina coriacea*, *Saccharina longipedalis*, *Saccharina religiosa*, *Laminaria digitata*, *Laminaria hyperborea*, *Laminaria solidungula*, *Laminaria rodriguezii*, and *Laminaria ephemera* and downloaded their mitochondrial genomes in a ".gb" and '.fasta' formats. Detailed species information and numbers are shown in Table 2.1 Data sources.

Table 2.1 Data sources

| **Species Name** | **Length** (bp) | **Accession Number** |
|---|---|---|
| *Saccharina japonica* | 37,657 | NC_013476.1 |
| *Saccharina angustata* | 37,605 | NC_013473.1 |
| *Saccharina coriacea* | 37,500 | NC_013475.1 |
| *Saccharina longipedalis* | 37,657 | NC_013484.1 |
| *Saccharina religiosa* | 37,657 | NC_013477.1 |
| *Laminaria digitata* | 38,007 | NC_004024.1 |
| *Laminaria hyperborea* | 37,976 | NC_021639.1 |
| *Laminaria solidungula* | 37,862 | NC_056140.1 |
| *Laminaria rodriguezii* | 38,047 | NC_057230.1 |
| *Laminaria ephemera* | 37,929 | MZ 156055.1 |

### 2.1.2 Data analysis tools and processes

#### 2.1.2.1 Mitochondrial genome analysis of algae in the genera Saccharina and Laminaria

Using Geneious, put the downloaded ".gb" files of the 10 species in the same folder, and then click the "Annotation and Tracks" section in the "Sequence View" window. Annotation and Tracks" in the 'Sequence View' window to get the genomic characterization information of the species, see Table 2-2.

Using the online mapping website OGDRAW (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html#), we uploaded all the information in the "GenBank/EMBL file to draw map" window according to the prompts in the window, and then uploaded the information in the "GenBank/EMBL file to draw map" window. In the "GenBank/EMBL file to draw map" window, follow the instructions in the window to upload the downloaded ".gb" files of *Saccharina* and *Laminaria* species. Then check mitochondria in "Sequence source" and set other parameters as default, finally click "Submit" to run the program, wait for a few moments to get the mitochondrial genome visualization map of the required species, download the map and save it. Download the map and save it.

#### 2.1.2.2 Construction of mitochondrial genome covariance maps for *Saccharina* and *Laminaria* spp.

In the upper left menu bar of Geneious software, click "Tools", then select "Plugins", choose Mauve plugin and open it. Import the downloaded mitochondrial genomes of the 10 species in ".gb" format into Geneious, select all the genomes, right-click, select "Align/Assemble", click "Align Whole Genomes", and then click "Align Whole Genomes". "Align Whole Genomes", wait for the end of the software to get the covariance map and save the result.

## 2.2 Results and Analysis

### 2.2.1 Mitochondrial Genome Characterization of 10 Kelp Strains

According to the information shown in Table 2.2, the mitochondrial genome length of the genus *Saccharina* is highly conserved (37,500–37,657 bp, with a range of only 157 bp), and its GC content remains stable (35.20–35.30%, with intra-genus variation ≤0.10%). In contrast, the genus *Laminaria* exhibits greater genome length variability (37,862–38,047 bp, range 185 bp) and lower overall GC content (34.20–35.20%). Regarding protein-coding genes, *Saccharina* species uniformly harbor 38

genes, though their types vary due to differences in open reading frames (ORFs). For example, *Saccharina angustata* contains *ORF*130 and *ORF*391, *Saccharina coriacea* contains *ORF*127 and *ORF*381, while *Saccharina japonica*, *Saccharina longipedalis*, and *Saccharina religiosa* share *ORF*130and *ORF*337. Within *Laminaria*, the number of protein-coding genes varies: *Laminaria digitata* has the highest count (39 genes), primarily differing due to the presence of *ORF*40, *ORF*157, and *ORF*384. *Laminaria ephemera* and *Laminaria hyperborea* each contain 38 protein-coding genes, with *L. ephemera* encoding *ORF*43 and *ORF*507, and *L. hyperborea* encoding *ORF*384 and *ORF*41. *Laminaria solidungula* has the fewest protein-coding genes (36), retaining only *ORF*1. tRNA gene numbers are stable across both genera (24–25), except in *Laminaria hyperborea* (23 tRNAs). Most species possess three rRNA genes (*rrn5*, *rrs*, *rnl*), but *Laminaria solidungula* and *Laminaria rodriguezii* lack *rrn5*, retaining only *rrs* and *rnl*.

| Species Name | Length (bp) | GC Content（%） | Protein-Coding Genes | tRNA Genes | rRNA Genes |
|---|---|---|---|---|---|
| *Saccharina japonica* | 37,657 | 35.30 | 38 | 25 | 3 |
| *Saccharina angustata* | 37,605 | 35.20 | 38 | 24 | 3 |
| *Saccharina coriacea* | 37,500 | 35.30 | 38 | 24 | 3 |
| *Saccharina longipedalis* | 37,657 | 35.30 | 38 | 24 | 3 |
| *Saccharina religiosa* | 37,657 | 35.30 | 38 | 24 | 3 |
| *Laminaria digitata* | 38,007 | 35.10 | 39 | 24 | 3 |
| *Laminaria hyperborea* | 37,976 | 35.20 | 38 | 23 | 3 |
| *Laminaria solidungula* | 37,862 | 34.90 | 36 | 24 | 2 |
| *Laminaria rodriguezii* | 38,047 | 34.20 | 37 | 25 | 2 |
| *Laminaria ephemera* | 37,929 | 34.50 | 38 | 25 | 3 |

Table 2.2 Size and number characterization of the whole mitochondrial genome of 10 kelp strains

In summary, the mitochondrial gene structure of the genus *Saccharina* is extremely conserved, with a small extreme difference in genome length, a stable number of protein-coding genes, and a consistent number of rRNAs, whereas the mitochondrial gene structure of the genus *Laminaria* exhibits flexibility, with a high

variability in length, differences in the number of protein genes, and deletion of rRNAs in some species, which may reflect different evolutionary strategies[28].

Figure 2.1 illustrates the mitochondrial genome circular mapping of five species each from the genera *Saccharina* and *Laminaria*, from which structural features such as genome size, gene distribution, GC content, and endemic genes can be fully understood. These mitochondrial genomes have a general preference for heavy chain coding, while *tat*C, *rpl*16, *rps*3, *rps*19, *rpl*2 and several *ORF* genes have a preference for light chain coding. The colors in the figure mark the different types of genes, while the dark and light gray areas in the inner ring represent the distribution of GC content, respectively, which facilitates the visualization of differences in base composition.

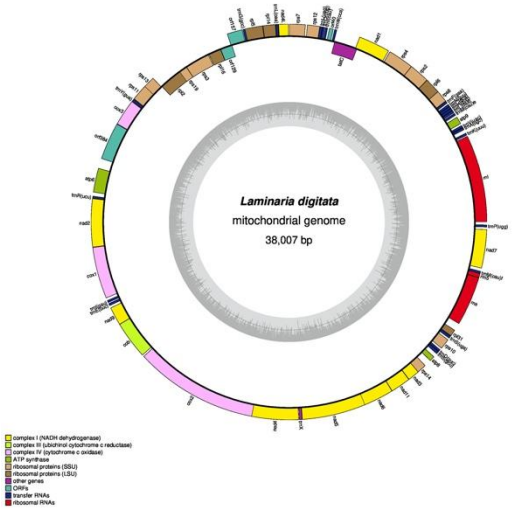
In the genus *Saccharina*, the mitochondrial genome of *Saccharina japonica* contained a total of 66 genes with a GC content of 35.30%, and a unique *trn*X gene was found between *nad*5 and *nad*4, which was further characterized by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) and tRNAscan-SE (https://lowelab.ucsc.edu/tRNAscan-SE/) analyses confirmed that it encodes tRNA- Lys, which is highly similar to *trn*K in *Saccharina japonica* × *Saccharina latissima* cultivar Sanhai (GenBank: MG712779.1) (E-value=2e-26, the closer to zero it is the higher the likelihood that the two are the same sequence, and P-value=100.00%). *Saccharina angustata* has a genome of 65 genes with a GC content of 35.20% and a unique ORF391 open reading frame.*Saccharina coriacea* contains 65 genes with a GC content of 35.30% and possesses the unique orf127. *Saccharina longipedalis* and *Saccharina religiosa* had no specific genes detected, but both contained the same number of 65 genes and both contained two open reading frames, *ORF*130 and *ORF*337, which may be unique to the genus *Saccharina*; they both had a GC content of 35.50%.

Within the genus *Laminaria*, *Laminaria digitata* possesses the highest number of genes (67), with a GC content of 35.10%, and harbors unique genes including *trnA*, *trnC*, *trnD*, *trnE*, *trnF*, *trnG*, *trnH*, *trnI*, *trnK*, *trnM*, *trnN*, *trnP*, *trnQ*, and *orf*157, which may enhance its adaptive fitness in specific

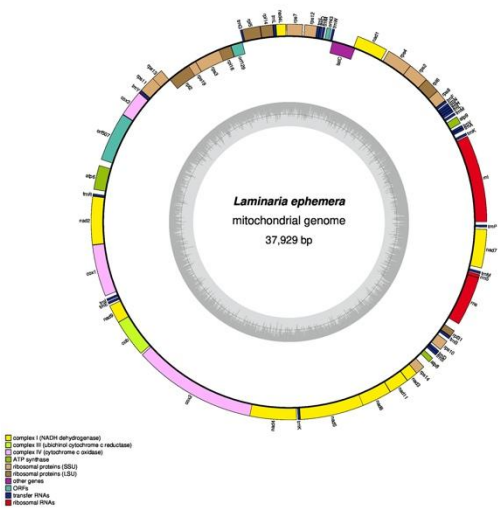
environments. *Laminaria hyperborea* contains 64 genes (GC content: 35.20%) and uniquely retains the *trn*M gene; *Laminaria solidungula* has the fewest genes (62) among all species, with a GC content of 34.90%, and uniquely carries the *ORF*1 gene. *Laminaria rodriguezii* and *Laminaria ephemera* contain 64 and 66 genes, with GC contents of 34.20% and 34.50%, respectively. The former uniquely possesses the tRNA-Ser gene, while the latter harbors four unique genes: *orf*43**,** *orf*507**,** *trn*K, and *trn*M.

Comparative analysis of mitochondrial genomes from 10 strains of *Saccharina* and *Laminaria* revealed that both genera share conserved genes, including *atp*6, *atp*8, *atp*9, *cob*, *cox*, *cox*2, *cox*3, *nad*1, *nad*11, *nad*2, *nad*3, *nad*4, *nad*4L, *nad*5, *nad*6, *nad*7, *nad*9, *rpl*14, *rpl*16, *rpl*2, *rpl*31, *rpl*5, *rpl*6, *rps*10, *rps*11, *rps*12, *rps*13, *rps*14, *rps*19, *rps*2, *rps*3, *rps*4, *rps*7, *rps*8, and *tat*C. However, a distinction was observed in the *rps*10 gene: the termination codon in *Saccharina* is TAG, whereas in *Laminaria*, it is TAA. Notably, only *Laminaria solidungula* exhibits a TGA termination codon for the *tat*C gene, while all other species retain TAA. Although the current dataset is limited, these differences remain stable across analyzed samples, suggesting their potential utility in intergeneric classification. Further validation with expanded sampling is warranted.

Overall, although the mitochondrial genomes of these 10 species of Phaeophyceae differed slightly in the number of genes (62-67), the gene structures remained highly consistent overall. Most of the unique genes were of the tRNA and *ORF* classes, reflecting the unique genetic features that may have been retained by different species during evolution as a result of adaptation to environmental changes. However, genes such as ATP synthase genes, genes related to respiration, and genes related to ribosomal small and large subunit proteins showed a high degree of conservatism among species, suggesting that their important functions in basic life activities cannot be altered. The above structural information provides basic data support for subsequent phylogenetic relationships and functional gene evolution studies.


Laminaria digitata
mitochondrial genome
38,007 bp

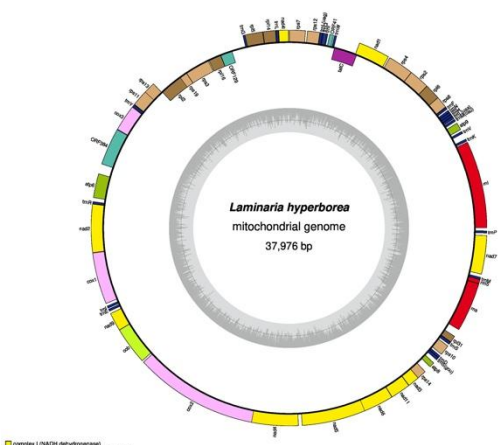
A

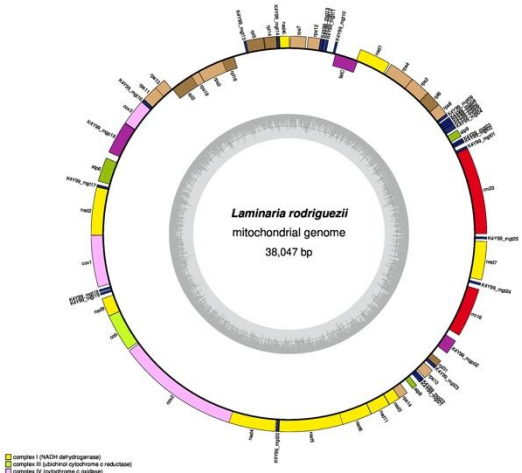

Laminaria ephemera
mitochondrial genome
37,929 bp

B

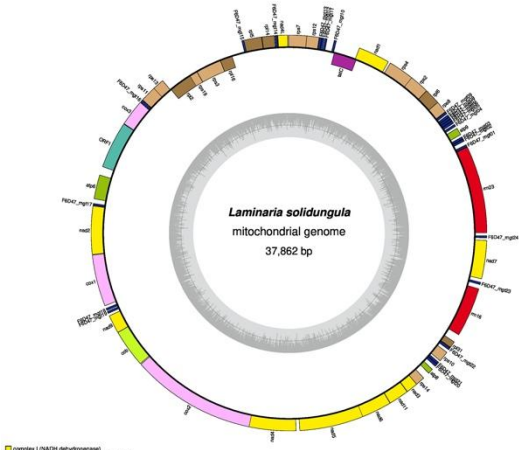
C

D

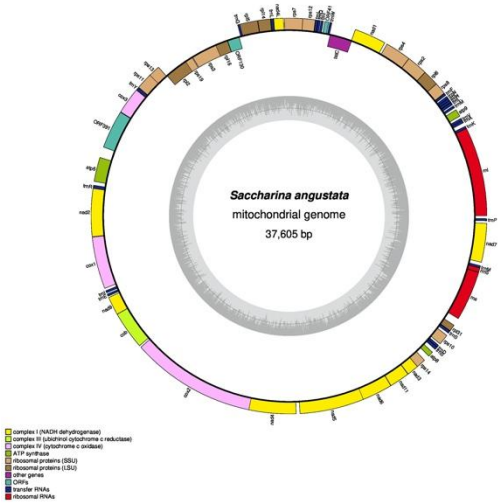

Laminaria hyperborea
mitochondrial genome
37,976 bp


Laminaria rodriguezii
mitochondrial genome
38,047 bp

F


Laminaria solidungula
mitochondrial genome
37,862 bp

E


Saccharina angustata
mitochondrial genome
37,605 bp

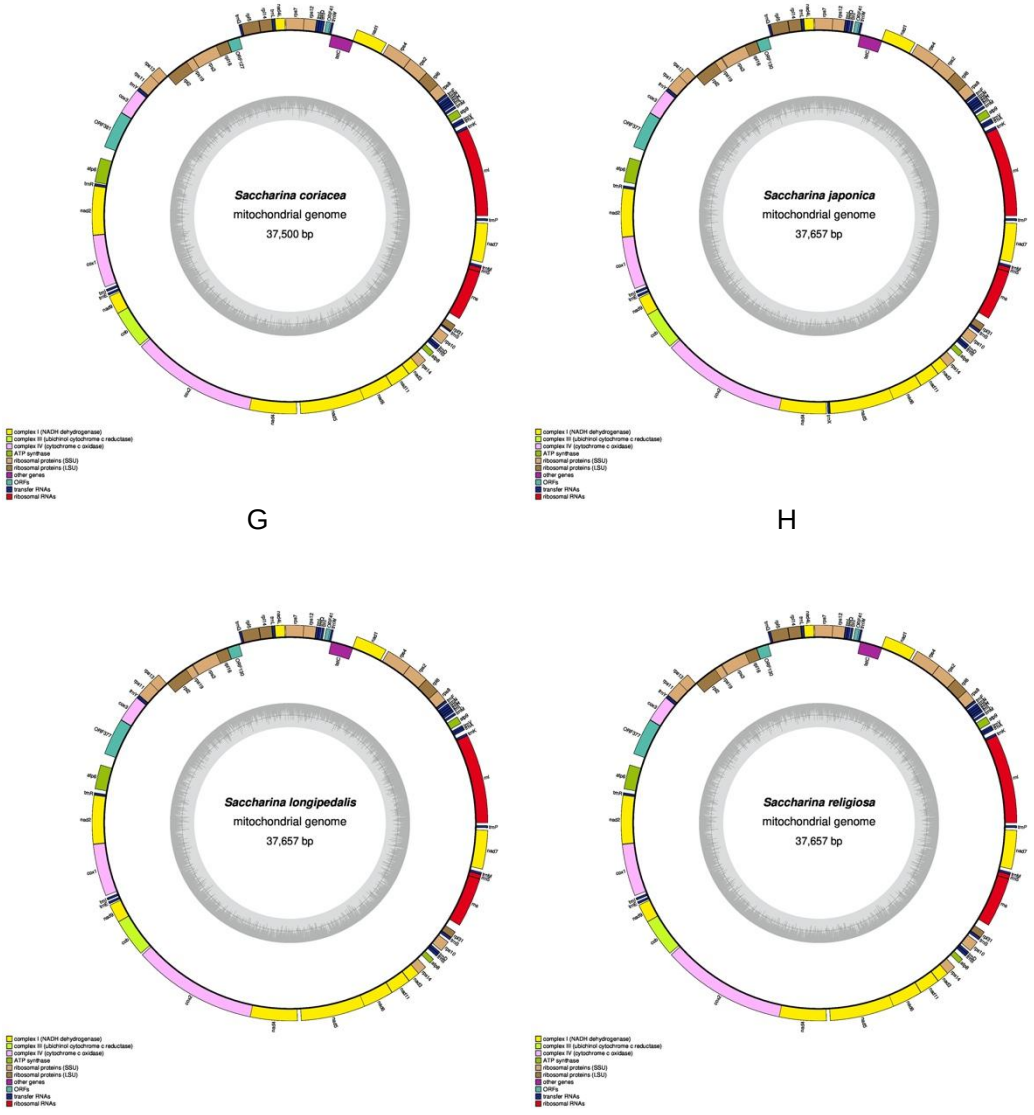



Figure 2.1 Mitochondrial genome mapping of 10 kelp species

Note:A: *Laminaria digitata*; B: *Laminaria ephemera*; C: *Laminaria hyperborea*; D: *Laminaria rodriguezii*; E: *Laminaria solidungula*; F: *Saccharina angustata*; G: *Saccharina coriacea*; H: *Saccharina japonica*; I: *Saccharina longipedalis*; J: *Saccharina religiosa*.

### 2.2.2 Covariance analysis of 10 kelp strains

According to the operation steps mentioned above, Figure 2.2 can be obtained.Through this covariance analysis figure, it can be seen that the gene arrangement in the two genera is generally conservative, and the structure of the

genome is relatively stable. However, some genes, such as tRNA genes, have gene deletions and substitutions within a certain range.

In the region spanning from *trnC* to *trnS*, frequent tRNA rearrangements, duplications, and deletions were observed (details in Table 2.3). The study revealed that gene arrangement in the genus *Saccharina* is highly conserved, with positions 3–6 and 10–14 being identical across species. In contrast, the genus *Laminaria* exhibited variations in these regions (highlighted in bold). For example, *Laminaria ephemera* showed duplications of the *trnM* gene at positions 5 and 6, and an insertion of *trnK*at position 14. Among the five *Laminaria* species analyzed, three displayed gene deletions or substitutions.

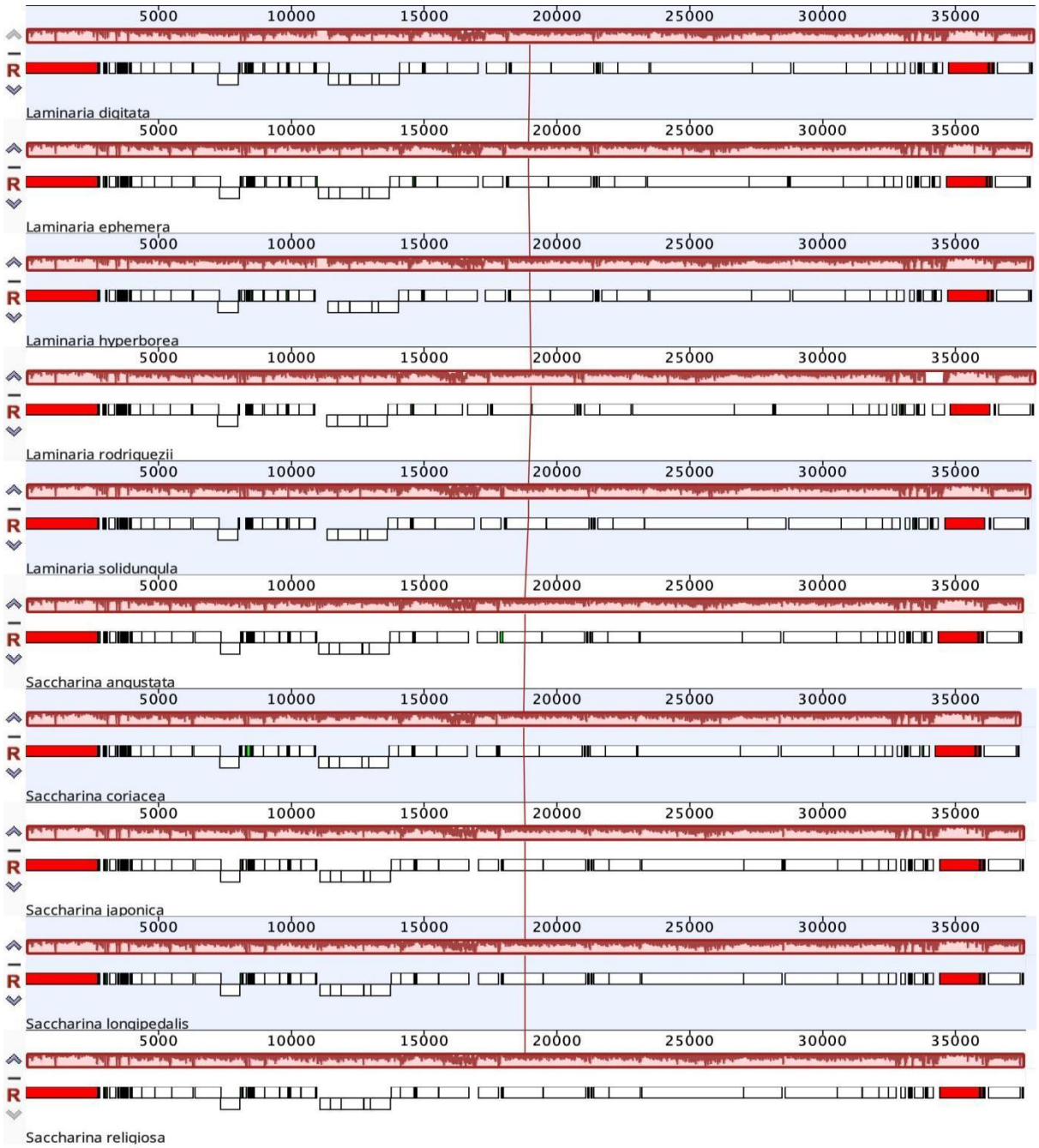



Figure 2.2 Covariance analysis of 10 species

| species | gene order | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| B | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| C | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| D | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| E | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| F | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| G | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| H | *trnC* | *trn*N | ***trn*F** | ***trn*W** | ***trn*M** | ***trn*M** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | ***trn*K** | *trn*S |
| I | *trnC* | *trn*A | ***trn*P** | ***trn*T** | ***trn*M** | ***trn*G** | *trn*L | *trn*L | *trn*G | ***trn*T** | ***trn*A** | ***trn*L** | ***trn*G** | ***trn*S** | *trn*S |
| J | *trn*C | *trn*A | ***trn*P** | ***trn*T** | ***trn*M** | ***trn*G** | *trn*L | *trn*L | *trn*G | ***trn*T** | ***trn*A** | ***trn*L** | ***trn*G** | | *trn*S |
| A | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| B | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| C | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| D | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| E | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| F | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| G | *trn*C | *trn*N | ***trn*F** | ***trn*W** | ***trn*L** | ***trn*Q** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | | *trn*S |
| H | *trnC* | *trn*N | ***trn*F** | ***trn*W** | ***trn*M** | ***trn*M** | *trn*L | *trn*L | *trn*G | ***trn*Y** | ***trn*R** | ***trn*L** | ***trn*E** | ***trn*K** | *trn*S |
| I | *trnC* | *trn*A | ***trn*P** | ***trn*T** | ***trn*M** | ***trn*G** | *trn*L | *trn*L | *trn*G | ***trn*T** | ***trn*A** | ***trn*L** | ***trn*G** | ***trn*S** | *trn*S |
| J | *trn*C | *trn*A | ***trn*P** | ***trn*T** | ***trn*M** | ***trn*G** | *trn*L | *trn*L | *trn*G | ***trn*T** | ***trn*A** | ***trn*L** | ***trn*G** | | *trn*S |

Note:A: *Laminaria digitata*; B: *Saccharina angustata*; C: *Saccharina coriacea*; D: *Saccharina japonica*; E: *Saccharina longipedalis*; F: *Saccharina religiosa*; G: *Laminaria hyperborea*; H: *Laminaria ephemera*; I: *Laminaria rodriguezii*; J: *Laminaria solidungula*.

## Summary of the chapter II

1.*Saccharina* genomes (37,500–37,657 bp) are shorter and more GC-stable (35.20–35.30%) than *Laminaria* (37,862–38,047 bp; GC 34.20–35.20%).

2.*Saccharina* exhibits conserved protein-coding gene numbers (38 genes), while *Laminaria* shows variability (36–39 genes) due to genus-specific ORFs (e.g., *ORF157*, *ORF384*).

3.The *rps*10 termination codon (*Saccharina*: TAG; *Laminaria*: TAA) serves as a genus-level molecular marker.

4.The *trnC–trnS* region displays rearrangements, such as *trnM* duplications in *Laminaria ephemera* and *trnK/trnS* deletions in *Saccharina*.

5.*Saccharina* maintains conserved gene order, whereas *Laminaria* shows insertions, deletions, or substitutions at critical sites (e.g., positions 3–6, 10–14).

# Chapter III
# EXPERIMENTAL PART

## 3.1 Data Sources and Methods

CDS sequences of 10 kelp species were downloaded from the NCBI database in ".fasta" format. The software CodonW was installed, and the CDS files of the 10 species were placed in the same directory as CodonW. The software was launched, and the following steps were executed in the interface:

1. Input "1" to load the sequence files.
2. Enter the "file name" containing the target sequences in the command line.
3. Input "4" to select codon indices for analysis.
4. Input "12" to select all indices.
5. Input "X" to return to the previous menu.
6. Input "R" to run the program.
7. Input "Q" to exit the program.

Two output files (".out" and ".blk") were generated, containing indices such as the *Codon Adaptation Index (CAI)*, *Effective Number of Codons (Nc)*, and *GC content at the third codon position (GC3)*. The data were tabulated, and scatter plots were constructed with *GC3s* as the independent variable and *ENc* (effective codon usage) as the dependent variable. A reference curve was generated using the standard formula:

Expected ENc= $2 + GC3 + 29/[GC32 + (1 - GC3)2]$

enabling *ENc-plot* analysis[29].

## 3.2 Results and Analysis

### 3.2.1 Codon Analysis

#### 3.2.1.1 ENc Value Analysis

Analysis of Table A1 revealed that the *ENc* values of the genus *Laminaria* range from 29.45 to 61.00, with a mean of 41.88, while those of *Saccharina* range from 27.13 to 55.58, with a mean of 44.15. The *ENc* (Effective Number of Codons) metric quantifies the degree of codon randomness, where lower values indicate stronger codon usage bias, and higher values reflect weaker bias. An *ENc* value ≤35 signifies statistically significant codon usage bias in a genome[29]. Although *Laminaria* exhibits marginally stronger codon usage bias than *Saccharina*, both genera display *ENc* values substantially above 35, indicating weak overall genomic codon bias. Furthermore, mitochondrial genomes of *Saccharina* demonstrate even weaker codon usage bias compared to *Laminaria*.

#### 3.2.1.2 CAI Value Analysis

Analysis of Table A2 indicates that the *Codon Adaptation Index (CAI)* values for the genus *Saccharina*range from 0.118 to 0.253, with a mean of 0.178, while those for *Laminaria* range from 0.121 to 0.228, with a mean of 0.180. The CAI metric reflects gene expression levels and translation efficiency, with values scaled between 0 and 1. Higher CAI values indicate stronger expression efficiency [31]. The negligible difference (0.002) between the two genera suggests highly similar mitochondrial gene expression efficiency and codon usage bias.

### 3.2.2 ENc-plot Analysis

The ENc-plot analysis revealed that mitochondrial genomes of both genera exhibited similar patterns, with most data points clustered near the standard curve, indicating that codon usage bias is primarily influenced by mutational pressure.

However, outliers deviating significantly from the curve—such as *Saccharina longipedalis* and *Saccharina religiosa*—suggest additional contributions from natural selection in shaping codon usage preferences.

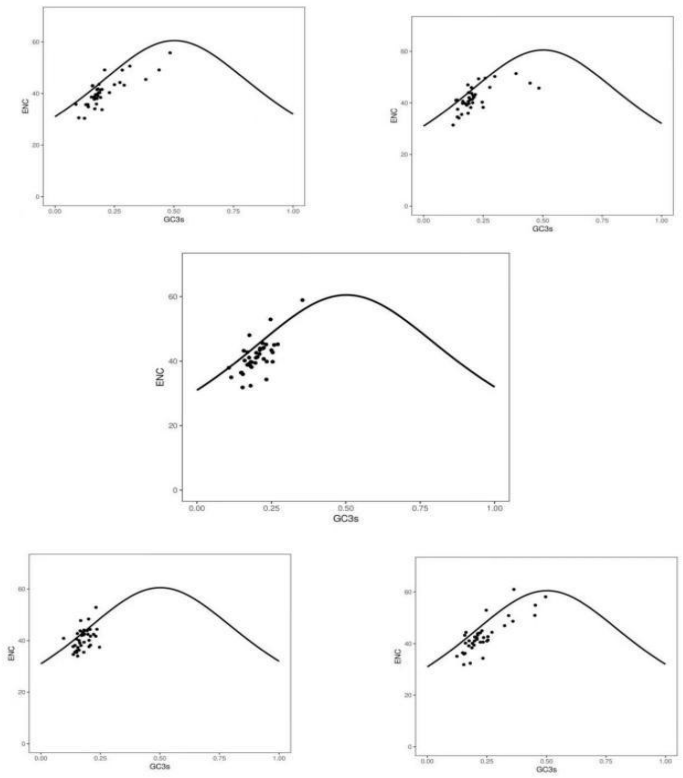



Figure 3.1 ENc-plot of mitochondrial genomes of five species of the genus Laminaria

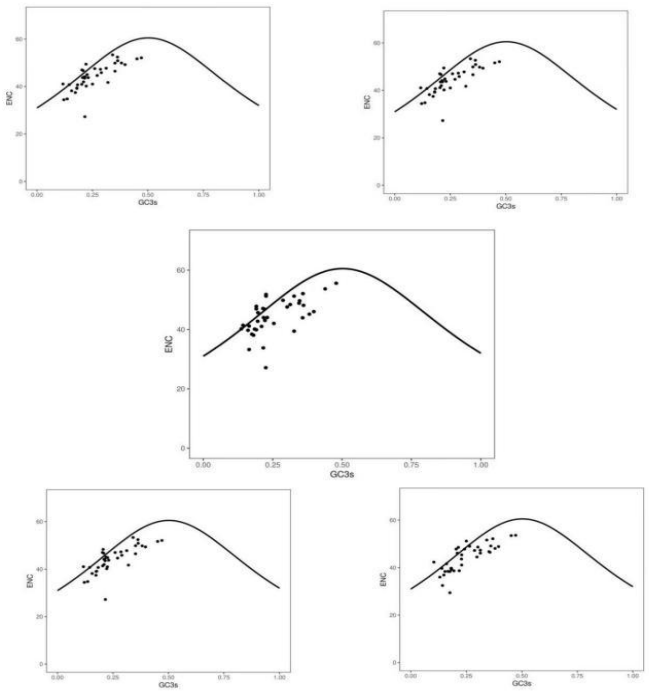



Figure 3.2 ENc-plot of mitochondrial genomes of five species of the genus Saccharina

## 3.3 Phylogenetic Methods

In current phylogenetic genomics research, analysis methods using likelihood ratio calculation are the most widely used research tools. Two categories are included, tree-building software using the maximum likelihood method, such as RAxML and IQ-TREE. tree-building software using the Bayesian method, such as the Mrbayes program and the PhyloBayes program [32]. In this study RAxML and Mrbayes programs will be used to construct phylogenetic trees.

## 3.4 Data Processing

The ".gb" format files of the 10 selected species and the outgroup species (*Ectocarpus siliculosus* NC_030223) were imported into PhyloSuite. All gene types were extracted, and the CDS_AA file was selected to identify protein-coding genes shared by all 11 species. These genes were aligned using MEGA software. The aligned

sequences were concatenated to integrate protein-coding genes for each species. Conserved regions were filtered using the online tool Gblocks (http://www.phylogeny.fr/one_task.cgi?task_type=gblocks). The "Cured alignment in PHYLIP Format" option was selected, and the output was saved as a text file named "ml.phy". The "Cured alignment in FASTA Format" output was converted into a ".nexus" file ("bi.nexus") using MEGA.

### 3.4.1 ML Tree Construction

The "ml.phy" file was placed in the RAxML software folder. The command-line window was opened by pressing "Win + R" and entering "cmd". The file path was navigated, and the following command was executed:

raxmlHPC -m PROTGAMMAAUTO -p 12345 -x 12345 -# 1000 -o NC_030223 -s ml.phy -f a -n outfile0421

This command sets the bootstrap value to 1000 iterations and exports the results to a file named "outfile0421". The output file was visualized and refined using FigTree software (http://tree.bio.ed.ac.uk/software/figtree/) to generate the final maximum likelihood (ML) tree.

### 3.4.2 BI Tree Construction

The "bi.nexus" file was opened in a text editor. The header was modified to specify 11 taxa and an amino acid sequence length of 8502. The footer was edited to include the outgroup name and set the MCMC (Markov chain Monte Carlo) run length to 1 million generations. The MrBayes program was executed with the commands:

sumt burnin=2500

After the analysis completed, the output file was imported into FigTree for visualization, resulting in the Bayesian inference (BI) tree.

## 3.5 Results and Analysis

This study analyzed concatenated amino acid sequences of 32 conserved protein-coding genes (*atp*6, *atp*8, *atp*9, *cox*1–3, *cob*, *nad*1–7, *nad*9, *nad*11, *nad*4L, *rpl*2, *rpl*5, *rpl*6, *rpl*31, *rps*3–4, *rps*7, *rps*10–11, *rps*13–14, *rps*19, *tat*C) from 11 species, resulting in a conserved region of 8,502 amino acids. Phylogenetic trees reconstructed by maximum likelihood (ML) and Bayesian inference (BI) methods exhibited highly congruent topologies, with all species divided into two major clades: the *Saccharina*clade and the *Laminaria* clade. Minor discrepancies were observed within the *Laminaria* clade, specifically in the branching of *Laminaria ephemera* and *Laminaria solidungula*.In the ML tree, *Laminaria hyperborea*, *Laminaria digitata*, and *Laminaria rodriguezii* clustered together, followed by sequential clustering with *Laminaria ephemera* and *Laminaria solidungula*. In contrast, the BI tree grouped *L. hyperborea*, *L. digitata*, and *L. rodriguezii* first with *L. solidungula*, then with *L. ephemera*. The node supporting this divergence showed weak phylogenetic signal, with a bootstrap value of 39% (ML) and a posterior probability of 0.7644 (BI). However, the close relationship between *L. hyperborea* and *L. digitata* was strongly supported (ML bootstrap = 100%, BI posterior probability = 1).

Within the *Saccharina* clade, *Saccharina longipedalis* and *Saccharina religiosa* formed a strongly supported sister group (ML bootstrap = 91%, BI posterior probability = 0.97), which further clustered with *Saccharina japonica*, followed by *Saccharina angustata*, and finally *Saccharina coriacea*, forming a monophyletic clade (genus *Saccharina*). This indicates that *S. longipedalis* and *S. religiosa* are most closely related, *S. japonica* shares a relatively close affinity with them, while *S. coriacea* is phylogenetically distinct from the other four *Saccharina* species.

These results align with the independent classification of *Saccharina* and *Laminaria* proposed by Lane et al. (2006)[13], further validating their division into distinct genera.

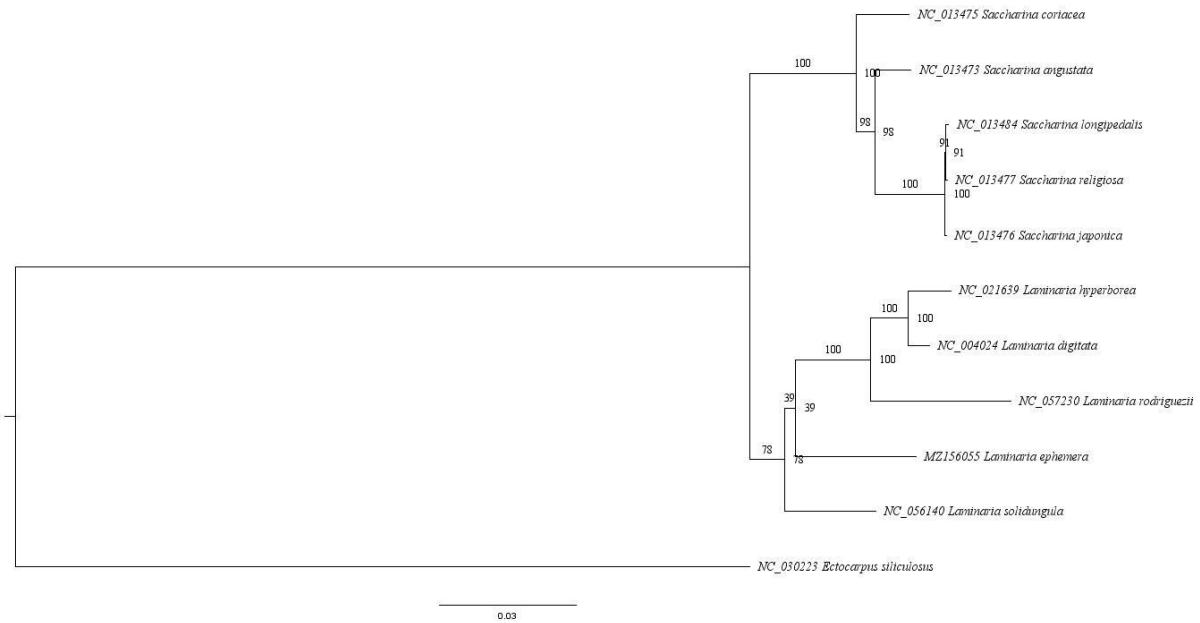



Figure 3.3 Maximum Likelihood Phylogenetic Tree

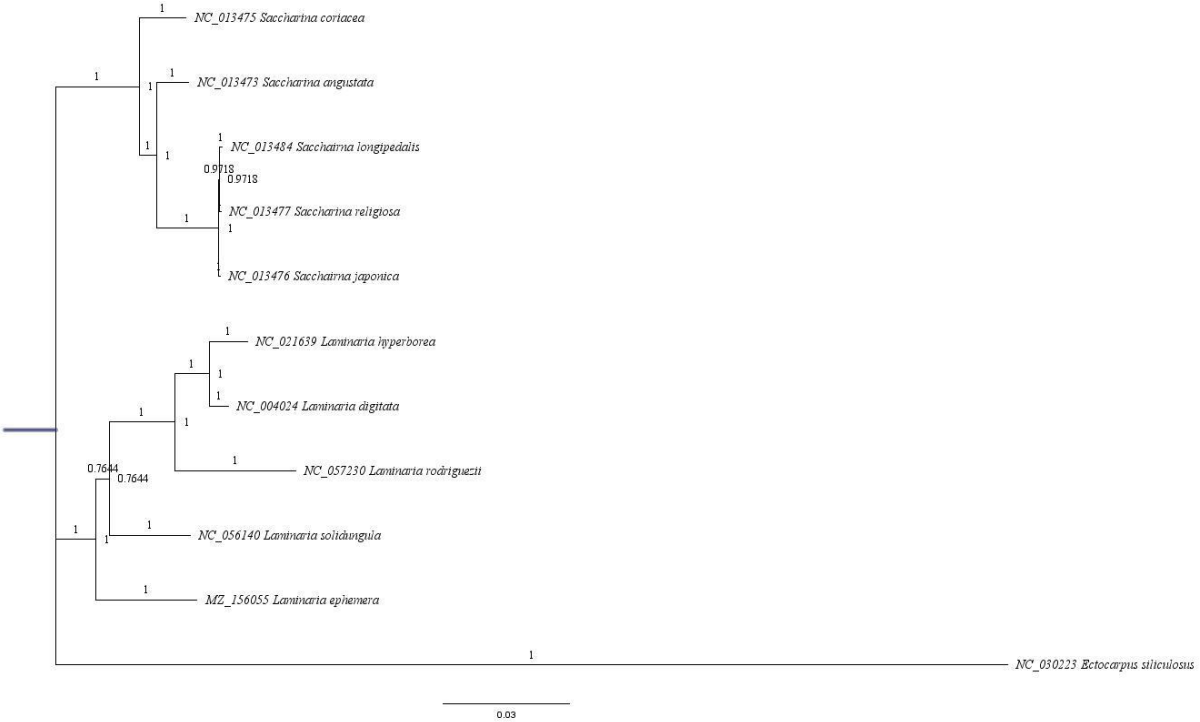



Figure 3.4 Bayesian Inference Phylogenetic Tree

## Summary of the chapter III

1.CDS sequences of 10 species were obtained from NCBI, and codon usage bias was analyzed using CodonW software. Parameters including *ENc* (Effective Number of Codons), *CAI* (Codon Adaptation Index), and *GC3* (GC content at the third codon position) were calculated. ENc-plot analysis was conducted to identify drivers of codon bias.

2.ENc Analysis: The *Laminaria* genus showed slightly lower *ENc* values (29.45–61.00, mean 41.88) compared to *Saccharina* (27.13–55.58, mean 44.15). Both genera exhibited weak overall codon bias (*ENc* >35), with *Saccharina* displaying weaker bias.

CAI Analysis: Similar *CAI* values were observed (*Saccharina*: 0.118–0.253, mean 0.178; *Laminaria*: 0.121–0.228, mean 0.180), with a negligible difference (0.002), indicating conserved mitochondrial gene expression efficiency.

3.Most data points clustered near the standard curve, suggesting mutational pressure as the primary driver of codon bias.

4.Phylogenetic trees were reconstructed using Maximum Likelihood (ML) **and** Bayesian Inference (BI) based on 32 shared protein-coding genes (e.g., *atp*6, *cox*1–3, *nad*1–7) from 11 species (including the outgroup *Ectocarpus siliculosus*).Sequences were aligned with MEGA, filtered for conserved regions (8,502 amino acids) via Gblocks, and concatenated into a single dataset.

5.Inter-genera Divergence: Both ML and BI trees strongly supported the independent clades of *Saccharina* and *Laminaria*, validating taxonomic revisions.

6.ntra-genera Relationships:Within *Laminaria*, *L. hyperborea* and *L. digitata* were closest relatives (ML bootstrap=100%, BI PP=1).

Within *Saccharina*, *S. longipedalis* and *S. religiosa* formed a strongly supported sister group (ML=91%, BI PP=0.97).

7.Controversial Nodes: The branching positions of *L. ephemera* and *L. solidungula* differed between ML and BI trees (ML bootstrap=39%, BI PP=0.7644), indicating weak phylogenetic signal at this node.

## Conclusion

1.This study conducted the first structural analysis of mitochondrial genomes across 10 kelp strains from two genera (*Saccharina* and *Laminaria*), revealing both shared and divergent features.

2.The two genera share 35 conserved protein-coding genes, but a key distinction lies in the termination codons of the *rps*10 gene: *Saccharina* species use TAG, while *Laminaria* species use TAA, providing a molecular marker for genus-level differentiation.

3.Codon usage bias analysis demonstrated weak overall bias in both genera, with *Saccharina* exhibiting even weaker bias.

4.Most codon preferences were influenced by mutational pressure, with minor contributions from natural selection.

5.Synteny maps constructed for the 10 strains identified gene duplications and deletions in the *trnC–trnS* regions. Phylogenetic analysis confirmed closer intra-genus relationships and distinct inter-genus divergence, robustly supporting the classification of *Saccharina* and *Laminaria* as separate genera.

In this study, we found that the mitochondrial genomes of the genera *Saccharina* and *Laminaria* differed in gene arrangement order, gene structure, and evolutionary development in some intervals, which provided new ideas for the classification and molecular evolutionary studies of Phaeophyceae. However, the existing findings still have some directions worth digging deeper. For example, the current study mainly focuses on 10 species of two genera. If the sample size can be further increased to include more genera, more molecular differences between genera may be found. Meanwhile, if the mitochondrial genome data can be analyzed in combination with the nuclear genome and chloroplast genome, a more three-dimensional taxonomic framework may be constructed, which can not only reduce the limitations of a single data source, but also resolve the phylogenetic relationships of Phaeophyceae more precisely.

## Reference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# APPENDIX

| gene | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| *tat*C | 47.83 | 46.73 | 46.73 | 46.73 | 47.82 | 58.93 | 48.35 | 50.87 | 44.25 | 50.25 |
| *rps*8 | 40.14 | 41.99 | 41.99 | 41.99 | 38.73 | 36.43 | 37.69 | 36.22 | 37.78 | 38.28 |
| *rps*7 | 39.74 | 40.71 | 40.71 | 40.71 | 41.63 | 41.11 | 43.18 | 41.11 | 40.31 | 43.96 |
| *rps*4 | 44.01 | 44.20 | 43.81 | 43.54 | 39.77 | 38.14 | 36.31 | 38.39 | 35.91 | 38.23 |
| *rps*3 | 48.15 | 52.43 | 52.43 | 52.71 | 52.18 | 42.80 | 42.75 | 44.36 | 43.07 | 45.94 |
| *rps*2 | 38.43 | 34.79 | 34.79 | 34.79 | 36.03 | 36.42 | 36.12 | 36.42 | 35.73 | 39.83 |
| *rps*19 | 41.42 | 41.05 | 41.05 | 41.05 | 42.35 | 37.92 | 40.89 | 46.95 | 43.21 | 46.02 |
| *rps*14 | 33.23 | 34.44 | 34.44 | 34.44 | 32.50 | 34.31 | 33.99 | 34.31 | 33.73 | 35.96 |
| *rps*13 | 42.04 | 44.68 | 44.68 | 44.68 | 41.13 | 42.71 | 37.48 | 42.71 | 40.33 | 40.28 |
| *rps*12 | 47.60 | 47.28 | 47.28 | 47.28 | 48.67 | 39.82 | 41.71 | 41.39 | 34.06 | 40.08 |
| *rps*11 | 43.04 | 38.20 | 38.20 | 38.20 | 36.92 | 39.44 | 38.16 | 39.44 | 41.42 | 40.63 |
| *rps*10 | 51.24 | 43.77 | 43.77 | 43.77 | 47.24 | 40.20 | 38.27 | 40.21 | 39.55 | 34.12 |
| *rpl*6 | 40.23 | 39.05 | 39.41 | 39.41 | 38.45 | 41.07 | 42.29 | 41.07 | 39.10 | 46.98 |
| *rpl*5 | 47.09 | 47.02 | 47.02 | 47.02 | 46.11 | 52.95 | 52.88 | 52.95 | 49.15 | 49.32 |
| *rpl*31 | 45.68 | 43.56 | 43.56 | 43.56 | 38.40 | 43.96 | 34.61 | 43.96 | 43.55 | 41.04 |
| *rpl*2 | 55.58 | 52.10 | 52.10 | 52.10 | 53.62 | 45.23 | 44.40 | 44.40 | 49.11 | 49.56 |
| *rpl*16 | 46.96 | 48.33 | 49.44 | 49.44 | 48.55 | 48.04 | 47.76 | 58.14 | 55.77 | 45.72 |
| *rpl*14 | 51.26 | 44.86 | 45.05 | 44.86 | 47.82 | 39.89 | 43.84 | 40.61 | 38.61 | 43.68 |
| *nad*9 | 43.94 | 50.90 | 50.90 | 50.90 | 46.48 | 39.17 | 38.13 | 39.17 | 35.70 | 39.72 |
| *nad*7 | 52.09 | 49.89 | 49.89 | 49.89 | 49.14 | 43.46 | 42.60 | 43.34 | 50.63 | 39.83 |
| *nad*6 | 48.42 | 47.80 | 47.74 | 47.80 | 47.37 | 45.18 | 41.99 | 45.00 | 41.52 | 41.47 |
| *nad*5 | 45.17 | 49.82 | 49.93 | 49.80 | 48.09 | 39.80 | 39.14 | 40.16 | 38.61 | 39.11 |
| *nad*4L | 49.84 | 45.86 | 45.86 | 45.86 | 44.51 | 31.85 | 37.35 | 31.85 | 30.36 | 31.38 |
| *nad*4 | 49.68 | 53.35 | 53.36 | 53.32 | 51.65 | 39.69 | 40.06 | 41.69 | 38.56 | 42.04 |
| *nad*3 | 39.45 | 41.73 | 41.73 | 41.73 | 46.12 | 34.96 | 35.39 | 35.07 | 35.81 | 34.54 |
| *nad*2 | 48.86 | 46.49 | 46.49 | 46.62 | 46.74 | 42.25 | 44.08 | 42.16 | 40.39 | 42.23 |
| *nad*11 | 46.88 | 47.02 | 47.56 | 47.02 | 49.01 | 45.59 | 43.80 | 54.89 | 49.15 | 47.65 |
| *nad*1 | 42.78 | 39.15 | 39.21 | 39.21 | 39.25 | 42.59 | 42.47 | 42.59 | 38.04 | 39.50 |
| *cox*3 | 41.03 | 37.46 | 37.46 | 37.46 | 39.73 | 38.85 | 42.46 | 50.94 | 45.42 | 51.40 |
| *cox*2 | 53.69 | 51.69 | 51.68 | 51.60 | 53.50 | 41.29 | 39.39 | 48.66 | 39.86 | 41.22 |
| *cox*1 | 46.04 | 49.38 | 49.25 | 49.38 | 48.88 | 40.70 | 40.14 | 40.58 | 38.46 | 40.61 |
| *cob* | 39.91 | 41.41 | 40.93 | 41.13 | 38.38 | 35.95 | 35.58 | 35.95 | 34.84 | 37.53 |
| *atp*9 | 38.10 | 40.19 | 40.19 | 40.19 | 38.69 | 43.41 | 41.70 | 42.35 | 43.41 | 42.78 |
| *atp*8 | 33.80 | 27.26 | 27.26 | 27.26 | 29.45 | 32.38 | 35.49 | 32.38 | 30.55 | 35.49 |
| *atp*6 | 41.16 | 40.77 | 40.77 | 40.77 | 39.71 | 43.24 | 40.66 | 43.24 | 42.94 | 41.13 |

| gene | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| *orf*41 | 27.13 | 41.01 | 41.01 | 41.01 | 51.21 | | | | | |
| *orf*127 | 51.81 | | | | | | | | | |
| *orf*130 | | 44.11 | 44.11 | 44.11 | 43.65 | | | | | |
| *orf*377 | | 45.11 | 44.16 | 44.06 | | | | | | |
| *orf*381 | 44.04 | | | | | | | | | |
| *orf*391 | | | | | 45.38 | | | | | |
| *orf*1 | | | | | | | | | | 43.22 |
| *orf*41 | | | | | | | | 41.01 | | |
| *orf*40 | | | | | | 38.54 | | | | |
| *orf*129 | | | | | | 43.98 | 37.72 | 61.00 | | |
| *orf*157 | | | | | | 45.03 | | | | |
| *orf*384 | | | | | | 44.17 | | 44.18 | | |
| *orf*507 | | | | | | | 44.34 | | | |

Table A1 Species ENC values

Note: A: *Saccharina coriacea*; B: *Saccharina japonica*; C: *Saccharina longipedalis*; D: *Saccharina religiosa*; E: *Saccharina angustata*; F: *Laminaria digitata*; G: *Laminaria ephemera*; H: *Laminaria hyperborea*; I: *Laminaria rodriguezii*; J: *Laminaria solidungula*.

| gene | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| atp6 | 0.162 | 0.172 | 0.172 | 0.172 | 0.178 | 0.160 | 0.168 | 0.160 | 0.162 | 0.174 |
| atp8 | 0.178 | 0.164 | 0.164 | 0.164 | 0.163 | 0.160 | 0.179 | 0.160 | 0.143 | 0.168 |
| atp9 | 0.248 | 0.251 | 0.251 | 0.251 | 0.253 | 0.242 | 0.248 | 0.255 | 0.239 | 0.249 |
| cob | 0.188 | 0.178 | 0.177 | 0.178 | 0.172 | 0.178 | 0.188 | 0.178 | 0.177 | 0.177 |
| cox1 | 0.202 | 0.207 | 0.207 | 0.207 | 0.207 | 0.189 | 0.189 | 0.189 | 0.192 | 0.195 |
| cox2 | 0.118 | 0.126 | 0.125 | 0.125 | 0.124 | 0.229 | 0.227 | 0.166 | 0.229 | 0.223 |
| cox3 | 0.207 | 0.212 | 0.212 | 0.212 | 0.215 | 0.208 | 0.206 | 0.127 | 0.174 | 0.180 |
| nad1 | 0.167 | 0.165 | 0.167 | 0.167 | 0.174 | 0.170 | 0.161 | 0.170 | 0.168 | 0.164 |
| nad11 | 0.167 | 0.162 | 0.161 | 0.162 | 0.163 | 0.147 | 0.153 | 0.135 | 0.159 | 0.157 |
| nad2 | 0.22 | 0.22 | 0.22 | 0.21 | 0.21 | 0.16 | 0.17 | 0.16 | 0.17 | 0.16 |

| gene | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7 | 0 | 0 | 9 | 9 | 9 | 1 | 8 | 2 | 7 |
| nad3 | 0.167 | 0.157 | 0.157 | 0.157 | 0.150 | 0.214 | 0.188 | 0.217 | 0.214 | 0.210 |
| nad4 | 0.191 | 0.209 | 0.209 | 0.210 | 0.203 | 0.157 | 0.155 | 0.150 | 0.155 | 0.162 |
| nad4L | 0.193 | 0.188 | 0.188 | 0.188 | 0.196 | 0.166 | 0.177 | 0.166 | 0.157 | 0.165 |
| nad5 | 0.172 | 0.182 | 0.181 | 0.182 | 0.180 | 0.180 | 0.183 | 0.178 | 0.180 | 0.184 |
| nad6 | 0.175 | 0.186 | 0.185 | 0.186 | 0.184 | 0.166 | 0.176 | 0.171 | 0.170 | 0.176 |
| nad7 | 0.149 | 0.137 | 0.137 | 0.137 | 0.141 | 0.199 | 0.206 | 0.196 | 0.164 | 0.202 |
| nad9 | 0.172 | 0.167 | 0.167 | 0.167 | 0.149 | 0.177 | 0.166 | 0.177 | 0.172 | 0.184 |
| rpl31 | 0.197 | 0.182 | 0.182 | 0.182 | 0.201 | 0.208 | 0.166 | 0.208 | 0.184 | 0.191 |
| rpl5 | 0.205 | 0.204 | 0.204 | 0.204 | 0.196 | 0.182 | 0.177 | 0.182 | 0.176 | 0.180 |
| *rpl*6 | 0.164 | 0.166 | 0.165 | 0.165 | 0.166 | 0.158 | 0.162 | 0.158 | 0.152 | 0.159 |
| *rps*10 | 0.209 | 0.174 | 0.174 | 0.174 | 0.183 | 0.164 | 0.159 | 0.164 | 0.168 | 0.152 |
| *rps*11 | 0.171 | 0.166 | 0.166 | 0.166 | 0.162 | 0.180 | 0.192 | 0.180 | 0.187 | 0.204 |
| *rps*12 | 0.178 | 0.182 | 0.182 | 0.182 | 0.187 | 0.213 | 0.211 | 0.215 | 0.192 | 0.188 |
| *rps*13 | 0.238 | 0.194 | 0.194 | 0.194 | 0.228 | 0.261 | 0.288 | 0.261 | 0.254 | 0.274 |
| *rps*14 | 0.206 | 0.191 | 0.191 | 0.191 | 0.207 | 0.169 | 0.166 | 0.169 | 0.177 | 0.172 |
| *rps*19 | 0.175 | 0.192 | 0.192 | 0.190 | 0.177 | 0.178 | 0.168 | 0.194 | 0.144 | 0.167 |
| *rps*2 | 0.171 | 0.172 | 0.172 | 0.172 | 0.176 | 0.161 | 0.160 | 0.161 | 0.159 | 0.155 |
| *rps*3 | 0.168 | 0.167 | 0.167 | 0.168 | 0.176 | 0.160 | 0.154 | 0.162 | 0.156 | 0.147 |
| *rps*4 | 0.160 | 0.171 | 0.172 | 0.173 | 0.165 | 0.156 | 0.160 | 0.156 | 0.162 | 0.170 |
| *rps*7 | 0.186 | 0.182 | 0.182 | 0.182 | 0.184 | 0.194 | 0.196 | 0.194 | 0.194 | 0.192 |
| *rps*8 | 0.17 | 0.17 | 0.17 | 0.17 | 0.18 | 0.17 | 0.19 | 0.18 | 0.17 | 0.20 |

| gene | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 7 | 7 | 7 | 1 | 9 | 3 | 3 | 4 | 6 |
| *tat*C | 0.141 | 0.151 | 0.151 | 0.151 | 0.152 | 0.158 | 0.155 | 0.146 | 0.174 | 0.167 |
| *rpl*16 | 0.166 | 0.178 | 0.185 | 0.185 | 0.164 | 0.197 | 0.184 | 0.168 | 0.165 | 0.177 |
| *rpl*2 | 0.181 | 0.163 | 0.163 | 0.163 | 0.187 | 0.176 | | 0.178 | 0.168 | 0.172 |
| *rpl*14 | 0.146 | 0.165 | 0.164 | 0.165 | 0.147 | 0.160 | 0.182 | 0.157 | 0.175 | 0.185 |
| *orf*1 | | | | | | | | | | 0.216 |
| *orf*40 | | | | | | 0.144 | | | | |
| *orf*41 | 0.210 | 0.126 | 0.121 | 0.121 | 0.199 | | | 0.121 | | |
| *orf*127 | 0.146 | | | | | | | | | |
| *orf*129 | | | | | | 0.156 | 0.137 | 0.193 | | |
| *orf*130 | | 0.150 | 0.150 | 0.150 | 0.144 | | | | | |
| *orf*157 | | | | | | 0.132 | | | | |
| *orf*377 | | 0.201 | 0.202 | 0.205 | | | | | | |
| *orf*381 | 0.176 | | | | | | | | | |
| *orf*384 | | | | | | 0.189 | | 0.186 | | |
| *orf*391 | | | | | 0.203 | | | | | |
| *orf*507 | | | | | | | 0.214 | | | |

Table A2 Species CAI values

Note: A: *Saccharina coriacea*; B: *Saccharina japonica*; C: *Saccharina longipedalis*; D: *Saccharina religiosa*; E: *Saccharina angustata*; F: *Laminaria digitata*; G: *Laminaria ephemera*; H: *Laminaria hyperborea*; I: *Laminaria rodriguezii*; J: *Laminaria solidungula*.