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QUALIFICATION THESIS

Mitochondrial genomes reveal the relationship in Gigartinales

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Completed: student of group BEBT-21
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
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Abstract

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Gigartinale is an important class of marine macroalgae in the phylum Rhodophyta, belonging to Florideophyceae, which has important ecological and economic values. In this study, we investigated 11 species of Gigartinales, and systematically analyzed the structural characteristics of the mitochondrial genomes of Gigartinales by using bioinformatics software, such as Geneious, CodonW, MEGA11, TBtools and Origin. We then constructed a phylogenetic tree to explore their evolutionary relationships. The phylogenetic tree was reconstructed to explore their evolutionary relationships.

The mitochondrial genome length of Gigartinales is concentrated between 25-26 kb, with a GC content of 27.9%-37.4%. It encodes 48-50 genes, mainly including cytochrome oxidase (*cox*1-*cox*3), NADH dehydrogenase (*nad*1-*nad*6), ATP synthase (*atp*4-*atp*9), and ribosomal protein genes, with the highest number of genes being 57 in *Chondrus crispus*, and *Sarcopeltis skottsbergii* had the lowest number of 48. The results of covariance analysis showed that the gene arrangement of the mitochondrial genome of Gigartinales was highly conserved, but there was a small rearrangement of the *trn*Y and *trn*R genomes among some species. The results of codon usage preference (RSCU) analysis showed that the mitochondrial genomes of Gigartinales prefer codons ending in A/T, among which the leucine codon UUA has the highest RSCU value (2.03-4.43), which fully indicates its obvious preference. 11 species of Gigartinale mitochondrial genomes have a smaller gap in RSCUs. Most of them are larger than 1, which suggests to some extent that the mitochondrial genomes of the Gigartinales are evolutionarily conservative. In addition, the phylogenetic trees constructed by maximum likelihood (ML) and Bayesian inference (BI) methods showed that the species of Gigartinales were divided into two branches and supported the traditional classification framework, while revealing some evolutionary differences among species.

Key words: *Gigartinale, mitochondrial genome, comparative genomic analysis, system evolution, codon preference*

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INTRODUCTION

Gigartinale, an ecologically and economically significant order of marine red algae (Rhodophyta), plays a critical role in marine ecosystems and biogeochemical cycles. Despite their importance, the evolutionary relationships and genomic adaptations within this group remain understudied. In this study, we conducted a comprehensive analysis of the mitochondrial genomes of 11 Gigartinales species to elucidate their structural characteristics, codon usage biases, and phylogenetic relationships. By integrating high-throughput sequencing, comparative genomics, and bioinformatics tools such as Geneious, CodonW, and MEGA11, we systematically explored the conserved and divergent features of these genomes. Our results revealed that the mitochondrial genomes of Gigartinales are highly compact (25–26 kb), with GC contents ranging from 27.9% to 37.4%, and encode 48–57 genes, including core oxidative phosphorylation components, ribosomal proteins, and tRNAs. Covariance analysis demonstrated remarkable conservation in gene arrangement, with minor rearrangements observed in *trn*Y and *trn*R orders among certain species. Codon usage bias analysis highlighted a strong preference for A/T-ending codons, particularly leucine (UUA, RSCU = 2.03–4.43), while ENC values (average 42.11) indicated weak codon bias influenced primarily by natural selection. Phylogenetic reconstruction using maximum likelihood (ML) and Bayesian inference (BI) methods resolved Gigartinales into two major clades: one comprising Solieriaceae, "*Caulacanthus okamurae*", and "*Hypnea edeniana*", and another including "*Chondrus crispus*", "*Sarcopeltis skottsbergii*", and "*Mastocarpus papillatus*". These findings align with traditional taxonomic frameworks while revealing lineage-specific evolutionary adaptations.

This study provides critical genomic insights into the evolutionary history and functional adaptations of Gigartinales. By establishing a foundation for comparative mitochondrial genomics, our work enhances understanding of their ecological roles, taxonomic relationships, and potential biotechnological applications. Furthermore, the identification of conserved genomic features and lineage-specific traits offers valuable data for future research on marine biodiversity conservation, algal resource utilization, and evolutionary biology.

The subject of the study eleven types of Gigartinales

Research methods the whole mitochondrial genome sequencing

The practical significance of the results obtained is the evolutionary dynamics and adaptive genomic features of Gigartinales, offering foundational insights to advance marine biodiversity conservation, biotechnological exploitation of algal resources, and the development of bioactive compounds for medical and industrial applications.

Chapter I
LITERATURE REVIEW

1.1 INTRODUCTION TO GIGARTINALES

The Gigartinale belongs to the class Florideophyceae under the phylum Rhodophyta and is a unique group of Marine algae. The algal forms of the species in this order are diverse, including shell-like, upright shrub-like and leaf-like. Their cells are usually small and dense, containing a large number of disc-shaped chloroplasts and without starch nuclei. Seaweeds of the Gigartinales are widely distributed in the temperate and northern seas of the world, especially in the intertidal zone and shallow sea areas[23]. Because its algal body shape is similar to the branches of a fir tree and the underwater landscape is like a miniature forest, it is named the Gigartinale. This type of seaweed not only has significant ecological value but also economic value.

1.2 BIOLOGICAL CHARACTERISTICS OF ALGAE IN THE GIGARTINALES

1.2.1 DISTRIBUTION AND MORPHOLOGICAL CHARACTERISTICS

The Gigartinale includes several families such as Gigartinaceae, Phyllophoraceae and Solieriaceae. The algae of this order grow in erect tufts or creeping, with cylindrical, compressed or blade-like forms, most of which are plump and fleshy, and some of which are branched with papillae or warty protuberances. The internal structure can be divided into uniaxial and multiaxial subtypes according to the number of main axes, and the cells are generally small and tightly arranged, containing small discoid pigment bodies and lacking protein nuclei. Auxiliary cell was usually near the basal part of the ampulla and generated several two to three celled carpogonial branches. Solieria robusta female thallus surface shows many scattered cystocarps that appeared as black coloured dots[49].The cells of the medulla are large and closely interconnected, or consist of parallel longitudinal filaments. The sporocysts and gametocysts of most species are

similar in appearance and difficult to distinguish[31]. Tetrasporangia are distributed throughout the alga, some are buried on the surface and are branched, some form apical branches, some are aggregated deep in the alga and are partly in chains, and some form special branches of the sporangia. The sporangia originate from the innermost cells of the cortex or develop from special cells of the medullary margin. In China, the algae of Gigartinales are mainly distributed in the waters of the Yellow Sea, Bohai Sea and East China Sea, which provide a favourable growth environment due to their clear water quality and abundant sunlight[43].

1.2.2 SCIENTIFIC VALUE AND APPLICATION PROSPECT OF THE MITOCHONDRIAL GENOME OF GIGARTINALES

Algae of the Gigartinales show diverse reproductive modes, including cruciform division, four-sided conical division and laminar division. Their spermatophores are born in the reproductive fossa, and the fruits are spherical or hemispherical, born in the body or slightly elevated, most of them with pericarp tissue . The auxiliary cells are common trophic filament interphase cells that produce juxtasporangia, and some or all of the cells develop into fruiting sporocysts. Gigartinale is rich in beneficial components such as proteins, carbohydrates, fatty acids, polyphenols and terpenoids with antibacterial, antifungal, antioxidant, and anti-inflammatory activities, which help to prevent chronic diseases[32].

As an important marine macroeconomic alga, Gigartinale has an irreplaceable role. Its in-depth study will promote the progress of ecology, medicine and agriculture in China, and make full use of marine resources to provide more production and living opportunities for people and used for decades in some countries as raw material for carrageenan extraction and in some cases destined for human consumption[28]. Mitochondrial genome research not only helps to understand the evolutionary history and ecological adaptations of Gigartinales, but also provides a rich resource for biotechnology and medicine[34]. Future studies will further reveal the complexity and

diversity of its mitochondrial genome and provide scientific support for the conservation and exploitation of marine resources.

1.3 PROGRESS OF ALGAL GENOME RESEARCH

In recent years, significant progress has been made in the study of algal genomes in Gigartinales. With the development of high-throughput sequencing technology, the whole genomes of several species have been determined, revealing genome size, gene number and species-based information. The mitochondrial genome of red algae is relatively small (25-36 kb) compared with other taxa, and the gene structure is also compact with sparse spacer regions; however, the mitochondrial genome conformation is very conserved and all circular, and the gene coding sequences are also conserved[3]. The mitochondrial genome varies greatly among eukaryotes, and many genes related to apoptosis and heme synthesis have been identified. In phylogenetic studies, genomic data provide strong support for the determination of relatedness among species. Following the taxonomic and nomenclatural updates, the taxonomic nomenclature was updated for Gigartinales and new taxonomic combinations were proposed[27]. However, there are still limitations in the current study, such as the lack of species genomic data and imperfect analysis of gene regulatory networks. In the future, with the advancement of technology and in-depth research, the study of algal genomes in Gigartinales can provide a more solid theoretical foundation in the fields of resource development, ecological conservation and evolutionary biology.

1.4 MITOCHONDRIA AND THEIR GENOMES

1.4.1 ORIGIN AND EVOLUTION OF MITOCHONDRIA

The origin of mitochondria has long been a focus of debate in the scientific community, mainly divided into the ‘endosymbiotic origin’ theory and the ‘non-endosymbiotic origin’ theory, while the current mainstream doctrine prefers the endosymbiotic origin theory. In 1970, Margulis proposed the theory of endosymbiotic

origin, which explained that large primitive eukaryotic cells with phagocytosis ability engulfed aerobic bacteria, which were not digested but parasitised in the host cell, gradually transformed into a symbiotic state, and eventually became mitochondria in the host cell[3]. The long-term symbiotic relationship led to the gradual loss of some of the original characteristics of these bacteria, shut down, lost or transferred some of the genes to the nucleus of the host cell, which led to the formation of the semi-autonomous nature of the mitochondria. According to this theory, the ancestor of eukaryotic cells was a bulky, anaerobic and phagocytic cell that relied on the glycolytic pathway for energy. In contrast, the ancestor of the mitochondrion was thought to be an α-amoeba, which, by virtue of its tricarboxylic acid cycle enzyme system and electron transport chain, further oxidises the glycolytic product pyruvate under aerobic conditions, releasing higher energy. However, subsequent reports have shown that the plastids of some organisms have a multilayered membrane structure, such as the presence of three to four membranes in the plastids of some unicellular organisms, which contradicts the idea of initial endosymbiosis.

Therefore, the researchers proposed the hypothesis of secondary endosymbiosis in plastids and suggested that they may have undergone two or three endosymbiotic processes. Palaeontological studies have shown that red algae differentiated about 1.4 billion years ago, while secondary endosymbiotic products, such as brown algae and diatoms, appeared in strata about 1 billion years ago. The theory of non-endosymbiotic origins suggests that the plasma membrane invaginated to form mitochondria, and that the functions of these organelles were gradually specialised during the course of evolution.In 1974, Uzzell et al. further elucidated the evolution of mitochondria by proposing the ‘theory of intracellular parthenogenetic chemistry’, which suggests that the structure of the multi-transmembranous plastid originated from the engulfment of other multi-transmembranous plastids by the plastid-containing eukaryotes. phagocytosis of other multi-membrane plastids. With the development of high-throughput sequencing technology, studies of more eukaryotic plastid genomes have revealed that plastids may have evolved after one or more endosymbioses.

1.4.2 CONSTRUCTION OF MITOCHONDRIA

Mitochondria are semi-autonomous organelles in eukaryotic cells with a variety of morphologies, including spherical, rod-shaped and thread-like. The basic structure of mitochondria consists of four core parts: the outer membrane, the inner membrane, the membrane gap and the matrix. The outer membrane consists of a double layer of phospholipid molecules in a closed state, allowing only small molecules to pass freely, while larger molecules need to use specific transport proteins. Pore proteins, such as porins, are present in the outer membrane and allow the free passage of small molecules (e.g., ions, ATP, NADH). The membrane gap, located between the outer and inner membranes, is connected to the cytoplasm, and the proteins within it originate mainly from the cytoplasm and enter via outer membrane transporter proteins. The inner membrane is one of the unique structures of mitochondria, and its permeability is much lower than that of the outer membrane, allowing only small molecules to pass through. Numerous protein complexes, such as respiratory chain complexes and ATP synthases, are distributed in the inner membrane and are responsible for electron transfer and ATP production. The stroma, located on the inner side of the inner membrane, is the main functional region of mitochondria and contains a large amount of DNA, RNA, ribosomes and enzymes involved in the tricarboxylic acid cycle (TCA cycle) and other metabolic pathways. Mitochondrial DNA (*mt*DNA) is highly variable in size, with the smallest mitochondrial DNA being only about 6,000 bp in size, while the largest mitochondria can be up to 11.30 Mbp[4], and is responsible for encoding a number of important mitochondrial proteins.

1.4.3 FUNCTIONS OF MITOCHONDRIA

Mitochondria are the energy centre of the cell and are mainly responsible for energy production, especially the synthesis of ATP through the process of oxidative phosphorylation.Oxidative phosphorylation is an important function of mitochondria,

where electrons released from the oxidation of nutrients (e.g. glucose, fatty acids) are transferred to oxygen through the electron transport chain to form water and release energy, which is used to drive the synthesis of ATP by the enzyme ATP synthase[5]. Mitochondria also carry out the tricarboxylic acid cycle, also known as the citric acid cycle, a series of biochemical reactions in the mitochondrial matrix that further oxidises pyruvate to carbon dioxide and releases large amounts of energy for oxidative phosphorylation. In addition, mitochondria are responsible for storing and releasing calcium ions, which are essential for processes such as cell signalling and muscle contraction. At the same time, mitochondria play a key role in the regulation of apoptosis by releasing factors such as cytochrome c, which initiates the apoptotic programme.

1.5 MITOCHONDRIAL GENOME RESEARCH METHODS

Currently, under the wave of new technological revolution, the rapid development of molecular biology and bioinformatics promotes the continuous breakthrough of modern biotechnology, and genomics has become an important research field in life sciences. Advances in genetic engineering have not only reshaped the landscape of biochemistry and molecular biology, but also had a profound impact on the entire field of biology. The mitochondrial genome has a wide range of applications in algal genetics and phylogenetic studies, which helps to facilitate the exploration of mitochondrial function and evolutionary history. Mitochondrial genome analysis can reconstruct the phylogenetic relationships among species and reveal the genetic variation and evolutionary pathways among species. Mitochondrial genome research not only improves our overall understanding of mitochondrial function and evolution, but also shows potential applications in medicine and chemistry, especially in agriculture and health care. As research progresses, new discoveries continue to emerge and new areas of study expand.

1.5.1 DIDEOXY TERMINATION SEQUENCING METHOD

Dideoxy termination sequencing, also known as Sanger sequencing, is a DNA sequencing method invented by Frederick Sanger in 1977. The principle is based on the replication of a target DNA fragment in vitro by DNA polymerase through the addition of dideoxyribonucleotide (ddNTP) labelled with a radioisotope or fluorescent dye. The incorporation of this special nucleotide into the DNA strand terminates the addition of subsequent nucleotides, ultimately leading to the termination of strand elongation.Sanger sequencing is widely used in biological research because of its simplicity, reliability and high accuracy, and has played a key role in early genome sequencing projects in particular. Due to the small size of the mitochondrial genome (the molecular weight of mitochondrial DNA in algae is generally between 15 kb and 70 kb, and that of Gigartinale species is around 25 kb) and the typical covalent, closed-loop structure, the application of Sanger sequencing is gradually decreasing with the emergence of the new generation of sequencing technologies, but it still has a unique advantage in the verification of sequencing results, etc., and it provides a comparative benchmark for third-generation sequencing, which is not an alternative for clinical diagnosis. benchmarks and is irreplaceable in clinical diagnosis.

1.5.2 PCR AMPLIFICATION PRODUCTS DIRECT SEQUENCING TECHNOLOGY

PCR (Polymerase Chain Reaction) is a technology for rapid amplification of specific DNA or RNA fragments in vitro, also known as cell-free molecular cloning or in vitro primer-directed enzymatic amplification of specific DNA sequences. The basic principle is the exponential amplification of target DNA fragments by DNA polymerase under specific conditions. The core of this technology lies in the design and development of specific primers, so that the primers can specifically bind to specific regions at both ends of the target DNA sequence to ensure that only the target DNA fragments are amplified, which makes the manipulation of trace amounts of nucleic acids simple and feasible, and is a major innovation in gene amplification technology. In view of the high copy number characteristics of mitochondrial DNA (*mt*DNA),

species-specific primers are usually designed to amplify the target region and directly sequence the PCR products, thus enabling rapid species identification or phylogenetic analyses[39]. For example, in the taxonomic study of Cynobacteria, mitochondrial *nad*5 or *cox*1 genes can be amplified to effectively distinguish morphologically related species[50,54]. The long PCR technique (long PCR), which amplifies DNA fragments of more than 5 kb, has also been applied to mitochondrial genome sequencing. This strategy greatly simplifies the process, but there is a limitation in the design of primers: the genomes of closely related species need to be available in the genetic database to inform the design of primers. This method has now been used in obtaining the complete mitochondrial genome sequences of the brown alga Gigartinales, and the Far North kelp *Laminaria hyperborea* [10].

1.5.3 APPLICATION OF HIGH-THROUGHPUT SEQUENCING TECHNOLOGY

High-throughput sequencing, or next-generation sequencing (NGS), is a mainstream sequencing technology that can rapidly sequence thousands of DNA or RNA molecules simultaneously. With higher sequencing throughput and lower cost than traditional Sanger sequencing, high-throughput sequencing is widely used in genomics, transcriptomics, and epigenetics, among other studies [6].NGS is capable of capturing mitochondrial sequences directly from total DNA without the need for pre-PCR amplification. Sequencing by whole-genome birdshot sequencing combined with bioinformatics screening (e.g., BLAST comparisons allow rapid assembly of high integrity mitochondrial genomes, significantly improving sequencing efficiency)[38]. High-throughput sequencing technology has revolutionised genomics and functional genomics by providing rapid and efficient access to massive sequences, and breakthroughs in large-scale, high-throughput sequencing have provided novel opportunities for algal mitochondrial genome research. In recent years, the complete organelle genome columns of e.g. *Eucheuma denticulatum*, *Kappaphycus alvarezii* and

Betaphycus gelatinus have also been mostly obtained using high-throughput sequencing.

The use of bioinformatics software to study the structure, size and content of the mitochondrial genome goes beyond the traditional morphological and structural classification methods, deepens the understanding of the germplasm identification of species in Gigartinales, and provides theoretical evidence for phylogenetic evolutionary studies.

1.6 RESEARCH PURPOSE AND SIGNIFICANCE

As an important part of the earth's ecosystem and a key carrier of elemental biogeochemical cycles, the diversity and function of algae play a positive role. With the in-depth study of modern molecular systematics, the range of algae has been expanding, and they have become the only group of organisms spanning both the prokaryotic and eukaryotic domains. As economically important red algae, studies on the structure and composition of their organelle genomes help to gain a deeper understanding of their taxonomic status, evolution of their origin and phylogeny of the red algal phylum Gigartinales. Meanwhile, mitochondrial genome studies play an indispensable role in revealing biological evolution, species and genetic characteristics. Mitochondrial DNA is short in length, abundant in content, evolves rapidly, replicates autonomously and is highly conserved. In recent years, mitochondrial genome sequencing has become increasingly popular with the development of genome sequencing technology. Using bioinformatics tools to analyse mitochondrial genomes can reveal the evolutionary history of organisms, inter-species relationships and genetic characteristics in a more in-depth manner than traditional methods. In addition, the study of codon preferences in mitochondrial genomes is important for understanding mutations in genetic material, natural selection and molecular evolution.

In this study, we intend to conduct structural characterisation and comparative genomics analysis of the genome of Gigartinales, which will provide abundant genomic information for subsequent molecular biology and genetics studies of Gigartinales. In

addition, based on the published mitochondrial genome data, a phylogenetic tree of mitochondria was reconstructed at the whole genome level to reveal the taxonomic status of the species and their evolutionary affinities, and to provide theoretical basis for more comprehensive and in-depth research on them in the future.

Summary of the chapter I

1. The mitochondrial genomes of Gigartinales are compact (25–26 kb), with conserved gene arrangements and a GC content of 27.9%–37.4%. They encode 48–50 genes, including “cox”, “nad”, “atp”, and ribosomal proteins, with minimal non-coding regions.

2. The study aims to analyze mitochondrial genome structure, codon usage bias, and evolutionary relationships using bioinformatics tools to enhance understanding of Gigartinales’ taxonomy, evolution, and biotechnological potential.

3. Gigartinales, a class of marine red algae (Rhodophyta), exhibits diverse morphological forms and ecological significance. It is distributed globally in temperate and northern seas, contributing to marine ecosystems and possessing economic value due to bioactive compounds like sulfated polysaccharides.

Chapter II
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 DATA SOURCE

The mitochondrial genomes of 11 species of Gigartinales have been published in the Genbank database[7], including the Solieriaceae (5): *Betaphycus gelatinus*, *Eucheuma denticulatum*, *Kappaphycus malesianus*, *Kappaphycus malesianus* and *Kappaphycus alvarezii*[24]; Gigartinaceae (2): *Chondrus crispus*, *Sarcopeltis skottsbergii*; Phyllophoraceae (1): *Mastocarpus papillatus*; Caulacanthaceae (1): *Caulacanthus okamurae*; Endocladiaceae (1): *Gloiopeltis furcata*[29]; and Cystocloniaceae (1): *Hypnea edeniana* [26,48].

2.2 EXPERIMENTAL METHODS

2.2.1 METHODS OF COMPARATIVE GENOMIC ANALYSIS

In this study, we systematically searched and downloaded the mitochondrial genome full sequence data (GenBank format files with genome annotation information) of 11 publicly available Gigartinale species from the NCBI public database. The protein coding genes, ribosomal RNA (rRNA) genes, length and transcription direction of the mitochondrial genomes were searched in the ‘Annotations and Tracks’ section of the Geneious Bioinformatics Analysis Platform (http://www.geneious.com/). In ‘Annotations and Tracks’, we searched and compared the protein coding genes, ribosomal RNA (rRNA) gene types, lengths, and transcription directions in the mitochondrial genome of Cynobacteria. Run tRNAscan-SE’ to get all the tRNA species, lengths and their corresponding amino acids, anticodons and other related information.

2.2.2 CO-LINEARITY ANALYSIS METHOD

The mitochondrial genomes of 11 species of Gigartinales were analysed by covariance analysis using the Mauve 2.4.0 plug-in in Geneious R11 software. Before comparison, the genomes were edited using the 'Allow Editing' function to ensure that all genomes were started from the same position. After editing, the 'Align Whole Genomes' function was used to perform the comparison, and the results were exported in 'File'[10].

Table 2-1 Experimental Sample Source Information

Sample name	*B.gelatinus*	*E.denticulatum*	*K.striatus*	*K.alvarezii*	*K.malesianus*	*C.crispus*	*S.skottsbergii*	*M.papillatus*	*C.okamurae*	*G.furcata*	*H.edenia na*
Genbank	NC_036431.1	NC_036432.1	NC_024265.1	NC_031814.1	NC_068226.1	NC_001677.2	NC_053772.1	NC_031166.1	NC_047435.1	NC_044414.1	NC_072907.1
Length (bp)	25,275	25,327	25,242	25,198	25,250	25,836	25,908	25,906	25,995	25,636	25,073
GC content (%)	29.70	30.30	30.06	29.86	30.25	27.89	28.51	35.00	30.65	37.38	28.10
Coding gene	50	50	50	49	50	57	48	50	50	49	50
tRNA gene	24	24	24	23	24	24	22	22	23	23	24
rRNA gene	2	2	2	2	2	2	2	2	2	2	2
Gene overlap region	4	2	1	1	0	8	5	4	4	1	3

Number of introns	1	1	1	1	0	1	0	0	0	1	0
ATG as start codon	23	24	23	23	23	28	23	25	25	23	28
GTG as start codon	0	0	0	0	0	0	0	0	0	*ymf*39	0
TTG as start codon	*tat*C	0	*tat*C	*tat*C	*tat*C	0	0	0	0	0	0
ATA as start codon	0	0	0	0	0	0	*tat*C	0	0	0	*tat*C
TAA as stop codon	22	21	22	22	22	25	24	22	19	22	29
TAG as stop codon	2	3	2	2	2	4	0	4	6	2	1

2.3 RESULTS AND ANALYSES

2.3.1 COMPARATIVE ANALYSIS OF THE MITOCHONDRIAL GENOMES OF ALGAE IN GIGARTINALES

The mitochondrial genomes of these 11 species in Gigartinales were basically the same length, concentrating between 25 kb-26 kb, with GC contents ranging from 27.89% to 37.38%, and the genes were all arranged in a relatively compact manner, with both the H-chain and the L-chain encoding genes. Most of them encoded 50 genes, of which the mitochondrial genome of *Chondrus crispus* encoded the most genes, 57; *Kappaphycus alvarezii* and *Gloiopeltis furcata*, 49, *Sarcopeltis skottsbergii* had the fewest with 48.

2.3.2 ANALYSIS OF PROTEIN CODING GENES

According to the data, the type and number of genes coding for all categories were broadly similar, all containing about 19-20 genes related to electron transport and oxidative phosphorylation systems, 5 ribosomal protein coding genes, 2-4 rRNA coding genes, and 22-24 tRNA coding genes[8]. Gene overlap is a common phenomenon in the mitochondrial genomes of *C. crispus,* with the number of overlapping groups ranging from 1-8, with *C. crispus* having the highest number of overlapping groups at eight. However, the same pattern of overlapping genes was not found in the mitochondrial genomes of these 11 *C. crispus* algae. The mitochondrial genomes of Gigartinales were more compactly coded, with gene coding regions accounting for 90.19%-95.88% of the genes. The *tat*C genes in the mitochondrial genomes of Gigartinales did not use ATG as the start codon, but other codons, except for *C. okamurae*, *B. gelatinus* , *E. denticulatum, K. alvarezii*, *K. striatus,* and *K. alvarezii*, which were all coded in the genomes. striatus) and *K. malesianus* use TTG, *C. crispus* uses GTT, *S. skottsbergii* uses ATA as an initiation codon, and *M. papillatus* uses ATC, which is rare in algae.It

is worth noting that ATC as an initiation codon is relatively rare in plants, but in certain animals such as *Trypetoptera punctulata*[36].

C. crispus encodes four additional genes (*orf*73, *orf*74, *orf*941, *orf*105) alone and one *orf*172 gene in common with *M. papillatus*. And the mitochondrial genomes of *C. okamurae* and *M. papillatus* also co-encoded an uncommon *tat*A gene, which was not found in the mitochondrial genomes of other red algae. Typically, the *tat*A and *tat*C genes are encoded in the mitochondrial genome and are not commonly found in the nuclear or plastid genomes, and the transfer of these two genes from the mitochondrial genome to the nuclear genome does not generally occur. However, available genome annotation results show that most red algal mitochondria encode only the *tat*C gene and not the *tat*A gene.

In this study, the protein-coding genes of the mitochondrial genomes of algae in Gigartinales were studied in detail, and all 11 species of Gigartinales encode the following 21 protein genes, including three cytochrome oxidase subunits (*cox*1, *cox*2, *cox*3,and Mitochondrial cytochrome c oxidase subunit 1 sequences can be used to explore patterns of phylogeography, lineage structure and population genetic differentiation)[25,30], one cytochrome b (*cob*), seven coenzyme dehydrogenase subunits (*nad*1, *nad*2, *nad*3, *nad*4, *nad*5 , *nad*6, *nad*4L), four ribosomal proteins (*rps*3, *rps*11, *rps*12, *rpl*16), and three ATP synthase subunits (*atp*6, *atp*8, *atp*9). In addition, three succinate dehydrogenase complexes (*sdh*2, *sdh*3, *sdh*4) were studied.

Table 2-2 Protein-coding genes in Gigartinales

gene product	Number Gene type	Gene type
Cytochrome oxidase	3	*cox*1, *cox*2, *cox*3
Apocytochrome b	1	*cob*
NADH dehydrogenase	7	*nad*1, *nad*2, *nad*3, *nad*4, *nad*5, *nad*6, *nad*4L

Ribosomal proteins	4	*rps*3, *rps*11, *rps*12, *rpl*16
ATPase subunits	3	*atp6*, *atp8*, *atp*9
Succinate dehydrogenase	3	*sdh2*, *sdh3*, *sdh4*

2.3.3 rRNA GENES

The mitochondrial genomes of all the algae in Gigartinales contain two rRNA genes, the *rnl* gene, the *rns* gene, and in the case of *Gloiopeltis furcata*, the *rrl* gene and the *rrs* gene. the two rRNA genes are very close to each other and are fixed in position, and they are all spaced apart by a *nad*4L gene.

Table 2-3 Four rRNAs in the mitochondrial genome of algae in Gigartinales

Gene	Length (bp)	coding chain
rnl	2619[a]/2637[b]/2581[c]/2583[d]/2584[e]/2583[f]/2577[g]/2592[h]/2613[i]/2633[j]/2589[j]	L
rns	1372[a]/1377[b]/1360[c]/1360[d]/1359[e]/1376[f]/1356[g]/1364[h]/1356[i]/1364[j]	L

a:*Betaphycus gelatinus ;* b:*Eucheuma denticulatum ;* c:*Kappaphycus striatus ;* d:*Kappaphycus alvarezii ;* e:*Kappaphycus malesianus ;* f:*Chondrus crispus ;* g:*Sarcopeltis skottsbergii ;* h:*Mastocarpus papillatus ;* i:*Caulacanthus okamurae ;* j:*Gloiopeltis furcata*

2.3.4 tRNA GENES

The sequences of the mitochondrial genomes of Gigartinales were studied by the online software tRNAscan-SE, and 25 tRNAs were identified and localised, with lengths ranging from 72-98 bp, with an average of 78 bp, of which only *Mastocarpus papillatus* encoded *trn*Sec and *Gloiopeltis furcata* encoded *trn*U. Most of the tRNAs such as *trn*S2 and *trn*L-UAG have small differences between species, some tRNAs such

as *trn*M have high stability, and the 25 tRNA genes encode on both the heavy (H) and light (L) strands.

Table 2-4 25 tRNAs in the mitochondrial genome of algae in Gigartinales

tRNA type	Amino acid	Anti-codon	Length
*trn*N	Asn	GTT	$72^{abdghijk}/73^{e}/75^{f}$
*trn*V	Val	TAC	$73^{abcdehijk}/76^{f}/74^{g}$
*trn*R1	Arg	ACG	$75^{abcdehijk}/76^{g}$
*trn*K	Lys	TTT	$74^{adehk}/75^{bcij}/72^{fg}$
*trn*E	Glu	TTC	$72^{ach}/74^{bdegjk}/75^{f}/73^{i}$
*trn*fM	Met	CAT	$74^{abehij}/73^{c}/75^{dgk}/76^{f}$
*trn*D	Asp	GTC	$72^{abdegijk}/73^{fh}$
*trn*G2	Gly	TCC	$75^{a}/74^{bcdefhij}$
*trn*Q	Gln	TTG	$72^{ac}/74^{bdeijk}/75^{fg}/73^{h}$
*trn*L-UAA	Leu	TAA	$84^{aghik}/86^{bdej}/82^{c}/85^{f}$
*trn*L-UAG	Leu	TAG	$81^{ach}/85^{bej}/84^{dgi}/82^{f}/86^{k}$
*trn*G	Gly	GCC	$73^{abcdefijk}/75^{g}/74^{hk}$
*trn*H	His	GTG	$75^{abeij}/76^{cdk}/78^{g}$
*trn*F	Phe	GAA	$72^{ach}/75^{bdeij}/74^{fgk}$
*trn*S	Ser	TGA	$84^{aik}/86^{bcdej}/83^{fh}/89^{g}/$
*trn*P	Pro	TGG	$74^{ahk}/75^{befgij}/72^{c}/76^{d}$
*trn*C	Cys	GCA	$72^{ac}/71^{bdeijk}/76^{f}/74^{gh}$
*trn*M	Met	CAT	$74^{abcdefghijk}$
*trn*W	Trp	TCA	$72^{abdeijk}/73^{gh}$
*Trn*A	Ala	TGC	$74^{abcdeghij}/75^{f}/72^{k}$
*trn*R	Arg	TCT	$76^{af}/73^{bcdegijk}/74^{f}/77^{h}$
*trn*Y	Tyr	GTA	$90^{ag}/84^{bdehijk}/82^{c}/85^{f}$
*trn*S2	Ser	GTC	$91^{b}/98^{c}/93^{d}/95^{e}/94^{gj}/92^{i}/93^{k}$

*trn*Sec	Sec	TCA	72[c]
*trn*U	Sec	TCA	74[f]

a:*Chondrus crispus ;* b:*Kappaphycus striatus ;* c:*Mastocarpus papillatus ;* d:*Betaphycus gelatinus ;* e:*Eucheuma denticulatum ;* f:*Gloiopeltis furcata ;* g:*Caulacanthus okamurae ;* h:*Sarcopeltis skottsbergii ;* i:*Kappaphycus malesianus ;* j:*Kappaphycus alvarezii ;* k:*H.edeniana*

2.3.5 CO-LINEARITY ANALYSIS

In general, the mitochondrial genomes of the species of the True Red Algae order are highly conserved and have significant sequence covariance. In this study, genome-wide covariance analysis was performed on the sequenced mitochondrial genomes of *B. gelatinus*, and the results showed that the gene order of the mitochondrial genomes of *B. gelatinus* was generally conserved, but there were some minor gene rearrangements, mainly reflected in the differences in the order of the *trn*Y and *trn*R genes in different species[33,35]. For example, the *trn*Y and *trn*R genes of *B. gelatinus*, *E. denticulatum*, *K. alvarezii*, *K. striatus*, *K. malesianus*, *C. okamurae*, and *G. furcata* were arranged in the order of *trn*Y and *trn*R genes. *trn*R genes are arranged in the order of *trn*Y-*trn*R, whereas in the mitochondrial genomes of *C. crispus*, *S. skottsbergii*, and *M. papillatus* it is *trn*R-*trn*Y. Similar rearrangements of *trn* genes have also been reported in Sargasso species, and studies have also shown that such rearrangements are prevalent in ferns[11].

In the present study, we analysed the mitochondrial genomes of three species of *C. crispus*, *M. papillatus*, and *C. okamurae* by fragmentation comparison, and the results showed that, in the region of the 12.50 kb gene cluster genomically localised to *ycf*21-*psa*M, the direction of the gene arrangement of the three species was the same as that of the family Solieriaceae (*Betaphycus gelatinus*, *Eucheuma denticulatum*, *Kappaphycus alvarezii* and *Kappaphycus striatus*) in the homologous gene interval. Further sequence comparisons confirmed that the inverse alignment was concentrated in the linear nucleotide sequence between the *ycf*21 stop codon and the *psa*M start codon

of the gene, and no local inversions or gene deletion events were detected within the 12.50 kb contiguous segment.

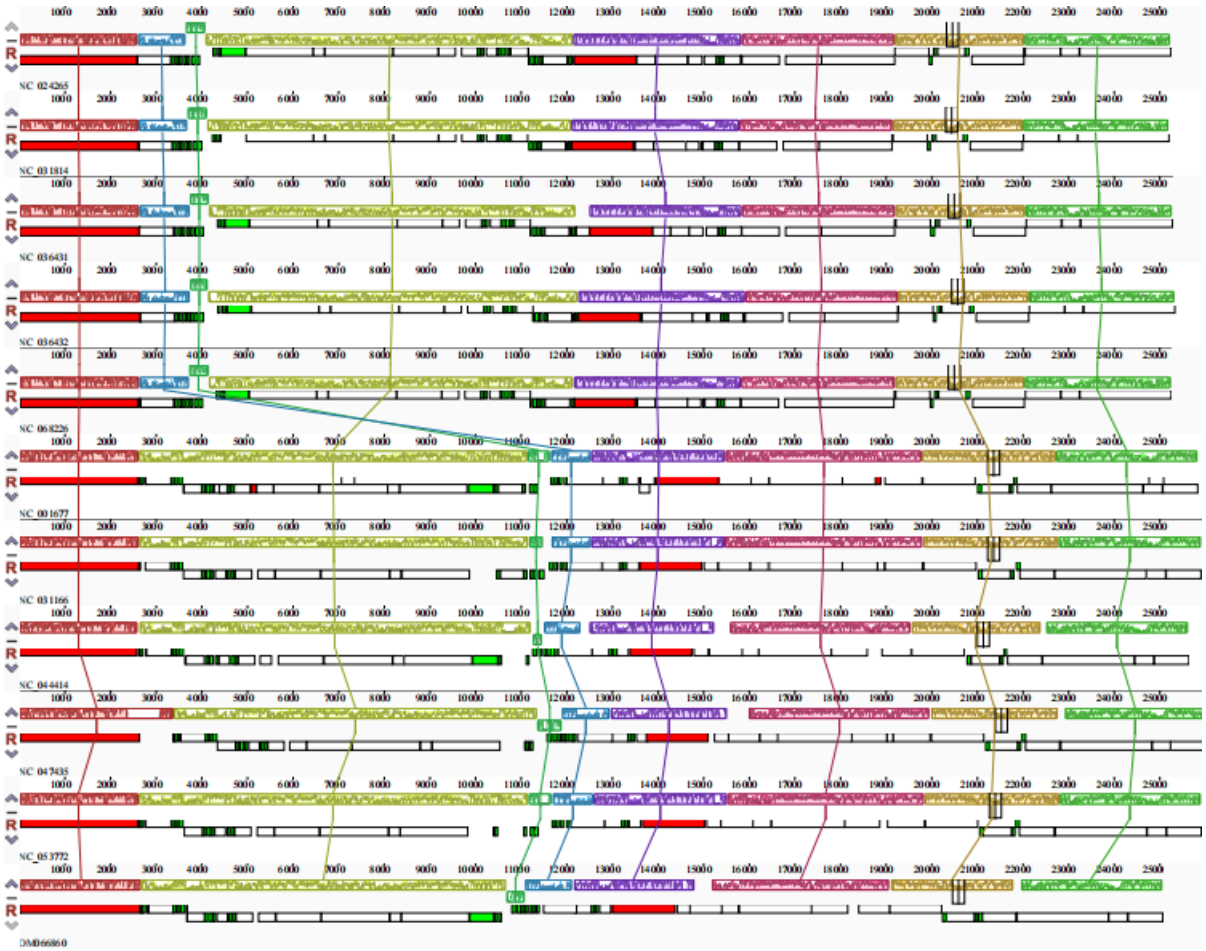

Figure 2-1 Collinearity analysis of mitochondrial genomes in the order Desmarestiales

Summary of chapter II

1.The mitochondrial genomes of 11 Gigartinales species are highly conserved in length (25–26 kb) and gene content (48–57 genes). Gene overlap and compact coding regions (90%–95%) highlight structural efficiency.

2. Minor rearrangements were observed, such as inverted “*trn*Y” and “*trn*R” gene orders in some species (e.g. “*Chondrus crispus*” vs. “*Betaphycus gelatinus*”).

Unique genes like “*tat*A” were identified in “*Caulacanthus okamurae*” and “*Mastocarpus papillatus*”.

3.Start codons for“*tat*C”genes varied across species (e.g. TTG in Solieriaceae, GTT in“*C. crispus*”), reflecting lineage-specific evolutionary adaptations.

Chapter III
EXPERIMENTAL PART
3.1 EXPERIMENTAL METHODS

Under the combined effects of natural selection, genetic mutation and environmental factors, different species have shown different codon usage frequencies during their long evolutionary history, i.e., each species has its own codon preference, and these characteristics can be used as molecular markers for exploring the laws and patterns of species evolution and inheritance. Therefore, codon preferences provide theoretical support for the in-depth study of mitochondrial genome evolution in algae of Gigartinales. The specific research methods are described as follows:

3.1.1 METHODS OF CODON COMPOSITION ANALYSIS

CodonW software was used to resolve the relative frequency of codon usage (RSCU) of the published species of Gigartinales. The steps include: firstly, all the CDS sequences of the same species were integrated into a '.fasta' file, which was placed in the CodonW folder, and then opened the Powershell window, and entered the command Enter. After the operation is completed, the corresponding '.out' and '.blk' files will be generated in the same folder. The '.out' file contains the values of parameters such as Codon Adaptation Index (CAI), Optimal Codon Frequency (Fop), Codon Bias Index (CBI), etc., while the '.blk' file displays the RSCU values of the codons.

3.1.2 ENC-PLOTTING

The difference between actual and expected ENC values can reflect the effect of mutation or selection pressure on codon usage preference. Based on the GC3 values measured by the CodonW run, the expected value of ENC can be calculated using the formula[16]. The formula is:$\mathrm{ENC\ Expected} = 2 + \mathrm{GC3} + 29/[\mathrm{GC3}^2 + (1 - \mathrm{GC3})^2]$

ENC-plot plotting: according to the data obtained in the table retain two decimal places, take GC3 as the X-axis and ENC as the Y-axis, use the RStudio program to run to plot the ENC plot and generate the corresponding standard curve, so as to study the effect of base composition on codon preference.

3.2 RESULTS AND ANALYSES

3.2.1 ANALYSIS OF CODON PREFERENCE IN THE MITOCHONDRIAL GENOME OF GIGARTINALES

In the process of protein synthesis, nucleic acids are converted to proteins through codons, and synonymous codons are codons that encode the same amino acids. Codon preference is the phenomenon of differences in the frequency of use of synonymous codons. Due to base variation, natural selection, genome size and other factors, the frequency of use of synonymous codons in different species during evolution shows an uneven distribution within genes[12]. As each species has unique codon preference characteristics, they can be used as indicators for assessing gene expression levels and predicting expression patterns, and have become an important molecular tool for exploring the evolutionary origins and genetic properties of species[22]. Therefore, the study of species synonymous codon preference can provide an important part of the theory in related fields. Codon usage preference is widely existed during the long-term evolution of organisms, and it has an important role in gene expression as well as the function of proteins[18]. In codon preference analysis, an RSCU value of 1 indicates no significant codon preference; greater than 1 indicates stronger preference; and less than 1 indicates weaker preference[13]. The RSCU values of the mitochondrial genomes of algae in Gigartinales have small differences, indicating that their mitochondrial genomes are relatively conserved in the evolutionary process. The experimental results of genes with RSCU>1 (i.e., the existence of preference) were selected for statistics, which showed that the percentage of codons with RSCU values greater than 1 was more than 3/4, indicating that the mitochondrial genes of the Gigartinales have a general preference for the use of amino acid codons. Table 3-1 shows that the RSCU values of

codons ending in A or U are mostly greater than 1, and the number of codons ending in A/U is significantly greater than that of G/C, suggesting that codons in the mitochondrial genome of Gigartinales are preferentially ending in A/U. Some codons ending in G have RSCU values equal to 1 and no preference among them, while codons ending in G or C mostly have RSCU values less than 1 and weaker preference. This suggests a preference for codons ending in A or U and less for codons ending in G or C in the mitochondrial genomes of the algal variants of the Gigartinales. Among the codons with RSCU>1, UUA encoding leucine (Leu) had the highest RSCU value, indicating that the mitochondrial genome of Sugozoa var. cinerea has a strong preference for UUA in encoding Leu compared to other amino acid codons[14,15].

In addition, analysis of the codon-related parameters of the mitochondrial genomes of Gigartinales showed that the maximum GC value was 40.82 and the minimum was 11.36, and the GC content at different positions varied, but not much. This feature was further confirmed by the fact that the mean CAI value of the mitochondrial genomes of both species was 0.149. The average CBI value was -0.072, the average Fop value was 0.394, and the average Gravy value was 0.278, indicating that the encoded protein may possess hydrophobic properties.

Table 3-1 Amino Acid Synonymous Codon Usage Levels

Amino acidse	codons synonyms	relative codon usag
Phe	UUU	$1.65^{a}/1.60^{b}/1.62^{c}/1.65^{d}/1.63^{e}/1.72^{f}/1.74^{g}/1.32^{h}/1.64^{i}/1.52^{j}$
Leu	UUA	$3.30^{a}/3.17^{b}/3.15^{c}/3.24^{d}/3.28^{e}/3.72^{f}/3.73^{g}/2.32^{h}/3.33^{i}/2.03^{j}$
	UUG	1.03^{j}
	CUU	$1.11^{f}/1.19^{g}/1.04^{h}/1.35^{i}$
	CUA	1.13^{h}
Ser	UCU	$2.13^{a}/2.08^{b}/2.33^{c}/2.10^{d}/2.10^{e}/2.12^{f}/2.10^{g}/1.51^{h}/1.78^{i}/1.75^{j}$
	UCA	$1.41^{a}/1.51^{b}/1.20^{c}/1.56^{d}/1.57^{e}/1.58^{f}/1.66^{g}/1.51^{h}/1.12^{i}/1.24^{j}$
	AGU	$1.36^{a}/1.25^{b}/1.16^{c}/1.30^{d}/1.25^{e}/1.20^{f}/1.29^{g}/1.46^{h}/1.02^{j}$

Cys	UGU	$1.36^{a}/1.56^{b}/1.54^{c}/1.56^{d}/1.43^{e}/1.57^{f}/1.68^{g}/1.20^{h}/1.33^{i}/1.13^{j}$
Trp	UGG	$1.00^{a}/1.00^{b}/1.00^{c}/1.00^{d}/1.00^{e}/1.00^{f}/1.00^{g}/1.00^{h}/1.00^{i}/1.00^{j}$
Pro	CCU	$2.85^{a}/2.52^{b}/2.37^{c}/2.62^{d}/2.68^{e}/2.00^{f}/1.87^{g}/1.39^{h}/2.32^{i}/1.61^{j}$
	CCA	$1.66^{f}/1.57^{g}/1.16^{h}/1.15^{j}$
His	CAU	$1.61^{a}/1.63^{b}/1.59^{c}/1.71^{d}/1.71^{e}/1.66^{f}/1.74^{g}/1.09^{h}/1.57^{i}/1.13^{j}$
Gln	CAA	$1.68^{a}/1.73^{b}/1.50^{c}/1.71^{d}/1.67^{e}/1.85^{f}/1.72^{g}/1.63^{h}/1.51^{i}/1.53^{j}$
Arg	CGU	$1.17^{a}/1.01^{b}/1.27^{c}/1.07^{e}/1.88^{f}/1.83^{g}/1.73^{h}/1.79^{i}/1.64^{j}$
	CGC	1.01^{j}
	CGA	$1.48^{f}/1.10^{h}/1.19^{j}$
	AGA	$2.94^{a}/2.87^{b}/2.78^{c}/2.92^{d}/2.66^{e}/2.12^{f}/2.13^{g}/1.80^{h}/2.21^{i}/1.60^{j}$
Ile	AUU	$1.38^{a}/1.24^{b}/1.33^{c}/1.28^{d}/1.25^{e}/1.89^{f}/2.00^{g}/1.68^{h}/1.26^{i}/1.50^{j}$
	AUA	$1.37^{a}/1.43^{b}/1.36^{c}/1.42^{d}/1.47^{i}$
Met	AUG	$1.00^{a}/1.00^{b}/1.00^{c}/1.00^{d}/1.00^{e}/1.00^{f}/1.00^{g}/1.00^{h}/1.00^{i}/1.00^{j}$
Thr	ACU	$1.92^{a}/1.95^{b}/1.80^{c}/2.02^{d}/1.86^{e}/2.04^{f}/1.94^{g}/1.58^{h}/1.84^{i}/1.87^{j}$
	ACA	$1.39^{a}/1.34^{b}/1.15^{c}/1.33^{d}/1.24^{e}/1.54^{f}/1.29^{g}/1.12^{i}/1.00^{j}$
Asn	AAU	$1.58^{a}/1.60^{b}/1.49^{c}/1.62^{d}/1.62^{e}/1.63^{f}/1.57^{g}/1.13^{h}/1.49^{i}/1.12^{j}$
Lys	AAA	$1.58^{a}/1.67^{b}/1.61^{c}/1.64^{d}/1.66^{e}/1.77^{f}/1.78^{g}/1.56^{h}/1.73^{i}/1.34^{j}$
Val	GUU	$1.84^{a}/1.81^{b}/1.90^{c}/1.81^{d}/1.69^{e}/2.37^{f}/2.23^{g}/1.86^{h}/1.64^{i}/1.92^{j}$
	GUA	$1.59^{a}/1.42^{b}/1.40^{c}/1.51^{d}/1.48^{e}/1.22^{f}/1.33^{g}/1.15^{h}/1.55^{i}/1.18^{j}$
Ala	GCU	$2.36^{a}/2.06^{b}/2.18^{c}/2.19^{d}/1.97^{e}/1.71^{f}/1.64^{g}/1.43^{h}/1.74^{i}/1.69^{j}$
	GCA	$1.06^{a}/1.13^{b}/1.20^{d}/1.29^{e}/1.87^{f}/1.81^{g}/1.22^{h}/1.27^{i}/1.15^{j}$
Asp	GAU	$1.66^{a}/1.66^{b}/1.64^{c}/1.70^{d}/1.65^{e}/1.61^{f}/1.81^{g}/1.21^{h}/1.60^{i}/1.12^{j}$
Glu	GAA	$1.65^{a}/1.67^{b}/1.64^{c}/1.66^{d}/1.66^{e}/1.78^{f}/1.68^{g}/1.54^{h}/1.77^{i}/1.40^{j}$
Gly	GGU	$1.72^{a}/1.74^{b}/1.60^{c}/1.73^{d}/1.78^{e}/1.88^{f}/1.87^{g}/1.58^{h}/1.56^{i}/1.52^{j}$
	GGA	$1.44^{a}/1.48^{b}/1.29^{c}/1.46^{d}/1.44^{e}/1.61^{f}/1.39^{g}/1.34^{h}/1.14^{i}/1.30^{j}$
Tyr	UAU	$1.65^{a}/1.58^{b}/1.53^{c}/1.59^{d}/1.50^{e}/1.53^{f}/1.60^{g}/1.43^{i}/1.04^{j}$
	UAC	1.04^{h}

a:*Betaphycus gelatinus*；b:*Kappaphycus striatus*；c:*Eucheuma denticulatum*；d:*Kappaphycus alvarezii*；e:*K. malesianus*；f:*Chondrus crispus*；g:*S. skottsbergii*；h:*M. papillatus*；i:*Caulacanthus okamurae*；j:*Gloiopeltis furcata*

3.2.2 ENC-PLOT ANALYSIS

After statistics, the average ENC value of codons in the mitochondrial genome of this experiment was 42.11. The fluctuation interval of ENC was 19.21-61.00, when a certain amino acid was encoded by only 1 codon, then the highest codon preference was 19.21; in the case of all the synonymous codons coding for a certain amino acid had equal chance of being used, it indicated that the lowest degree of codon preference was 61.00.The average ENC value is usually 35 to assess the strength of codon preference; if the ENC value is more than 35, it indicates a weak codon preference; conversely, if the ENC value is less than 35, it indicates a strong codon preference[8,18]. According to Table 3-2, most of the codons in the mitochondrial genome sequencing have ENC values greater than 35, and the results obtained in this experiment indicate a weak codon preference for the mitochondrial genome in Gigartinales.

The ENC-plot was used to determine the main factors affecting codon preference by analysing the distribution of ENC in relation to GC3, and by scattering the points at a distance from the standard curve. If the points of the gene are located near the standard curve, it indicates that the codon preference is mainly controlled by mutational factors; if the gene deviates from the standard curve and the measured ENC value is significantly different from the predicted value, it indicates that the base mutation is not the dominant factor determining its codon preference, and other factors may be involved, such as the existence of the base mutation or other factors that may play a role, such as natural selection [21]. The ENC-plot diagrams of the mitochondrial genomes of algae in Gigartinales were broadly similar, with some genes distributed along the standard curve or falling near the bottom of the curve, suggesting that the ENC measurements of these genes were similar to the predicted values and that their codon

preferences were mainly controlled by base mutations. Among them, only a few genes such as *atp*9, *orf*73, *rpl*20 followed the distribution of the standard curve, while most of the genes *rpl*16, *tat*C, *sdh*3 fell below the standard curve, i.e., most of the genes measured ENC values lower than the predicted values, which suggests that selective pressures are the main factor influencing codon usage preference for most of the protein-coding genes of Gigartinales, whereas, for some of the genes, the selection pressure has a greater effect on mutation.

Table 3-2 Codon preference analyses of mitochondrial genomes of algae in Gigartinales

Gene	ENC Expected	GC3%	ENC Actual
*rps*11	50.40[a]/39.90[b]/44.82[c]/50.40[d]/100.79[e]/34.16[f]/49.26[g]/53.94[h]/49.26[i]/79.21[j]/37.76[k]/	23.73[a]/15.38[b]/19.49[c]/23.73[d]/40.83[e]/12.40[f]/22.88[g]/27.12[h]/22.88[i]/36.67[j]/14.29[k]/	40.83[a]/38.59[b]/47.43[c]/30.08[d]/50.87[e]/38.92[f]/58.03[g]/60.93[h]/54.49[i]/59.22[j]/38.40[k]/
*nad*6	43.48[a]/45.00[b]/53.00[c]/43.09[d]/51.77[e]/45.30[f]/48.20[g]/54.12[h]/48.91[i]/73.14[j]/43.09[k]/	18.72[a]/20.00[b]/27.45[c]/18.54[d]/26.34[e]/20.20[f]/21.78[g]/27.23[h]/22.77[i]/35.44[j]/18.54[k]/	34.18[a]/39.16[b]/42.93[c]/40.71[d]/45.67[e]/42.11[f]/44.44[g]/51.76[h]/47.93[i]/49.88[j]/39.10[k]/
cob	42.81[a]/39.73[b]/48.67[c]/52.17[d]/60.59[e]/37.61[f]/42.38[g]/45.04[h]/43.67[i]/61.17[j]/39.13[k]/	18.32[a]/15.45[b]/22.71[c]/24.48[d]/31.41[e]/14.17[f]/16.75[g]/19.63[h]/18.85[i]/31.94[j]/15.18[k]/	38.25[a]/37.62[b]/44.15[c]/42.62[d]/49.92[e]/36.14[f]/37.70[g]/40.57[h]/40.12[i]/48.07[j]/38.70[k]/
ymf16	36.46[b]/90.17[j]/	14.07[b]/38.61[j]/	34.39[b]/61.00[j]/
*cox*3	42.31[a]/32.30[b]/47.81[c]/52.41[d]/58.20[e]/37.76[f]/46.12[g]/44.64[h]/42.94[i]/79.14[j]/39.90[k]/	17.22[a]/11.36[b]/21.61[c]/26.01[d]/29.72[e]/14.29[f]/20.51[g]/19.41[h]/17.95[i]/36.63[j]/15.38[k]/	38.91[a]/32.72[b]/42.17[c]/45.17[d]/43.94[e]/37.42[f]/46.43[g]/46.50[h]/42.47[i]/49.51[j]/40.96[k]/
*cox*2	42.26[a]/38.07[b]/52.85[c]/54.07[d]/67.01[e]/42.10[f]/43.60[g]/43.07[h]/45.34[i]/60.08[j]/42.29	17.18[a]/14.51[b]/24.71[c]/25.48[d]/33.33[e]/16.73[f]/18.70[g]/18.32[h]/20.23[i]/31.13[j]/16.86[k]/	36.59[a]/41.12[b]/42.83[c]/47.54[d]/49.60[e]/36.71[f]/39.84[g]/41.61[h]/42.07[i]/

	[k]/		51.81[j]/37.01[k]/
*cox*1	45.41[a]/36.91[b]/53.84[c]/50.79[d]/65.11[e]/43.87[f]/47.46[g]/47.46[h]/46.04[i]/63.77[j]/38.83[k]/	20.30[a]/14.07[b]/25.32[c]/23.84[d]/32.83[e]/18.94[f]/21.23[g]/21.23[h]/20.86[i]/32.46[j]/14.90[k]/	39.52[a]/36.79[b]/44.04[c]/42.17[d]/51.57[e]/37.26[f]/39.86[g]/40.89[h]/40.80[i]/50.07[j]/35.78[k]/
*rpl*16	42.11[a]/33.83[b]/44.65[c]/50.56[d]/76.07[e]/45.30[f]/49.38[g]/49.38[h]/44.34[i]/52.80[j]/47.70[k]/	17.04[a]/12.23[b]/19.42[c]/23.70[d]/36.03[e]/19.85[f]/22.96[g]/22.96[h]/19.26[i]/26.28[j]/21.58[k]/	39.10[a]/36.48[b]/43.74[c]/44.39[d]/51.74[e]/36.13[f]/42.30[g]/45.87[h]/39.60[i]/44.68[j]/45.00[k]/
*rps*3	45.64[a]/38.89[b]/46.28[c]/48.65[d]/61.47[e]/45.02[f]/45.11[g]/51.28[h]/53.84[i]/58.66[j]/42.88[k]/	20.60[a]/14.94[b]/20.96[c]/22.75[d]/31.67[e]/20.09[f]/19.74[g]/24.46[h]/25.32[i]/29.44[j]/16.46[k]/	36.92[a]/40.91[b]/39.79[c]/38.30[d]/57.52[e]/38.96[f]/42.86[g]/46.60[h]/46.03[i]/51.53[j]/41.11[k]/
*nad*4L	50.39[a]/42.26[b]/50.39[c]/72.57[d]/59.00[e]/55.70[f]/84.21[g]/78.38[h]/67.01[i]/55.70[j]/44.12[k]/	23.53[a]/16.67[b]/23.53[c]/35.29[d]/30.10[e]/28.43[f]/37.25[g]/36.27[h]/33.33[i]/28.43[j]/19.61[k]/	41.54[a]/33.49[b]/33.43[c]/48.60[d]/45.14[e]/45.64[f]/46.07[g]/40.06[h]/50.45[i]/33.34[j]/33.05[k]/
*rps*12	41.07[a]/32.78[b]/51.73[c]/47.03[d]/80.67[e]/42.29[f]/44.75[g]/43.60[h]/44.75[i]/57.49[j]/43.67[k]/	16.00[a]/11.72[b]/24.39[c]/21.14[d]/37.90[e]/17.07[f]/19.51[g]/18.70[h]/19.51[i]/28.91[j]/18.75[k]/	36.14[a]/36.35[b]/46.64[c]/44.94[d]/52.55[e]/33.92[f]/38.29[g]/38.61[h]/41.98[i]/50.55[j]/36.89[k]/
*orf*73	38.30[b]/	14.86[b]/	38.17[b]/
*rpl*20	45.00[a]/40.49[b]/57.46[c]/54.07[d]/55.27[e]/45.12[f]/57.29[g]/57.29[h]/64.70[i]/66.17[j]/39.44[k]/	20.00[a]/15.79[b]/28.92[c]/25.58[d]/26.51[e]/19.75[f]/28.74[g]/28.74[h]/32.18[i]/32.95[j]/15.48[k]/	33.89[a]/35.50[b]/38.27[c]/49.03[d]/51.96[e]/42.42[f]/43.14[g]/44.83[h]/49.46[i]/47.64[j]/33.23[k]/
*tat*C	43.09[a]/53.00[c]/48.40[d]/64.49[e]/42.10[f]/52.48[g]/52.48[h]/50.07[i]/47.64[k]/	18.58[a]/25.00[c]/22.69[d]/32.11[e]/16.53[f]/26.14[g]/26.14[h]/23.65[i]/21.46[k]/	43.84[a]/53.41[c]/46.37[d]/54.05[e]/41.46[f]/47.47[g]/42.19[h]/43.48[i]/50.32[k]/

*sdh*4	43.67[a]/42.98[b]/42.83[c]/48.24[d]/74.74[e]/41.37[f]/53.00[g]/59.71[h]/65.57[j]/34.16[k]/	18.75[a]/17.65[b]/17.50[c]/22.50[d]/35.90[e]/16.25[f]/25.00[g]/30.00[h]/32.50[j]/12.50[k]/	37.08[a]/32.11[b]/29.05[c]/38.55[d]/44.08[e]/38.23[f]/47.46[g]/43.39[h]/47.51[i]/57.12[j]/39.30[k]/
*atp*9	42.43[a]/40.26[b]/42.43[c]/42.43[d]/47.33[e]/32.37[f]/52.17[g]/52.17[h]/49.61[i]/56.04[j]/37.76[k]/	16.88[a]/15.58[b]/16.88[c]/16.88[d]/22.08[e]/11.69[f]/24.68[g]/24.68[h]/23.38[i]/25.97[j]/14.29[k]/	31.18[a]/32.63[b]/28.56[c]/30.58[d]/35.71[e]/27.09[f]/37.44[g]/36.65[h]/37.81[i]/36.61[j]/31.82[k]/
*sdh*3	48.24[a]/44.85[b]/53.00[c]/42.71[d]/54.74[e]/33.42[f]/47.76[g]/43.44[h]/50.86[i]/54.17[j]/43.19[k]/	22.22[a]/19.53[b]/25.00[c]/17.46[d]/27.61[e]/12.00[f]/21.43[g]/19.05[h]/23.81[i]/27.27[j]/17.91[k]/	46.45[a]/43.93[b]/39.60[c]/46.10[d]/57.66[e]/34.12[f]/37.87[g]/35.46[h]/36.26[i]/55.20[j]/49.11[k]/
*sdh*2	48.80[a]/42.86[b]/51.67[c]/55.15[d]/63.96[e]/42.04[f]/45.70[g]/49.93[h]/48.80[i]/70.47[j]/42.76[k]/	22.62[a]/17.93[b]/24.30[c]/25,69[d]/32.54[e]/16.40[f]/20.63[g]/23.41[h]/22.62[i]/34.26[j]/16.93[k]/	38.07[a]/33.79[b]/41.42[c]/36.96[d]/52.58[e]/33.11[f]/40.08[g]/44.30[h]/41.74[i]/53.33[j]/38.12[k]/
*atp*4	46.15[a]/47.28[c]/46.15[d]/34.51[f]/45.39[g]/45.76[h]/42.02[i]/35.07[k]/	20.99[a]/21.55[c]/20.99[d]/12.71[f]/19.89[g]/20.44[h]/18.23[i]/13.04[k]/	43.80[a]/44.94[c]/43.73[d]/35.95[f]/33.89[g]/38.76[h]/36.26[i]/33.55[k]/
*ymf*39	35.07[b]/69.22[e]/57.87[j]./	13.04[b]/33.70[e]/29.35[j]/	32.19[b]/57.37[e]/48.50[j]/
*orf*172	34.54[b]/67.01[j]/	12.72[b]/33.33[j]/	40.10[b]/51.97[j]/
*tat*A	37.18[c]/72.57[j]/	13.95[c]/35.29[j]/	19.21[c]/43.44[j]/

a:*Betaphycus gelatinus* ; b:*Chondrus crispus* ; c:*Caulacanthus okamurae* ; d:*Eucheuma denticulatum* ; e:*Gloiopeltis furcata* ; f:*Hypnea edeniana ;* g:*Kappaphycus alvarezii* ; h:*Kappaphycus malesianus ;* i:*Kappaphycus striatus* ; j:*Mastocarpus papillatus ;* k:*Sarcopeltis skottsbergii*

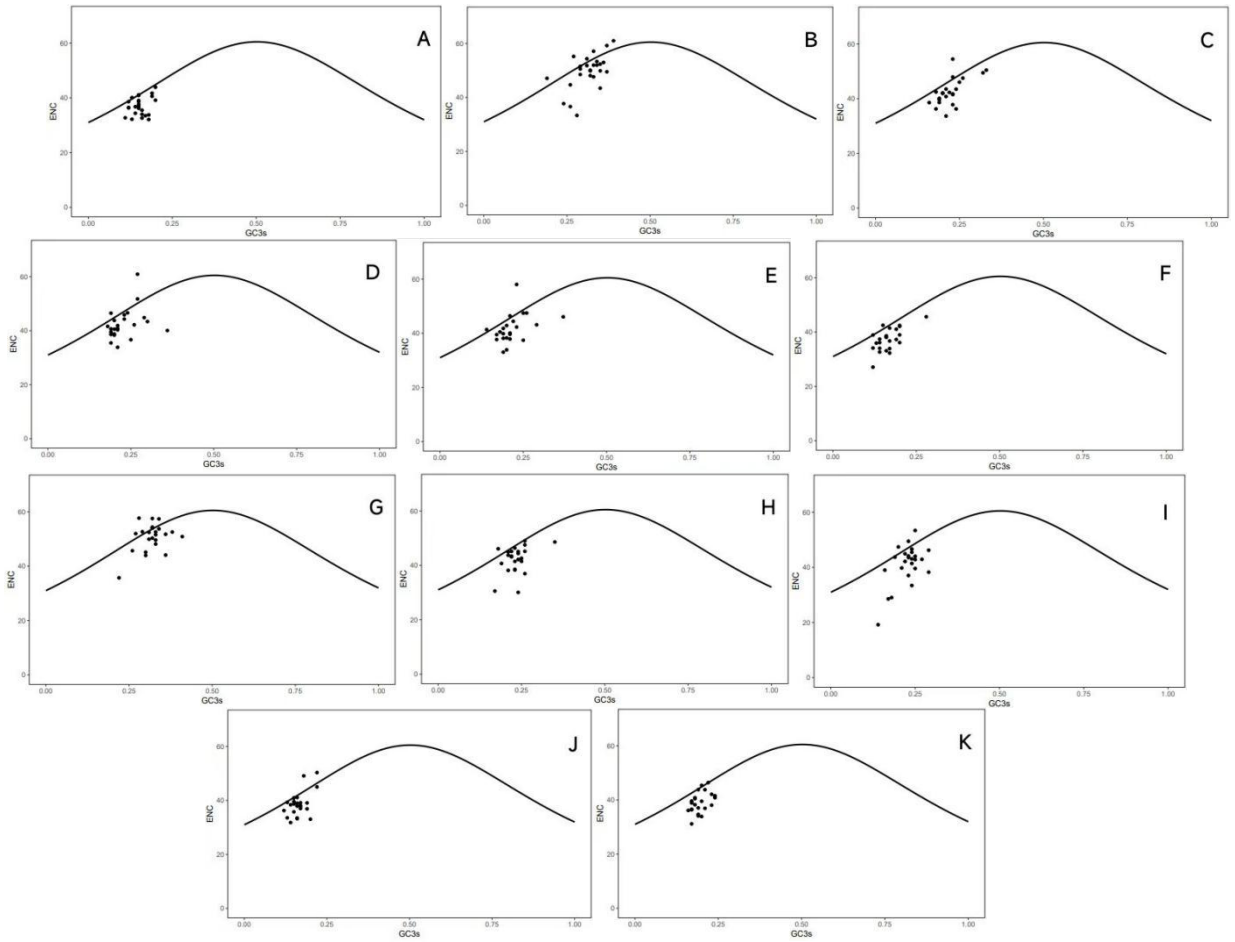

Figure 3-1 ENC-plot of 11 species in Gigartinales.

A:*C.crispus*; B:*M.papillatus*; C:*K.striatus;* D:*K.malesianus*; E:*K.alvarezii*; F:*H.edeniana*; G:*G.furcata*; H:*E.denticulatum*; I:*C.okamurae*; J:*S.skottsbergii*; K:*B.gelatinus*

3.3 EXPERIMENTAL METHODS

3.3.1 ML PHYLOGENETIC TREE CONSTRUCTION

In this study, the phylogenetic tree was constructed using the maximum likelihood (ML) method in the following steps: firstly, the genes of the 11 species to be analysed, as well as *Gracilaria chilense* as an outgroup (Genbank accession no. MF401962), were downloaded into gb format and imported into PhyloSuite to screen out the common protein-coding genes, which comprised a total of 18 genes (*atp*6, *atp*8, *atp*9, *cob*, *cox*1, *cox*2, *cox*3, *nad*1, *nad*2, *nad*3, *nad*4, *nad*4L, *nad*5, *nad*6, *rpl*16, *rps*3, *rps*11, and *rps*12), opened the screened shared protein coding genes in MEGA, and used

Muscle for sequence After merging the files, use Alignment-Gblocks in PhyloSuite function area to screen the conserved regions, you can check the number of amino acid sites before and after screening, and how much the conserved regions accounted for the total sequences, and the file with suffix 'gb.fasta' is the file required for tree construction, and then use gb.fasta format file for the file. The saved fasta format file was opened with bioedit and exported to phy format, the phy file was put into the folder of [Standard-RaxML-master], and the evolution tree file was modified and opened with figtree to get the systematic evolution tree, the tree shape was modified and edited, and saved as a picture for analysis.

3.3.2 BAYESIAN PHYLOGENETIC TREE CONSTRUCTION

The phylogenetic tree was constructed by Bayesian inference (BI) method:11 species of species as well as Gracilaria chilense outgroup genes were imported into PhyloSuite in gb format file data, and the common protein coding genes were extracted, with a total of 18 genes (*atp*6, *atp*8, *atp*9, *cob*, *cox*1, *cox*2, *cox*3, *nad*1, *nad*2, *nad*3, *nad*4, *nad*4L, *nad*5, *nad*6, *rpl*16, *rps*3, *rps*11, and *rps*12) were batch-aligned using the [Alignment-MUSCLE] software, and then the script FASconCAT_v1.0 was placed into the folder after the pair of them, and the script was executed in order to merge the sequences by clicking the Alignment-Gblocks in the PhyloSuite ribbon to filter the conservative area, open the saved fasta format file with Mega, and then output it as a nexus format file, modify the nexus file command, put the saved file into Bayesian and run it, and then use the figtree software to view the results and beautify them.

3.4 RESULTS AND ANALYSES

Of all taxonomic characteristics proposed by earlier studies, the only ones that have taxonomic value considering the species analyzed in this work were: (1) habit of the thallus, (2) main axis, (3) apex shape, (4) form of the branchlets and (5) tetrasporangial sori position in the branches[40]. The study of phylogeny can use

sequences of 5′ region of the cytochrome oxidase subunit 1 gene, large subunit rDNA, and ribulose- 1,5- bisphosphate carboxylase/oxygenase large subunit gene as genetic markers to elucidate their phylogenetic positions[46].In order to improve the accuracy and reliability of algal species classification, molecular phylogenetic analysis was used as the core method in this study. Traditional morphological identification has limitations due to the susceptibility of algal morphological features to environmental fluctuations (e.g., light, temperature, nutrient salts) and developmental stages[41], whereas molecular biology techniques can effectively compensate for this deficiency by resolving genomic genetic information, providing a more reliable basis for accurate classification and identification of algae[17]. Phylogenetic relationships are considered to be in the form of tree-like branches, and the tree illustrations that represent the phylogenetic relationships between organisms are called phylogenetic tree or evolutionary tree, which are used to reconstruct the phylogenetic analyses by searching for the common ancestor and ancestry relationships. Among the phylogenetic tree construction methods, Hall (2005) compared the Bayesian inference (BI) method as the optimal algorithm, followed by the ML method, which is based on the principle of maximum likelihood and searches for the topology and parameters of the evolutionary tree that maximises the probability of occurrence of the observed data. The method does not rely on a priori information, can handle large datasets quickly, and yields accurate evolutionary trees when the model assumptions are reasonable[19]. It is worth noting that when using the ML method to construct the phylogenetic tree[52], the computation time will be extended accordingly with the increase in the number of species and the length of sequences.The BI method is based on the Markov Chain Monte Carlo algorithm (MCMC) to estimate the posterior probability of the phylogenetic tree, and it can deal with a wide range of data forms, including nucleotide and amino acid sequence data at the molecular level. Therefore, in this study, ML and BI were used to reconstruct the phylogenetic relationships of the Gigartinales.

The length of branches reflects the genetic distance between species, and the longer the branches, the greater the genetic differences. In this study, we used ML and BI to reconstruct the phylogenetic relationship of Gigartinales.

By analysing the phylogenetic tree of Gigartinales obtained in this study, the following conclusions can be drawn:

The obtained ML evolutionary tree and the BI evolutionary tree have similar topology of each branch and the same trend of grouping of some species, and the algae of Gigartinales are clearly divided into two sub-branches. The five species of Solieriaceae, together with *Caulacanthus okamurae* and *Hypnea edeniana*[37], form a large evolutionary branch, while *C. crispus*, *S. skottsbergii*, *M. papillatus*, and *G. furcata* form a second sub-branch. second sub-branch. However, there was a slight difference in the branch position association of *G. furcata* in the two figures[55].

The phylogenetic trees constructed by both methods supported the clustering of *Chondrus crispus* and *Sarcopeltis skottsbergii* into a single branch in the phylogenetic trees constructed by both methods[42,44], suggesting that they are more closely related, whereas *Mastocarpus papillatus* separated from it earlier. This result confirms the high degree of differentiation between species and the effectiveness of the mitochondrial genome in revealing these relationships.Algae of the genus Kappaphycus (e.g., *K. malesianus*, *K. striatus*, and *K. alvarezii*) are tightly clustered in the phylogenetic trees constructed by both methods[51,53], indicating closer affinities and suggesting that they may share a common evolutionary history. However, there were also differences in the branch positions and support of some species: *Caulacanthus okamurai* and *Hypnea edeniana* were different in the phylogenetic tree constructed by the ML method[45,47], whereas they were more closely clustered in the phylogenetic tree constructed by the BI method, which reflected the effects of the different analytical methods or parameter settings on the construction of the phylogenetic tree.

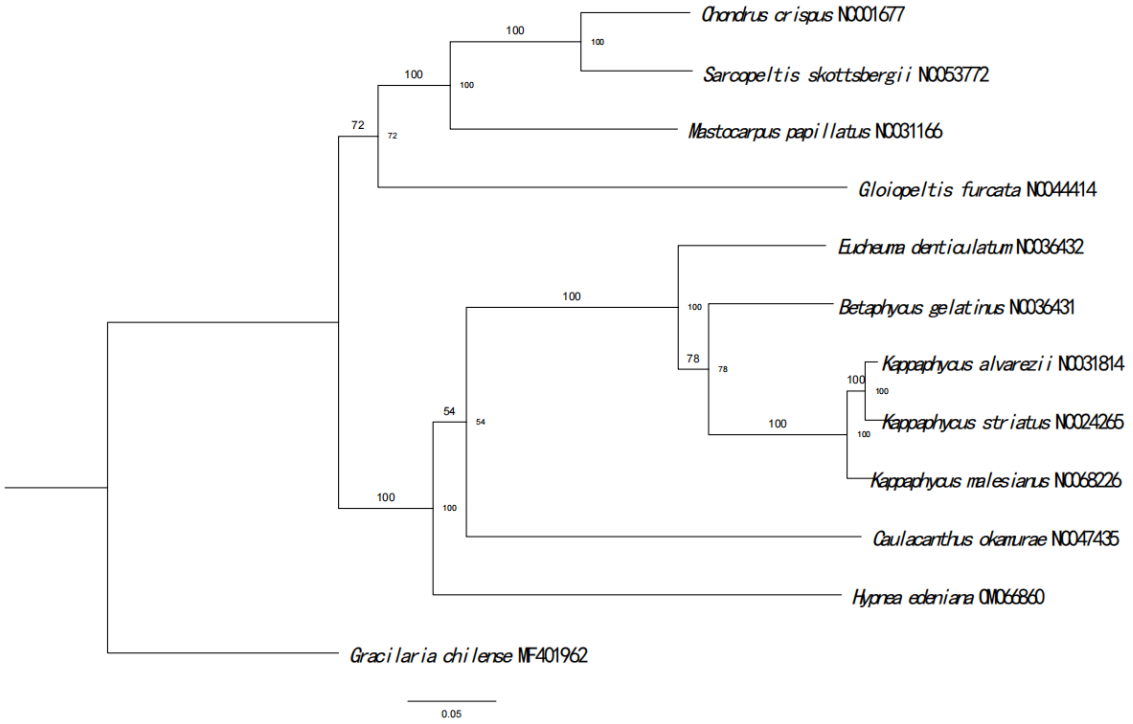

Figure 3-2 Reconstruction of the phylogenetic tree of Gigartinales based on the mitochondrial genome ML approach.

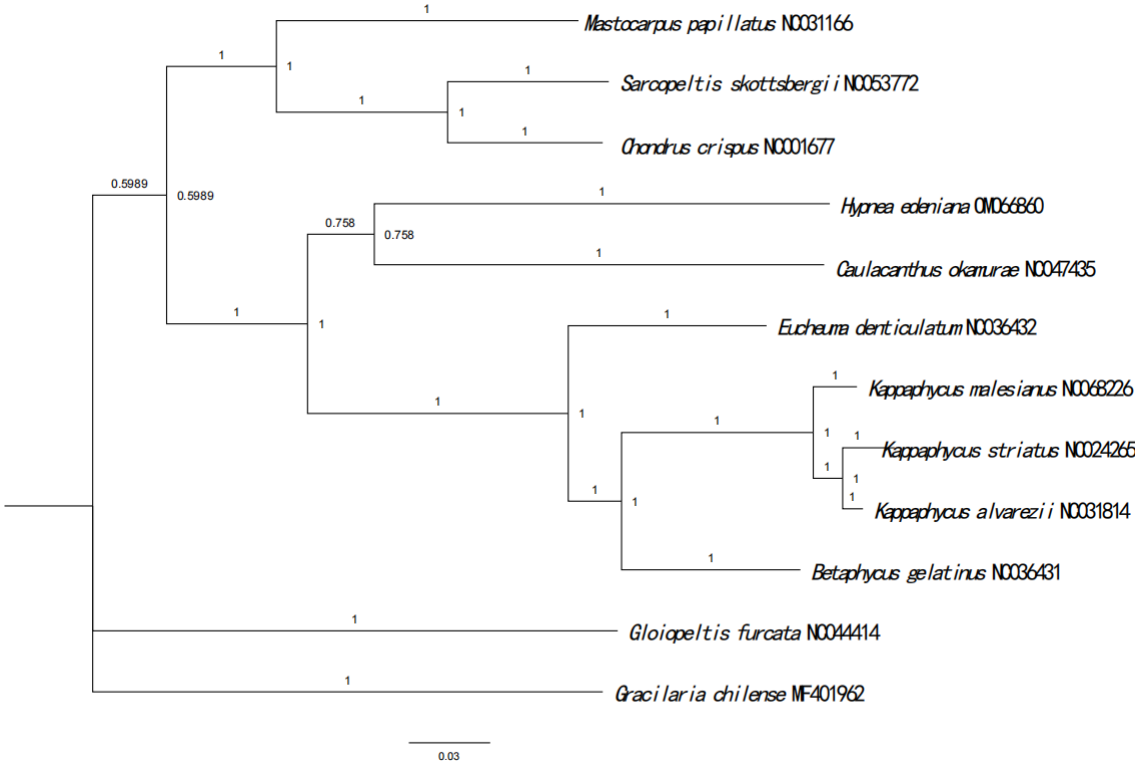

Figure 3-3 Reconstruction of phylogenetic tree based on the mitochondrial genome BI approach

Summary of chapter III

1. Mitochondrial genomes strongly prefer codons ending in A/T, with leucine (UUA) showing the highest RSCU values (2.03–4.43). Codons ending in G/C were underutilized (RSCU < 1).

2. ENC values (average 42.11) indicated weak codon usage bias, influenced primarily by natural selection rather than mutational pressure. ENC-plot deviations suggested selective forces shape codon preferences.

3. Gravy values (average 0.278) implied mitochondrial proteins are hydrophobic, consistent with their functional roles in oxidative phosphorylation and membrane-associated processes.

4.ML and BI trees divided Gigartinales into two branches: one comprising Solieriaceae, "*Caulacanthus okamurae*", and "*Hypnea edeniana*", and the other including"*Chondrus crispus*","*Sarcopeltis skottsbergii*", and "*Mastocarpus papillatu»*

5."*Chondrus crispus*" and "*S. skottsbergii*"clustered tightly, while "*Kappaphycus*"species (e.g.,"*K. alvarezii*") formed a distinct monophyletic group, supporting their taxonomic coherence.

6. Both ML and BI methods produced congruent topologies, though minor discrepancies (e.g."*Gloiopeltis furcata*"placement) highlighted the impact of analytical parameters on phylogenetic resolution.

CONCLUSION

In this study, we focused on the mitochondrial genomes of Gigartinales, analysed their structural features, codon preferences and evolutionary relationships, and revealed the genetic differences and evolutionary significance of Gigartinales at the mitochondrial genome level through high-throughput sequencing, bioinformatics analysis and molecular evolutionary studies, which provided important theoretical bases for the molecular identification and phylogenetic studies.

According to the basic genome characteristics, the mitochondrial genomes of the 11 species of Sarcopeltis were concentrated in the 25-26 kb range, with about 50 genes, including 57 genes in *Chondrus crispus* and 48 genes in *Sarcopeltis skottsbergii*, and the GC content ranged from 27.89% to 37.38%, and the genes were highly conserved in the order. The GC content ranged from 27% to 37.38%, and the order of genes was highly conserved, although there were still minor rearrangements such as *trn*Y and *trn*R genes that differed in order among some species. Analysis of protein-coding genes showed that the types and numbers of genes encoded by these algae were highly similar, with a general overlap of 1-8 groups of genes and a high percentage of gene coding regions ranging from 90.19% to 95.88%. In terms of start codon usage, the start codons of *tat*C gene showed diversification, such as the use of TTG by agaricus, etc. and the use of GTT by angiospermum, etc., which were more specific among algae, except for Okamura stem spiny algae.

Codon preference analysis showed that the mitochondrial genomes of the algae in Gigartinales had small RSCU gaps, with most genes having RSCU>1, and preferred codons ending in A/T, with the highest RSCU value for the leucine codon UUA. The fluctuation interval of ENC in the sequenced mitochondrial genome of Gigartinale was 19.21-61.00, and the average value of ENC was 42.11, and the ENC value was greater than 35 indicating a weak codon preference in the mitochondrial genome of Gigartinales. There were slight differences in the CAI, CBI and Fop parameters of the

genomes, suggesting that the encoded proteins may be hydrophobic.The ENC-plot showed that the measured ENC values of most genes were lower than the expected values, suggesting that selective pressures are the main factors influencing the codon usage preference of the genes coding for most of the proteins of Gigartinales.

Phylogenetic trees were constructed using maximum likelihood and Bayesian inference methods, and *C. crispus* showed closer affinities with algae of the genera *S. skottsbergii* and Kappaphycus (e.g., *K. malesianus*, *K. striatus*, and *K. alvarezii*), respectively, suggesting that they may share a common evolutionary history. At the same time, evolutionary differences between some species were precisely revealed.

In summary, the present study comprehensively and deeply analysed the mitochondrial genome features of algae in Gigartinales, and provided key basic data and theoretical support for the fields of algal biology, marine ecology and evolutionary biology, which is of great significance for further studies on ecological adaptations, species evolution and resource utilisation of algae, as well as pointing out the direction of further studies, such as in-depth research on the functional significance of gene overlap, codon preference regulation, and the role of codon preference. It also points out the direction for further research, such as in-depth study of the functional significance of gene overlap, the regulatory mechanism of codon preference, and the evolutionary driving force among different genera.

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Exhibit A1 Main parameters of mitochondrial genome codons in Gigartinales

Gene	CAI	CBI	Fop	Nc	GC3s	Gravy
*rps*3	0.189[a]/0.161[b]/0.191[c]/0.158[d]/0.189[e]/0.131[f]/0.174[g]/0.197[h]/0.184[i]/0.152[j]/0.183[k]/	-0.038[a]/-0.068[b]/10.045[c]/-0.09[d]/-0.122[e]/-0.255[f]/-0.15[g]/-0.077[h]/-0.068[i]/-0.122[j]/-0.158[k]/	0.405[a]/0.37[b]/0.384[c]/0.362[d]/0.341[e]/0.265[f]/0.325[g]/0.37[h]/0.378[i]/0.329[j]/0.326[k]/	39.51[a]/36.48[b]/39,79[c]/38.3[d]/57.52[e]/38.96[f]/45.05[g]/46.05[h]/47.52[i]/51.53[j]/41.11[k]/	0.26[a]/0.111[b]/0.187[c]/0.204[d]/0.31[e]/0.186[f]/0.265[g]/0.24[h]/0.249[i]/0.288[j]/0.15[k]/	-0.327273[a]/-0.113[b]/-0.048[c]/-0.084[d]/-0.252[e]/0.090[f]/0.201[g]/0.052[h]/-0.344[i]/-0.081[j]/-0.056[k]/
*nad*4L	0.161[a]/0.168[b]/0.145[c]/0.152[d]/0.23[e]/0.125[f]/0.186[g]/0.19[h]/0.196[i]/0.129[j]/0.195[k]	-0.139[a]/-0.183[b]/-0.282[c]/-0.053[d]/-0.081[e]/-0.195[f]/-0.049[g]/-0.038[h]/-0.046[i]/-0.172[j]/-0.217[k]	0.297[a]/0.325[b]/0.258[c]/0.351[d]/0.383[e]/0.272[f]/0.368[g]/0.368[h]/0.373[i]/0.28[j]/0.319[k]	39.88[a]/40.91[b]/41.07[c]/41.53[d]/44.14[e]/45.64[f]/52.24[g]/51.19[h]/50.79[i]/33.34[j]/37.08[k]	0.23[a]/0.13[b]/0.215[c]/0.234[d]/0.266[e]/0.217[f]/0.224[g]/0.211[h]/0.213[i]/0.226[j]/0.266[k]	1.35974[a]/-0.154[b]/-0.178[c]/0.993[d]/-0.233[e]/1.171[f]/1.084[g]/1.109[h]/1.035[i]/1.159[j]/
*rpl*20	0.132[a]/0.212[b]/0.205[c]/0.119[d]/0.283[e]/0.164[f]/0.198[g]/0.12[h]/0.136[i]/0.237[j]/0.258[k]	-0.119[a]/-0.169[b]/0.032[c]/-0.16[d]/0.098[e]/-0.173[f]/-0.014[g]/-0.155[h]/-0.209[i]/0.093[j]/-0.06[k]	0.324[a]/0.358[b]/0.43[c]/0.276[d]/0.494[e]/0.312[f]/0.406[g]/0.32[h]/0.28[i]/0.482[j]/0.405[k]	61[a]/47.91[b]/38.27[c]/44.97[d]/51.96[e]/42.42[f]/58[g]/42.81[h]/49.97[i]/47.64[j]/33.23[k]	0.243[a]/0.222[b]/0.266[c]/0.237[d]/0.266[e]/0.169[f]/0.232[g]/0.2[h]/0.213[i]/0.301[j]/0.139[k]	0.680263[a]/-0.388[b]/-0.187[c]/1.508[d]/-0.648[e]/-0.01[f]/-0.147[g]/0.319[h]/1.035[i]/-0.528[j]/-0.553[k]
*rps*12	0.133[a]/0.029[b]/0.174[c]/0.181[d]/0.197[e]/0.163[f]/0.165[g]/0.152[h]/0.17[i]/0.193[j]/0.263[k]	-0.106[a]/0.115[b]/0.069[c]/-0.036[d]/0.073[e]/-0.077[f]/-0.081[g]/-0.112[h]/-0.111[i]/0.065[j]/0.152[k]	0.324[a]/0.48[b]/0.45[c]/0.376[d]/0.451[e]/0.372[f]/0.38[g]/0.33[h]/0.333[i]/0.452[j]/0.504[k]	39.08[a]/36.35[b]/46.64[c]/44.42[d]/52.55[e]/33.92[f]/47.41[g]/52.86[h]/50.96[i]/50.55[j]/36.89[k]	0.396[a]/0.104[b]/0.225[c]/0.413[d]/0.369[e]/0.165[f]/0.34[g]/0.376[h]/0.398[i]/0.286[j]/0.176[k]	0.164035[a]/-0.406[b]/-0.723[c]/0.028[d]/-0.917[e]/-0.752[f]/-0.147[g]/-0.037[h]/-0.110[i]/-0.669[j]/-0.420[k]
tatC	0.18[a]/0.178[c]/0.183[d]/0.164[e]/0.12	-0.104[a]/-0.139[c]/-0.108[d]/-	0.332[a]/0.315[c]/0,336[d]/0.305[e]/0.2	44.85[a]/50.77[c]/44.93[d]/50.55[e]/41.	0.264[a]/0.261[c]/0.226[d]/0.291[e]/0.1	1.039111[a]/0.935[c]/0.785[d]/0.624

	7[f]/0.171[g]/0.176[h]/0.173[i]/0.194[k]	0.151[e]/-0.296[f]/-0.152[g]/-0.147[h]/-0.159[i]/-0.123[k]	19[f]/0.32[g]/0.323[h]/0.319[i]/0.344[k]	46[f]/39.15[g]/39.58[h]/38.45[i]/44.99[k]	59[f]/0.204[g]/0.202[h]/0.221[i]/0.269[k]	[e]/1.047[f]/0.408[g]/0.507[h]/0.425[i]/1.084[k]
*atp*6	0.121[a]/0.133[b]/0.154[c]/0.139[d]/0.141[e]/0.113[f]/0.139[g]/0.127[h]/0.13[i]/0.157[j]/0.114[k]	-0.186[a]/-0.204[b]/-0.086[c]/-0.144[d]/-0.067[e]/-0.191[f]/-0.105[g]/-0.16[h]/-0.099[i]/-0.107[j]/-0.187[k]	0.271[a]/0.242[b]/0.314[c]/0.3[d]/0.339[e]/0.272[f]/0.325[g]/0.293[h]/0.331[i]/0.321[j]/0.242[k]	40.83[a]/59.99[b]/52.2[c]/45.07[d]/61.00[e]/32.65[f]/41.79[g]/41.89[h]/42.29[i]/55.16[j]/60.71[k]	0.154[a]/0.359[b]/0.324[c]/0.227[d]/0.385[e]/0.128[f]/0.171[g]/0.191[h]/0.192[i]/0.398[j]/0.347[k]	1.173228[a]/1.187[b]/1.103[c]/0.144[d]/0.767[e]/1.148[f]/1.194[g]/1.232[h]/1.175[i]/0.521[j]/1.394[k]
*atp*8	0.126[a]/0.172[b]/0.181[c]/0.159[d]/0.175[e]/0.135[f]/1.141[g]/0.121[h]/0.132[i]/0.186[j]/0.152[k]	-0.115[a]/-0.159[b]/-0.129[c]/-0.06[d]/-0.124[e]/-0.272[f]/-0.165[g]/-0.187[h]/-0.172[i]/0.027[j]/-0.178[k]	0.331[a]/0.35[b]/0.33[c]/0.357[d]/0.322[e]/0.235[f]/0.3[g]/0.275[h]/0.3[i]/0.413[j]/0.27[k]	39.59[a]/45.57[b]/57,97[c]/45.17[d]/53.51[e]/42.5[f]/41.38[g]/39.49[h]/38.57[i]/47.54[j]/45.29[k]	0.157[a]/0.16[b]/0.25[c]/0.198[d]/0.339[e]/0.136[f]/0.138[g]/0.191[h]/0.162[i]/0.38[j]/0.306[k]	0.317693[a]/0.135[b]/0.406[c]/0.418[d]/0.208[e]/0.380[f]/0.253[g]/0.435[h]/0.277[i]/0.348[j]/1.041[k]
*nad*5	0.181[a]/0.145[b]/0.162[c]/0.161[d]/0.185[e]/0.139[f]/0.143[g]/0.142[h]/0.143[i]/0.177[j]/0.174[k]	-0.159[a]/-0.186[b]/-0.149[c]/-0.109[d]/-0.055[e]/-0.225[f]/-0.164[g]/-0.17[h]/-0.149[i]/-0.038[j]/-0.133[k]	0.331[a]/0.274[b]/0.29[c]/0.334[d]/0.354[e]/0.271[f]/0.3[g]/0.299[h]/0.309[i]/0.363[j]/0.298[k]	50.52[a]/43.28[b]/52.55[c]/41.46[d]/56.52[e]/41.06[f]/38.15[g]/38.37[h]/41.52[i]/51.32[j]/48.44[k]	0.345[a]/0.282[b]/0.319[c]/0.198[d]/0.396[e]/0.166[f]/0.156[g]/0.169[h]/0.2[i]/0.371[j]/0.31[k]	0.299203[a]/0.902[b]/0.982[c]/0.968[d]/0.727[e]/0.931[f]/0.961[g]/0.909[h]/0.971[i]/0.607[j]/0.994[k]
*nad*4	0.173[a]/0.194/0.228[c]/0.136[d]/0.184[e]/0.135[f]/0.123[g]/0.126[h]/0.123[i]/0.202[j]/0.194[k]	-0.148[a]/-0.207[b]/-0.064[c]/-0.166[d]/-0.043[e]/-0.205[f]/-0.212[g]/-0.208[h]/-0.23[i]/-0.058[j]/-0.152[k]	0.303[a]/0.319[b]/0.368[c]/0.292[d]/0.362[e]/0.272[f]/0.262[g]/0.264[h]/0.253[i]/0.373[j]/0.309[k]	57.74[a]/50.79[b]/48.42[c]/43.16[d]/61.00[e]/38.62[f]/39.63[g]/40.29[h]/38.62[i]/52.06[j]/52.87[k]	0.286[a]/0.338[b]/0.317[c]/0.189[d]/0.421[e]/0.137[f]/0.178[g]/0.189[h]/0.167[i]/0.361[j]/0.314[k]	0.839437[a]/0.568[b]/0.533[c]/1.102[d]/0.661[e]/1.018[f]/1.089[g]/1.117[h]/1.093[i]/0.405[j]/0.888[k]

*sdh*4	0.135[a]/0.185[b]/0.117[c]/0.135[d]/0.179[e]/0.136[f]/0.162[g]/0.173[h]/0.184[i]/0.159[j]/0.113[k]	-0.014[a]/-0.177[b]/-0.175[c]/-0.031[d]/0.063[e]/-0.189[f]/-0.144[g]/-0.138[h]/-0.107[i]/-0.189[j]/-0.262[k]	0.355[a]/0.337[b]/0.253[c]/0.342[d]/0.408[e]/0.267[f]/0.318[g]/0.333[h]/0.338[i]/0.306[j]/0.197[k]	37.08[a]/45.29[b]/29.05[c]/38.55[d]/44.08[e]/38.23[f]/32.03[g]/32.8[h]/33.77[i]/61[j]/39.3[k]	0.171[a]/0.321[b]/0.133[c]/0.211[d]/0.338[e]/0.133[f]/0.258[g]/0.273[h]/0.246[i]/0.387[j]/0.105[k]	1.50641[a]/0.219[b]/1.522[c]/1.658[d]/1.32[e]/1.477[f]/0.682[g]/0.569[h]/0.738[i]/0.309[j]/1.563[k]
nad2	0.137[a]/0.24[b]/0.166[c]/0.129[d]/0.175[e]/0.136[f]/0.127[g]/0.137[h]/0.131[i]/0.174[j]/0.161[k]	-0.208[a]/-0.123[b]/-0.194[c]/-0.213[d]/-0.057[e]/-0.291[f]/-0.128[g]/-0.2[h]/-0.205[i]/-0.066[j]/-0.17[k]	0.274[a]/0.324[b]/0.278[c]/0.273[d]/0.348[e]/0.222[f]/0.265[g]/0.276[h]/0.273[i]/0.354[j]/0.278[k]	45.41[a]/27,72[b]/51.49[c]/43.41[d]/59.1[e]/38.06[f]/39.5[g]/40.64[h]/42[i]/52.36[j]/49.4[k]	0.168[a]/0.189[b]/0.293[c]/0.197[d]/0.373[e]/0.134[f]/0.149[g]/0.171[h]/0.179[i]/0.324[j]/0.262[k]	1.038241[a]/-0.403[b]/0.814[c]/0.983[d]/0.811[e]/1.127[f]/1.028[g]/0.989[h]/1.038[i]/0.581[j]/0.925[k]
*nad*1	0.126[a]/0.199[b]/0.174[c]/0.155[d]/0.181[e]/0.131[f]/0.128[g]/0.129[h]/0.128[i]/0.189[j]/0.189[k]	-0.165[a]/-0.183[b]/-0.089[c]/-0.039[d]/-0.015[e]/-0.172[f]/-0.124[g]/-0.135[h]/-0.118[i]/-0.076[j]/-0.128[k]	0.28[a]/0.322[b]/0.336[c]/0.36[d]/0.37[e]/0.277[f]/0.308[g]/0.302[h]/0.311[i]/0.371[j]/0.319[k]	40.5[a]/45,24[b]/48.54[c]/41.49[d]/60.87[e]/34.04[f]/40.52[g]/43.79[h]/43.52[i]/54.32[j]/50.69[k]	0.151[a]/0.318[b]/0.376[c]/0.215[d]/0.453[e]/0.096[f]/0.151[g]/0.174[h]/0.19[i]/0.421[j]/0.344[k]	1.022468[a]/0.444[b]/0.546[c]/1.013[d]/0.609[e]/1.086[f]/1.027[g]/1.024[h]/1.056[i]/0.072[j]/0.654[k]
*nad*3	0.149[a]/0.177[b]/0.176[c]/0.152[d]/0.19[e]/0.126[f]/0.157[g]/0.162[h]/0.154[i]/0.182[j]/0.14[k]	-0.146[a]/-0.156[b]/-0.207[c]/-0.178[d]/-0.004[e]/-0.312[f]/-0.13[g]/-0.172[h]/-0.094[i]/-0.211[j]/-0.335[k]	0.313[a]/0.349[b]/0.263[c]/0.296[d]/0.397[e]/0.209[f]/0.322[g]/0.298[h]/0.348[i]/0.286[j]/0.188[k]	34.69[a]/46.96[b]/52.82[c]/38.15[d]/41.31[e]/32.28[f]/33.02[g]/33.85[h]/33.65[i]/55.99[j]/30.49[k]	0.174[a]/0.366[b]/0.354[c]/0.2[d]/0.384[e]/0.13[f]/0.174[g]/0.175[h]/0.174[i]/0.362[j]/0.287[k]	1.05[a]/0.181[b]/0.898[c]/0.973[d]/1.142[e]/1.016[f]/0.923[g]/0.892[h]/0.842[i]/0.275[j]/0.812[k]
*rps*11	0.169[a]/0.156[b]/0.117[c]/0.183[d]/0.098[e]/0.181[f]/0.173[g]/0.191[h]/0.174[i]/0.208[j]/0.129[k]	-0.214[a]/-0.253[b]/-0.112[c]/-0.138[d]/-0.221[e]/-0.173[f]/-0.101[g]/-0.025[h]/-0.083[i]/-0.06[j]/-0.11[k]	0.301[a]/0.292[b]/0.308[c]/0.342[d]/0.234[e]/0.325[f]/0.36[g]/0.404[h]/0.368[i]/0.394[j]/0.333[k]	40.83[a]/38.87[b]/43.83[c]/30.08[d]/49.00[e]/38,92[f]/58.03[g]/60.93[h]/54.49[i]/52.12[j]/35.63[k]	0.221[a]/0.283[b]/0.221[c]/0.228[d]/0.308[e]/0.111[f]/0.219[g]/0.263[h]/0.219[i]/0.349[j]/0.248[k]	-0.156897[a]/0.395[b]/0.478[c]/-0.108[d]/0.275[e]/-0.029[f]/0.027[g]/-0.107[h]/0.022[i]/-0.458[j]/0.173[k]

*atp*9	0.265[a]/0.132[b]/0.146[c]/0.246[d]/0.151[e]/0.162[f]/0.239[g]/0.209[h]/0.243[i]/0.137[j]/0.156[k]	0.101[a]/-0.194[b]/-0.081[c]/0.077[d]/-0.061[e]/-0.183[f]/0.077[g]/0.006[h]/0.101[i]/-0.054[j]/-0.024[k]	0.465[a]/0.31[b]/0.281[c]/0.451[d]/0.323[e]/0.296[f]/0.451[g]/0.408[h]/0.465[i]/0.311[j]/0.328[k]	31.18[a]/38.59[b]/59.24[c]/30.58[d]/57.19[e]/27.09[f]/37.44[g]/36.65[h]/37.81[i]/42.41[j]/55.9[k]	0.113[a]/0.15[b]/0.456[c]/0.113[d]/0.5[e]/0.056[f]/0.197[g]/0.197[h]/0.183[i]/0.525[j]/0.466[k]	1.201316[a]/-0.296[b]/1.448[c]/1.201[d]/0.805[e]/1.201[f]/1.201[g]/1.201[h]/1.201[i]/0.808[j]/0.915[k]
*sdh*3	0.17[a]/0.176[b]/0.116[c]/0.178[d]/0.168[e]/0.136[f]/0.201[g]/0.18[h]/1.104[i]/0.169[j]/0.118[k]	-0.215[a]/-0.045[b]/-0.195[c]/-0.287[d]/-0.124[e]/-0.232[f]/-0.145[g]/-0.192[h]/-0.257[i]/-0.14[j]/-0.16[k]	0.299[a]/0.328[b]/0.262[c]/0.265[d]/0.323[e]/0.265[f]/0.342[g]/0.319[h]/0.235[i]/0.327[j]/0.272[k]	49.51[a]/40,17[b]/39.60[c]/38.89[d]/55.75[e]/34.12[f]/49.37[g]/61[h]/36.26[i]/44.89[j]/49.11[k]	0.274[a]/0.448[b]/0.23[c]/0.221[d]/0.358[e]/0.094[f]/0.281[g]/0.267[h]/0.218[i]/0.363[j]/0.152[k]	0.413559[a]/0.977[b]/0.001[c]/0.576[d]/0.286[e]/0.901[f]/0.678[g]/0.703[h]/0.776[i]/0.524[j]/1.145[k]
*sdh*2	0.175[a]/0.184[b]/0.137[c]/0.153[d]/0.193[e]/0.142[f]/0.141[g]/0.131[h]/0.135[i]/0.17[j]/0.162[k]	-0.109[a]/-0.22[b]/-0.178[c]/-0.119[d]/-0.072[e]/-0.22[f]/-0.092[g]/-0.198[h]/-0.067[i]/-0.056[j]/-0.129[k]	0.364[a]/0.304[b]/0.308[c]/0.343[d]/0.363[e]/0.287[f]/0.327[g]/0.301[h]/0.338[i]/0.367[j]/0.312[k]/	46.5[a]/34.8[b]/41.42[c]/49.21[d]/55.75[e]/33.11[f]/59.88[g]/44.3[h]/55.19[i]/57.05[j]/53.11[k]	0.346[a]/0.261[b]/0.207[c]/0.324[d]/0.385[e]/0.135[f]/0.281[g]/0.209[h]/0.278[i]/0.34[j]/0.271[k]	-0.054955[a]/-0.733[b]/-0.276[c]/-0.234[d]/0.286[e]/-0.263[f]/0.875[g]/-0.352[h]/0.914[i]/0.126[j]/0.646[k]
*nad*6	0.121[a]/0.17[b]/0.15[c]/0.118[d]/0.193[e]/0.123[f]/0.132[g]/0.124[h]/0.136[i]/0.191[j]/0.147[k]/	-0.183[a]/-0.07[b]/-0.099[c]/-0.184[d]/-0.072[e]/-0.195[f]/-0.193[g]/-0.168[h]/-0.134[i]/-0.127[j]/-0.229[k]/	0.264[a]/0.383[b]/0.346[c]/0.273[d]/0.363[e]/0.251[f]/0.274[g]/0.279[h]/0.307[i]/0.331[j]/0.254[k]/	34.12[a]/47.67[b]/52.92[c]/40.7[d]/55.75[e]/42.11[f]/53.6[g]/51.76[h]/47.93[i]/61[j]/57.8[k]/	0.15[a]/0.355[b]/0.352[c]/0.144[d]/0.358[e]/0.162[f]/0.372[g]/0.237[h]/0.185[i]/0.419[j]/0.391[k]/	1.14802[a]/-0.232/0.603[c]/1.046[d]/0.286[e]/1.266[f]/1.02[g]/1.102[h]/1.055[i]/0.459[j]/0.828[k]/
cob	0.148[a]/0.233[b]/0.113[c]/0.149[d]/0.153[e]/0.131[f]/0.123[g]/0.164[h]/0.12[i]/0.181[j]/0.164[k]/	-0.269[a]/-0.188[b]/-0.218[c]/-0.201[d]/-0.084[e]/-0.31[f]/-0.195[g]/-0.219[h]/-0.229[i]/-0.078[j]/-0.212[k]/	0.277[a]/0.337[b]/0.263[c]/0.31[d]/0.338[e]/0.207[f]/0.269[g]/0.309[h]/0.247[i]/0.341[j]/0.264[k]/	45.87[a]/41.14[b]/44.15[c]/48.63[d]/49.92[e]/36.14[f]/37.7[g]/49.64[h]/40.12[i]/48.07[j]/38.7[k]/	0.317[a]/0.232[b]/0.205[c]/0.321[d]/0.288[e]/0.112[f]/0.147[g]/0.321[h]/0.167[i]/0.299[j]/0.124[k]/	0.097765[a]/0.242[b]/0.881[c]/-0.064[d]/0.842[e]/0.824[f]/0.886[g]/-0.023[h]/0.894[i]/0.857[j]/0.836[k]/
*atp*4	0.21[a]/0.168[c]/0.127[d]/0.133[f]/0.16	-0.064[a]/-0.029[c]/-0.181[d]/-	0.38[a]/0.386[c]/0.287[d]/0.246[f]/0.3[g]/	48.3[a]/44.94[c]/44.74[d]/35.95[f]/45.1	0.293[a]/0.205[c]/0.267[d]/0.123[f]/0.2	0.170064[a]/0.330[c]/0.405[d]/0.418[f]

	8[g]/0.169[h]/0.157[i] /0.179[k]/	0.276[f]/-0.162[g]/-0.138[h]/-0.112[i]/-0.154[k]/	0.316[h]/0.329[i]/0.309[k]/	1[g]/45.11[h]/46.24[i] /33.55[k]/	47[g]/0.263[h]/0.25[i] /0.122[k]/	/0.615[g]/0.610[h]/ 0.630[i]/0.348[k]/
*cox*3	0.168[a]/0.18[b]/0.166[c]/0.184[d]/0.233[e]/0.163[f]/0.179[g] /0.187[h]/0.178[i]/0.179[j]/0.188[k]/	-0.16[a]/-0.145[b]/-0.116[c]/-0.112[d]/-0.053[e]/-0.266[f]/-0.092[g]/-0.087[h]/-0.121[i]/-0.079[j]/-0.15[k]/	0.291[a]/0.313[b]/0.315[c]/0.332[d]/0.381[e]/0.252[f]/0.328[g]/0.332[h]/0.109[i] /0.354[j]/0.295[k]/	57.29[a]/32.19[b]/56.89[c]/55.34[d]/51.23[e]/37.42[f]/61[g]/58.55[h]/59.52[i]/51.53[j]/57.89[k]/	0.363[a]/0.126[b]/0.361[c]/0.353[d]/0.418[e]/0.177[f]/0.381[g]/0.357[h]/0.366[i] /0.405[j]/0.359[k]/	0.774409[a]/0.240[b]/0.774[c]/0.653[d]/0.405[e]/0.834[f]/ 0.843[g]/0.942[h]/0.861[i]/0.138[j]/0.933[k]/
*cox*2	0.149[a]/0.182[b]/0.171[c]/0.153[d]/0.19[e]/0.159[f]/0.154[g] /0.164[h]/0.156[i]/0.136[j]/0.156[k]/	-0.103[a]/-0.185[b]/-0.076[c]/-0.125[d]/0.02[e]/-0.245[f]/-0.111[g]/-0.052[h]/-0.093[i]/-0.09[j]/-0.081[k]/	0.31[a]/0.299[b]/0.335[c]/0.319[d]/0.412[e]/0.261[f]/0.316[g] /0.349[h]/0.324[i]/0.364[j]/0.327[k]/	57.63[a]/32.72[b]/61[c]/55.87[d]/56.6[e]/ 36.72[f]/61[g]/61[h]/ 61[i]/61[j]/57.65[k]/	0.395[a]/0.091[b]/0.392[c]/0.403[d]/0.465[e]/0.135[f]/0.383[g]/0.388[h]/0.4[i]/0.377[j]/0.379[k]/	0.840265[a]/0.773[b]/0.733[c]/0.464[d]/0.444[e]/0.401[f]/ 0.738[g]/0.664[h]/0.683[i]/- 0.434[j]/0.851[k]/
*cox*1	0.165[a]/0.19[b]/0.061[c]/0.164[d]/0.187[e]/0.164[f]/0.164[g] /0.16[h]/0.161[i]/0.173[j]/0.163[k]/	-0.076[a]/-0.227[b]/-0.115[c]/-0.074[d]/-0.044[e]/-0.177[f]/-0.072[g]/-0.076[h]/-0.071[i]/-0.118[j]/-0.084[k]/	0.333[a]/0.278[b]/0.32[c]/0.328[d]/0.369[e]/0.198[f]/0.326[g] /0.322[h]/0.326[i]/0.354[j]/0.32[k]/	54.84[a]/41.12[b]/52.95[c]/56.58[d]/56.06[e]/37.26[f]/54.22[g]/55.7[h]/57.52[i]/ 53.06[j]/54.76[k]/	0.384[a]/0.118[b]/0.37[c]/0.394[d]/0.41[e]/0.144[f]/0.387[g]/ 0.383[h]/0.389[i]/0.38[j]/0.39[k]/	0.93444[a]/0.211[b]/0.770[c]/0.148[d]/ 0.525[e]/0.749[f]/1.079[g]/1.090[h]/1.026[i]/- 0.153[j]/1.227[k]/
*rpl*16	0.182[a]/0.182[b]/0.161[c]/0.139[d]/0.243[e]/0.193[f]/0.126[g]/0.171[h]/0.137[i] /0.215[j]/0.147[k]/	-0.036[a]/-0.174[b]/-0.117[c]/-0.115[d]/0.156[e]/-0.012[f]/-0.142[g]/-0.123[h]/- 0.114[i]/0.034[j]/-0.086[k]/	0.368[a]/0.305[b]/0.348[c]/0.342[d]/0.511[e]/0.412[f]/0.307[g]/0.325[h]/0.327[i] /0.439[j]/0.363[k]/	51.28[a]/36.79[b]/43.74[c]/54.95[d]/51.73[e]/36.13[f]/40.97[g]/55.04[h]/46.52[i] /44.68[j]/45.01[k]/	0.342[a]/0.098[b]/0.159[c]/0.27[d]/0.344[e]/0.176[f]/0.289[g] /0.307[h]/0.319[i]/0.25[j]/0.207[k]/	0.528814[a]/0.740[b]/- 0.234[c]/0.023[d]/- 0.176[e]/- 0.182[f]/0.480[g]/0.297[h]/0.494[i]/- 0.187[j]/-0.023[k]/
*ymf*16	0.158[b]/0.187[j]/	-0.176[b]/0.018[j]/	0.288[b]/0.422[j]/	40.03[b]/45.29[j]/	0.216[b]/0.304[j]/	1.289167[b]/0.343[j]/
*orf*73	0.102[b]/	-0.295[b]/	0,202[b]/	33.49[b]/	0.106[b]/	1.155[b]/
*ymf*39	0.169[b]/0.157[e]/0.	-0.197[b]/-	0.272[b]/0.372[e]/0.	37.62[b]/55.68[e]/5	0.126[b]/0.404[e]/0.	0.878[b]/0.442[e]/0.

	158[j]/	0.026[e]/-0.047[j]/	366[j]/	4.84[j]/	378[j]/	460[j]/
*orf*172	0.154[b]/0.204[j]/	-0.224[b]/-0.034[j]/	0.287[b]/0.406[j]/	40.1[b]/49.76[j]/	0.117[b]/0.284[j]/	-0.152[b]/-0.124[j]/
tatA	0.122[c]/0.145[j]/	-0.236[c]/-0.122[j]/	0.244[c]/0.327[j]/	43.44[j]/	0.122[c]/0.347[j]/	0.831[c]/0.308[j]/

a:*B.gelatinus ;* b:*C.crispus ;* c:*C.okamurae ;* d:*E.denticulatum ;* e:*G.furcata ;* f:*H.edeniana ;* g:*K.alvarezii ;* h:*K.malesianus ;* i:*K.striatus ;* j:*M.papillatus ;* k:*S.skottsbergii*