

Comprehensive analysis of chloroplast genomes from 15 mangrove species (Rhizophoraceae): codon usage, phylogenetic relationships, and evolutionary insights

Rui XU, Xinyue GUAN, Jing LIU, Xiujuan YIN, Liqiang WANG*

College of Pharmacy, Heze University, Heze 274015, China

Received Jan. 26, 2026; accepted in principle Mar. 25, 2026; accepted for publication Apr. 25, 2026

© Chinese Society for Oceanology and Limnology, Science Press and Springer-Verlag GmbH Germany, part of Springer Nature 2026

Abstract Mangrove plants, serving as critical blue carbon ecosystems and biodiversity hotspots, play critical roles in coastal protection, water purification, and climate change mitigation. In this paper, we present the first comprehensive analysis of chloroplast genomes from 15 mangrove species within the Rhizophoraceae family, with particular emphasis on their codon usage bias (CUB), phylogenetic connections, and evolutionary dynamics. The total GC contents of protein-coding genes (PCGs) span from 36.79% to 38.13%, with a distribution trend of 50%>1st-site GC (GC1)>2nd-site GC (GC2)>total GC (GCall)>3rd-site GC (GC3)>synonymous codon 3rd GC (GC3s). The average values of effective number of codons (ENC) range from 46.43 to 47.18, reflecting a relatively weak CUB across the genomes. This bias is shaped by both mutational pressure and natural selection, with mutation exerting a more significant influence. We identified 13–19 optimal codons across these species, with ACU, AUU, CAA, CGU, GGU, GUA, UGC, and UUA being the most common optimal codons. Among all the genes analyzed, the *rps14* gene demonstrated the strongest CUB (ENC<35), primarily driven by natural selection that optimizes its expression to ensure the efficient and accurate synthesis of the ribosome, whereas *rps18* gene exhibited clear signals of positive selection across all species against their terrestrial relative by the ratio of non-synonymous and synonymous substitution (Ka/Ks) analysis, suggesting it may be undergoing potential adaptive evolution within Rhizophoraceae mangrove species. Phylogenetic analysis based on common coding sequences (CDSs), concatenated sequences of *rps14* and *rps18*, and relative synonymous codon usage (RSCU) values strongly supported monophyletic groups of four true mangrove genera. This study elucidated the synonymous codon usage patterns within the Rhizophoraceae family, offering valuable insights into their phylogenetic relationships, evolutionary processes, and potential applications in genetic engineering of Rhizophoraceae mangrove species.

Keyword: Rhizophoraceae; chloroplast genome; codon usage bias; optimal codon; phylogenetic analysis

1 INTRODUCTION

Mangroves, as vascular plant communities inhabiting marine intertidal zones, constitute critical habitats for a wide range of coastal organisms. Together with their surrounding environment, they create a unique transitional zone between terrestrial and marine systems. These ecosystems are vital for stabilizing coastlines, serving as significant reservoirs for atmospheric carbon storage, and providing critical habitats that support numerous species (Lee et al., 2014; Rahman et al., 2024).

Despite accounting for less than 2% of the global marine ecosystem area, mangroves are among the most productive and vital “blue carbon” sinks, burying over 40% of the organic carbon circulating in marine systems annually (Kelleway et al., 2016; Sidik et al., 2018; Soeprbowati et al., 2024). Nevertheless, these vital habitats face escalating threats from anthropogenic pressures and climate change, highlighting the critical importance of

* Corresponding author: lys832000@163.com

effective conservation strategies, particularly for endangered species (Akram et al., 2023; Howai, 2023). Accordingly, gaining molecular insights into mangrove adaptive mechanisms is critical for successful restoration efforts, with chloroplast genomes emerging as essential tools due to their roles in photosynthesis, environmental stress responses, and evolutionary studies (Asaf et al., 2021).

To delve deeper into the molecular underpinnings of mangrove adaptation, it is crucial to focus on specific mangrove-associated plant families. Among these, the Rhizophoraceae family stands out as a group of flowering plants consisting of approximately 16–18 genera, primarily distributed in the intertidal zones along tropical and subtropical coastlines across the Old World (Van Vliet, 1976). While the Rhizophoraceae is often recognized as a mangrove family, it is important to note that only four of its genera—*Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora*—are exclusively mangrove-dwelling (true mangrove), with the remaining members either terrestrial species or mangrove associates (Juncosa and Tomlinson, 1988; Zhang et al., 2023).

Chloroplasts, as essential organelles in plants, are fundamental to photosynthesis and adaptation to environmental stress. Studies have shown that salt stress induces alterations in chloroplast ultrastructure and modulates the expression of chloroplast-encoded proteins (Hameed et al., 2021; Wang et al., 2024). Due to the relatively small size, conserved structure, and low nucleotide substitution rates, chloroplast genomes have become a valuable tool for comparative evolutionary studies in plants (Yuan et al., 2025). As the critical interface between nucleic acid sequences and protein synthesis in chloroplast genomes, codons play an essential role in the faithful transmission of genetic information. Among the 64 codons, methionine (Met) and tryptophan (Trp) are each encoded by a single codon, AUG and UGG, respectively, while the remaining amino acids are specified by two or more synonymous codons. Notably, the utilization of these synonymous codons is not random across chloroplast genomes; this preferential usage pattern, termed codon usage bias (CUB), can reflect evolutionary pressures from mutation, natural selection, and environmental adaptation (Zhang et al., 2021; Wu et al., 2023).

In plants, chloroplast CUB influences gene expression efficiency, translational accuracy, and the efficacy of chloroplast genetic engineering (Fages-Lartaud et al., 2022; Wu et al., 2024). An analysis of

Kandelia obovata (Rhizophoraceae) revealed weak CUB (average effective number of codons, $ENC > 45$) predominantly driven by mutational pressure (Zhao et al., 2020). However, research on three Combretaceae mangroves (*Laguncularia racemosa*, *Lumnitzera littorea*, and *Lumnitzera racemosa*) demonstrated a weak CUB with natural selection as the primary force rather than mutation (Zhong et al., 2024). These observations underscore the variability in codon usage across different mangrove species and highlight the urgent need for further comparative studies to elucidate the phylogenetic relationships and evolutionary dynamics at work.

Although some chloroplast genomes of mangrove species have been reported, the CUB analysis in their chloroplast genomes remains limited (Zhang et al., 2023; Fang et al., 2024). In this study, we characterized codon usage bias and its driving forces by analyzing chloroplast genomes from 15 Rhizophoraceae mangrove species belonging to *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora* (Supplementary Table S1). We further performed comprehensive phylogenetic analyses and evaluated intrafamily selection pressure, providing insights into the evolution, genetic adaptation, and species differentiation within the Rhizophoraceae family.

2 MATERIAL AND METHOD

2.1 Sequence processing

We downloaded the complete chloroplast genome sequences of 15 Rhizophoraceae mangrove species were downloaded from the NCBI database, with comprehensive details listed in Supplementary Table S1. To enhance the precision of the CUB analysis, we conducted the coding sequences (CDSs) filtration with a custom Python script according to the following criteria: (1) duplicate genes and sequences shorter than 300 bp were removed; (2) only sequences initiating with ATG and terminating with a canonical stop codon (TAA, TAG, or TGA) were retained; (3) those containing internal stop codons were excluded to prevent inclusion of truncated open reading frames (Geng et al., 2022; Wang et al., 2022b; Li et al., 2025).

2.2 Codon usage index and their calculation

Codon usage indices include ENC, codon adaptation index (CAI), codon bias index (CBI), optimal codon frequency (Fop), relative synonymous codon usage (RSCU), general average hydropathicity (GRAVY), aromaticity (AROMO) and the GC

contents in filtered coding sequences (Zhao and Zhang, 2024). We calculated the total GC content (GCall) and the GC content at the first, second, and third positions (GC1, GC2, GC3) with EMBOSS-cusp (<https://www.bioinformatics.nl/cgi-bin/emboss/cusp>). ENC, CAI, CBI, Fop, RSCU, and GC content at the third position of synonymous codons (GC3s) were subsequently estimated using CodonW 1.4.2 (Wright, 1990).

2.3 Determination of optimal codons

The RSCU serves as a metric for evaluating the comparative prevalence of each synonymous codon in genetic encoding. Specifically, codons exhibiting RSCU values exceeding 1 were identified as high-frequency codons (Wang et al., 2022b). First, we sorted the genes by ENC value and then divided them into two groups: those with high expression level (the bottom 10% of sequences) and those with low expression (the top 10% of sequences). And then, we calculated the difference in RSCU values between the two sets (Δ RSCU). Codons with both Δ RSCU > 0.08 and RSCU > 1 were classified as optimal codons (Duret and Mouchiroud, 1999).

2.4 Neutral plot analysis

The neutral plot, the average GC content of the first and second codon positions (GC12) vs. GC3, is a widely used method to assess the relative contributions of mutational pressure and natural selection in shaping codon usage bias (Tyagi et al., 2020). According to neutral evolution theory, nucleotide substitutions are expected to occur randomly without affecting organismal fitness. In practice, however, selective constraints can distort this pattern, leading to systematic biases in codon usage that deviate from neutral expectations (Orr, 1999). Consequently, a significant correlation between GC12 and GC3 suggests that mutation pressure is the dominant force shaping codon usage. In contrast, a weak or absent correlation indicates that natural selection plays a more important role in driving CUB (Hu et al., 2024).

2.5 ENC-plot analysis

The ENC-plot (ENC vs. GC3s) is commonly used to evaluate the relative influence of mutational pressure and natural selection on codon usage bias. The theoretical expectation under pure mutational bias (ENC_{exp}) is represented by a predicted curve, which was calculated according to the established formula $ENC_{exp} = 2 + GC3s + 29/[GC3s^2 + (1 - GC3s)^2]$

(Wright, 1990; Gao et al., 2024). Points clustering near the curve suggest dominance of mutation pressure, while significant deviations below the curve indicate additional influences such as natural selection. Furthermore, we evaluated the discrepancies between the observed ENC values (ENC_{obs}) and ENC_{exp} using the ENC_{ratio} index by the formula: $ENC_{ratio} = (ENC_{exp} - ENC_{obs})/ENC_{exp}$ (Zhao and Zhang, 2024).

2.6 PR2-plot analysis

According to parity rule 2 (PR2), the relative frequencies of adenine (A) and thymine (T), as well as cytosine (C) and guanine (G), are expected to be equal within a DNA strand, providing insights into mutation bias and evolutionary constraints. The PR2-plot analysis based on $[A3s/(A3s+T3s) \text{ vs. } G3s/(G3s+C3s)]$ (A/T/G/C content at the synonymous third codon position) serves as a useful tool for assessing both the degree and direction of CUB. The center point (0.5, 0.5) of the graph indicates that double-stranded DNA follows the PR2, which means the codon has no usage bias (Sueoka, 1999, 2001). We identified amino acids encoded by four synonymous codons and recalculated the base compositions at the third position for each gene with a custom Python script.

2.7 Correspondence analysis

Correspondence analysis (COA) is a widely used multivariate statistical method to study patterns of synonymous codon usage (Clarke and Greenacre, 1985; Li et al., 2016). We carried out the analysis using CodonW (v1.4.2) based on RSCU values in a 59-dimensional space, corresponding to 59 sense codons after excluding three stop codons, AUG (encoding Met) and UGG (encoding Trp). This approach identifies major axes that capture the principal trends in codon usage bias and accounts for the highest proportion of variation among genes. The positions of genes within the multidimensional COA plot were compared using Euler square distances based on RSCU values, enabling visualization and interpretation of the underlying factors influencing codon preference. We performed correlation analysis of Axis 1 with GC contents and ENC was performed to further investigate the factors shaping CUB (Hu et al., 2024).

2.8 Selection pressure analysis

Ka/Ks, the ratio of non-synonymous (Ka) and synonymous (Ks) substitution, functions as a crucial

measure of selective pressure: values exceeding 1.0 indicate adaptive or positive selection, those less than 1.0 suggest purifying or negative selection, and values approximately equal to 1.0 imply neutral evolution (Xue et al., 2025). We calculated the Ka, Ks, and Ka/Ks ratios of orthologous genes between 15 mangrove Rhizophoraceae species and their terrestrial non-mangrove relative in Rhizophoraceae (*Pellacalix yunnanensis*, MN106253) using KaKs_Calculator (v3.0) (Zhang, 2022), with the MYN (modified Yang-Nielsen) method.

2.9 Phylogenetic analysis and cluster analysis

To gain deeper insights into the evolutionary relationships among selected species in the Rhizophoraceae family, we performed comprehensive phylogenetic and clustering analyses on the basis of CDSs from 15 selected Rhizophoraceae chloroplast genomes. We reconstructed phylogenetic trees using the related software embedded in PhyloSuite v1.2.3, with MAFFT for multiple sequence alignment, Gblocks for sequence trimming, ModelFinder for the best-fit model, and IQtree for phylogenetic tree construction with the maximum likelihood (ML) method and default parameters (Zhang et al., 2020). For cluster analysis, the complete linkage method and Euclidean distance were applied on the CNSknowall platform (<https://cnsknowall.com>), based on RSCU values with stop codons, AUG, and UGG removed.

3 RESULT

3.1 Codon usage indices calculation

The number of CDSs after filtering for all

selected species is shown in Supplementary Table S1, ranging from 48 to 52. Analysis of GC contents and codon usage indices revealed that chloroplast genomes of 15 Rhizophoraceae species share very similar nucleotide compositions and codon usage patterns (Fig.1; Supplementary Table S2). The total GC content varied between 36.79% and 38.13%, implying a preference for A or T in Rhizophoraceae chloroplast genomes. The GC contents at different codon positions were all less than 50%, with a distribution following a descending order: 50%>GC1>GC2>GCall>GC3>GC3s. This site-specific variation indicates a pronounced imbalance in codon base composition, with the third codon position demonstrating a statistically significant bias toward terminating in A or T.

ENC is a metric used to evaluate the degree of codon usage bias and is considered the most informative and comprehensive indicator for assessing such bias. The range of ENC values typically falls between 20 and 61, with a lower ENC value signifying a stronger preference for some certain codons. As a general guideline, an ENC value below 35 suggests the presence of significant CUB, while a value exceeding 35 indicates a relatively weak preference for certain codons (Wright, 1990; Shen et al., 2024). In this study, the ENC values of genes from all 15 Rhizophoraceae chloroplast genomes fell within the range of 33–61 (Fig.1), with average ENC values of 46.43–47.18 (Supplementary Table S2), suggesting a relatively weak CUB.

Consistent with the observed codon preference patterns, the CAI, CBI, and Fop indices further

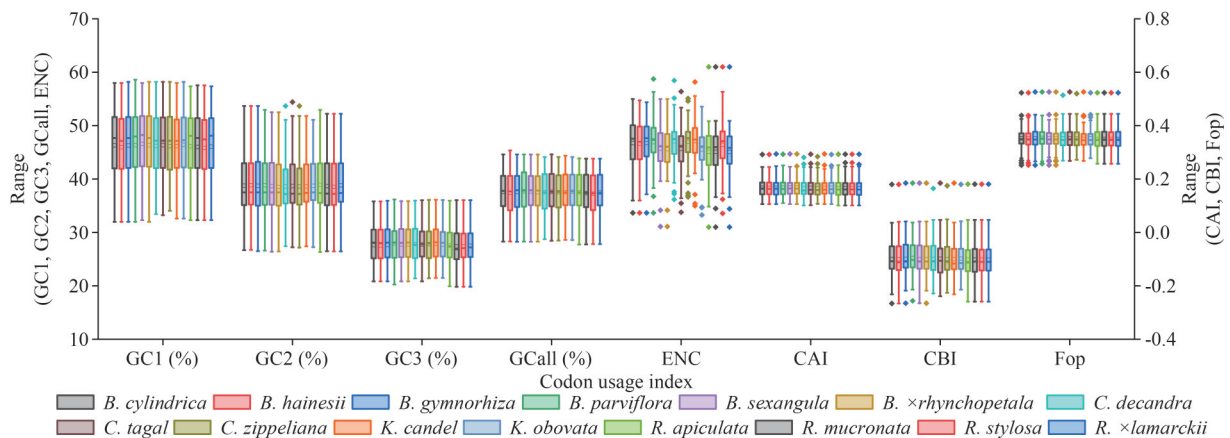


Fig.1 Codon usage indices in the chloroplast genomes of 15 mangrove species within the Rhizophoraceae family

GC1, GC2, GC3, GCall, and ENC refer to the left y-axis, while CAI, CBI, and Fop refer to the right y-axis. GC1, GC2, GC3: the GC content at the first, second, and third positions; GCall: the total GC content; ENC: effective number of codons; CAI: codon adaptation index; CBI: codon bias index; Fop: optimal codon frequency.

supported the presence of weak codon usage bias. The CAI measures gene expression level, with higher values reflecting greater expression. The CBI evaluates expression based on the proportion of optimal codons, where 1 means full use and negative values indicate underuse. The Fop ranges from 0 to 1. A value of 0 suggests no optimal codons are used during translation, whereas a value of 1 indicates that all optimal codons are fully employed (Li et al., 2025). The average CAI, CBI, and Fop values for 15 Rhizophoraceae chloroplast genomes were 0.17, -0.10, and 0.35, respectively (Supplementary Table S2), implying relatively low genome expression levels and underutilization of optimal codons (Shen et al., 2024).

3.2 RSCU analysis and optimal codon

RSCU analysis across the 15 Rhizophoraceae chloroplast genomes indicated highly conserved codon usage patterns (Fig.2). 30–31 high-frequency codons (RSCU>1) were identified in these species (Fig.2a; Supplementary Table S3). Among all the high-frequency codons, 16 end with uracil (U), 13 end with A, one ends with G (UUG, encoding leucine), indicating a preference for codons that end with A or U. The only high-frequency codon ending

in C is UCC, which is shared by 12 chloroplast genomes except for *C. tagal*, *C. zippeliana*, and *K. candel*. In all 15 Rhizophoraceae mangrove species, UUA (encoding leucine) is most strongly preferred (RSCU>2.0), followed by AGA (RSCU>1.8, encoding arginine) and GCU (RSCU>1.7, encoding alanine).

Based on these identified high-frequency codons, we further constructed high- and low-expression gene datasets, with 13–19 optimal codons identified across 15 Rhizophoraceae chloroplast genomes (Supplementary Table S3). *Rhizophora mucronata* and *R. ×lamarckii* exhibit the highest number of optimal codons, totaling 19 codons, whereas *K. candel* displays the fewest, with just 13 codons (Fig.2b). 92%–100% of optimal codons end with A or U, while UUG is the only codon ending with G or C that is shared by 7 out of 15 analyzed species. ACU, AUU, CAA, CGU, GGU, GUA, UGU, and UUA are the most commonly used optimal codons across 15 Rhizophoraceae chloroplast genomes, followed by AAA, AGU, GAA, and UUU, which are optimal codons in 13 species, except for *K. candel* and *K. obovata*. Furthermore, CAU serves as an optimal codon exclusively in *Kandelia*, while UAU is specific to *Ceriops*. The analysis revealed that the general patterns of codon usage in the

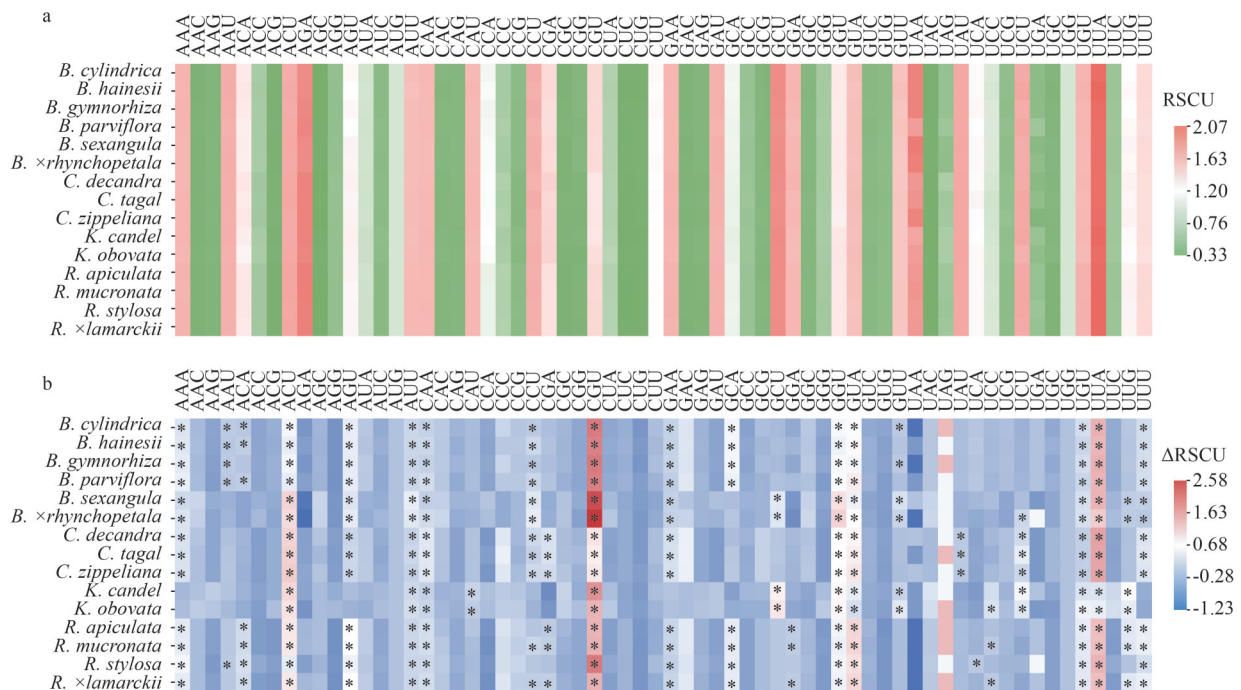


Fig.2 RSCU and optimal codon analysis in the chloroplast genomes of 15 mangrove species within the Rhizophoraceae family

a. heatmap of RSCU values; b. heatmap of Δ RSCU values. *: optimal codons with both Δ RSCU>0.08 and RSCU>1; RSCU: relative synonymous codon usage; Δ RSCU: the difference in RSCU values between high-expression and low-expression sets.

chloroplast genomes of Rhizophoraceae mangroves are highly conserved, and individual genera exhibit specific and distinguishable features in their codon usage preferences.

3.3 Neutral plot analysis

To provide further insights into how mutation pressure and natural selection affect codon usage patterns in Rhizophoraceae chloroplast genomes, we explored the relationship between GC12 and GC3 values across all filtered genes via neutral plot analysis (Fig.3; Supplementary Table S4). The black line indicates the best-fit curve, and the surrounding grey shaded region corresponds to the 95% confidence interval. The results revealed statistically significant positive correlations between GC12 and GC3 across all 15 Rhizophoraceae mangrove species ($P < 0.01$), with Pearson correlation coefficients ranging from 0.412 8 to 0.502 8, and regression coefficients ranging from 0.629 5 to 0.732 2, indicating that mutation pressure may represent a major contributing factor to the codon usage bias. These results indicate that mutation may have a more significant influence than natural

selection in driving CUB across the 15 Rhizophoraceae chloroplast genomes (Fig.3).

3.4 ENC-GC3s analysis

To further characterize the factors governing CUB, scatter plots of ENC and GC3s were constructed, with an expected ENC curve included to assess the impact of mutation on CUB. In the ENC-GC3s plot, genes clustered into small groups on the left side (Fig.4). Most of these genes distribute near or along the standard curve, whereas several others fall below and far away. Additionally, ENC_{ratio} was calculated and displayed in stacked bar graphs (Fig.5). 48.98%–60% of genes showed ENC_{ratio} values of -0.05 – 0.05 , 77.27%–85.11% of genes showed ENC_{ratio} of -0.1 – 0.1 . These findings suggest that CUB in the chloroplast genomes of the Rhizophoraceae family was mainly influenced by mutation pressure, which aligns with the results of neutrality plot analysis.

Notably, among the analyzed genes, *rps14* exhibited an ENC consistently below 35, demonstrating a strong bias in its synonymous codon usage. Furthermore, the *rps14* gene exhibits

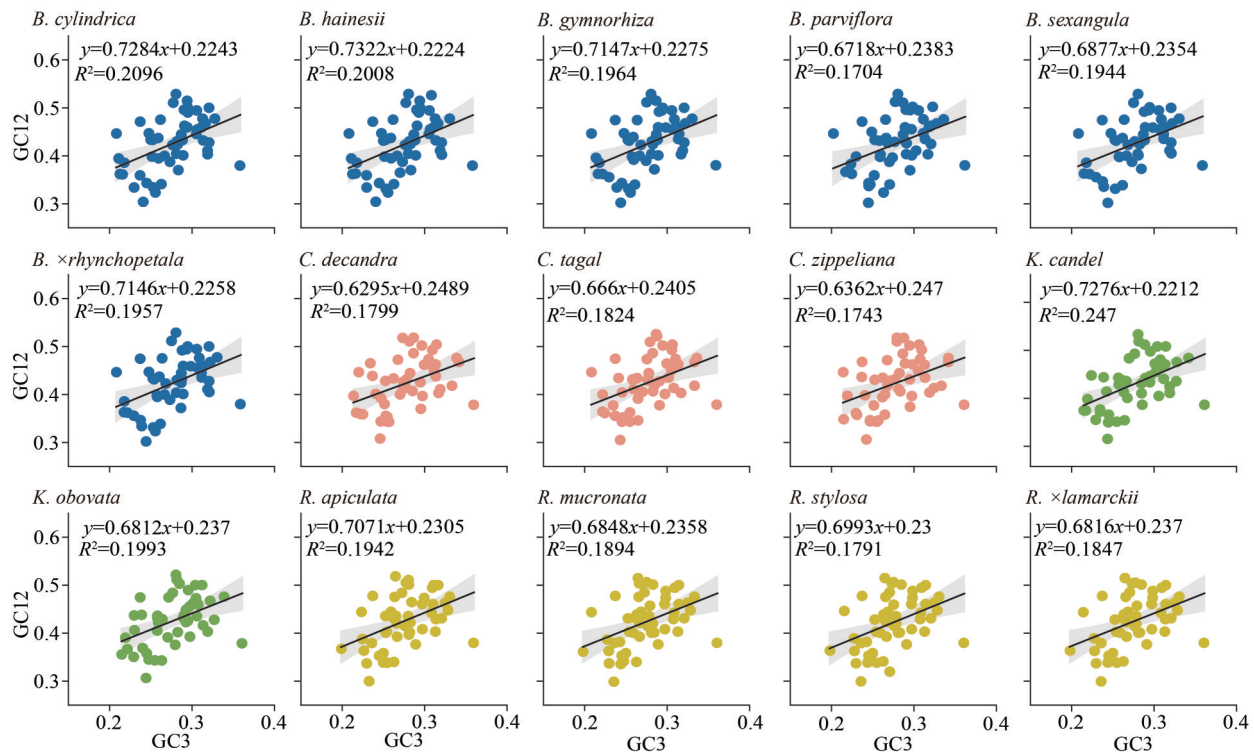


Fig.3 Neutral analysis of chloroplast CDSs from 15 mangrove species within the Rhizophoraceae family

Species belonging to the same genus are represented by the same color. In each coordinate system, dot colors (blue, pinkish-orange, green, yellow) correspond to *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora*, respectively. The black line indicates the best-fit curve, and the surrounding grey shaded region corresponds to the 95% confidence interval. GC12: the average GC content of the first and second codon positions; GC3: GC content of the third codon position.

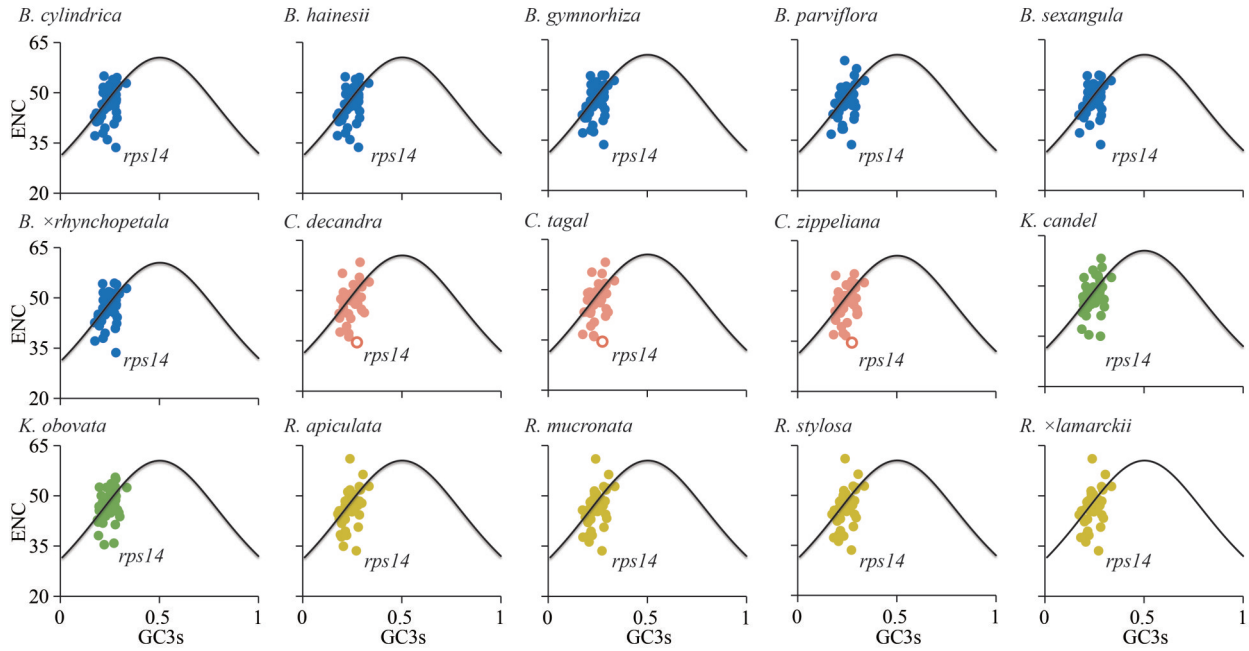


Fig.4 ENC-GC3s analysis of chloroplast CDSs from 15 mangrove species within the Rhizophoraceae family

Species belonging to the same genus are represented by the same color. In each coordinate system, dot colors (blue, pinkish-orange, green, yellow) correspond to *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora*, respectively. The solid line depicts the standard curve when CUB is influenced only by mutation. The hollow circle represents the outlier gene *rps14* from the *Ceriops* species, while solid dots indicate other genes. ENC: effective number of codons; GC3s: GC content at the third position of synonymous codons.

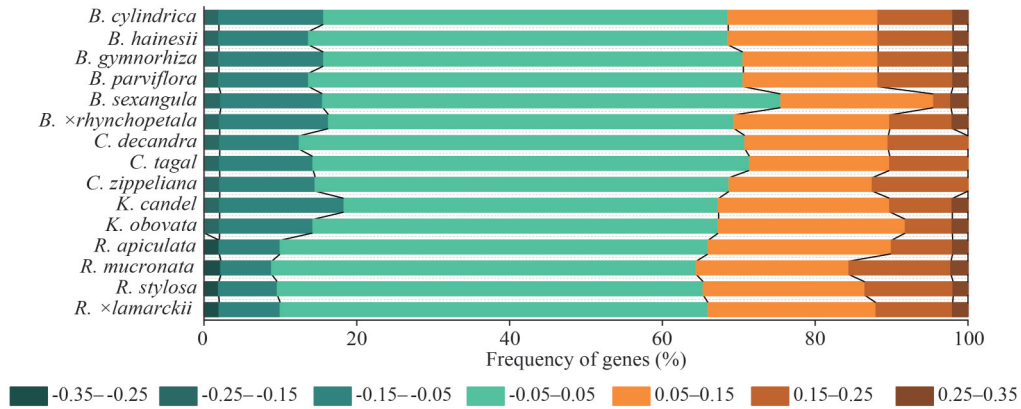


Fig.5 ENC_{ratio} distribution of 15 Rhizophoraceae chloroplast genomes

The x-axis represents the proportion of genes in different ENC_{ratio} intervals. Most genes showed ENC_{ratio} of -0.1–0.1. $ENC_{ratio} = (ENC_{exp} - ENC_{obs}) / ENC_{exp}$. ENC: effective number of codons; ENC_{exp} : theoretical expectation under pure mutational bias; ENC_{obs} : observed ENC values.

the greatest deviation from the standard curve, with ENC_{ratio} values ranging from 0.28 to 0.33, suggesting that it is more strongly influenced by natural selection. In three *Ceriops* species, the *rps14* gene was excluded from further analysis due to its length being less than 300 bp. Thus, we separately analyzed the ENC values and GC3s contents of this gene in *Ceriops* species and obtained results consistent with those observed in other species (Fig.4), confirming the conclusion that *rps14* is subject to strong selective pressures.

3.5 PR2-plot analysis

We performed PR2-plot analysis across CDSs of selected Rhizophoraceae chloroplast genomes to evaluate potential bias at the third codon position of amino acids with four degenerate codons. At the third codon position, random mutations without any selective pressure would theoretically lead to equal frequencies of the four nucleotides—A, T, C, and G. Consequently, the genes would be distributed evenly around the center point in the PR2-plot graph. However, in reality, natural selection frequently

influences base usage preferences, leading to non-uniform distributions of four bases at this position.

As shown in Fig.6, most genes of the 15 Rhizophoraceae chloroplast genomes were located in the third quadrant, characterized by $G3s/(G3s+C3s) < 0.5$ and $A3s/(A3s+T3s) < 0.5$, indicating a pronounced preference for C/T over G/A at the third position. Therefore, the CUB in the chloroplast genomes of the 15 Rhizophoraceae mangrove species is influenced not only by mutations but also significantly by natural selection. However, unlike other chloroplast genes, the *rps14* gene was consistently located in the second quadrant across all species, including *C. decandra*, *C. tagal*, and *C. zippeliana*, where both $G3s/(G3s+C3s)$ and $A3s/(A3s+T3s)$ exceed 0.5, suggesting a preference for G and A. Furthermore, the specific $G3s/(G3s+C3s)$ and $A3s/(A3s+T3s)$ values of the *rps14* gene exhibited variations among different genera within the Rhizophoraceae family, suggesting potential genus-specific patterns in codon usage.

3.6 Correspondence analysis

We further performed correspondence analysis based on RSCU values from the CDSs of 15 Rhizophoraceae chloroplast genomes to investigate their codon usage bias (Fig.7). Most genes grouped

along the first two principal axes, suggesting that there was little variation in the preference for synonymous codons in each species. Axis 1 accounted for 9.88%–11.69% of the total variance, while Axis 2 contributed 8.84%–10.06%, suggesting that Axis 1 is the primary driving force of CUB, with Axis 2 playing a secondary role. The relatively low percentages of explained variance indicate that patterns of codon usage in the chloroplast genomes of the Rhizophoraceae family are likely shaped by multiple interrelated factors, rather than driven by a single dominant evolutionary mechanism.

Moreover, Spearman correlation analysis of Axis 1 with ENC and nucleotide composition demonstrates a clear correlation between Axis 1 and the GC3s, especially with the content of C3s, except for *B. parviflora* and *C. tagal* (Table 1). The absolute values of the correlation coefficients between Axis 1 and C3s ranged from 0.354 to 0.533. In addition, correlation was also observed between Axis 1 and GCall in *K. candel*, *K. obovata*, *R. apiculata*, and *R. ×lamarckii*, as well as correlation between Axis 1 and T3s in *B. sexangula*, *B. ×rhynchoptala*, *C. decandra*, and *C. zippeliana*.

3.7 Selection pressure analysis

To detect potential genes under positive

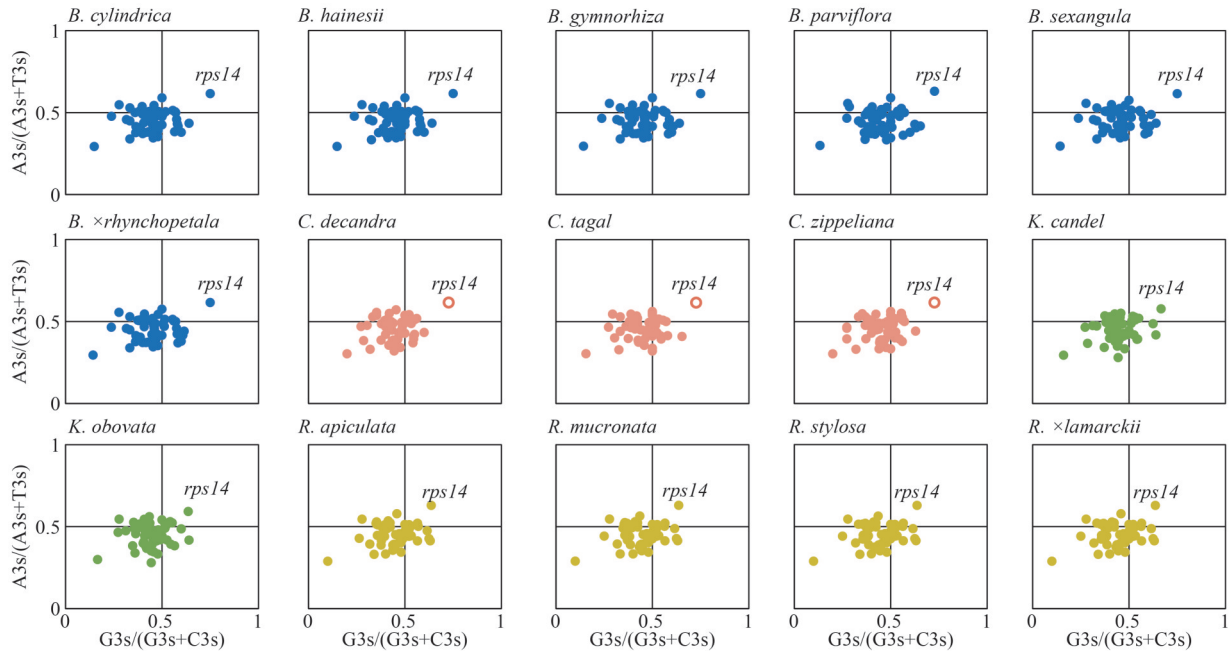


Fig.6 PR2-plot analysis of chloroplast CDSs from 15 Rhizophoraceae mangrove species

Species belonging to the same genus are represented by the same color. In each coordinate system, dot colors (blue, pinkish-orange, green, yellow) correspond to *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora*, respectively. The center point (0.5, 0.5) indicates theoretical neutral equilibrium. The hollow circle represents the outlier gene *rps14* from the *Ceriops* species, while solid dots indicate other genes. A3s/T3s/G3s/C3s: A/T/G/C content at the synonymous third codon position.

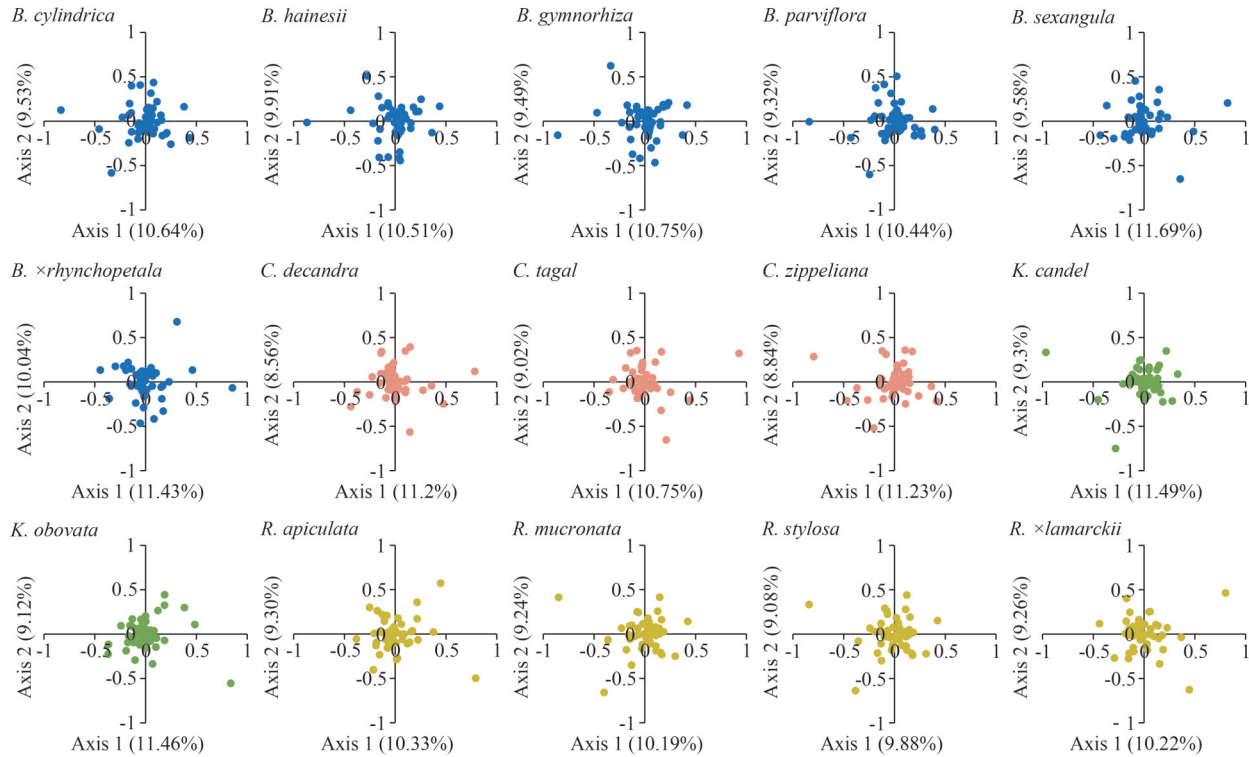


Fig.7 Correspondence analysis of chloroplast CDSs from 15 mangrove species within the Rhizophoraceae family

Species belonging to the same genus are represented by the same color. In each coordinate system, dot colors (blue, pinkish-orange, green, yellow) correspond to *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora*, respectively. Most genes clustered along with Axis 1 and Axis 2. The percentages represent the contributions of Axis 1 and Axis 2 to codon usage bias, respectively.

Table 1 Spearman correlation of Axis 1 with ENC and nucleotide composition

Axis 1	ENC	GCall	GC3s	A3s	C3s	G3s	T3s
<i>B. cylindrica</i>	0.246	0.209	0.299*	-0.002	0.399**	-0.046	-0.217
<i>B. hainesii</i>	0.012	0.230	0.173	0.057	0.372**	-0.187	-0.181
<i>B. gymnorhiza</i>	0.259	0.246	0.337*	-0.042	0.381**	-0.060	-0.201
<i>B. parviflora</i>	0.315*	0.141	0.228	0.136	0.213	0.060	-0.266
<i>B. sexangula</i>	-0.205	-0.280	-0.357*	0.007	-0.497***	0.134	0.309*
<i>B. ×rhynchoptala</i>	-0.254	-0.243	-0.311*	-0.071	-0.366**	0.057	0.305*
<i>C. decandra</i>	-0.210	-0.187	-0.377**	-0.020	-0.354*	-0.156	0.310*
<i>C. tagal</i>	-0.225	-0.080	-0.330*	-0.006	-0.242	-0.220	0.212
<i>C. zippeliana</i>	0.230	0.166	0.371**	0.017	0.396**	0.092	-0.326*
<i>K. candel</i>	-0.157	0.375**	0.092	-0.021	0.378**	-0.301*	-0.123
<i>K. obovata</i>	-0.039	-0.394**	-0.234	0.137	-0.511***	0.220	0.086
<i>R. apiculata</i>	-0.226	-0.344*	-0.380**	0.18	0.533***	0.040	0.120
<i>R. mucronata</i>	0.156	0.253	0.297*	-0.07	0.448***	-0.068	-0.173
<i>R. stylosa</i>	0.210	0.239	0.292*	-0.00	0.383**	-0.018	-0.215
<i>R. ×lamarckii</i>	-0.184	-0.319*	-0.345*	0.13	-0.484***	0.050	0.148

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; GC1, GC2, GC3: the GC content at the first, second, and third positions; GCall: the total GC content; GC3s: GC content at the third synonymous codon position; A3s, T3s, G3s, C3s: A/T/G/C content at the synonymous third codon position; ENC: effective number of codons..

selection, we computed Ka/Ks ratios for 72 conserved chloroplast genome PCGs between 15 mangrove species and *Pellacalix yunnanensis*, their non-mangrove relative in Rhizophoraceae. The

Ka/Ks scatter plot and overall Ka/Ks distribution histogram demonstrated that most genes display Ka/Ks ratios below 0.5, suggesting strong functional constraints and purifying selection in most chloroplast genome genes evolution within the Rhizophoraceae mangrove species (Fig.8a & b). In contrast, the Ka/Ks ratios of *atpF*, *rpl20*, *rpl33*, *rps8*, *rps14*, *rps15*, *rps18*, *rps19*, and *ycf2* were greater than one in some certain species pairs, indicating of putative positive selection during their evolutionary process (Fig.8c). Particularly, the Ka/Ks ratios of gene *rps18* between all 15 Rhizophoraceae mangroves and *P. yunnanensis* ranged from 1.153 to 1.807, with the average value of 1.367, suggesting the gene *rps18* being under positive selection and potential adaptive evolution in Rhizophoraceae mangrove species against their terrestrial relative.

3.8 Phylogenetic analysis and cluster analysis

Phylogenetic reconstruction based on common protein-coding genes from 15 Rhizophoraceae chloroplast genomes revealed well-established

evolutionary relationships and highly supported monophyletic groups for the genera *Bruguiera*, *Ceriops*, *Kandelia*, and *Rhizophora* (Fig.9a), respectively. All species within the same genus clustered together into well-defined clades, distinctly separated from one another, with the outgroup taxon *Excoecaria agallocha* exhibiting the expected phylogenetic relationships. *Ceriops* and *Kandelia* species presented a close genetic relationship and formed a sister branch, then clustered together with *Rhizophora* species, while *Bruguiera* species were more distantly related to these species. In addition, the origin and evolution of hybrid species were also elucidated in this phylogenetic tree, with close relationships between *B. ×rhynchoptala* and *B. sexangular*, and between *R. ×lamarckii* and *R. stylosa*. Considering the distinct codon usage performance of *rps14* and *rps18*, a phylogenetic tree based on the concatenated sequences of *rps14* and *rps18* was also constructed, with a very similar topological structure (Fig.9a), demonstrating the potential application of *rps14* and

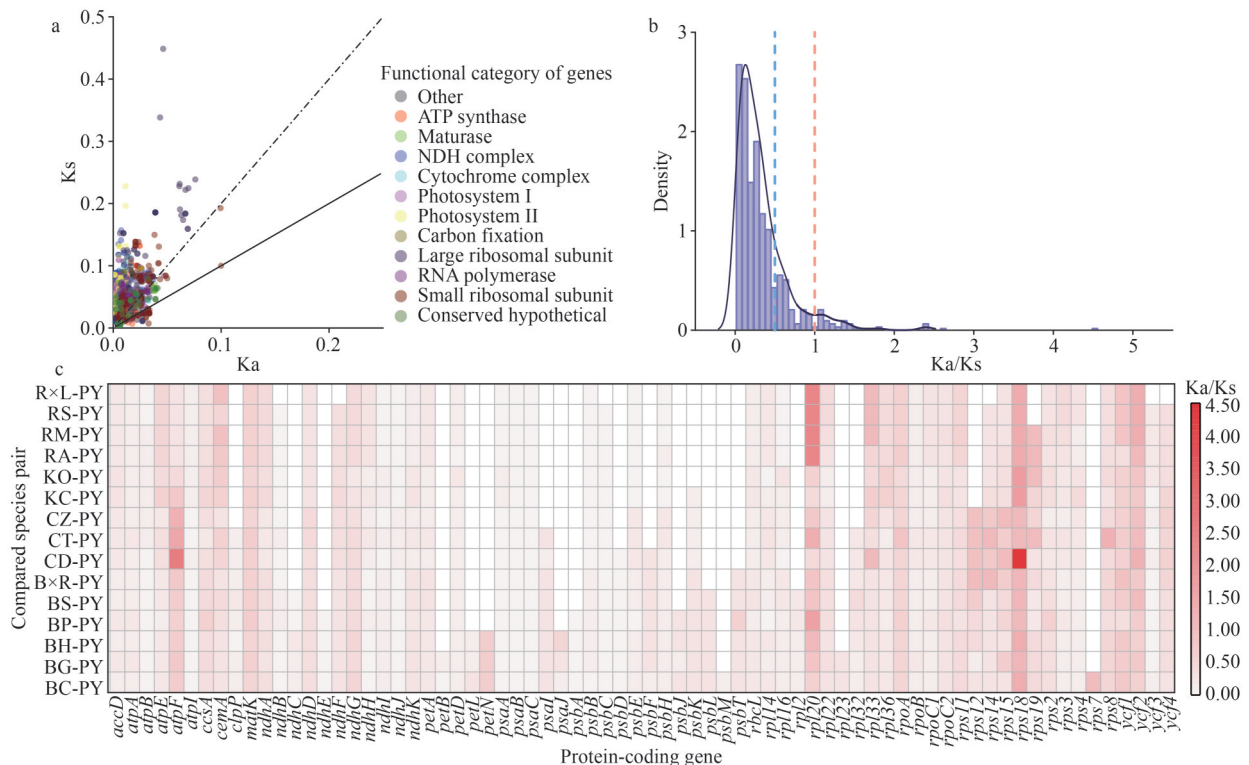


Fig.8 Evolutionary selection pressure analysis of chloroplast protein-coding genes (PCGs)

a. Ks versus Ka scatter plot with points colored by gene family. Genes with Ka/Ks exceeding 1 are positioned above the solid line; while those with a Ka/Ks ratio ranging from 0.5 to 1 are situated between the solid and dashed lines; b. overall Ka/Ks distribution histogram. The dashed lines represent the Ka/Ks ratios of 0.5 and 1, respectively; c. Ka/Ks ratios of chloroplast PCGs between Rhizophoraceae mangrove species and *Pellacalix yunnanensis*. BC: *B. cylindrica*; BH: *B. hainesii*; BG: *B. gymnorrhiza*; BP: *B. parviflora*; BS: *B. sexangular*; B×R: *B. ×rhynchoptala*; CD: *C. decandra*; CT: *C. tagal*; CZ: *C. zippeliana*; KC: *K. candel*; KO: *K. obovate*; RA: *R. apiculata*; RM: *R. mucronata*; RS: *R. stylosa*; R×L: *R. ×lamarckii*; PY: *P. yunnanensis*. Ka: non-synonymous substitution; Ks: synonymous (Ks) substitution, Ka/Ks: the ratio of non-synonymous and synonymous substitution.

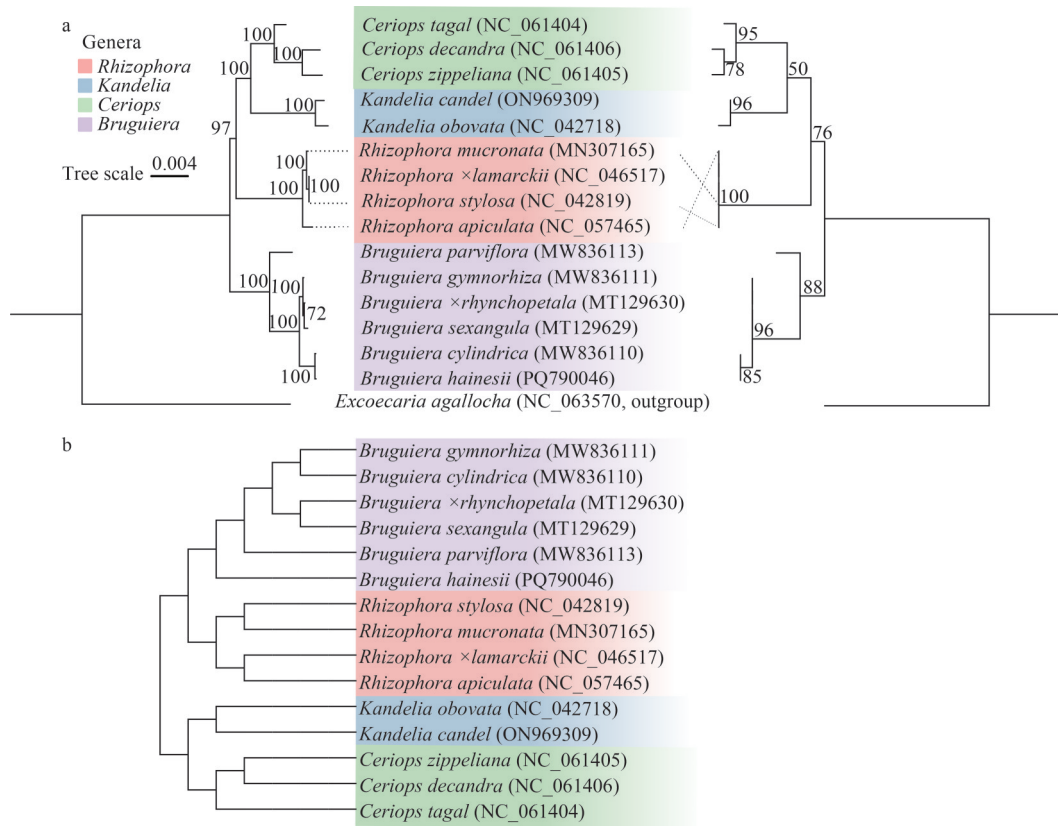


Fig.9 Phylogenetic and cluster analysis of 15 Rhizophoraceae mangrove species

a. phylogenetic analysis of all shared chloroplast protein-coding sequences (left) and concatenated sequence of *rps14* and *rps18* (right). The numbers displayed on each branch node represent the bootstrap support values (1 000 replicates). Dashed lines highlight branches with topological differences between the two trees; b. cluster analysis based on the RSCU values of selected chloroplast CDSs.

rps18 in evolutionary relationship research among species within the Rhizophoraceae family.

Complementing the phylogenetic tree topology, the clustering tree based on RSCU values divided 15 Rhizophoraceae species into four primary branches (Fig.9b). Species from the same genus all clustered together, suggesting conserved patterns of codon usage among closely related taxa. *C. decandra* clustered together with *C. zippeliana*, and then clustered with *C. tagal*, forming the *Ceriops* branch. The larger group formed by *Ceriops* and *Kandelia*, as well as by *Bruguiera* and *Rhizophora*, indicated similarities in the codon usage patterns within these pairs. However, there were some differences between the clustering tree and the phylogenetic tree. For example, in the clustering tree, the hybrid species *B. ×rhynchoptala* consistently clustered with *B. sexangular*, whereas *R. ×lamarckii* formed a subclade with *R. apiculata* rather than *R. stylosa*. Taken together, the results of phylogenetic and cluster analyses based on common CDSs, combined *rps14* and *rps18*, and RSCU values complement each other, providing a more robust and authentic

representation of the evolutionary relationships within Rhizophoraceae species.

4 DISCUSSION

Codons serve as the fundamental link that translates genetic information from nucleic acids into amino acid sequences. Investigation of the codon usage bias offers valuable insights into gene expression, protein translation, and genome evolution (Wang et al., 2018). Codon usage patterns have been well characterized in diverse organisms, including animals, green plants, algae, fungi, and viruses (Singh and Tyagi, 2017; Wu et al., 2023; Zhao and Zhang, 2024; Fang et al., 2025; Xue et al., 2025). The availability of chloroplast genome sequences for Rhizophoraceae mangrove plants has established a fundamental basis for comprehensive analyses in their chloroplast genomes. This study presents the first comprehensive analysis of chloroplast genomes from 15 Rhizophoraceae mangrove species, delivering substantial understanding of codon usage tendencies and the evolutionary forces shaping their

genetic architecture.

To characterize their basic features, we first analyzed nucleotide composition and RSCU values. Our results demonstrated that the GC composition followed a descending trend of $0.5 > GC1 > GC2 > GC3 > GC4$. The GC1 remained consistently below 40%, while the GC3 was consistently below 30% across all 15 mangrove species. This low GC content, particularly at the third position of codons, suggests a pronounced bias toward nucleotides A and U at the end of codons. This aligns with the overall A/U bias commonly noted in the chloroplast genomes across a wide range of plant species, such as those in the Combretaceae mangroves, Gracilariaceae, and Rutaceae families (Li et al., 2024; Shen et al., 2024; Zhong et al., 2024). RSCU analysis identified 30–31 high-frequency codons and 13–19 optimal codons, revealing a highly conserved pattern of codon usage, which supports the hypothesis that closely related species or those inhabiting similar environments tend to exhibit comparable codon preferences (Sharp et al., 1988; Parvathy et al., 2022). Despite these overall similarities, certain optimal codons, namely AAA, AGU, GAA, and UUU, were shared among 13 species but were absent in two species of the genus *Kandelia*. Instead, CAU serves as an optimal codon exclusively in *Kandelia*, while UAU is uniquely preferred as an optimal codon in *Ceriops*. Overall, the analysis indicates that although Rhizophoraceae species maintain broadly consistent codon usage patterns in their chloroplast genomes, distinct genera within this family display unique and identifiable preferences in codon selection, reflecting genus-specific evolutionary adaptations.

Such codon usage patterns can be influenced by multiple biological factors, such as neutral mutation, natural selection, tRNA abundance, GC composition, and gene expression levels (Camiolo et al., 2015; Meyer, 2021; Li et al., 2024). Among these, mutation and natural selection are considered as the major driving forces (Hershberg and Petrov, 2008). Our results suggest that the CUB observed in these species arises from a combination of mutation pressure and natural selection, with mutation pressure playing a dominant role in shaping the overall pattern. Interestingly, the *rps14* gene, which showed the most pronounced CUB ($ENC < 35$), consistently demonstrated that the dominant evolutionary force shaping its CUB is natural selection, not mutation. Similar patterns have been reported in the chloroplast genomes of other plants,

such as Oil-Tea *Camellia* species and Willo *Salix matsudana* (Chen et al., 2023; Zou et al., 2026). This pronounced bias suggests that the evolutionary constraints acting on *rps14* are distinctly different from those on other chloroplast genes.

The selection pressure analysis of Rhizophoraceae mangroves compared to their non-mangrove relative within the same family revealed a heterogeneous pattern of selective pressures across chloroplast genomes. Several genes (*atpF*, *rpl20*, *rpl33*, *rps8*, *rps14*, *rps15*, *rps18*, *rps19*, *ycf2*) with Ka/Ks ratios > 1 in certain species pairs were identified, suggesting relaxed selective constraints during their evolution. This set of genes differs from those previously reported under positive selection in this mangrove lineage when compared to their assumed ancestors (e.g., *Erythroxylum novogranatense*, *Ctenolophon englerianus*, *Averrhoa carambola*, *Vitis rotundifolia*), where *rps7*, *rpl23*, and *rpl36* were highlighted (Han et al., 2020; Ruang-Areerate et al., 2021). Our intra-family comparison might capture recent adaptations specific to this mangrove lineage, whereas the cited studies, involving deeper evolutionary comparisons to ancestral nodes, may reflect more ancient adaptive events during the initial transition to mangrove habitats.

Particularly noteworthy is the *rps18* gene, which exhibited $Ka/Ks > 1$ in all selected species, indicating a strong and conserved selective pressure on this ribosomal protein gene in the Rhizophoraceae mangrove lineage. The positive selection signal detected in *rps18*, as well as the pronounced codon usage bias in *rps14*, may carry meaningful functional implications for mangrove adaptation to stressful environments. Both genes encode core components of the chloroplast translational apparatus. RPS14 is a 30S ribosomal subunit protein that binds directly to 16S rRNA and is essential for the assembly of 30S particles; In chloroplasts, *rps14* is co-transcribed with the photosystem I core genes *psaA* and *psaB* as part of a tricistronic operon, linking ribosomal biogenesis with photosynthetic complex assembly (Kirsch et al., 1986). RPS18, also a component of the chloroplast small ribosomal subunit, has been confirmed to be indispensable for plastid ribosome function—deletion of *rps18* in tobacco resulted in non-viable homoplasmic plants, indicating that this gene is essential for cell survival (Rogalski et al., 2006). The functional centrality of these ribosomal proteins likely places them under stringent selective pressure to maintain translational efficiency and accuracy, especially in mangrove

habitats characterized by high salinity, hypoxia, and fluctuating tidal conditions (Robles and Quesada, 2022). Previous studies have shown that ribosomal protein genes, including plastid ribosomal proteins (PRPs), are key contributors to salinity tolerance in plants (Omidbakhshfard et al., 2012; Xu et al., 2013; Lelekami et al., 2025). Under such conditions, even minor enhancements in translation efficiency can confer a significant adaptive advantage.

Building upon the pronounced adaptive signature of *rps14*, together with the signal of positive selection detected in *rps18*, it becomes evident that these two ribosomal protein genes, while functionally associated, have likely followed distinct evolutionary trajectories to contribute to mangrove environmental adaptation. The strong codon preference in *rps14*, shaped by natural selection, suggests a strategy centered on translational optimization by enhancing the efficiency and accuracy of protein synthesis under stress. In contrast, the positive selection acting on *rps18* in Rhizophoraceae mangroves, compared with their closely related terrestrial species, points to an adaptive mechanism driven by structural or functional innovation at the protein level. This divergence highlights the complex and multifaceted adaptive response within the chloroplast genomes of Rhizophoraceae mangroves. Further investigations are required to elucidate how such complementary evolutionary strategies collectively enhance fitness in dynamic intertidal habitats.

Turn to the whole-genome level, the observed A/U-rich and overall weak codon usage bias may further complement the adaptive strategies exemplified by *rps14* and *rps18*. The prevalence of A/U-ending codons likely reduces mRNA secondary structure stability, which could facilitate ribosome binding and elongation under energy-limited conditions, for instance, during tidal submergence when photosynthesis is suppressed (Fages-Lartaud et al., 2022; Wang et al., 2022a). On the other hand, a weak codon usage bias allows the use of a broader range of tRNAs during protein synthesis, rather than relying on a few preferred tRNA species. This feature may help mangroves sustain translation flexibility under fluctuating intertidal conditions (Gingold et al., 2012; Chan et al., 2018). Tyagi et al. (2023) showed that in rice, stress conditions are associated with distinct codon usage patterns correlated with gene expression levels. Thus, while certain genes such as *rps14* have evolved a strong codon preference to enhance translational efficiency under stress, and *rps18* shows signs of structural or

functional innovation at the protein level, the A/U-rich and overall weak codon usage bias across the chloroplast genomes within the Rhizophoraceae family could make their translation system more tolerant to sudden stresses in the intertidal zone—such as salinity shifts, hypoxia, or high UV exposure. This combination likely represents a strategy that balances translational efficiency with functional robustness in response to recurring environmental challenges.

We then reconstructed phylogenetic trees using the concatenated CDSs of chloroplast genomes shared by all 15 Rhizophoraceae mangrove species, as well as the concatenated sequence of *rps14* and *rps18*. Both trees exhibited congruent topologies at the genus level, strongly supporting the established taxonomic relationships within the Rhizophoraceae family. To assess the robustness of our topology, we compared them with previously published phylogenies of Rhizophoraceae based on various molecular markers (e.g., complete chloroplast genomes, nuclear ITS, and multi-gene datasets). Xu et al. (2017) and Shi et al. (2020) recovered a monophyletic *Rhizophora* and a close relationship between *Ceriops* and *Kandelia*, both of which are consistent with our topology. Zhang et al. (2023) further confirmed that *Bruguiera* is more distantly related to the other three genera, a pattern also observed in our trees. This consistency further supports the reliability of using chloroplast protein-coding genes for phylogenetic inference in Rhizophoraceae. To our knowledge, this is the first report employing the *rps14* and *rps18* gene for phylogenetic analysis within the Rhizophoraceae family. The robust phylogenetic tree obtained validates the effectiveness of this combined sequence as a molecular marker for evolutionary studies in this group, indicating its broad potential for application in the systematics of this family.

Cluster analysis based on RSCU values across chloroplast genomes revealed a clustering pattern highly consistent with the phylogenies inferred from sequence data. All species from the same genus formed distinct clusters, confirming that closely related species tend to share similar patterns of codon usage (Wang et al., 2022b). This congruence demonstrates that synonymous codon usage patterns contain phylogenetic signals comparable to nucleotide substitutions and can serve as a reliable indicator for inferring evolutionary relationships at a higher taxonomic level. Therefore, the clustering analysis based on RSCU values can serve as a

complementary approach to traditional molecular taxonomy and phylogenetic studies of Rhizophoraceae species.

5 CONCLUSION

This study presents the first comparative and comprehensive analysis of codon usage patterns, phylogenetic relationships, and evolutionary dynamics in the chloroplast genomes of 15 Rhizophoraceae mangrove species. Our results revealed an overall weak codon usage bias, which is driven by multiple factors, primarily mutation pressure and natural selection. In addition, we identified 30–31 high-frequency codons and 13–19 optimal codons, most of which end with A/U. Among all the genes analyzed, the *rps14* gene demonstrated the strongest codon usage bias ($ENC < 35$), primarily driven by natural selection that optimizes its expression to ensure the efficient and accurate synthesis of the ribosome, whereas the *rps18* gene exhibited significant positive selection across all species relative to their terrestrial relatives based on Ka/Ks analysis, suggesting it may be undergoing potential adaptive evolution within Rhizophoraceae mangrove species. Phylogenetic analysis based on common CDSs, concatenated sequences of *rps14* and *rps18*, as well as cluster analysis RSCU values, complement each other and provide a more comprehensive and reliable depiction of the evolutionary relationships within the Rhizophoraceae family, suggesting the potential application of *rps14* and *rps18* genes, along with codon usage patterns of chloroplast genomes in molecular taxonomy and phylogenetic studies. These findings could provide valuable information for further evolutionary analyses of chloroplast genomes in mangrove species.

6 DATA AVAILABILITY STATEMENT

The chloroplast genomic sequences involved in this paper are publicly accessible on NCBI (<https://www.ncbi.nlm.nih.gov/>) and listed in the Supplementary Table S1. Other data generated or analyzed during this research are available within the main text and supplementary materials.

References

- Akram H, Hussain S, Mazumdar P et al. 2023. Mangrove health: a review of functions, threats, and challenges associated with mangrove management practices. *Forests*, **14**(9): 1698, <https://doi.org/10.3390/f14091698>.
- Asaf S, Khan A L, Numan M et al. 2021. Mangrove tree (*Avicennia marina*): insight into chloroplast genome evolutionary divergence and its comparison with related species from family Acanthaceae. *Scientific Reports*, **11**(1): 3586, <https://doi.org/10.1038/s41598-021-83060-z>.
- Camiolo S, Melito S, Porceddu A. 2015. New insights into the interplay between codon bias determinants in plants. *DNA Research*, **22**(6): 461–470, <https://doi.org/10.1093/dnares/dsv027>.
- Chan C, Pham P, Dedon P C et al. 2018. Lifestyle modifications: coordinating the tRNA epitranscriptome with codon bias to adapt translation during stress responses. *Genome Biology*, **19**(1): 228, <https://doi.org/10.1186/s13059-018-1611-1>.
- Chen J, Ma W Q, Hu X W et al. 2023. Synonymous codon usage bias in the chloroplast genomes of 13 oil-tea *Camellia* samples from South China. *Forests*, **14**(4): 794, <https://doi.org/10.3390/f14040794>.
- Clarke R T, Greenacre M J. 1985. Theory and applications of correspondence analysis. *Journal of Animal Ecology*, **54**(3): 1031, <https://doi.org/10.2307/4399>.
- Duret L, Mouchiroud D. 1999. Expression pattern and, surprisingly, gene length shape codon usage in *Caenorhabditis*, *Drosophila*, and *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **96**(8): 4482–4487, <https://doi.org/10.1073/pnas.96.8.4482>.
- Fages-Lartaud M, Hundvin K, Hohmann-Marriott M F. 2022. Mechanisms governing codon usage bias and the implications for protein expression in the chloroplast of *Chlamydomonas reinhardtii*. *The Plant Journal*, **112**(4): 919–945, <https://doi.org/10.1111/tpj.15970>.
- Fang J, Qin L M, Liu H N et al. 2025. Analysis of codon usage bias of 30 chloroplast genomes in *Ulva* (Ulvophyceae, Chlorophyta). *Genes*, **16**(5): 608, <https://doi.org/10.3390/genes16050608>.
- Fang Z S, Li D F, Murong H E et al. 2024. Comparative plastome analysis between endangered mangrove species *Acanthus ebracteatus* and *Acanthus* relatives provides insights into its origin and adaptive evolution. *Ecology and Evolution*, **14**(11): e70566, <https://doi.org/10.1002/ece3.70566>.
- Gao W, Chen X D, He J et al. 2024. Intraspecific and interspecific variations in the synonymous codon usage in mitochondrial genomes of 8 *Pleurotus* strains. *BMC Genomics*, **25**(1): 456, <https://doi.org/10.1186/s12864-024-10374-3>.
- Geng X S, Huang N, Zhu Y L et al. 2022. Codon usage bias analysis of the chloroplast genome of cassava. *South African Journal of Botany*, **151**: 970–975, <https://doi.org/10.1016/j.sajb.2022.11.022>.
- Gingold H, Dahan O, Pilpel Y. 2012. Dynamic changes in translational efficiency are deduced from codon usage of the transcriptome. *Nucleic Acids Research*, **40**(20): 10053–10063, <https://doi.org/10.1093/nar/gks772>.
- Hameed A, Ahmed M Z, Hussain T et al. 2021. Effects of salinity stress on chloroplast structure and function. *Cells*, **10**(8): 2023, <https://doi.org/10.3390/cells10082023>.
- Han K, Shi C C, Li L W et al. 2020. Lineage-specific

- evolution of mangrove plastid genomes. *The Plant Genome*, **13**(2): e20019, <https://doi.org/10.1002/tpg2.20019>.
- Hershberg R, Petrov D A. 2008. Selection on codon bias. *Annual Review of Genetics*, **42**: 287-299, <https://doi.org/10.1146/annurev.genet.42.110807.091442>.
- Howai N. 2023. Mangrove ecosystem services in Tobago: challenges, uses and future prospects. *Farm and Business-The Journal of the Caribbean Agro-Economic Society*, **15**(1): 10-21, <https://doi.org/10.22004/ag.econ.339780>.
- Hu X, Li Y Q, Meng F X et al. 2024. Analysis of chloroplast genome characteristics and codon usage bias in 14 species of Annonaceae. *Functional & Integrative Genomics*, **24**(3): 109, <https://doi.org/10.1007/s10142-024-01389-w>.
- Juncosa A M, Tomlinson P B. 1988. Systematic comparison and some biological characteristics of Rhizophoraceae and Anisophylleaceae. *Annals of the Missouri Botanical Garden*, **75**: 1296-1318, <https://doi.org/10.2307/2399287>.
- Kelleway J J, Saintilan N, Macreadie P I et al. 2016. Seventy years of continuous encroachment substantially increases 'blue carbon' capacity as mangroves replace intertidal salt marshes. *Global Change Biology*, **22**(3): 1097-1109, <https://doi.org/10.1111/gcb.13158>.
- Kirsch W, Seyer P, Herrmann R G. 1986. Nucleotide sequence of the clustered genes for two P₇₀₀ chlorophyll *a* apoproteins of the photosystem I reaction center and the ribosomal protein S14 of the spinach plastid chromosome. *Current Genetics*, **10**(11): 843-855, <https://doi.org/10.1007/BF00418531>.
- Lee S Y, Primavera J H, Dahdouh-Guebas F et al. 2014. Ecological role and services of tropical mangrove ecosystems: a reassessment. *Global Ecology and Biogeography*, **23**(7): 726-743, <https://doi.org/10.1111/geb.12155>.
- Lelekami M A, Pahlevani M H, Zaynali Nezhad K et al. 2025. Transcriptome and network analysis pinpoint ABA and plastid ribosomal proteins as main contributors to salinity tolerance in the rice variety, CSR28. *PLoS One*, **20**(4): e0321181, <https://doi.org/10.1371/journal.pone.0321181>.
- Li N, Li Y Y, Zheng C C et al. 2016. Genome-wide comparative analysis of the codon usage patterns in plants. *Genes & Genomics*, **38**(8): 723-731, <https://doi.org/10.1007/s13258-016-0417-3>.
- Li T T, Ma Z, Ding T M et al. 2024. Codon usage bias and phylogenetic analysis of chloroplast genome in 36 gracilariaceae species. *Functional & Integrative Genomics*, **24**(2): 45, <https://doi.org/10.1007/s10142-024-01316-z>.
- Li X J, Liu L, Ren Q D et al. 2025. Analysis of synonymous codon usage bias in the chloroplast genome of five *Caragana*. *BMC Plant Biology*, **25**(1): 322, <https://doi.org/10.1186/s12870-025-06351-x>.
- Meyer M M. 2021. Revisiting the relationships between genomic G+C content, RNA secondary structures, and optimal growth temperature. *Journal of Molecular Evolution*, **89**(3): 165-171, <https://doi.org/10.1007/s00239-020-09974-w>.
- Omidbakhshfard M A, Omranian N, Ahmadi F S et al. 2012. Effect of salt stress on genes encoding translation-associated proteins in *Arabidopsis thaliana*. *Plant Signaling & Behavior*, **7**(9): 1095-1102, <https://doi.org/10.4161/psb.21218>.
- Orr H A. 1999. The evolutionary genetics of adaptation: a simulation study. *Genetics Research*, **74**(3): 207-214, <https://doi.org/10.1017/S0016672399004164>.
- Parvathy S T, Udayasuriyan V, Bhadana V. 2022. Codon usage bias. *Molecular Biology Reports*, **49**(1): 539-565, <https://doi.org/10.1007/s11033-021-06749-4>.
- Rahman, Lokollo F F, Manuputty G D et al. 2024. A review on the biodiversity and conservation of mangrove ecosystems in Indonesia. *Biodiversity and Conservation*, **33**(3): 875-903, <https://doi.org/10.1007/s10531-023-02767-9>.
- Robles P, Quesada V. 2022. Unveiling the functions of plastid ribosomal proteins in plant development and abiotic stress tolerance. *Plant Physiology and Biochemistry*, **189**: 35-45, <https://doi.org/10.1016/j.plaphy.2022.07.029>.
- Rogalski M, Ruf S, Bock R. 2006. Tobacco plastid ribosomal protein S18 is essential for cell survival. *Nucleic Acids Research*, **34**(16): 4537-4545, <https://doi.org/10.1093/nar/gkl634>.
- Ruang-Areerate P, Kongkachana W, Naktang C et al. 2021. Complete chloroplast genome sequences of five *Bruguiera* species (Rhizophoraceae): comparative analysis and phylogenetic relationships. *PeerJ*, **9**: e12268, <https://doi.org/10.7717/peerj.12268>.
- Sharp P M, Cowe E, Higgins D G et al. 1988. Codon usage patterns in *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila melanogaster* and *Homo sapiens*; a review of the considerable within-species diversity. *Nucleic Acids Research*, **16**(17): 8207-8211, <https://doi.org/10.1093/nar/16.17.8207>.
- Shen L W, Chen S Q, Liang M et al. 2024. Comparative analysis of codon usage bias in chloroplast genomes of ten medicinal species of Rutaceae. *BMC Plant Biology*, **24**(1): 424, <https://doi.org/10.1186/s12870-024-04999-5>.
- Shi C C, Han K, Li L W et al. 2020. Complete chloroplast genomes of 14 mangroves: phylogenetic and comparative genomic analyses. *BioMed Research International*, **2020**(1): 8731857, <https://doi.org/10.1155/2020/8731857>.
- Sidik F, Supriyanto B, Krisnawati H et al. 2018. Mangrove conservation for climate change mitigation in Indonesia. *WIREs Climate Change*, **9**(5): e529, <https://doi.org/10.1002/wcc.529>.
- Singh N K, Tyagi A. 2017. A detailed analysis of codon usage patterns and influencing factors in Zika virus. *Archives of Virology*, **162**(7): 1963-1973, <https://doi.org/10.1007/s00705-017-3324-2>.
- Soeprbowati T R, Sularto R B, Hadiyanto H et al. 2024. The carbon stock potential of the restored mangrove ecosystem of Pasarbanggi, Rembang, Central Java. *Marine Environmental Research*, **193**: 106257, <https://doi.org/10.1016/j.marenvres.2023.106257>.
- Sueoka N. 1999. Translation-coupled violation of Parity Rule 2 in human genes is not the cause of heterogeneity of the DNA G+C content of third codon position. *Gene*, **238**(1): 53-58, [https://doi.org/10.1016/s0378-1119\(99\)00320-0](https://doi.org/10.1016/s0378-1119(99)00320-0).
- Sueoka N. 2001. Near homogeneity of PR2-bias fingerprints in

- the human genome and their implications in phylogenetic analyses. *Journal of Molecular Evolution*, **53**(4-5): 469-476, <https://doi.org/10.1007/s002390010237>.
- Tyagi S, Jung J A, Kim J S et al. 2020. Comparative analysis of the complete chloroplast genome of mainland *Aster spathulifolius* and other *Aster* species. *Plants*, **9**(5): 568, <https://doi.org/10.3390/plants9050568>.
- Tyagi S, Kabade P G, Gnanapragasam N et al. 2023. Codon usage provide insights into the adaptation of rice genes under stress condition. *International Journal of Molecular Sciences*, **24**(2): 1098, <https://doi.org/10.3390/ijms24021098>.
- Van Vliet G J C M. 1976. Wood anatomy of the Rhizophoraceae. *Leiden Botanical Series*, **3**(1): 20-75, <https://repository.naturalis.nl/pub/508220>.
- Wang C W, Wong S L, Liao T S et al. 2022a. Photosynthesis in response to salinity and submergence in two Rhizophoraceae mangroves adapted to different tidal elevations. *Tree Physiology*, **42**(5): 1016-1028, <https://doi.org/10.1093/treephys/tpab167>.
- Wang L Y, Xing H X, Yuan Y C et al. 2018. Genome-wide analysis of codon usage bias in four sequenced cotton species. *PLoS One*, **13**(3): e0194372, <https://doi.org/10.1371/journal.pone.0194372>.
- Wang X M, Chen Z T, Sui N. 2024. Sensitivity and responses of chloroplasts to salt stress in plants. *Frontiers in Plant Science*, **15**: 1374086, <https://doi.org/10.3389/fpls.2024.1374086>.
- Wang Z J, Cai Q W, Wang Y et al. 2022b. Comparative analysis of codon bias in the chloroplast genomes of Theaceae species. *Frontiers in Genetics*, **13**: 824610, <https://doi.org/10.3389/fgene.2022.824610>.
- Wright F. 1990. The 'effective number of codons' used in a gene. *Gene*, **87**(1): 23-29, [https://doi.org/10.1016/0378-1119\(90\)90491-9](https://doi.org/10.1016/0378-1119(90)90491-9).
- Wu P, Xiao W Q, Luo Y Y et al. 2023. Comprehensive analysis of codon bias in 13 *Ganoderma* mitochondrial genomes. *Frontiers in Microbiology*, **14**: 1170790, <https://doi.org/10.3389/fmicb.2023.1170790>.
- Wu X K, Xu M Z, Yang J R et al. 2024. Genome-wide impact of codon usage bias on translation optimization in *Drosophila melanogaster*. *Nature Communications*, **15**(1): 8329, <https://doi.org/10.1038/s41467-024-52660-4>.
- Xu S H, He Z W, Zhang Z et al. 2017. The origin, diversification and adaptation of a major mangrove clade (Rhizophoraceae) revealed by whole-genome sequencing. *National Science Review*, **4**(5): 721-734, <https://doi.org/10.1093/nsr/nwx065>.
- Xu T, Lee K, Gu L L et al. 2013. Functional characterization of a plastid-specific ribosomal protein PSRP2 in *Arabidopsis thaliana* under abiotic stress conditions. *Plant Physiology and Biochemistry*, **73**: 405-411, <https://doi.org/10.1016/j.plaphy.2013.10.027>.
- Xue Y, Qin S W, Xianyu Z C et al. 2025. Comparative plastome analysis reveals evolutionary dynamics and codon usage patterns in *Bidens* (Asteraceae). *Functional & Integrative Genomics*, **25**(1): 175, <https://doi.org/10.1007/s10142-025-01699-7>.
- Yuan J G, Gu W, Yang H J et al. 2025. Comparative and phylogenetic analyses of the chloroplast genomes of mangrove plants. *Scientific Reports*, **15**(1): 3915, <https://doi.org/10.1038/s41598-024-81894-x>.
- Zhang D, Gao F L, Jakovlić I et al. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, **20**(1): 348-355, <https://doi.org/10.1111/1755-0998.13096>.
- Zhang P P, Xu W B, Lu X et al. 2021. Analysis of codon usage bias of chloroplast genomes in *Gynostemma* species. *Physiology and Molecular Biology of Plants*, **27**(12): 2727-2737, <https://doi.org/10.1007/s12298-021-01105-z>.
- Zhang Y, Yang Y C, He M et al. 2023. Comparative chloroplast genome analyses provide insights into evolutionary history of Rhizophoraceae mangroves. *PeerJ*, **11**: e16400, <https://doi.org/10.7717/peerj.16400>.
- Zhang Z. 2022. KaKs_Calculator 3.0: calculating selective pressure on coding and non-coding sequences. *Genomics, Proteomics & Bioinformatics*, **20**(3): 536-540, <https://doi.org/10.1016/j.gpb.2021.12.002>.
- Zhao S, Deng L H, Chen F. 2020. Codon usage bias of chloroplast genome in *Kandelia obovata*. *Journal of Forest and Environment*, **40**(5): 534-541, <https://doi.org/10.13324/j.cnki.jfcf.2020.05.011>. (in Chinese with English abstract)
- Zhao Y, Zhang S C. 2024. Comparative analysis of codon usage bias in six *Eimeria* genomes. *International Journal of Molecular Sciences*, **25**(15): 8398, <https://doi.org/10.3390/ijms25158398>.
- Zhong C R, He S M, Fang Z S. 2024. Analysis on codon usage bias of chloroplast genomes of three species of mangroves in Combretaceae. *Journal of Plant Resources and Environment*, **33**(2): 1-12, <https://doi.org/10.3969/j.issn.1674-7895.2024.02.01>. (in Chinese with English abstract)
- Zou D J, Guo B R, Liu J Q et al. 2026. Characterization of codon usage bias in the chloroplast genome of *Salix matsudana*. *Journal of Southwest Forestry University*, **46**(1): 74-80, <https://doi.org/10.11929/j.swfu.202503017>. (in Chinese with English abstract)

Electronic supplementary material

Supplementary material (Supplementary Tables S1–S4) is available in the online version of this article at <https://doi.org/10.1007/s00343-026-6037-0>.