Research Article

Chloroplast Genome Sequences and Comparative Analyses of Combretaceae Mangroves with Related Species

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In the Combretaceae family, only two species of Lumnitzera and one species of Laguncularia belong to mangroves. Among them, Lumnitzera littorea (Jack) Voigt. is an endangered mangrove plant in China for the limited occurrence and seed abortion. In contrast, Lumnitzera racemosa Willd., known as the most widespread mangrove plant in China. Laguncularia racemosa C. F. Gaertn., an exotic mangrove in China, has the fast growth and high adaptation ability. To better understand the phylogenetic positions of these mangroves in Combretaceae and in Myrtales and to provide information for studies on evolutionary adaptation for intertidal habitat, the complete chloroplast (cp) genomes of Lu. racemosa and La. racemosa were sequenced. Furthermore, we present here the results from the assembly and annotation of the two cp genomes, which were further subjected to the comparative analysis with Lu. littorea cp genomes we published before and other eleven closely related species within Myrtales. The chloroplast genomes of the three Combretaceae mangrove species: Lu. littorea, Lu. racemosa, and La. racemosa are 159,687 bp, 159,473 bp, and 158,311 bp in size. All three cp genomes host 130 genes including 85 protein-coding genes, 37 tRNAs, and 4 rRNAs. A comparative analysis of those three genomes revealed the high similarity of genes in coding-regions and conserved gene order in the IR and LSC/SSC regions. The differences between Lumnitzera and Laguncularia cp genomes are the locations of rps19 and rpl2 genes in the IR/SC boundary regions. Investigating the effects of selection events on shared protein-coding genes showed a relaxed selection had acted on the ycf2, ycf1, and matK genes of Combretaceae mangroves compared to the nonmangrove species Eucalyptus aromaphloia. The phylogenetic analysis based on the whole chloroplast genome sequence with one outgroup species strongly supported three Combretaceae mangroves together with other two Combretaceae species formed a cluster in Combretaceae. This study is the first report on the comparative analysis of three Combretaceae mangrove chloroplast genomes, which will provide the significant information for understanding photosynthesis and evolution in Combretaceae mangrove plants.

1. Introduction

Mangroves are critical marine resources for their remarkable ability to tolerate seawater and are uniquely adapted to tropical and subtropical coasts [1]. Mangrove forests played an important role in ecosystem services and supported coastal livelihoods, yet they are relatively low in the number of species [2]. In the world, the increasing number of endangered mangrove species has made a huge destruction on the global economy and environment [3, 4]. In the family Combretaceae, only two genera are typical mangrove constituents: Lumnitzera and Laguncularia. Lumnitzera, including 2 species Lumnitzera littorea and Lumnitzera racemosa, is an Asian genus most characteristic of back mangal and ranges from India and Sri Lanka, through the Malesian region to China [5]. Due to natural and human impacts, populations of the two species in this genus have been isolated, fragmented, and highly disturbed [6]. Laguncularia is a monotypic genus and locally dominant in the West African and Caribbean regions of tropical America [5]. In China, two Lumnitzera species are both native mangrove species. Lu. racemosa can be found in almost all mangrove locations with the extensive adaptation [7], but Lu. littorea occurs only on Hainan Island and has become an endangered mangrove species due to its small population size caused by seed abortion [8, 9]. The only species La. racemosa from the other genus was
firstly transplanted in Dongzhai harbor, Hainan, China [10]. Having the fast growing and high adaptation ability, it was used as the pioneer specie for the mangrove restoration in estuarine and coastal regions [11]. However, its invasiveness and the possibility of replacing native mangrove species have been subjects of debate in China and there are disagreements on whether La. racemosa should be planted [12]. Despite the obviously different adaptive capacities to the environment of those three mangrove species, Lumnitzera and Laguncularia are considered to be closely related and could have evolved from a common ancestor [5, 13].

In plants, chloroplast plays an important role in many cell functions, including photosynthesis, carbon fixation, and stress response [14], and is one of three major genetic systems [15]. A large amount of genetic information can get from the chloroplast genome to explore the occurrence, development, evolution of species, and to develop the fields of plant genomics and bioinformatics due to its self-replication mechanism and relatively independent evolution [16]. In angiosperms, the chloroplast genome was found to be a conserved quadripartite structure composing of two copies of inverted repeat (IR), one large single copy (LSC), and one small single copy (SSC) [14]. The chloroplast genome includes 120-130 genes, primarily participating in photosynthesis, transcription, and translation [17]. Recent studies have identified considerable diversity within noncoding intergenic spacer regions, which often include important regulatory sequences [18]. Similar to the genes, the introns in chloroplast genomes of land plants are generally conserved, but the loss of introns within protein-coding genes and tRNA genes has been reported in several plant species [19]. Until now, over 3,665 plants have been sequenced, which has greatly improved chloroplast (cp) genome research (https://www.ncbi.nlm.nih.gov/genome/browse/#/plasmids/). However, there is little genetic and genomic research on plants from the family Combretaceae. Lu. littorea and La. racemosa separately. DNA extraction was isolated from fresh leaf tissue of one individual plant of Lu. racemosa and La. racemosa separately. DNA extraction was used by purelink genomic plant DNA purification kit (Thermo Fisher, China). The extracted DNA was sent to a sequencing company TGS (Shenzhen, China) and sequenced with 350 bp pair-end reads by using an Illumina Hiseq-2000 platform according to the standard protocol at TGS.

2. Materials and Methods

2.1. Plant Materials, DNA Extraction, and Sequencing. Two Combretaceae mangrove samples were collected from Tielu Bay, Sanya, China (18°17' N, 109°44' E). Cp DNA was isolated from fresh leaf tissue of one individual plant of Lu. racemosa and La. racemosa separately. DNA extraction was used by purelink genomic plant DNA purification kit (Thermo Fisher, China). The extracted DNA was sent to a sequencing company TGS (Shenzhen, China) and sequenced with 350 bp pair-end reads by using an Illumina Hiseq-2000 platform according to the standard protocol at TGS.

2.2. De Novo Assembly, Gap Filling, and Genome Annotation. Raw reads were first filtered to obtain the high-quality clean data by removing adaptor sequences and low-quality reads with Q value ≤ 20. SOAP de novo 2.04 (http://soap.denovo.html) was used to perform the initial assembly and obtain the contig sequences. The software GapCloser 1.12 (http://soap.genomics.org.cn/soapdenovo.html) was used to fill the gaps in the frame sequences diagram. The whole framework maps of the cp genomes were obtained by using the reference genes of Lu. littorea (MH551146) with the methods of [16]. The cp genome sequences were annotated using CpgAVAS software with parameters that use default values [23]. The genes in the cp genome were annotated using the DOGMA software. The circular cp genome map was drawn using OGDRAW [26]. Primers were designed (Table S1) to test for correct sequence assembly. PCR amplification was performed according to a previously reported procedure [27].

2.3. Sequence Divergence Analysis. The complete cp genomes of Lu. racemosa and La. racemosa were compared with Lu. littorea by using the mVISTA program in Shuffle-LAGAN mode [28]. We set Lu. littorea as the reference sequence for these comparisons. The borders between single-copy regions (LSC and SSC) and inverted repeats (IR) regions among three Combretaceae cp sequences were compared by using Geneious v11.0.4 software. The sequence divergences in protein-coding genes between the three Combretaceae species were evaluated by using MEGA 7 [29]. To estimate the nucleotide diversity (Pi) values of LSC, SSC, and IR regions of three cp genomes, a sliding window analysis was conducted by using DnaSP 6 [30]. The step size was set to 200 bp, the window length was 600 bp, and the Tamura 3-parameter (T92) model was selected to test the pairwise sequence divergences [31].

In this study, two whole cp genomes of Combretaceae mangroves, Lu. racemosa and La. racemosa, were sequenced by using next-generation sequencing and applying a combination of de novo and reference guided assembly (Lu. littorea (MH551146) as a reference). Using a comparative genomics approach, we analyzed the characteristics of the cp genomes of the three mangrove species from Combretaceae. Nonsynonymous (Ka) and synonymous (Ks) substitution rates of conservative protein-coding genes among the three Combretaceae mangrove cp genomes were calculated to evaluate selection pressures. The coding sequences (CDSs) under selective events were also detected. Furthermore, the phylo-
2.4. Selection Pressure Analysis. Selective pressures were analyzed for consensus protein-coding genes among four Myrtales species (Lu. littorea, Lu. racemosa, La. racemosa, and Eucalyptus aromaphloia (NC_022396.1)). Seventy-six coding sequences (CDSs) longer than 300 bp were kept for the identification of codon usage patterns and then used for the estimation of codon usage using Codon W (http://codonw.sourceforge.net) [32]. Ka/Ks value for each gene was calculated by using the Ka/Ks calculator, and the settings were listed as previous description [32].

2.5. Phylogenomic Analysis. Phylogenomic analysis was performed for seventeen cp genomes including three Combretaceae mangroves in this study. Multiple sequence alignment was performed by using MAFFT software [33] to obtain the aligned chloroplast genomes before constructing the phylogenetic tree. The maximum likelihood tree, neighbor-joining tree, UPGMA tree, and test maximum parsimony tree were constructed using MEGA X [34] with Rhizophora stylosa as outgroup, and a bootstrap test was set to 1000 replicates to calculate each bootstrap value.

3. Results and Discussion

3.1. Assembly and Features of cp Genomes for Three Species. The complete cp genomes of Lu. racemosa and La. racemosa are 159,473 bp and 158,311 bp in length (GenBank accession number: MH551146, MH551145), respectively (Figure 1). The minor differences in length of cp genomes are no more than 214 bp in genus Lumnitzera. The maximum difference in length of cp genomes between the three Combretaceae mangroves is 1,376 bp (Table 1). The typical quadripartite structure of most angiosperms was found in both of the two cp genomes, which comprise a pair of IRs (26,402 bp for Lu. racemosa and 26,156 bp for La. racemosa) separated by the LSC (88,056 bp for Lu. racemosa and 87,023 bp for La. racemosa) and SSC (18,613 bp for Lu. racemosa and 18,887 bp for La. racemosa) regions (Table 1). The cp
genomes of the three Combretaceae mangroves are consider-
able conservation in length, and a previous study showed that
the IR sequence length of some species in the Myrtales is
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the IR sequence length of some species in the Myrtales is
able conservation in length, and a previous study showed that
for the translation initiation factor gene, 
was absent in the three cp 
genomes to nuclear or mitochondrial genomes [17, 37].
were the most reported genes are found from the chloro-
plasts to the nuclear genome [17], is kept in three cp 
genomes to have been deleted from the chloroplast and
assemble into the photosystem I complex to mediate cyclic
electron transport in chloroplasts and play an important role
in facilitating chlororespiration, and in some autotrophic
plants which were found to be lost in cp genomes [24]. In
the three Combretaceae mangroves, all the eleven 
ndh genes

gene trnR-AGG with one intron is found in the cp genome of
Lu. racemosa, but no intron in the cp genome of La. racemosa
(Tables S2 and S3). The majority of reported intron losses
have been observed in specific plant groups or species
including monocots, eudicots, and gymnosperms [17].

3.2. Comparison of cp Genomes of Three Combretaceae 
Mangroves. Gene content and structure are found to be con-
served in the chloroplast genome sequences of most autotro-
phic land plants, but there are still some protein-encoding
genes being absent in some specific species. InfA, rpl22, and
ndh were the most reported genes are found from the chloro-
plast genomes to nuclear or mitochondrial genomes [17, 37].
But in this study, only gene infA was absent in the three cp 
genomes, and the same loss events are found in other Myr-
tales plants [38]. For the translation initiation factor gene,
infA in cp has been lost independently at least 24 times in
angiosperms and evidence provided from some cases sug-
gested functional replacement by a nucleus copy [39]. The
essential gene rpl22, which is reported in 57 chloroplast
organisms to have been deleted from the chloroplast and
transferred to the nuclear genome [17], is kept in three cp
organisms of Combretaceae mangroves. The ndh proteins
assemble into the photosystem I complex to mediate cyclic
electron transport in chloroplasts and play an important role
in facilitating chlororespiration, and in some autotrophic
plants which were found to be lost in cp genomes [24]. In
the three Combretaceae mangroves, all the eleven ndh genes

| Table 2: Genes in the cp genomes of three Combretaceae mangrove species. |
| --- | --- | --- |
| Category | Gene group | Gene name |
| Photosynthesis | Photosystem I | psaA, psaB, psaC, psaI, psaJ |
| | Photosystem II | psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ |
| | Cytochrome b/f complex | petA, petB, petD, petG, petL, petN |
| | ATP synthase | atpA, atpB, atpE, atpF, atpH, atpI |
| | Large subunit of RuBisCo | rbcL |
| | NADH dehydrogenase | ndhA, ndhB, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK |
| | Ribosomal RNA genes | rnr4.5, rnr5, rnr16, rnr23 |
| | Ribosomal RNA genes (SSU) | rps2, rps3, rps4, rps7, rps8, rps12 ’, rps14, rps15, rps16, rps18, rps19 |
| | Ribosomal RNA genes (LSU) | rpl2, rpl14, rpl16, rpl20, rpl22, rpl23, rpl32, rpl33, rpl36 |
| Self-replication | Transfer RNA genes | rpoA, rpoB, rpoC1, rpoC2 |
| | Maturase | trnA-UGC, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnJ-MAU, trnG-UCC, trnH-GUG, trnL-CAU, trnK-UUU, trnL-UAA, trnL-UAG, trnM-CAU, trnN-GUU, trnP-UAG, trnQ-UUG, trnR-AGG, trnR-UCC, trnS-GCU, trnS-GGA, trnT-GGU, trnT-UAG, trnV-GAC, trnV-UCU, trnW-CCA, trnY-GUA |
| | Envelope membrane factor | ccaA |
| | Subunit of acetyl-CoA | accD |
| Other genes | C-type cytochrome synthesis gene | ccsA |
| | Protease | clpP |
| | Hypothetical chloroplast reading | ycf1, ycf2, ycf3, ycf4 |

* Pseudogene.
in cp genomes encoding ndh subunits and involved in photosynthesis [40] were present, consistent with other Myrtle plants such as those in Lythraceae [40].

The whole-genome alignment revealed the high sequence similarity across the three cp genomes, suggesting that Combrutaceae mangrove cp genomes are all conserved (Figure 2). All three cp genomes showed that the single-copy regions are more divergent than the IR regions as observed in other angiosperms [31], which is possibly due to error correction occurring via gene conversion between IRs [41]. Furthermore, the coding regions are more conserved than noncoding regions, as seen in other plants [31]. As found in most other green terrestrial plants [42], the maturase K (matK) in the cp genomes of the three Combretaceae mangrove species is also located within the trnK intron. rpl2 gene is found to be a transspliced gene in the three Combretaceae cp genomes, which was observed to be intron loss in three Lagerstroemia cp genomes and considered to be one of the important evolutionary events in the Lythraceae of the Rosids [40]. The four rRNA genes and two tRNA genes of trnI and trnA are clustered as 16S-trnA-23S-4.5S-SS in the IR region in these three cp genomes and in most other green terrestrial plants [32, 43]. The most divergent coding regions in the three cp genomes were ycf1, rps19, and ndhF. In noncoding regions, the highest sequence divergence among these three cp genomes is regions trnG-UCC/atpA, atpH/apl, ndhC/trnM-CAU, and trnL-UAG/ccsA. These hotspot regions can furnish valuable information for exploring molecular markers for phylogenetic studies and identification of Combretaceae species.

The angiosperms chloroplast genomes are highly conserved, but slightly vary as a result of either expansion or contraction of the single-copy (SC) and IR boundary regions [44]. The IR/SC junction position change caused by the expansion and contraction of IR/SC boundary regions was usually considered as a primary mechanism in creating the length variation of the higher plant cp genomes [40]. In this study, we found the IR/SC junction position change among the cp genomes between Lu. littorea and Lu. racemosa and the high conservation in Lu. littorea genus (Figure 3). The functional ycf1 gene crossed the IRA/SSC boundary creating ycf1 pseudogene fragment at the IRb region in all the genomes as in other land plants [44]. All the ycf1 pseudogene overlapped with the ndhF gene in the SSC and IRA junctions with a stretch of 15 bp, and the ndhF gene is located in SSC regions in all cp genomes. Genes rps19
crossed the IRB/LSC boundary creating \textit{rps19} pseudogene fragment were only found in \textit{Lu. littorea}. However, in \textit{Lu. racemosa}, the \textit{rps19} gene was located in the LSC region, 5 bp apart from the IRB/LSC border. The \textit{rpl2} genes crossed the LSC/IRB and LSC/IRA junction in \textit{Lumnitzera} cp genome but the distances between \textit{rpl2} and the border is 106 bp in both of the two \textit{Lumnitzera} species. Those variations at the IR/SC borders in these three cp genomes contribute to the differences in length of the cp genome sequence as a whole and were found in other plants [31].

3.3. Genome Sequence Divergence among Combretaceae Species. The sequence divergence among the three Combretaceae mangrove cp genomes was investigated by calculating the nucleotide variability (Pi) values within 600 bp windows (200 bp stepwise moving) in LSC, SSC, and IR regions (Figure 4). In the LSC, SSC, and IR regions, the values were found to vary from 0 to 0.22 with a mean of 0.085, from 0 to 0.095 with a mean of 0.063 and from 0 to 0.0169 with a mean of 0.082 separately. These results mean conservation between the three Combretaceae mangrove cp genomes. However, the certain highly variable regions have also been found in \textit{rpoC2} and \textit{ndhC/trnV} with Pi > 0.02 in LSC regions, \textit{trnK/matK} with Pi > 0.08 in SSC regions, and \textit{rpoC1} and \textit{rpoB} with Pi > 0.01 in IRs regions (Figure 4). All those regions or some of them have also been identified as highly variable in other plants, which showed great potential as sources of useful phylogenetic markers for Combretaceae and other plant species [31, 45].

3.4. Selection Events in Protein-Coding Genes. The nonsynonymous (Ka) and synonymous (Ks) substitution ratios (Ka/Ks) were calculated for 78 consensus protein-coding genes to estimate selective pressures in cp genomes of the three Combretaceae and one reference species \textit{E. aromaphloia}, which is a normal land and nonhalophytes plant, to evaluate the selective pressure (Table S4). The average Ka/Ks ratio of the shared genes analyzed across the three cp genomes was 0.1638. Although all of the Ka/Ks values were all less than 1.0 in Figure 5, the Ka/Ks ratio of three genes (\textit{ycf1}, \textit{ycf2}, and \textit{matK}) in all Combretaceae...
mangroves were within the range of 0.5 to 1.0 indicating a relaxed selection [31]. Furthermore, there was one gene rpl22 only found in La. racemosa with the Ka/Ks ration within the range of 0.5 to 1.0. Another gene accD with the Ka/Ks ration is closed to 0.5 in La. racemosa and not in the other two mangroves in Figure 5. The most conserved genes with Ka/Ks values of 0 in the three cp genomes were atpH, petG, petN, psaC, psbE, psbM, psbN, psbT, and rps7, suggesting very strong purifying selection (Table S4). For the none positive selection gene was found in
Combretaceae mangroves cp genome, this may be because adaptive modifications to salt stresses targeting genes in the nucleus were sufficient to maintain homeostasis for photosynthesis since there are a variety of strategies for plants to adapt to the environment, so there is no need for adaptive evolution of chloroplast-encoded genes [46]. But genes under relative selection in cp genomes may play some roles in Combretaceae mangroves evolution and environment adaptation. Ycf genes have proved useful for analyzing cp genome variation in higher plants and algae, even though the function is not thoroughly known [47]. Among Ycf genes, ycf1 and ycf2 are the two largest genes and are located in IR/SC junction and IR region, respectively, almost in all plants [48]. The gene ycf1 encodes a component of the chloroplast’s inner envelope membrane protein translocation [49]. It is also highly variable in terms of phylogenetic information at the level of species, has also been shown to be subject to relative selection with rps12 and matK genes in three Combretaceae mangroves, and has also been identified in many plant lineages [50]. The ycf2 gene in the cp genome is regarded as having one of the fastest evolutionary rates within the cp genome for the lost events [51]. Even though a giant reading frame of ycf2 is believed as unknown function in land plants, which is responsible for the differences in the competitive behavior of plastid genotypes [52]. The nucleotide sequence similarity of ycf2 in land plant is extraordinarily low compared to other plastid-encoded genes, being less than 50% across bryophytes, ferns, and seed plants [53]. The gene matK (maturase K gene) is a plant chloroplast gene [54] and encodes an intron maturase involved in the cutting and splicing of Group II RNA transcriptional introns [55]. By sequence analysis, there are one well-conserved domain X and the remnants of a reverse transcriptase domain in the coding sequence of matK [55]. The matK gene is always found to be located within the intron of the trnK gene (Figure 1) same with some land plants [56]. MatK gene is identified to be essential for plant cell survival, and the expression of it needs to be tightly regulated to prevent detrimental effects and establishes another link between leaf variegation and chloroplast translation [57]. For the studies of plant systematics, the matK gene is the high selectively used gene as the DNA barcoding fragment for plants [58].

Gene rpl5 are ribosome genes that have been proven to be essential for the chloroplast ribosome development in plants [59]. Among them, rpl22 is always found to have a frameshift mutation generated premature termination codons within it in land plants. In some species, rpl22 was also found to be truncated with considerable length variation [60]. For the plastid gene, accD, which encodes the β-carboxyl transferase subunit of acetyl-CoA carboxylase, is an essential and required component for plant leaf development [61]. The same site-specific selection events of it have been observed in other plants [8], and which have been shown to affect the plant fitness by altering the acetyl-CoA carboxylase production [62]. Both genes rpl22 and accD were only found to be under different selection between La. racemosa and two Lumnitzera species, and for La. racemosa, which is an introductive mangrove species in China and having the different original habitat other two Lumnitzera mangroves [5].

3.5. Phylogenetic Analysis of Three Combretaceae Mangrove cp Genomes and Related Myrtale Species. Cp genome sequences are useful for deciphering phylogenetic relationships among closely related taxa and for clarifying the evolutionary patterns of plant species [63]. To evaluate the phylogenetic position of Combretaceae mangrove species in the Myrtales, the whole chloroplast genome sequences of seven Combretaceae species with other nine plants in Myrtales were used to infer their phylogenetic relationships (Figure 6). Among those cp genomes, one mangrove plant Rhizophora stylosa was set as outgroup. By the phylogenetic analysis, 16 Myrtle species were clustered into five families, six species in Myrtaceae, two species in Onagraceae, and five Combretaceae mangroves were clustered into their own groups. Furthermore, two Lumnitzera genera were clustered into one subclade in the Combretaceae group. Within the family Combretaceae, Laguncularia and Lumnitzera are quite closely related in the field in China and are considered to be evolved from a common ancestor [5]. In this study, the position of Combretaceae mangroves are more closed with
Figure 6: Continued.
Lythraceae and Punicaceae species in Myrtales, which was showed in all the four polygenetic trees. In Myrtales, there included three significant shifts in diversification rates, one of them contributed from Combretaceae [64]. The chloroplast genome of those three Combretaceae mangroves will provide valuable and essential genetic information to further the phylogenetic resolution among angiosperms [63].

4. Conclusions

With the help of high-throughput sequencing technology, we comparatively analyzed the complete cp genomes of three Combretaceae mangroves. The gene contents and gene orders of the three cp genomes were highly conserved. Gene ycf1, rps19, and ndhF were found to be the most divergent coding regions, and there are still some other noncoding regions with the high sequence divergence, which could potentially serve as molecular markers in phylogenetic studies. Three genes ycf1, ycf2, and matK were found to be under relaxed selection in the cp genomes of three Combretaceae mangroves. Phylogenetic analysis showed that the position of Combretaceae mangroves is closest to Punicaceae and Lythraceae species in Myrtales.

Data Availability

The analysis and experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

Y.Z. and C.-C.Y. analyzed the data and wrote the manuscript; J.-D.Z. and Y.W. conceived the experiments; J.-D.Z. performed the experiments. All authors read and approved the final manuscript. Ying Zhang and Hai-Li Li are co-authors.

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Supplementary Materials

The following are available online at https://www.mdpi.com/xxx/s1. Table S1: primers used for gap closing and sequencing verification in Laguncularia racemosa and Lumnitzera racemosa. Table S2: functional classification of Lumnitzera racemosa chloroplast genome genes. Table S3: functional classification of Laguncularia racemosa chloroplast genome genes. Table S4: Ka/Ks value of the consensus protein-coding genes in three cp genomes of Combretaceae mangroves. (Supplementary Materials)

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