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FIRST COMPLETE CHLOROPLAST GENOME OF *CYANOTIS NYCTITROPA* DEFLERS AND ITS PHYLOGENETIC RELATIONSHIPS WITHIN THE TRIBE CYANOTINAE (COMMELINACEAE)

Widad Saleem Al-Juhani*

Department of Biology, College of Science, Umm Al-Qura University,
24381 Makkah, Saudi Arabia.

*Keywords***:** *Cyanotis*; Chloroplast genome, Molecular phylogeny; Taxonomy.

Abstract

Cyanotis nyctitropa Deflers is an herb native to the southern Arabian Peninsula. Little is known about this species, and no genomic sequences are available in the public databases. This is the first report on the cpDNA data for the endemic species *C. nyctitropa* and includes a comparative assessment with relatives in the Cyanotinae subtribe. The *C. nyctitropa* plastome has 157,472 base pairs (bp) and exhibits a typical quadripartite structure, comprising a large single-copy (LSC) region, a small single-copy (SSC) region, and a pair of inverted repeats (IRa and IRb). The lengths of the LSC and SSC regions are 88,395 bp and 17,693 bp, respectively, whereas the lengths of the IRa and IRb segments are 25,692 bp each. A significant reduction in the length of the IR region was observed in *C. nyctitropa*, as well as the variation in the positional distribution of genes at the boundaries. The overall number of simple sequence repeats (SSRs) identified within the Cyanotinae species ranged from 396 to 466. Nucleotide diversity analysis revealed that *trnQ-UUG, trnT-UGU, atpB, rpI33, psbB, petB, ycf1,* and *ndhD* sites with the highest Pi values. The construction of DNA markers on the basis of these high-nucleotide-diversity sites is recommended. Bayesian phylogenetic analysis of Cyanotinae chloroplast yielded a monophyletic clade. These results highlight the usefulness of plastome sequence data as a resource for determining the relationships among Commelinaceae taxa.

Introduction

Commelinaceae Mirb., a member of the order Commelinales, comprises 36 genera and 810 species and is primarily found in tropical and subtropical regions (Jung *et al*., 2025). The genus *Cyanotis* D. Don belongs to the tribe Tradescantieae–Hexandrae (Commelinaceae). This genus is characterised by compact cincinni, connate petals, six fertile moniliform hairy stamens, and generally swollen filaments and styles. There are approximately 56 species of *Cyanotis* worldwide, most of which are found in Asia (Faden, 2000) and Africa (Nandikar and Gurav, 2014).

They are cultivated for ornamental and therapeutic purposes, including *Cyanotis axillaris* (L.) D. Don *ex* Sweet and *Cyanotis cristata* (L.) D. Don (Saensouk and Saensouk, 2021). The roots of several species, such as *C. axillaris*, are used by the Man people in Northwest China because they can stop swelling and cure snake bites. In addition, leaves from *C. cristata* can counteract oxidation. Phytochemical studies have indicated the presence of flavonoids and tannins in these plants. By enhancing tensile strength in wounds, the leaf extract of *C. cristata* has been shown to accelerate the healing process of incisions (Pathak *et al*., 2017).

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*Corresponding author: wsjuhani@uqu.edu.sa

Cyanotis nyctitropa Deflers is a perennial herbaceous plant. Its basal leaves have 3 green angles and a sharp tip, all approximately 10 cm long, and pubescent margins (Fig. 1). The inflorescences are purple, and the alternative flower forms can be yellow, each as wide as 10 mm (Al-Sarur, 2019). This species originated in the southwestern Arabian Peninsula, specifically in Saudi Arabia and Yemen.

Fig. 1. *Cyanotis nyctitropa* Deflers growing in its natural habitat in the Faifa region in southwestern Saudi Arabia. (A) The top view shows the inflorescence shape. (B) The side view shows the stem and leaf shape.

The chloroplast (cp) is a crucial organelle that plays major roles in photosynthesis and starch production and is involved in several metabolic processes. The conserved architecture of cp genomes, attributable to their gradual evolutionary rate and maternal transmission, offers significant insights into plant phylogenetics via comparative assessments among closely related taxa (Cheng *et al*., 2020; Song *et al*., 2024). This is the first time that an extensive analysis of cp genome data has been performed on *C. nyctitropa*, and its taxonomic position has been evaluated. Moreover, the results of this study offer a comparative evaluation between *C. nyctitropa* and members of other genera attributed to Cyanotinae.

Materials and Methods

DNA extraction and genomic library preparation

DNA was isolated from dried leaves of *C. nyctitropa* using the CTAB method (Doyle and Doyle, 1987). A total of 1.0 μg of DNA was employed as the fundamental substrate for the sample preparation. An Illumina TruSeq Nano DNA Kit was used for library construction following the manufacturer's instructions.

Genome sequencing and annotation

Sequencing was conducted using an Illumina sequencer (NextSeq 500) through the application of sequencing by synthesis (SBS) technology. The raw data were analysed, resulting in the acquisition of 12 Gb of filtered reads. NOVOPlasty version 4.3.1 (Dierckxsens *et al*., 2020) was used to assemble the cp genome sequence of *C. nyctitropa*. For the assembly process, a previously published sequence of *C. ciliata* (accession number: MK133255.1) was used as a reference point. The cp genome of *C. nyctitropa* was predicted and annotated following the GeSeq pipeline (Tillich *et al*., 2017). All the tRNA genes within these genomes were identified using tRNAscan-SE version 2.0 (Chan and Lowe, 2019). Circular gene maps for the cp genomes were generated using OGDRAW v. 1.3.1 (Greiner *et al*., 2019). The genomic sequence of the cp from *C. nyctitropa* has been submitted to GenBank under the accession number PV890880 and NCBI SRA accession number SAMN60678617.

Codon usage and simple sequence repeats analyses

The relative synonymous codon usage (RSCU) indices were evaluated using Graphical Codon Usage Analyser (GCUA) v. 1.2 (McInerney, 1998). Predictions regarding RNA-editing loci within protein-coding sequences were generated through the PREPACT3 suite (Lenz *et al*., 2018). This analysis adhered to a stipulated minimum threshold criterion of 0.8

Simple sequence repeats (SSRs) were investigated using MIcroSAtellite (version 2.0) in the cp genomes of *C. nyctitropa* and four additional species belonging to the Cyanotinae subtribe. The parameters used during the SSR analysis were eight for mononucleotide repeats, five for dinucleotide repeats, and four each for trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats. REPuter (Kurtz *et al*., 2001) was employed to detect palindromic, forward, reverse, and complementary repeats within the plastid genomes of *C.* and the four species of the Cyanotinae lineage.

Plastome comparison

The plastome of *C. nyctitropa* was subjected to alignment and comparison with the genomes of four species from the Cyanotinae subtribe via mVISTA (Mayor *et al*., 2000). Additionally, the junctions of the IR regions and the small-copy (SC) segments were identified using IRplus (Díez Menéndez *et al*., 2023).

Nucleotide diversity analysis

Nucleotide variations were subsequently examined using the sliding window approach in DnaSP v.611.01, with a window dimension of 600 bp and a step size of 200 bp (Rozas *et al*., 2017). In accordance with the GBseq annotation, the delineations of the IR, LSC, and SSC regions were identified. IRplus (Díez Menéndez *et al*., 2023) was used to conduct comparative analyses of cp genomes among Cyanotinae taxa, with *C. nyctitropa* designated as the reference species.

Phylogenetic analysis

For phylogenetic relationships of *C. nyctitropa* within the Commelinaceae, a MrBayes tree was constructed using the plastomes of 31 taxa, with *Zingiber officinale* from the Zingiberaceae family serving as the outgroup. A Bayesian inference (BI) tree was constructed in MrBayes v3.2.6 (Huelsenbeck and Ronquist, 2001). The resulting BI tree was visualised using the iTOL tool (Letunic and Bork, 2010).

Results and Discussion

The complete cp genome sequence of *C. nyctitropa*, with a total length of 157,472 bp, is described in this work. The plastome size of *C. nyctitropa* falls within the typical plastome sizes among angiosperms, with the cp genomes of flowering plants ranging from 107–218 kb (Li *et al*., 2022). The genome of *C. nyctitropa* was divided into LSC, SSC, and two IR (IRa and IRb) regions (Fig. 2). The lengths of the LSC, SSC, and IR regions were 88,395 bp, 17,693 bp, and 25,692 bp, respectively, and the GC content was 37.82%. These findings are consistent with the angiosperm cp genome structure (Wu *et al*., 2023).

In this study, 130 genes were identified including 113 unique genes. This collection of genes contains 79 protein-coding genes, 30 tRNAs, and 4 rRNAs. These results are consistent with the gene numbers of the Commelinaceae family (Jung *et al*., 2025).

Table 1 presents the functional annotation of the *C. nyctitropa* genome. These genes were systematically classified into categories relevant to photosynthetic functions, as well as other essential functions related to the plastid. A total of 20 genes from *C. nyctitropa* contained introns, with 12 protein-coding genes and 8 tRNA genes encoding them. The exons and introns within genes yield critical insights into the investigation of cp genomes (Fan *et al*., 2021). The genes *ycf3* and *clpP* each contain two exons, whereas the other genes are characterised by a single exon in *C. nyctitrop,* which is consistent with the findings of Asiri *et al*. (2026) in the cp genome of *Lycium shawiia*. The *trnK-UUU* gene harbours the longest intron, measuring 2510 bp. This observation aligns with findings reported within the Commelinaceae family (Jung *et al*., 2021).

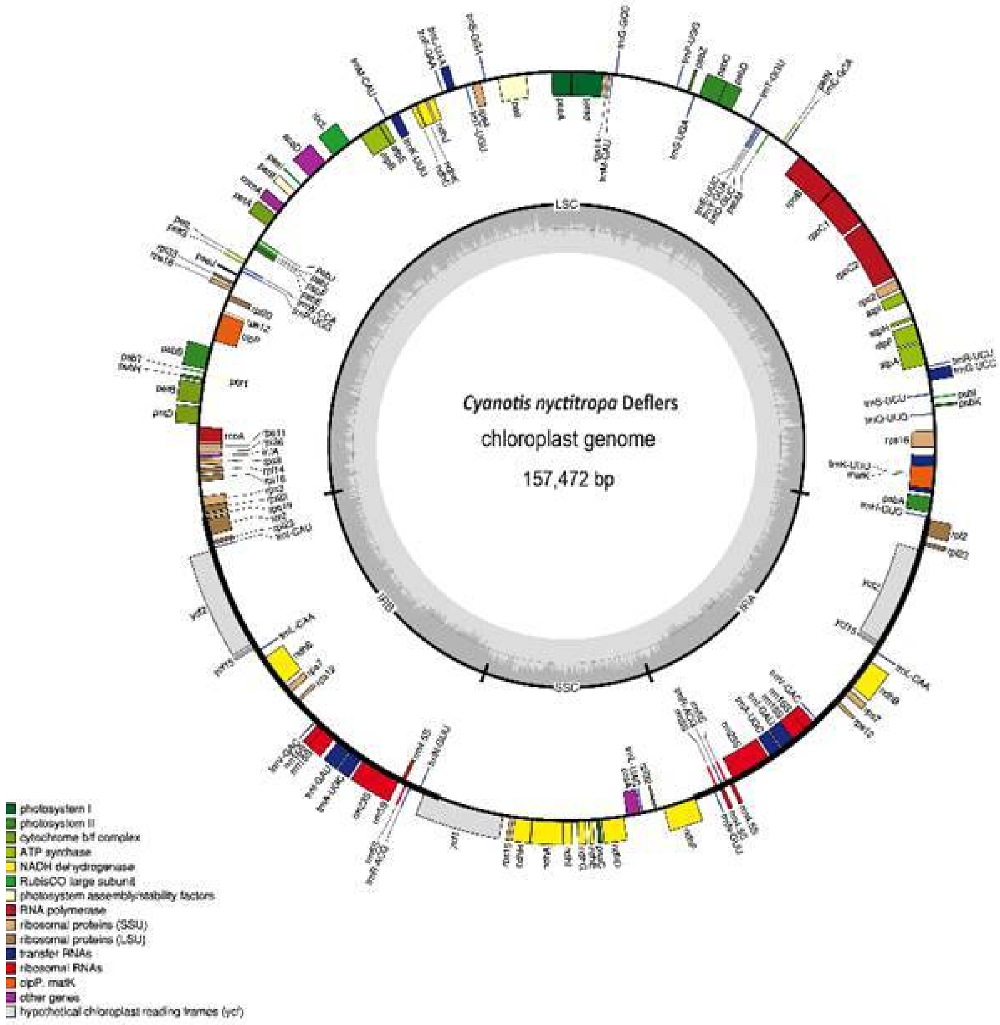

Fig. 2. Gene map illustrating the chloroplast genome of *C. nyctitropa*.

Table 1. Genes identified within the plastid genome of *Cyanotis nyctitropa*.

Group of genes	Name of genes
Small subunit of ribosome	*rpl14, rpl16, rpl2*(a+), *rpl20, rpl22, rpl23*(a), *rpl33, rpl36*
Large subunit of ribosome	*rps11, rps12*(a+), *rps14, rps15, rps16* (+), *rps18, rps19*(a), *rps2, rps3, rps4, rps7*(a), *rps8*
DNA dependent RNA polymerase	*rpoA, rpoB, rpoC1*(+), *rpoC2*
Photosystem I	*psaB, psaC, psaI, psaJ, paf* I (++), *pafII*
Photosystem II	*psbA, psbB, psbC, psbD, psbE, psbF, psbI, psbJ, psbK, psbL, psbM, pbf1, psbT, psbZ*
Subunit of cytochrome	*petA, petB*(+), *petD*(+), *petG, petL, petN*
Subunit of synthase	*atpA, atpB, atpE, atpF*(+), *atpH, atpI*
Large subunit of RUBISCO	*rbcL*
NADH-dehydrogenase	*NdhA*(+), *ndhB*(a+), *ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK*
Subunits of ATP synthase	*atpA, atpB, atpE, atpF, atpH, atpI*
Maturase	*matK*
Subunit acetyl-coA carboxylase	*accD*
C-type cytochrome synthesis	*ccsA*
Hypothetical proteins	*ycf1, ycf2*(a)
Protease	*ClpP* (++)
Envelope membrane protein	*cemA*
Translational initiation factor	*infA*

(+) genes with one intron, (++) genes with two introns, and (a) genes with a copy number.

The findings from the RSCU analysis indicated that the amino acids arginine, serine and leucine had the greatest RSCU values; specifically, leucine was the most prevalent amino acid, accounting for 10.68%. Ahmed and Rahman (2024) reported similar results in a study on the *Meconopsis torquata* Prain cp genome, indicating that these amino acids are essential for persistence and could contribute to the survival and adaptation of these organisms. Codons are among the critical components of the translation of genetic information into proteins from mRNA (Tao *et al*., 2023). One remarkable preference for codon usage strongly influences the evolutionary trajectory of cp genomes driven by selection pressure and gene manipulation (Li *et al*., 2017). Within *C. nyctitropa*, 28 codons had RSCU values exceeding 1, with A/U-ending codons manifesting a bias that is prevalent in various angiosperms. AT-rich sequences within the plastid genome have been shown to facilitate gene transfer efficacy through the stabilisation of transcript formation (Stegemann and Bock, 2006). Numerous studies have suggested that elevated AT content serves as a principal factor contributing to the predominance of synonymous codons terminating in A/U bases, a phenomenon potentially associated with natural selection and mutational events occurring throughout evolutionary history (Liu *et al*., 2019; Wang *et al*., 2021).

RNA editing represents a category of posttranscriptional alteration that can affect modifications within the coding regions of primary RNA transcripts. Therefore, understanding evolutionary phenomena is valuable (Li *et al*., 2022). The most common changes in the coding sequence resulting from RNA editing in the *C. nyctitropa* plastome were conversions to the amino acids tyrosine (Y), phenylalanine (F), isoleucine (I), leucine (L), and valine (V) across the 70

RNA-editing sites. Within *C. nyctitropa*, 54 of these edits resulted in amino acid substitutions to hydrophobic amino acids. This evolutionary strategy of the *Cyanotis* genome may reflect adaptation to the arid and semiarid habitat of the Arabian Peninsula. The *ycf2* gene harboured the greatest number of editing sites, totalling eight. These observations are likely related to acclimatisation to arid environments, which may be important for cell survival (Feng *et al*., 2022). Alofi *et al*. (2025) reported that the protein modification pathway in the herb *Aizoon canariense* L. exhibits the greatest number of genes, which could facilitate their adaptation to arid environments and resistance to environmental stress.

The cumulative number of long repeats *in C. nyctitropa* was 44, whereas the total number of long repeats in both *C. natalensis* and *C. ciliata* was 48. In contrast, *C.* speciosa and *A. hispida* presented a total of 49 long repeats. *C. nyctitropa* (Fig. 3A) presented the greatest number of palindromic repeats (17). These repetitive sequences could play a role in the stabilisation of the genome and the evolution of the plastome. The highest frequency of SSRs was detected predominantly within the LSC and SSC regions, whereas small numbers of SSRs were identified in the IR regions. The total numbers of SSRs identified across various Cyanotinae species were 405, 410, 396, 427, and 470 for *C. nyctitropa, C. natalensis, A. hispida, C. speciosa,* and *C. ciliata, respectively* (Fig. 3B).

Single-nucleotide SSRs were the most prevalent (295, 338, 325, 362, and 359) in *C. nyctitropa, C. natalensis, A. hispida, C. speciosa,* and *C. ciliata,* constituting 72.84, 82.44, 82.07, 84.78 and 77.66%, respectively (Fig. 3B). The mononucleotides were predominantly A/T repeats. This type of SSR nucleotide is common in the cp genome (Asiri *et al*., 2026). The cpSSR markers exhibit polymorphisms and are transferable among phylogenetically related taxa owing to the conserved characteristics of the flanking regions adjacent to cpSSR loci, thus rendering them advantageous for studies pertaining to population genetics, species identification, and the evaluation of genetic diversity (Pan *et al*., 2014).

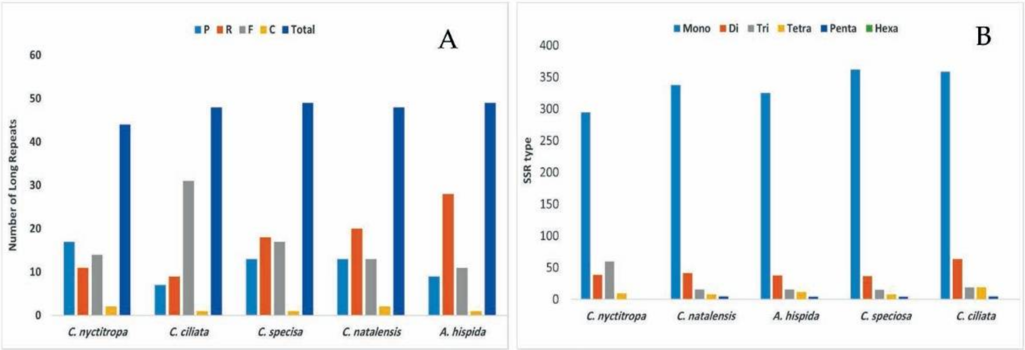

Fig. 3. Overview of long repeats and SSRs identified within the plastomes of *C. nyctitropa* and Cyanotinae, A, Longer Repeats, B. Simple Sequence Repeats.

The mVISTA analytical framework was used to assess the sequence divergence of *C. nyctitropa* relative to four cp genomes within the Cyanotinae. Overall, compared with their noncoding counterparts, the protein-coding genes displayed greater degrees of conservation (Fig. 4).

This study demonstrated that the boundaries of Cyanotinae taxa included *trnH, psb, ycf1, ndhF, rps19,* and *rpl22* (Fig. 5). Furthermore, an analysis of the five Cyanotinae species revealed discrepancies in the dimensions of the IR, LSC, and SSC regions. In addition, all plastid genomes, except those of *C. nyctitropa*, had a duplicated *ycf1* gene (pseudogene) in the IRb–SSC junctions. This may be related to the decrease in the IR of the *C. nyctitropa* plastome. Observations have indicated that the boundary sequences of the IR region can undergo either expansion or contraction, resulting in variations in the copy numbers of neighbouring genes or the development of pseudogenes (Hai *et al*., 2024).

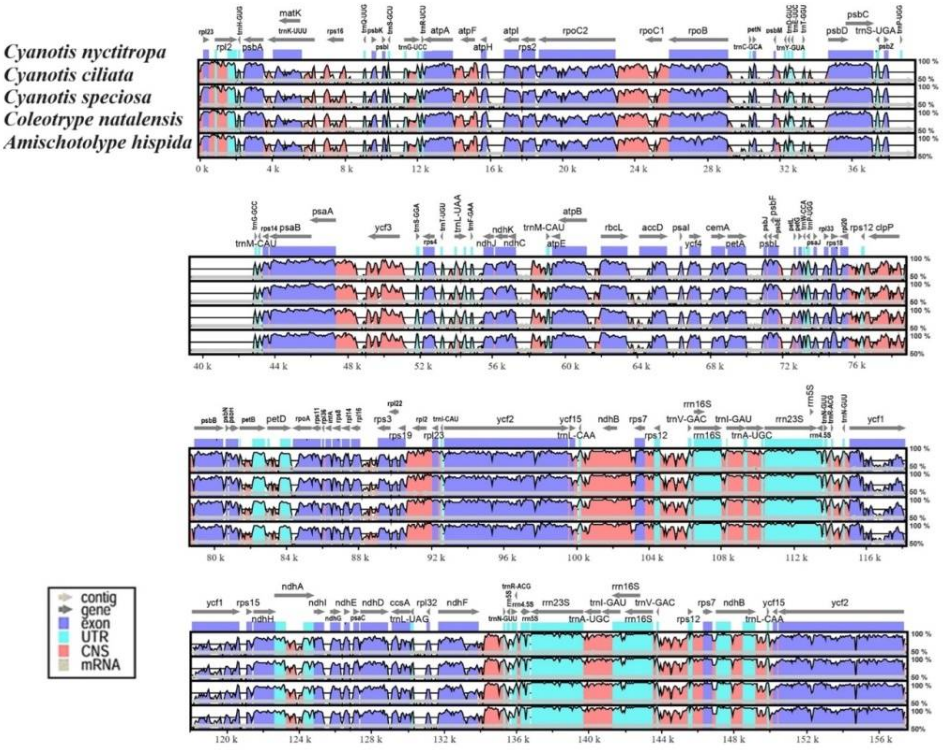

Fig. 4. mVISTA representation of the entire chloroplast genomic sequences and their corresponding genomic rearrangements for five species within the subtribe Cyanotinae using *C. nyctitropa* as reference.

Nucleotide diversity analysis of Cyanotinae (Fig. 6) revealed 34 polymorphic loci predominantly located within the LSC and SSC regions, corroborating previous results (Ahmed and Rahman, 2024). IR regions are generally characterised by a relatively higher degree of conservation because of their essential function in preserving the structural integrity of the genome. The heightened divergence in the LSC and SSC regions may be ascribed to elevated mutation frequencies and diminished selective pressures in comparison to those in the IR regions (Ahmed and Rahman, 2024). The degree of nucleotide variability ranged from 0.0204–0.0558, with a mean Pi of 0.0411. Precisely, *trnQ-UUG*, *trnT-UGU*, *atpB*, *rpI33*, *psbB*, *petB*, *ycf1*, and *ndhD* sites represented the most degree of nucleotide variability. Because these loci carry much genetic variability, which is important for phylogenetic analysis and preserving biodiversity, priority should be given to these genes during the selection or design of new molecular markers for species of the Cyanotinae subfamily.

The results of the current phylogenetic analysis of the Commelinaceae family revealed a split into two subfamilies that established the main lineages, namely, Commelinoideae and

Cartonematoideae (Fig. 7). This aligns with the contemporary phylogenetic framework of the Commelinaceae. The subfamily Commelinoideae is divided into two clades (tribes): Commelineae and Tradescantieae. These findings are in accordance with earlier research utilising nuclear and plastid regions, such as the 5S nontranscribed spacer (NTS) and plastid *trnL-F* (Burns *et al*., 2011). In the phylogenetic framework established herein, Palisoteae is located as a sister group to Commelinoideae, with a high support value (PP=1). Taxonomic assignment of the genus *Palisota* has shown a substantial lack of stability. However, recent work (Jung *et al*., 2025) has confirmed that it is a sister to Commelinoideae.

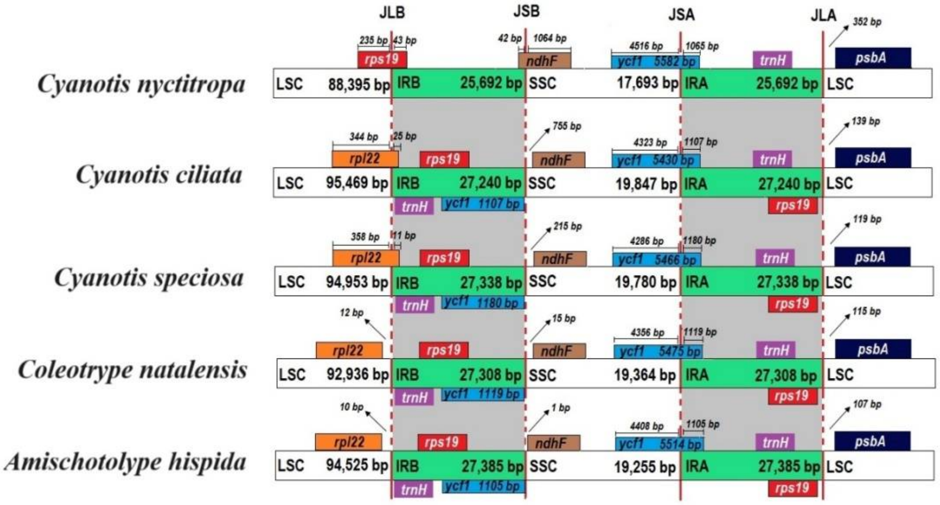

Fig. 5. Comparative analysis of the LSC, SSC, and IR regions across five chloroplast genomes belonging to the Cyanotinae subtribe.

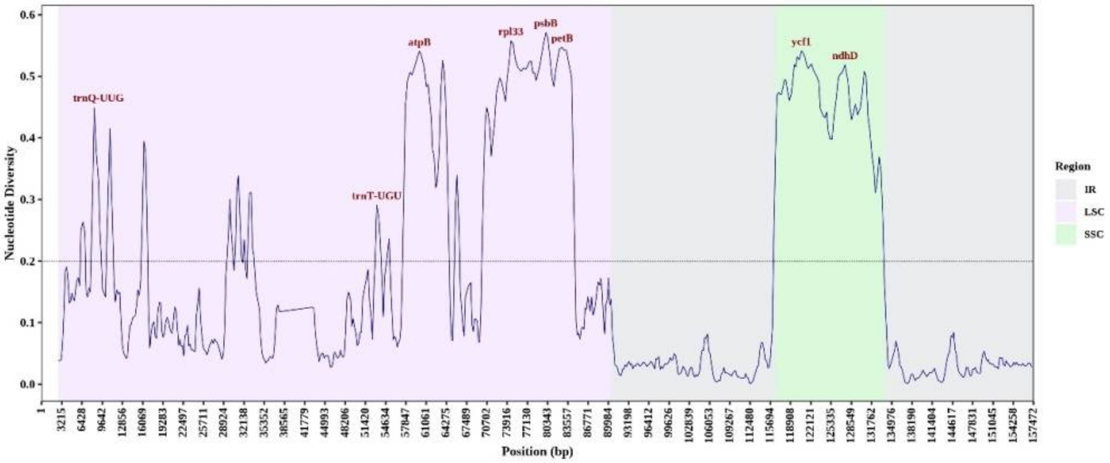

Fig. 6. Nucleotide variability of the Cyanotinae evaluated using DnaSP.

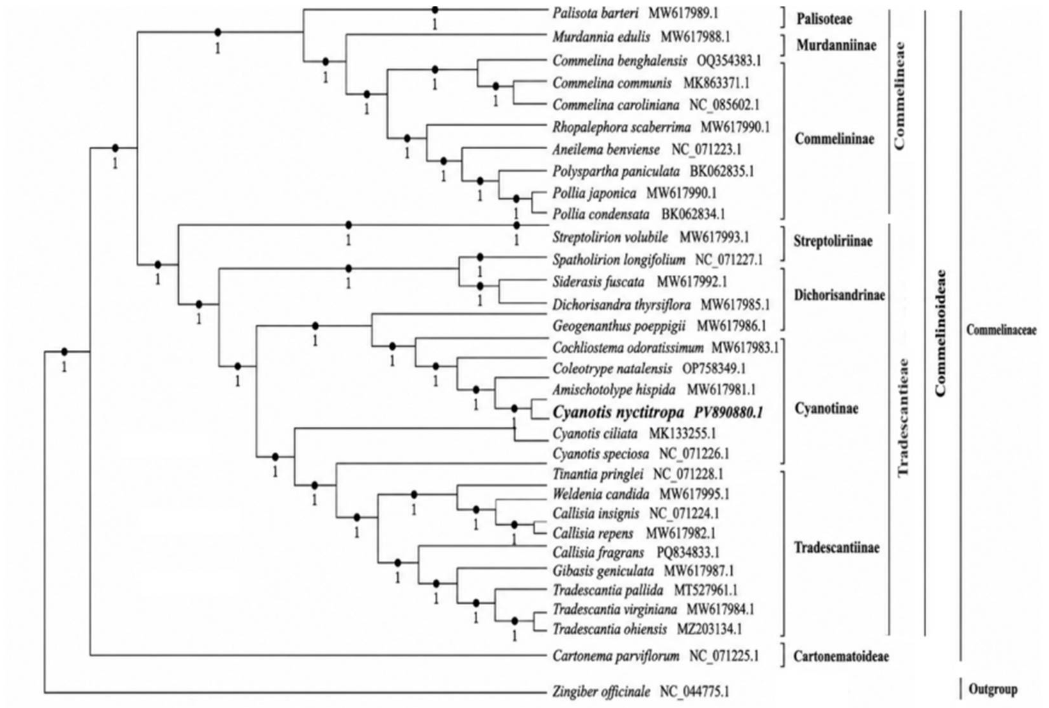

Fig. 7. Phylogenetic tree of the chloroplast genomes of the Commelinaceae family. The posterior probability (PP) values are represented at the branching nodes.

In the present tree, the tribe Tradescantieae includes four separate subtribes: Streptoliriinae, Cyanotinae (with Coleotrypinae as a synonym of Cyanotinae), Dichorisandrinae, and Tradescantiinae. A close phylogenetic relationship (PP=1) was found between the three species groups *Cyanotis* and *Liparidiopsis miaoluana*; together, they formed a monophyletic group. *Cyanotis speciosa* had the closest connection with *Cyanotis ciliata*, and they formed a combination with *C. nyctitropa* (PP=1). In addition, Cyanotis is a close relative of *Amischotolype hispida*, with a high support (PP=1). *Amischotolype* and *Cyanotis* constitute a clade that is sister to the *Coleotrype*. The subtribe Coleotrypinae is nonmonophyletic, which supports the hypothesis that it is synonymous with Cyanotiinae. These results are consistent with those of previous studies on cp genomes (Jin *et al*., 2025).

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