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PLASTOME REPORT

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The complete chloroplast genome and phylogenetic analysis of *Cyperus malaccensis* Lam (Cyperaceae)

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ABSTRACT

Cyperus malaccensis Lam is a perennial herbaceous plant that is distributed over a large area along the southern coast of China. Some plants of the Cyperaceae family are highly similar morphologically, which makes them difficult to classify and identify. In this study, the complete chloroplast genome of *C. malaccensis* was sequenced and assembled. The chloroplast genome is 186,098 bp long with a 33.18% content of GC. The structure of chloroplast genome includes a quadripartite structure that is composed of a pair of inverted repeats (IRs) of 37,434 bp, a small single copy (SSC) region of 10,296 bp, and a large single copy (LSC) region of 100,934 bp. The genome contains 141 genes, including 94 protein-coding genes, 39 tRNA genes, and 8 rRNA genes. A phylogenetic analysis showed that *C. malaccensis* is the most closely related to the congeneric species *C. rotundus*. These results enrich the genetic resources of the Cyperaceae and provide a molecular basis for further study on the phylogeny of this family.

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Cyperus malaccensis Lam; complete chloroplast genome; phylogenetic analysis; Cyperaceae

Introduction

Cyperus malaccensis Lam (1791) (Figure 1), a perennial herb in the family Cyperaceae (Institute of Botany, Chinese Academy of Sciences 1961), is the most widely distributed native coastal salt marsh herb along the coast of southern China. It is also distributed in India, Myanmar, Vietnam, Malaysia, Indonesia, and the Ryukyu Islands of Japan (Institute of Botany, Chinese Academy of Sciences 1961). As a typical salt marsh plant, *C. malaccensis* is highly valuable ecologically and economically since it can protect beaches and shores, promote siltation and land formation, improve saline land and the primary productivity of mudflats, and expand the amount of space available to reduce carbon emissions. The previous inhabitants of the southern coast of China used *C. malaccensis* to weave mats and prepare various daily handicrafts, and now, owing to its characteristics of producing rich, natural fiber, *C. malaccensis* is being studied for use in reinforcing an epoxy matrix (Neuba et al. 2023).

The coastal salt marsh, mangrove, and seagrass ecosystems are important components of the "Blue Carbon Sink" owing to their high rate of carbon sequestration and potential role in mitigating climate change (Nellemann et al. 2009, Douvere 2021). The "saltmarsh grass - mangrove cooperating ecological conservation system" can effectively break the bottleneck of mangrove afforestation technology (He et al. 2014). Native salt marsh plants are an important option for future coastal ecological conservation and restoration. However, there have only been limited studies on native salt marsh plants in southeast China.

The *Cyperus* salt marsh plants are highly similar morphologically and thus, difficult to classify and identify (Wu et al. 2021). With the development of high-throughput sequencing technology, chloroplast genomic information has become the basis of species research and evolutionary analysis. Chloroplast genomes have been widely used in the delimitation and phylogeny of species because of their uniparental inheritance and lower substitution rates compared with those of nuclear genomes (Wei et al. 2020, Gu et al. 2022). However, there is only limited genomic information on *Cyperus*, which limits the identification of its species by molecular methods. In this study, we sequenced the complete chloroplast genome of *C. malaccensis* using high-throughput sequencing technology and reconstructed the phylogenetic relationships of the Cyperaceae family.

Materials and methods

Plant material, DNA extraction, and sequencing

Samples of *C. malaccensis* were collected from Maoweihai, Qinnan District, Qinzhou City, Guangxi, China (108°34′43.80″E,

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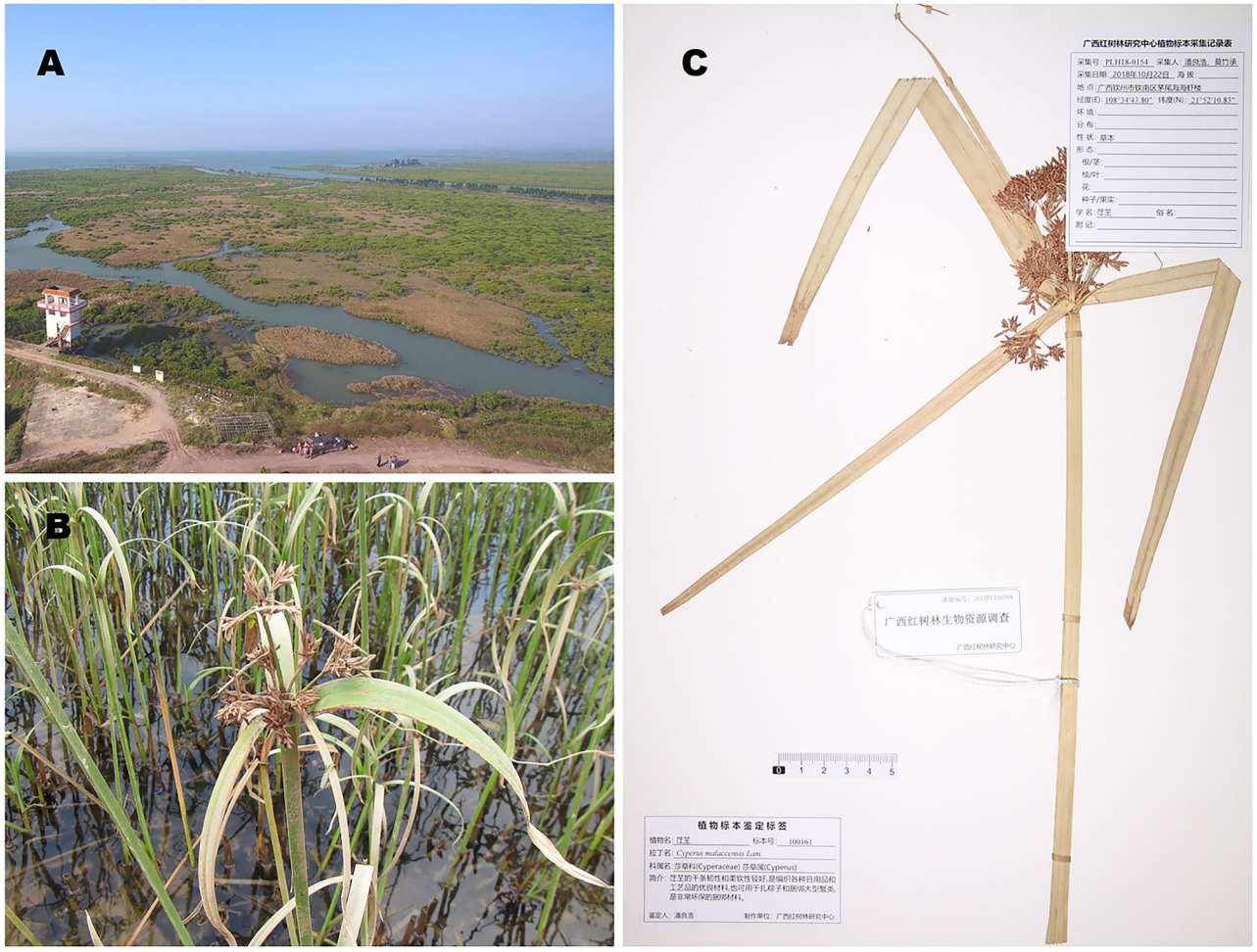

Figure 1. Habitat and morphology of *Cyperus malaccensis*. A. Coast salt marsh composed of *Cyperus malaccensis*. B. Flowers, leafy bracts, and aboveground stems. C. Herbarium specimen prepared by Lianghao Pan. A and B were photographed at Maoweihai, Qinnan District, Qinzhou City, Guangxi, China, and C was photographed in the specimen room of the Guangxi Mangrove Research Center. All the images were photographed by Lianghao Pan, and they are not subject to copyright.

21°52′10.83″N) and vouchered in the specimen room of the Guangxi Mangrove Research Center (Guangxi Academy of Marine Sciences, Beihai, China). Its accession number is PLH18-0154. The voucher can be accessed by contacting Lianghao Pan at panlh86@163.com.

The total genomic DNA was isolated from fresh, healthy leaves of three samples using a modified CTAB method. After DNA quality and quantity testing, a paired-end library with an insert size of 450 bp was constructed and sequenced using an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

Genome assembly and annotation

Trimmomatic v. 0.38 (Bolger et al. 2014) was used to clean the sequencing adapters and low-quality reads. The genome sequence was assembled by NOVOPlasty v. 4.2 (Dierckxsens et al. 2017). GeSeq software (Tillich et al. 2017) was used to predict the protein coding, tRNA, and rRNA genes in the chloroplast genome. CPGView was used to generate the gene structure of the cis-/trans-splicing genes (Liu et al. 2023). Finally, OGDRAW v. 1.3.1 (Greiner et al. 2019) was used to draw a chloroplast genome map for *C. malaccensis*. The genome sequence of *C. malaccensis* was deposited in GenBank (accession number: OR238391).

Phylogenetic analysis

The chloroplast genome sequences of *C. malaccensis* and the other 11 plants in the Cyperaceae were downloaded from GenBank and used to construct phylogenetic trees using *Sorghum bicolor* as an outgroup. A phylogenetic tree was constructed based on the chloroplast single nucleotide polymorphism (SNP) matrix, and the SNP sites were obtained as previously described (Li et al. 2019). The maximum likelihood (ML) tree was then generated by PhyML v. 3.0 (http://www.atgc-montpellier.fr/phyml/) with 1000 bootstrap replicates.

Results

The complete chloroplast genome sequence of *C. malaccensis* is 186,098 bp in length with a GC content of 33.18%. It has four regions composed of two IRs of 37,434 bp that are separated by LSC (100,934 bp) and SSC (10,296 bp) regions (Figure 2 and Table S1). The read depth map and coverage map are shown in Figure S1, and they yielded an average

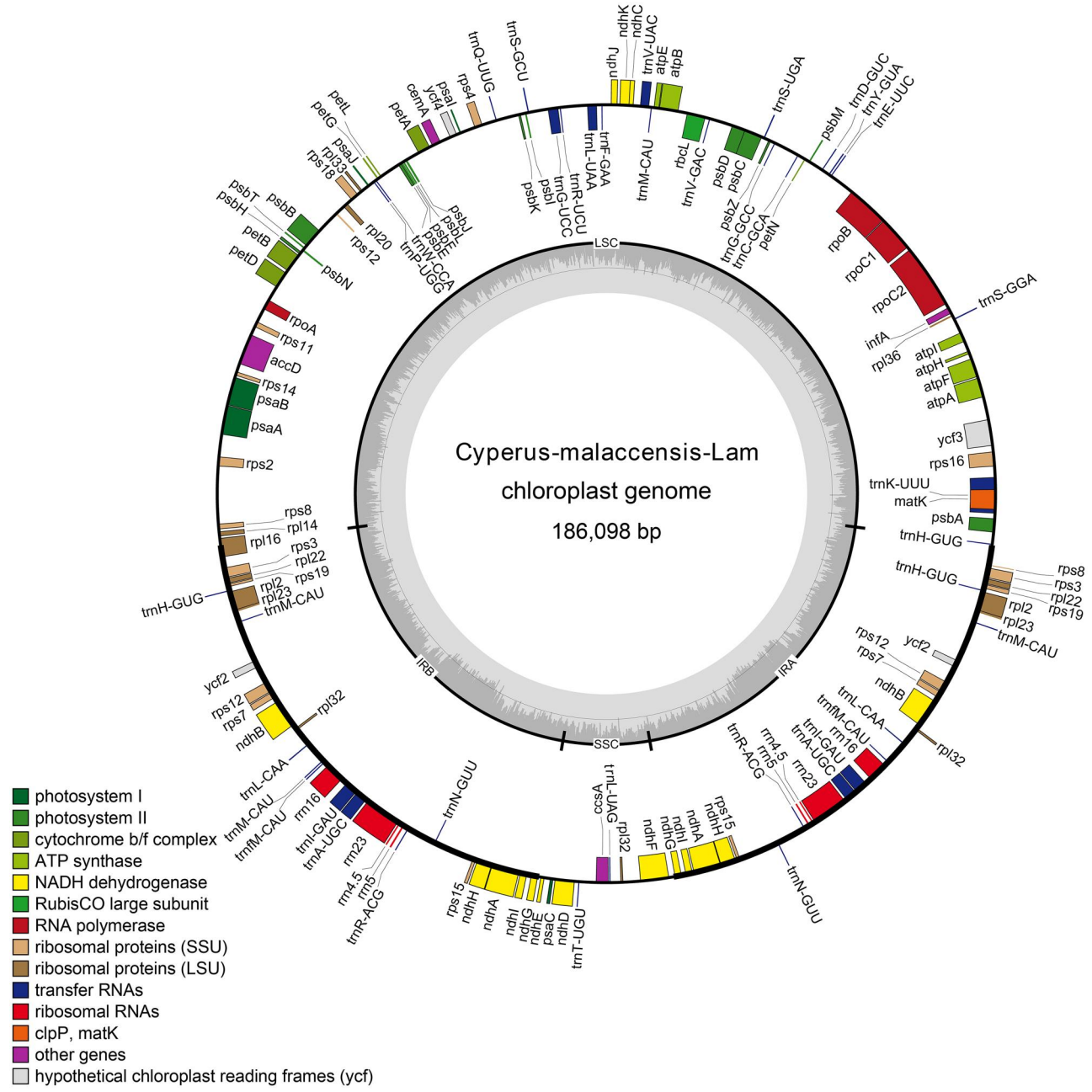

Figure 2. The circular map of the *Cyperus malaccensis* chloroplast genome. The Central circle indicates the four functional groups of the chloroplast genome. In the outer circle, the transcription direction for the inner genes is clockwise, and the outside is counterclockwise. The functional classification of the genes is shown as the figure legend in the bottom left corner. IRA, inverted repeat A; IRB, inverted repeat B; LSC, large single copy; SSC, small single copy.

coverage of 261×. The gene structure maps of cis- and trans-splicing gene are shown in Figure S2 and S3. The chloroplast genome contains 141 genes that are composed of 94 protein-coding genes, 39 tRNA genes, and 8 rRNA genes. Among these, 27 genes were repeated in the IR regions, including 15 protein-coding genes (*rps3*, *rpl22*, *rps19*, *rpl2*, *rpl23*, *ycf2*, *rps12*, *rps7*, *ndhB*, *rpl32*, *rps15*, *ndhH*, *ndhA*, *ndhI*, and *ndhG*), 8 tRNA genes (*trnN-GUU*, *trnR-ACG*, *trnA-UGC*, *trnI-GAU*, *trnH-GUG*, *trnM-CAC*, *trnL-CAA,* and *trnI-CAU*), and 4 rRNA genes (*rrn4.5*, *rrn5*, *rrn16*, and *rrn23*) (Table S2).

To further study the genetic background and taxonomic relationship of *C. malaccensis*, the chloroplast genome sequence of *C. malaccensis* was compared with that of the other 11 plants in the Cyperaceae, and *Sorghum bicolor* was used as an outgroup. The phylogenetic tree indicated that *C. malaccensis* exhibited the closest relationship with *C. rotundus* with a high bootstrap value of 99 (Figure 3), and a cladogram was shown in Figure S4.

Discussion and conclusion

The chloroplast genome can provide information for the evolutionary relationship of species owing to its high degree of conservation. Many herbaceous plants of the Cyperaceae family are highly similar morphologically. Thus, a study of the chloroplast genome can help to identify the species of Cyperaceae. In this study, the chloroplast genome of *C.*

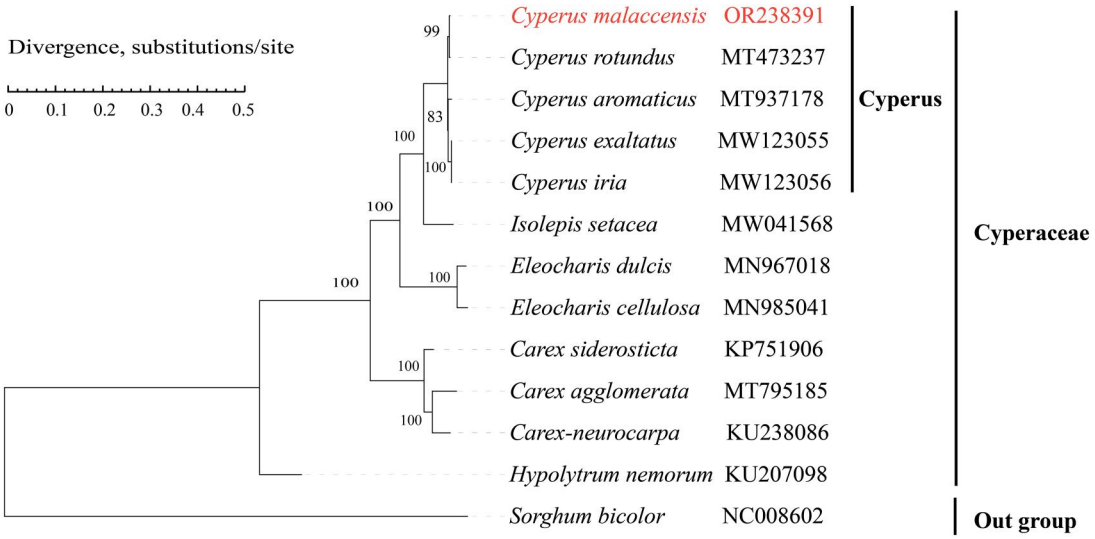

Figure 3. The phylogenetic tree was constructed based on the SNP matrix from 13 chloroplast genomes. The following sequences were used: *Carex agglomerate* (MT795185), *Carex neurocarpa* (KU238086), *Carex siderosticta* (KP751906), *Cyperus aromaticus* (MT937178), *Cyperus exaltatus* (MW123055), *Cyperus iria* (MW123056), *Cyperus rotundus* (MT473237), *Eleocharis cellulose* (MN985041), *Eleocharis dulcis* (MN967018), *Hypolytrum nemorum* (KU207098), and *Isolepis setacea* (MW041568) with *Sorghum bicolor* (NC 008602) as an outgroup. The numbers on the branches represent the bootstrap values based on 1000 replicates. The scale bar shows the expected number of nucleotide substitutions per site.

malaccensis was sequenced, assembled, and annotated for the first time. The phylogenetic results indicate that *C. malaccensis* exhibits the closest relationship with *C. rotundus,* which is a widespread perennial herb species of Cyperus and shares a highly similar morphology with *C. malaccensis.* The ML trees indicate that the chloroplast SNP-based phylogenetic analyses could be used to identify the *Cyperus* genus and the Cyperaceae family. In summary, our study enriched the basic information for the molecular evolution of the Cyperaceae family.

Authors' contributions

LHP collected, identified and processed plant samples. KC constructed the phylogenetic tree and drafted the manuscript. XL designed and conceived the experiment, performed the data analysis and edited the manuscript. All authors read and approved the final manuscript.

Ethical approval

C. malaccensis in this study is an unprotected species. We confirm that all research complies with ethical guidelines and local legislation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank under accession no. OR238391. The associated BioProject, SRA, and BioSample numbers are PRJNA992942, SRR25210280, and SAMN36377775, respectively.

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