Insights into Phylogenetic Relationships and Genome Evolution of Subfamily Commelinoideae (Commelinaceae Mirb.) Inferred from Complete Chloroplast Genomes

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Research Article

Keywords: Commelinoideae, Commelinaceae, chloroplast genome, nucleotide diversity, phylogenomics

Posted Date: December 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-116972/v1

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Version of Record: A version of this preprint was published at BMC Genomics on April 2nd, 2021. See the published version at https://doi.org/10.1186/s12864-021-07541-1.
Abstract

Background

Commelinaceae (Commelinales) comprise 41 genera and widely distributed in both the Old and New Worlds except Europe. The relationships among genera in this family have been suggested in several morphological and molecular studies. However, it is difficult to explain their relationships due to high morphological variations and low support values. Nowadays, many researchers are commonly using complete chloroplast genome data for inferring evolution of land plants. In this study, we completed 15 new chloroplast genome sequences of subfamily Commelinoideae using Mi-seq platform. We utilized genome data for the first time to reveal the structural variations and reconstruct the problematic positions of genera.

Results

All examined species of Commelinoideae have three pseudogenes (accD, rpoA, and ycf15) and former two genes might be a synapomorphy within the Commelinales. Only four species in tribe Commelineae appear IR expansion which affected duplication of rpl22 gene. We identified inversions which range from approximately 3 to 15 kb from four taxa (Murdannia, Streptolirion, Amischotolype, and Belosynopsis). The phylogenetic analysis using 77 chloroplast protein coding genes with maximum parsimony, maximum likelihood, and the Bayesian inference suggest that Palisota connected with tribe Commelinoideae with high support values, differ from recent classification of Commelinaceae. Also, we resolved unclear position of Streptoliriniae and monophyly of Dichorisandriniae.

Conclusions

In this study, we provide detailed information of the 15 plastid genomes of Commelinaceae taxa. We identified characteristic pseudogenes and nucleotide diversity, which can be used for inferring evolutionary history about this family. Also, we need a further research to revise position of Palisota in recent classification.

Introduction

Commelinaceae Mirb., commonly known as dayflower and spiderwort group, are the largest family in the order Commelinales Mirb. ex Bercht. & J.Presl, which comprised four more families: Hanguanaceae, Haemodoraceae, Pontederiaceae, and Philydraceae [1, 2]. The Commelinaceae consist approximately 730 species of 41 genera and widely distributed in both the Old and New Worlds except Europe [2-4]. In this family, we are commonly using Callisia Loeffl. and Tradescantia L. as an ornamental and Commelina L. for vegetables. The species of Commelinaceae is usually succulent and distinct with others by having closed sheathed leaves, raphide-canals and three celled glandular microhairs [3, 4]. Additionally, flowers of Commelinaceae species are mainly insect-pollinated or autogamous which have short blooming times and lack of nectar [5, 6]. The flowering unit (inflorescence) of Commelinaceae is single or compound, commonly panicle-like thyrses composed of several to many scorpoid-cymose (cincinni) branches, sometimes reduced to a single cincinnus or single flower [4, 7].

Previous classifications of Commelinaceae emphasized on floral and anatomical characters. In the first classification, Commelinaceae were divided into two tribes, Commelineae Meisner and Tradescantieae Meisner, based on number of stamens and their reproductive capacity [8]. Then, Bruckner [9] used flower symmetry and Pichon [10] used anatomical characters to exclude Cartonema from Commelinaceae. In 1966, 15 genera of Commelinales were defined by using various flower morphological characters [11]. In the recent classification, Commelinaceae were divided into two subfamilies, Cartonematoidae (Pichon) Faden ex G. C. Tucker and Commelinoideae Faden & D. R. Hunt, by existence of raphide-canals and glandular microhairs [4]. Cartonematoidae consists two genera (Cartonema R.Br. and Triceratella Brenan) whereas Commelinoideae includes 39 genera, which are divided into two tribes by palynological characters, Commelinae (Meisner) Faden & D. R. Hunt and Tradescantieae (Meisner). Faden & D. R. Hunt. The latter tribe was arranged into seven subtribes by morphological and cytological characters [4, 12]. However, it is difficult to interpret relationships among genera due to morphological variations. Morphological cladistic result was homoplasy and incongruent with recent classification [13]. To clarify relationships of Commelinaceae, several phylogenetic studies have been conducted [14-20]. In plastid rbcl phylogenetic analysis, Cartonema was in basal clade and both Commelinae and Tradescantieae were monophyletic except Palisota Rchb. which had low support values [15]. Additionally, plastid ndhF from previous research suggested that subtribe Tradescantiniae were paraphyletic whereas Thyrasanthineae and Dichorisandriniae were polyphyletic [16]. Combined data of nuclear 5S NTS and plastid trnL-F regions resulted in a well-supported relationship between Commelinae and Tradescantieae, however the positions of Palisota and Spatholirion Ridl. were ambiguous [17]. These confused relationships between genera require further research.

Chloroplast genome or plastid genome (cpDNA) is highly conserved and has a typical quadripartite structure containing a large single copy (LSC) and a small single copy (SSC) separated by two inverted repeats (IRs). The size of cpDNA ranges from 19,400 bp (Cytinus hypochistis) to 242,575 bp (Pelargonium transvaalense) and generally contains 120-130 genes, which performs important roles of photosynthesis, translation, and transcription [21, 22]. Raphid development of next-generation sequencing (NGS) enables many studies on completing plastid genomes with high quality of raw reads at low costs. Due to its conserved characteristics, chloroplast protein-coding genes were used to reconstruct the phylogenetic relationships in other monocot groups [23-25]. Also, these data are useful to infer biogeography, molecular evolution, and age estimation [26-28]. The aims of this study are to 1) explore genome evolution in Commelinaceae through analyses of sequence variation, and gene content and order; 2) find latent phylogenetically informative genes through high nucleotide diversity; 3) reconstruct the phylogenetic relationships among members of Commelinoideae with other monocot groups using 77 chloroplast protein-coding genes data, especially the relationships among seven subtribes of Tradescantieae.

Materials And Methods
Comparative chloroplast genome structure and nucleotide diversity identified regions (Table 2). These three genes contained several internal stop codons due to insertions and deletions, thus are identified as pseudogenes. Also, we three CDS (77 protein-coding genes (CDS), 30 transfer RNA (tRNA) genes and 4 ribosomal RNA (rRNA) genes in examined Commelinoideae taxa (Table 2). In these genes, GC content was in SSC region about 3.4 % between content (36.2 %) (Table 1). The highest length difference was observed in LSC region about 8,801 bp between (Fig. 1). Plastid genome sequences of plastid genomes, including Chloroplast genome assembly and annotation

Results

Taxon sampling and DNA extraction

Fresh leaf samples were collected in the field and dried directly with silica gel in room temperature until extraction of DNA (Table 1). The samples covered four out of 14 genera in tribe Commelinae and 11 out of 25 genera which include six subtribes of tribe Tradescantiae. We prepared the voucher specimens for all used samples and deposited them in the Gachon University Herbarium (GCU) with the accession numbers. We used modified CTAB method to extract total DNA [44] and checked quality using spectrophotometer (Biospec-nano, Shimadzu) and assessed by agarose gel electrophoresis.

Genome sequencing, assembly, and annotation

Next-generation sequencing (NGS) was conducted using the Illuma MiSeq sequencing system (Illumina, Seoul, Korea). We imported NGS raw data and trimmed ends limited 5% error probability to remove poor quality of reads using Gineous prime 2020.1.2 [45]. Then, we performed 'map to reference' using Hanguana malayana chloroplast genome (GenBank accession = NC_029962.1) as a reference to isolate cpDNA reads. De novo assembly was implemented to reassemble reads using Gineous prime 2020.1.2 [45]. We used newly generated sequences as a reference to reassemble raw reads. We repeated this step until quadripartite structures were completed. Gaps were filled by Sanger sequencing using specific primers. Gene content and order were annotated using Hanguana malayana as a reference using 80% similarity to identify genes in Gineous. All tRNAs were checked by tRNAscan-SE [46] with default search mode. Illustration of plastomes were produced using OGDraw [47].

Comparative genome analysis

We compared genome structure, size, gene content across all 16 species including Belosynapsis ciliata (GenBank accession = MK133255.1) to cover lacking subtribe, Cyanotinae. The GC content was calculated and compared using Gineous. The whole chloroplast genome sequences of Commelinoideae species were aligned using MUSCLE embedded in Gineous and visualized using LAGAN mode in mVISTA [48, 49]. For the mVISTA plot, we used the annotated cpDNA of Hanguana malayana as a reference. We also examined the nucleotide diversity (Pi) of chloroplast protein coding genes, transfer RNA genes and ribosomal RNA genes among the 16 Commelinoideae species through a sliding window analysis using DnaSP v. 6.0 [50]. For the sequence divergence analysis, we applied the window size of 100 bp with a 25 bp step size. The IR and SC boundaries of the 16 Commelinoideae species were compared and illustrated using IRscope [51].

Phylogenetic analysis

A total of 42 chloroplast genome sequences (including 15 new chloroplast genomes of Commelinoideae) were used (Table S2). We extracted 77 protein coding genes and aligned using the MUSCLE embedded in Gineous prime 2020.1.2 [45]. For the data set, Acorus calamus (Acoraceae) was designated as an outgroup. We performed maximum parsimony (MP), maximum likelihood (ML), and Bayesian inferences (BI) to infer relationships of Commelinoideae and related taxa. The MP analyses were carried out in PAUP* v4.0a [52] with all characters equally weighted and unordered. Gaps were treated by missing data. Searches of 1000 random taxon addition replicates used tree-bisection-reconnection (TBR) branch swapping and MulTrees permitted 10 trees to be held at each step. Bootstrap analyses (PBP, parsimony bootstrap percentages, 1000 pseudoreplicates) were conducted to examine internal support with the same parameters. We used jModelTest version 2.1.7 [53, 54] to find the best model with Akaikes information criterion (AIC) before running the ML and BI analyses. The GTR + I + G was the best model for the concatenated data sets. We used the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) to make the ML searches [55]. Support value (MBP, mean bootstrap percentage) was calculated with 1000 replicates of ultrafast bootstrap [56]. MrBayes v3.2.7 [57] was used for BI analyses. Two simultaneous runs were performed starting from random trees for at least 1,000,000 generations. One tree was sampled every 1,000 generations. In total, 25% of trees were discarded as burn-in samples. The remaining trees were used to construct a 50% majority-rule consensus tree, with the proportion of bifurcations found in this consensus tree given as posterior probability (PP) to estimate robustness of the half of BI tree. Then, the effective sample size values (ESS) were checked for model parameters (at least 200). The phylogenetic trees were edited using FigTree v1.4.4 program [58].

Comparative chloroplast genome structure and nucleotide diversity

We completed 15 new plastid genomes in this study listed in Table 1 through 9 to 21 million raw reads for each species (Fig. S1, Table S1). A total of 16 plastid genomes, including Belosynapsis ciliata, exhibit the typical quadripartite structure containing LSC and SSC regions separated by two inverted repeats (Fig. 1). Plastid genome sequences of Murdannia edulis and Belosynapsis ciliata are over 170 kb in length whereas that of Commelina communis is 160,116 bp in length (Table 1). In addition, Murdannia edulis and Belosynapsis ciliata have the lowest GC content (34.5 %) whereas Palisota bari onis has the highest GC content (36.2 %) (Table 1). The highest length difference was observed in LSC region about 8,801 bp between Belosynapsis ciliata and Commelina communis, GC content was in SSC region about 3.4 % between Dichorisandra thysiflora and Murdannia edulis (Table 1). Plastid genomes of Commelinoideae have 131 genes, of which 111 are unique and 20 are duplicated in the IR regions (Table 2), except np22 gene which was not duplicated in tribe Tradescantiae. There are 77 protein-coding genes (CDS), 30 transfer RNA (tRNA) genes and 4 ribosomal RNA (rRNA) genes in examined Commelinoideae taxa (Table 2). In these genes, three CDS (ps12, clpP, and ycf3) have two introns, nine CDS (atpF, ndhA, ndhB, petB, petD, rpl2, rpl16, rpoC1, and rps16) and six tRNA (trnK-UUU, trnG-UCC, trnL-UAA, trnV-UAC, trnR-GAU, and trnM-UGC) have one intron (Table 2). The ps12 gene was trans-spliced, which has 5’ exon in LSC and 3’ exon and intron in the IR regions. Three pseudogenes (accD, rpoA, and ycf15) were identified from all Commelinoideae species, one (ycf15) of which was duplicated in the IR regions (Table 2). These genes contained several internal stop codons due to insertions and deletions, thus are identified as pseudogenes. Also, we identified ndhB as pseudogene in two species (Pollia japonica and Rhopalephora scaberrima) in sequence of point mutation.
The aligned data of whole plastid genomes showed high similarities in coding genes, and high variations in non-coding genes (Fig. 3). We found several genome structure variations among Commelinoideae species. *Murdannia edulis* and *Streptolirion volubile* had one inversion from *rbcL* to *psal* intergenic spacer (approximately 3 kb) and *petN* to tmE-UUC (approximately 2.8 kb), respectively. *Amischotolype hispida* and *Belosynapsis ciliata* had two large inversions from tmV-UAC to *rbcL*, and *psbl* to *petD* about approximately 5 kb and 16 kb, respectively. The IR-SSC boundary was similar among species of Commelinoideae (Fig. 4). All plastid genomes have incomplete duplicated *ycf1* gene in the IR5-SSC junctions. We also found an expansion of IR regions in Commelinoideae species which resulted duplication of *rpl22* genes (Fig. 4).

We analysed nucleotide divergences of CDS, tRNA, and rRNA to explain variant characteristics among the 16 Commelinoideae plastid genomes (Fig. 2, Table S3). Nucleotide diversity (Pi) for each CDS ranges from 0.00427 (*psbl*) to 0.09543 (*ycf1*) with an average of 0.03473. Nine CDS (rps3, ndhG, ndhD, ccsA, rps15, rpl32, ndhF, matK, and *ycf1*) have remarkably high values (Pi > 0.05) and seven CDS (psbl, rpl23, rps19, ndhB, rpl2, rps7, rps12) have low values (Pi < 0.01; Fig. 2). Compared with Tradescantieae, Commelinoideae have relatively higher values in almost CDS (Fig. 2). In Tradescantieae, however, the *rpl22* gene has higher value (Pi = 0.04655) in comparison with Commelinoideae. In tRNA and rRNA, Pi value ranges from 0 (tmT-UGU, tmH-GUG, tmV-GAC, tmT-GAU) to 0.02697 (tmQ-UUG) with an average of 0.006. Commelinoideae have the highest value in the tmL-UAA (Pi = 0.02941) while Tradescantieae have no value in this gene. We tried to find latent phylogenetically informative genes for the Commelinoideae by checking individual CDS with high values (Pi > 0.045) and over 500 bp length. Ten CDS (ndhH, rpoC2, ndhA, rps3, ndhG, ndhD, ccsA, ndhF, matK, and *ycf1*) were checked respectively with ML analysis and compared positions among 16 genera of Commelinoideae with Fig. 5. Total four CDS (ndhH, rpoC2, matK, *ycf1*) have similar topology in Commelinoideae even though the other monocot groups were unclear.

**Phylogenetic analysis**

The aligned 77 chloroplast protein-coding genes had 65,481 characters, of which 16,380 were parsimony informative. The MP analysis produced single mostly-parsimonious tree (tree length = 72,586, CI = 0.488, RI = 0.626). The tree topologies from among MP, ML, and BI were found to be congruent with each other with 100% bootstrap (PBP, MBP) values and 1.00 Bayesian posterior probabilities (PP) supporting in almost all nodes except *Palisota* which was unresolved in MP analysis (not shown) (Fig. 5). The result suggested that *Palisota* was sister to the group consisting of the rest of Commelinoideae (Fig. 5). In Tradescantieae, *Streptolirion* was positioned at the basal node. Then, *Dichorisandrinae* divided into two clades (*Dichorisandra, Siderasis, (Cochliostema, Geogenanthus)* with relatively low support values in both MP and ML analysis (PBP = 74, MBP = 84, PP = 1) (Fig. 5). Among remain four subtribes, where two clades (*Coleotrypinae and Cyanotinae*), (*Tradescantiae and Thyrsantheminae*) were formed with high support values (PBP = 100, MBP = 100, PP = 1), respectively (Fig. 5).

**Discussion**

**Chloroplast genome structure**

In this study, we completed 15 new plastid genomes of Commelinoideae taxa (Table 1). Plastid genomes have typical quadripartite structures, including LSC, SSC and two IR regions. Plastid genomes of Commelinoideae have variable total length and GC content. The LSC and SSC regions have relatively higher length and AT-content difference rather than IR region. The IR-SSC boundary was similar among species of Commelinoideae (Fig. 4). All plastid genomes have incomplete duplicated *ycf1* gene in the IR5-SSC junctions. We also found an expansion of IR regions in Commelinoideae species which resulted duplication of *rpl22* genes (Fig. 4).

Within Commelinoideae plastid genomes, three protein coding genes (accD, rpoA, and *ycf1*) were found as pseudogenes (Fig. S2). The *ycf1* gene has several abnormal stop codons caused by insertions and deletions (indel) of bases, which are similar with other monocots. We also identified that all examined species have indels at the front part of *accD* gene (until 400 bp) and terminal part of *rpoA* gene (after 700 bp; Fig. S2). The *accD* gene, encoding the beta-carboxyl transferase subunit of acetyl-CoA carboxylase, is in most flowering plants and synthesize fatty acid within the chloroplast. It was suggested as an essential gene that related with maintaining chloroplast structure [33]. However, it was reported as a gene loss or pseudogenization in Acoraceae, and Poaceae [34, 35]. Recent studies suggested that *accD* gene was found in nucleus originated from chloroplasts in several eudicots [36, 37]. The *rpoA* gene, encoding the alpha subunit of RNA polymerase, is also in most flowering plants but recorded gene loss in the chloroplast genome of mosses [38]. One of species, *Physcomitrella patens* (Funariaeae), *rpoA* gene has transferred to the nucleus [39]. We need a further study whether these two genes transferred to the nuclear or not in the Commelinoideae. We identified that these pseudogened *accD* and *rpoA* only appeared in the Commelinoideae among the Commelinales. It might be a specific character of gene composition in the Commelinales. We also found point mutated base in the third codon of *ndhB* gene in both *Pollia japonica* and *Rhopalephora scaberima*, which formed a clade together in this study (Fig. 5).
We measured the nucleotide diversity of CDS, tRNA, and rRNA to identify the genetic divergence between 16 Commelinoideae plastid genomes. We found that the CDS in the IR regions have lower nucleotide diversity than that of the LSC and SSC regions (Fig. 2). This result has also been identified in the other monocots [40-42]. It may possibly be attributed to copy correction of the IR regions via gene conversion [43]. Especially, we can see this result in the rpl22 gene. Only Commelinae species have duplicated one due to IR expansion mentioned above while remain 12 taxa have one gene in the LSC or LSC-IR junction (Fig. 4). Difference of nucleotide diversity in this gene between Commelinae (Pi = 0.015) and Tradescantieae (Pi = 0.0466) is 0.0316. It might be phylogenetically useful information for Tradescantieae only.

### Implication of phylogenomic study using plastomes data

In the first phylogenetic analysis of Commelinaceae based on plastid rbcL, they revealed a relationship of 32 species representing 30 genera of Commelinaceae [15]. Cartonematoideae was in a basal clade connected with Commelinoideae as a sister consisting of all remain species [15]. Except 
*Palisota*, Commelinoideae was divided into two tribes, Commelinae and Tradescantieae, with the low bootstrap support value due to insufficient information [15]. Although several phylogenetic studies were conducted, Commelinaceae still have unresolved relationships between genera. First, the position of 
*Palisota* had been problematic that 1) sister to all genera of Commelinoideae with high bootstrap values [15]; 2) support low bootstrap value with other Tradescantieae species [16], and; 3) belong to Tradescantieae as a basal group [19]. Second, Streptolirini was placed with Commelinae species in tml-tmf analysis [17].

Third, subtribe Dichorisandriinae seemed polyphyletic in the previous researches [15, 16, 19]. These results are most likely due to limited taxon sampling and/or used few informative genetic markers. The aligned 77 chloroplast coding genes data in this study suggests improved relationship of each genera (Fig. 5). We identified that Commelinoideae divided into two clades, tribe Commelinae and Tradescantieae, with high support values (Fig. 5). However, 
*Palisota*, which belongs to Tradescantieae in recent classification [3], is connected with Commelinae species as basal group in this study (Fig. 5). Both ML and BI results are supported with high values even though unresolved in MP (data not shown). Compared with recent classification, it seems like that subsidiary cells in stomata and pollen exine are not key characters at least for 
*Palisota* [3]. In the Commelinaceae, fruits are commonly loculicidally dehiscent capsules while 
*Palisota, Pollia, Tapheocarpa*, and some 
*Aneilema* species have indehiscent type [3]. The latter three genera are groups of Commelinae in recent classification. Also, indehiscent fruit was distinctive character in previous research to place 
*Pollia* and 
*Palisota* as a same group [11]. Other four Commelinae species (*Murdannia, (Commelina, (Pollia, Rhopalophora)))* are connected with high support values, which have similar relationships with previous research [15]. Within Tradescantieae, Streptoliriinae was diverged in the first and Dichorisandriinae was divided into two clades with relatively low support values (PBP = 77/MBP = 84/PP = 1) (Fig. 5). After that, Coleotrypiniae and Cyanotinae were diverged, which formed a sister with remain Thysantheriniae and Tradescantieae. Interestingly, the Asian and African subtribe Coleotrypinia and Cyanotinae were nested well within the New World subtribes (Fig. 5). This result is similar with previous research [15] and shows questions of biogeographic history.

### Conclusion

Our study revealed genome structural characteristics, nucleotide diversity, improved relationships between genera using 15 newly complete chloroplast genomes of Commelinaceae. Compared with other Commelinaceae species, we found two characteristic pseudogenes in all members of Commelinoideae and this might be a synapomorphy within the Commelinales. Four genes (*ndhH, rpoC2, matK, ycf3*) seem to provide phylogenetically useful information for Commelinoideae due to similar topology with Fig. 5. We also reconstruct the phylogenetic relationships using 77 chloroplast protein coding genes. Although we cannot explain of whole Commelinaceae due to loss of subfamily Cartonematoideae, we identified relationships of Commelinoideae taxa especially seven subtribes of Tradescantieae. One interesting result was that 
*Palisota* (Palisotinae) relates to Commelinae clade with high support values. This result is incongruent with the latest classification and we need a further research about that [3]. Also, we resolved the position of Streptoliriinae and monophyly of Dichorisandriinae. Future studies might use the information of chloroplast genomes that we provided in this study and make sure the evolutionary history of the Commelinaceae.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Availability of data and materials

The 15 chloroplast genomes sequences we obtained from this study were archived in NCBI. The accession numbers are presented in Table 1.

#### Competing interests

The authors declare that they have no competing interests.

#### Funding

This work was supported by the Gachon University research fund of 2019(GCU-2019-0821) and the National Research Foundation of Korea (NRF) Grant Fund(NRF- 2017R1D1A1B06029326).

#### Authors' contributions

[Further details on authors' contributions can be added here if necessary.]
JJ performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft. CK authored or reviewed drafts of the paper, approved the final draft. JHK conceived and designed the experiments, contributed reagents / materials / analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Acknowledgements

We would like to thank Gerardo A. Salazar at Universidad Nacional Autónoma de México (Mexico), Claudia T. Hornung-Leoni at Autonomous University of Hidalgo (Mexico), Manuel González Ledesma at Autonomous University of Hidalgo (Mexico), Kenneth M. Cameron at University of Wisconsin–Madison (United States of America), Chien-Ti Chao at National Taiwan Normal University (Taiwan), David Warming at Cairns Botanic Gardens (Australia), Carlos Gustavo Espejo Zurita at Jardín Botánico Histórico La Concepción (Spain) for collecting and providing the plant material for this study.

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Tables

Table 1 Comparison of the features of plastomes from 16 genera of Commelinaceae.
| Taxa                      | Tribe       | Subtribe       | Length and G+C content | GenBank accession number | Voucher                                      |
|---------------------------|-------------|----------------|-------------------------|--------------------------|----------------------------------------------|
|                           |             |                | LSC bp (G+C%) | SSC bp (G+C%) | IR bp (G+C%) | Total bp (G+C%) |                                    |
| Gibasis geniculata        | Tradescantiae | Tradescantinae | 89,154(33.3) | 18,278(30.5) | 26,953(42.5) | 161,338(36.1) | This study JH200402001              |
| Tradescantia virginiana   | Tradescantiae | Tradescantinae | 91,991(32.7) | 18,462(30.2) | 27,236(42.3) | 164,925(35.6) | This study JH170813001              |
| Callisia repens           | Tradescantiae | Tradescantinae | 89,446(33.2) | 18,252(30.3) | 27,078(42.5) | 161,854(36.0) | This study                         |
| Weldenia candida          | Tradescantiae | Thyrsantheminae| 95,029(32.6) | 19,024(30.3) | 27,233(42.6) | 168,519(35.5) | This study JH190730001              |
| Amischotolype hispida     | Tradescantiae | Coleotrypinae  | 94,525(32.9) | 19,255(30.4) | 27,385(42.4) | 168,550(35.7) | This study JH191109002              |
| Belosynapsis ciliata      | Tradescantiae | Cyanotinae     | 96,164(31.3) | 20,224(28.0) | 27,241(42.6) | 170,870(34.5) | MK133255.1                         |
| Cochliostema odoratissimum| Tradescantiae | Dichorisandrinae | 92,560(33.2) | 18,856(30.4) | 27,276(42.5) | 165,968(35.9) | This study                          |
| Geogenanthus poeppigi     | Tradescantiae | Dichorisandrinae | 94,583(32.8) | 18,612(30.7) | 27,098(42.5) | 167,391(35.7) | This study JH190803001              |
| Dichorisandra thrysiflora | Tradescantiae | Dichorisandrinae | 94,347(32.9) | 18,348(31.1) | 27,194(42.6) | 167,083(35.8) | This study JH190616001              |
| Siderasis fuscata         | Tradescantiae | Dichorisandrinae | 94,389(32.9) | 18,606(31.0) | 27,196(42.6) | 167,387(35.8) | This study XX-0-GENT-19822394     |
| Streptolirion volubile    | Tradescantiae | Streptoliriinae | 91,528(33.1) | 19,595(29.3) | 27,447(42.0) | 166,017(35.6) | This study JH180919003              |
| Palisota barteri          | Tradescantiae | Palisotinae     | 93,315(33.5) | 18,905(30.8) | 27,074(42.7) | 166,368(36.2) | This study JH190222001              |
| Pollia japonica           | Commelineae  |                | 90,295(33.2) | 19,151(29.7) | 27,604(42.2) | 164,654(35.8) | This study JH180805001              |
| Rhopalephora scaberina    | Commelineae  |                | 87,602(33.2) | 18,354(29.5) | 27,487(42.1) | 160,930(35.8) | This study JH191109014              |
| Commelina communis        | Commelineae  |                | 87,363(33.0) | 18,561(29.1) | 27,096(42.3) | 160,116(35.7) | This study JH180709001              |
| Murdannia edulis          | Commelineae  |                | 96,248(31.4) | 20,798(27.7) | 27,464(42.1) | 171,974(34.4) | This study JH191110010              |

**Table 2** Gene composition within chloroplast genomes of Commelinaceae species.
| Groups of genes | Names of genes | No. |
|-----------------|----------------|-----|
| RNA genes | **Ribosomal RNAs** | *rn4.5*, *rn8*, *rn16*, *rn23* | 8 |
| | **Transfer RNAs** | *tmK*, *tmQ*, *tmS*, *tmG*, *tmC*, *tmU*, *tmGUC*, *tm39GY*, *tm40UC*, *tmE*, *tmT*, *tmG*, | 38 |
| | | *tmSUGA*, *tm39GCA*, *tm9UGA*, | |
| | Protein genes | **Photosystem I** | 5 |
| | | **Photosystem II** | 15 |
| | | **Cytochrome** | 6 |
| | | **ATP synthases** | 6 |
| | | **Large unit of Rubisco** | 1 |
| | | **NADH dehydrogenase** | 12 |
| | | **ATP-dependent protease subunit P** | 1 |
| | | **Envelope membrane protein** | 1 |
| | Ribosomal proteins | **Large units of ribosome** | 12 |
| | | **Small units of ribosome** | 15 |
| | Transcription/translation | **RNA polymerase** | 3 |
| | | **Initiation factor** | 1 |
| | | **Miscellaneous protein** | 2 |
| | | **Hypothetical proteins and conserved reading frames** | 5 |
| | Total | | 131 |

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a: gene with one intron; b: gene with two introns; X2: duplicated gene; : pseudogene

**Figures**
Representative chloroplast genome of Commelinaceae. The colored boxes represent conserved chloroplast genes. Genes shown inside the circle are transcribed clockwise, whereas genes outside the circle are transcribed counter-clockwise. The small grey bar graphs inner circle shows the GC contents.
Figure 1

Representative chloroplast genome of Commelinaceae. The colored boxes represent conserved chloroplast genes. Genes shown inside the circle are transcribed clockwise, whereas genes outside the circle are transcribed counter-clockwise. The small grey bar graphs inner circle shows the GC contents.
Figure 2

Nucleotide diversity (Pi) values in protein coding genes, tRNA, and rRNA in 16 Commelinaceae species. The dashed lines are the borders of LSC, IR and SSC regions.
Figure 2

Nucleotide diversity (Pi) values in protein coding genes, tRNA, and rRNA in 16 Commelinaceae species. The dashed lines are the borders of LSC, IR and SSC regions.
Figure 3

Plots of percent sequence identity of the chloroplast genomes of 16 Commelinaeae species with Hanguana malayana as a reference. The percentage of sequence identities were estimated and the plots were visualized in mVISTA.
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Plots of percent sequence identity of the chloroplast genomes of 16 Commelinaceae species with Hanguana malayana as a reference. The percentage of sequence identities were estimated and the plots were visualized in mVISTA.
Figure 4

Comparisons of LSC, SSC, and IR regions boundaries between 16 Commelinaceae species.
Figure 4

Comparisons of LSC, SSC, and IR regions boundaries between 16 Commelinaceae species.
Figure 5

The Maximum Likelihood tree of 42 monocots inferred from 77 chloroplast protein coding genes. Numbers indicate support (maximum parsimony bootstrap (PBP)/maximum likelihood bootstrap (MBP)/posterior probability (PP)). Only support under PBP = 90/MBP = 100/PP = 1.00 is shown. The dashes "−" indicate incongruence between MP and ML/BI trees.
The Maximum Likelihood tree of 42 monocots inferred from 77 chloroplast protein coding genes. Numbers indicate support (maximum parsimony bootstrap (PBP)/maximum likelihood bootstrap (MBP)/posterior probability (PP)). Only support under PBP = 90/MBP = 100/PP = 1.00 is shown. The dashes "-" indicate incongruence between MP and ML/BI trees.

**Supplementary Files**

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