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The initial complete chloroplast genome of *Ludisia discolor* (Orchidaceae) in Vietnam

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Abstract. *Ho VT, Nguyen MP, Trinh TH. 2023. The initial complete chloroplast genome of* Ludisia discolor *(Orchidaceae) in Vietnam. Nusantara Bioscience 15: 232-237. Ludisia discolor* (Ker Gawl.) Blume is traditionally utilized as an effective herb to treat various diseases. Due to its high medicinal value, this herb has been overharvested and faces extinction in a wild population. Morphological characterization to DNA barcode-based techniques are applied to accurately identify this plant for conservation and breeding purposes; nevertheless, they show limitations. This study aimed to characterize complete chloroplast (cp) sequences for effectiveness in plant species' classification and phylogenetic analysis. The cp genome of *L. discolor* collected from Vietnam was initially sequenced, annotated, and compared with published cp genomes from NCBI GenBank to understand its genetic makeup better. The results reveal that the complete cp genome of *L. discolor* is 159,043 bp, consisting of 96 protein-coding genes, 12 rRNA, and 33 tRNA genes. Moreover, 53 simple sequence repeats (SSR) were detected in this genome, most of which are polyA and polyT. Phylogenetic analysis also revealed a significant distance between the cp genomes of *L. discolor* from Vietnam with other *L. discolor* accessions from China. These findings could provide valuable insights into the taxonomy, plant identification, breeding, protection, and conservation programs of *L. discolor* in Vietnam.

Keywords: Chloroplast genome, *Ludisia discolor*, medicinal plant, next-generation sequencing, simple sequence repeat

Abbreviations: LSC: Large Single Copy, NGS: Next-Generation Sequencing, SSC: Small Single Copy, SSR: microsatellite

INTRODUCTION

Ludisia discolor (Ker Gawl.) Blume, an enchanting jewel orchid distributed in East and Southeast Asia, is rich in medicinal compounds used to treat several diseases, such as tuberculosis, hemoptysis, and loss of appetite (Liu et al. 2021). The increasing demand and inadequate management practices have led to its scarcity due to overharvesting (Poobathy et al. 2019). Consequently, wild *L. discolor* is dying out due to overexploitation in recent years and has been reported in several countries, such as China (Su et al. 2017), Malaysia (Burkhan et al. 2022), and Vietnam (Nguyen and Phung 2023). Moreover, the powder of *L. discolor* in the market was mixed with powders of other jewel orchid species, such as *Anoectochilus roxburghii*, *A. formosanus*, or *Goodyera schlechtendaliana* leading the reduced effectiveness of treatment efficacy (Chai et al. 2022).

Traditionally, the morphological identification of jewel orchids has been employed for conservation purposes. Nonetheless, this approach has limitations, particularly when closely related species share similar morphological characteristics, and the morphological identification is mainly based on personal experience and unproducible due to several jewel orchids possessing resemble features (Ho et al. 2021). Furthermore, different jewel orchid species display a high degree of morphological feature overlap, thereby, the reliability of morphological identification in question, especially for closely related species sharing similar morphological characteristics.

Numerous researchers applied DNA barcodes to identify several jewel orchid species based on standardized DNA regions to compensate for the drawback of morphological identification. Huynh et al. (2019) applied nine DNA barcode regions to identify six jewel orchid accessions belonging to three species collected from Vietnam. Their obtained data show only ITS 1 and ITS2 regions are applicable to discriminate *Anoectochilus* and *Lusidia* species (Huynh et al. 2019). After screening about 7,000 sequences of 380 medicinal orchid species in Asia, ITS is the best barcode, and the combination of ITS and matK will produce higher discrimination results (Raskoti and Ale 2021). Nevertheless, DNA barcode also shows drawbacks since this technique only applies short DNA sequences, limiting the discrimination power. Gao et al. (2009) reported that ITS sequence could not determine species level in the genus *Anoectochilus* since it is highly assembled to *L. discolor*. Chen and Shiau (2015) reported that ITS and matK did not effectively distinguish *L. discolor* from its hybrid cultivars. The accuracy of this method is also highly dependent on the availability of corresponding sequences in the database.

In recent years, the widespread adoption and increased affordability of next-generation sequencing (NGS) technology have greatly facilitated the comprehensive characterization of the complete chloroplast (cp) genome in

numerous plant species in the family Orchidaceae, such as *Dendrobium nobile* (Konhar et al. 2019), *Paphiopedilum delenatii* (Vu et al. 2020), and *Vanda coerulescens* (Wang et al. 2020). The high discrimination power of complete cp genomes comes from significant variation within and between plant species in their structure and sequence (Daniell et al. 2016). This technique has proven to be highly effective in identifying and classifying jewel orchids, as it enables thorough examination of the entire cp sequences. Therefore, Oh et al. (2019a) found the effectiveness in phylogeographic and phylogenetic studies of species in the genus *Goodyera* using NGS. Despite the wide distribution of *L. discolor* across East and Southeast Asia, only a limited number of cp genomes from this plant in China have been sequenced (Yu et al. 2019). In this study, we sequenced, annotated, and compared the cp genome of *L. discolor* originating from Vietnam with published cp sequences. The obtained data could provide valuable information for taxonomy and conservation programs concerning this plant species in Vietnam.

MATERIALS AND METHODS

Plant materials

Ludisia discolor samples were provided by the Biotechnology Center of Ho Chi Minh City (HCMBIOTECH), Vietnam. The remaining cloned plants continue to be cultivated for further studies and conservation.

Procedures

Five grams of fresh leaves were utilized to extract total DNA using the Isolate II Plant DNA Kit (Bioline, UK), following the manufacturer's protocol. The quality and quantity of DNA were assessed through 1% gel electrophoresis and Nanodrop (ThermoScientific, Delaware, USA), respectively.

For library construction, 100-1,000 ng of DNA was fragmented using Covaris S220 through acoustic disruption. The fragmented DNA underwent final repair, dA tailing, adapter ligation, and purification before being selected for library construction. Before sequencing, the effective concentration of each sample library in the library mixture was determined using qPCR to ensure accurate sample concentration and reliable sequencing data. Base calling was performed using the RTA software integrated with the sequencer, which converted the four fluorescence signals obtained from the Charge-Coupled Device (CCD) into binary bcl data in real-time. The bcl data were subsequently transformed into a fastq file using bcl2fastq (v.2.17), a part of the software package provided by Illumina. Concurrent demultiplexing of the data was carried out based on the index information. Primary analysis was conducted using the built-in High-Content Screening (HCS) sequencer software to determine whether the read data passed the chastity filter based on the signal quality of the first 25 cycles. To assess the quality of the raw reads, FastQC was initially used in the Galaxy portal (http://usegalaxy.org). Subsequently, the raw reads were submitted to the Sequence Read Archive (SRA) database in NCBI under the PRJNA965922 project.

Data analysis

The Geseq program (https://chlorobox.mpimp-golm.mpg.de/geseq.html) was utilized for gene annotation and localization in the chloroplast genomes (Tillich et al. 2017). Simple sequence repeat (SSR) motifs were identified using MISA (accessible at http://pgrc.ipk-gatersleben.de/misa/misa.html) with default parameters (Beier et al. 2017). In addition, to identify the location of extended repetitive regions, commonly known as minisatellites. In DNA sequences, the REPuter software (http://bibiserv.cebitec.uni-bielefeld.de/reputer) was with default settings employed as described by Kurtz et al. (2001). This software detected long repeat regions with Hamming distance = 3, a minimum size of 30 base pairs, and a minimum identity of 90%. It categorized the repeats into four types, namely forward (F), reverse (R), complement (C), and palindromic (P).

The MAFFT program (accessible at https://mafft.cbrc.jp/alignment/server/) was employed to align the obtained cp sequence with three other cp sequences of *L. discolor* downloaded from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) with default parameters (Katoh et al. 2019). The alignment was then utilized to determine the phylogenetic relationship among the genomes. Phylogenetic trees of the obtained cp sequence and three cp sequences of *L. discolor* downloaded from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) consisting of MN317571.1, MN880628.1, and NC_030540.1 were constructed using the Neighbor-Joining (NJ) method, a distance-based approach, by using MEGA X software (accessible at https://www.megasoftware.net/) with 500 bootstrap replicates and default parameters (Kang et al. 2017). The chloroplast genome of *Dendrobium sinense* (OM792979.1), a common orchid species for ornamental purposes in Vietnam and China, was used as an outgroup.

RESULTS AND DISCUSSION

Chloroplast genomes own notable advantages in phylogenetic and taxonomic analysis, such as moderate rate of nucleotide replacement, significant variation in coding and non-coding genes, moderate genome size, and collinear properties among different plant species make these genomes an important source for genomic comparison (Su et al. 2020). This study generated a dataset of 7.01 GB of 150 bp pair-end reads, resulting in 19,634,198 raw reads. These reads had a Phred score of approximately 35. From this dataset, 19,388,976 clean reads (98.75% of the total) were selected for further analysis. The assembly of the cp genome map revealed a conserved circular structure including four typical structures, namely Large Single Copy (LSC), Small Single Copy (SSC), and two inverted repeats (IRA and IRB) with a total length of 159,043 bp. The genes located in cp genome are depicted inside and outside the cp map (Figure 1), with those outside being

transcribed in a clockwise direction and those inside in a counterclockwise direction. The functions of genes are described in different colors and explained in figure legend. The size of this cp genome is slightly different from three previously published complete cp genomes in this species, namely MN317571.1, MN880628.1, and NC_030540.1, with cp genome lengths of 153,324 bp, 153,373 bp, and 153,054 bp, respectively. So far, the sizes of sequenced cp genomes range from 120 kb to 160 kb, and different explanations for the variation in cp genomes have been proposed, such as variation in intergenic regions within a genus, variation in inverted repeat regions, and gene loss (Zheng et al. 2017). Park et al. (2020) reported the different cp genome sizes between two *Viburnum erosum* accessions from Korea. By analyzing genomes of 17 accessions in *Aegilops tauschii* species collected across large geographic areas from Turkey, Georgia, Iran, Turkmenistan, Kazakhstan, Tajikistan, Afghanistan, Pakistan, and India to Xinjiang, Shannxi and Henan Provinces in China, Su et al. (2020) found that the cp genome length of these accessions varies from 135,551 to 136,009 bp. Although these genomes were found to be relatively conserved, several structure variations were also noticed among these cp genomes, and this difference was mostly detected in non-coding regions. The variations in cp genome size have also been reported in other jewel orchid species, such as *Goodyera schlechtendaliana* (Oh et al. 2019a). The variations in cp genome size were also reported in two accessions of this species collected from China and Korea by Kim and Kim (2022) and in peach (Amar et al. 2019).

Microsatellites, also known as simple sequence repeats (SSRs), are distributed with high frequency in the cp genomes and are considered to play important roles in the evolution and adaptation of plants. These repeat motifs are commonly employed to detect genomic variations among species. This study applied the MISA program to identify the presence and abundance of SSRs within the obtained cp genome. The program utilized default parameters, focusing on tandem repeat sequences comprising 1-6 nucleotide repeat units.

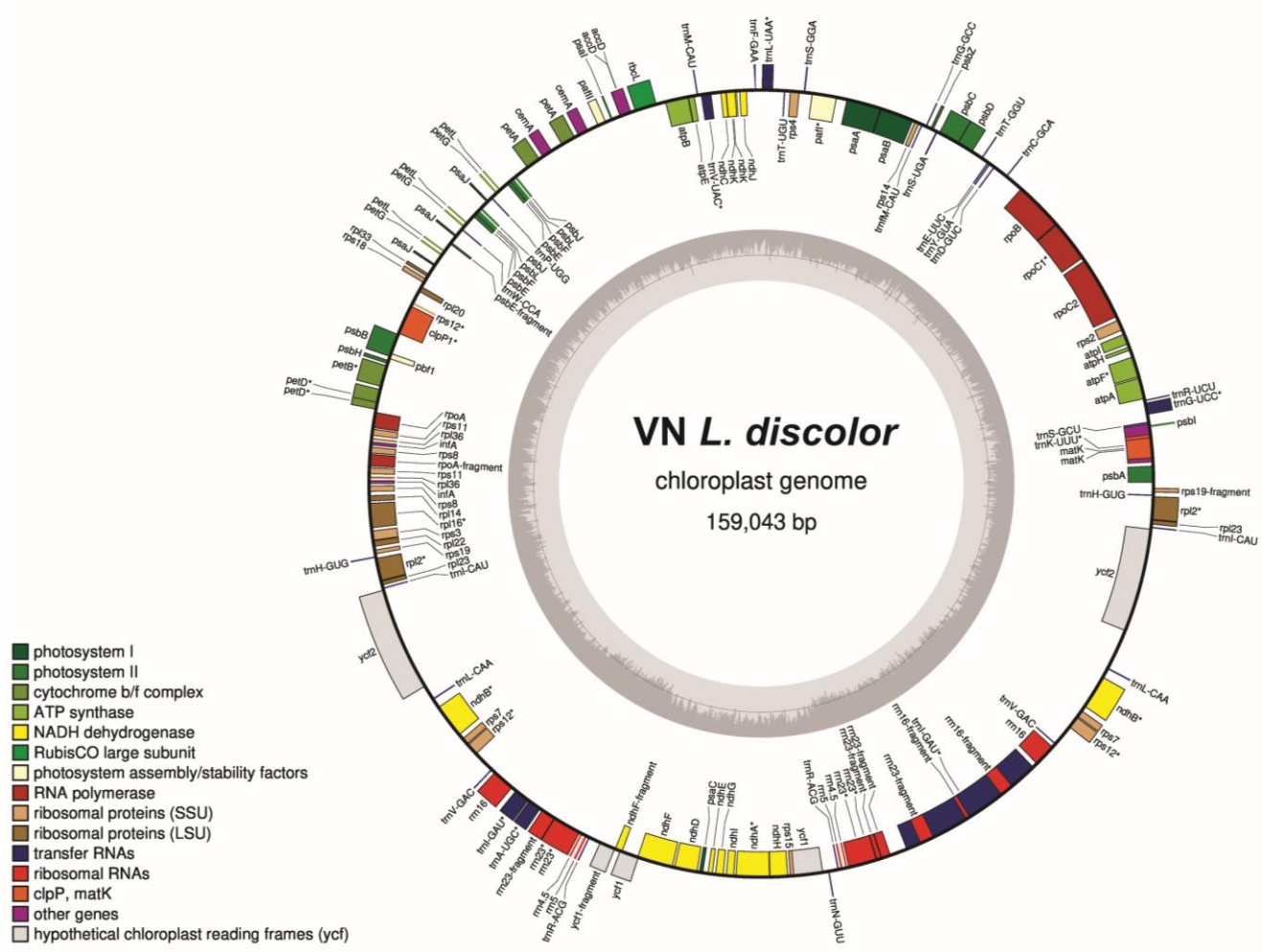

Figure 1. The circular representation of *L. discolor* chloroplast (cp) genome from Vietnam. The genes are depicted inside and outside the circle, with those outside being transcribed in a clockwise direction and those inside in a counterclockwise direction. The inner circle is divided into two regions, with the dark gray color indicating the GC content and the light gray color representing the AT content. Furthermore, the genes are color-coded based on their functional groups.

Moreover, 53 SSRs were detected in the cp genome (Table 1). These SSR motifs encompassed five types: A, T, C, AT, and TA. Among them, mononucleotide motifs consisting of T and A were the most prevalent, accounting for 69.8% (37 SSRs) and 28.3% (15 SSRs) of the total, respectively. Conversely, the genome contained only one PolyC in NC_030540.1, while no polyG was detected. Interestingly, only *L. discolor* from Vietnam shows the presence of the TA motif. It is worth noting that short polyA and polyT repeats are commonly observed in cp genomes, while polyG or polyC repeats are relatively rare (Lei et al. 2016). The different SSR patterns within the cp genome of a plant species have been reported previously. *In silico* comparative analysis, up to polymorphic 49 SSR loci were detected among cp genomes of four *Liriodendron chinense* accessions (Li et al. 2020). The difference in the number and types of SSR motifs was also reported recently in different accessions of *Tetrastigma hemsleyanum* in the family Vitaceae (Dong et al. 2022). Furthermore, this study also reported the variation of SSR numbers corresponding to the regions samples collected. Microsatellites in the cp genome are inherited from a single parent and are frequently employed as molecular markers in evolutionary studies, including investigations of genetic diversity and species identification. The TA motif identified in this study has the potential for evolutionary research on *L. discolor* from Vietnam and helps identify and conserve different species within this genus.

The REPuter tool applied in this study shows the possibility of identifying long repeated regions, known as minisatellites, containing 10 to 100 nucleotides in the cp genome and being proven suitable for analyzing repeat motifs in complete genomes (Kurtz et al. 2001). The difference in length of long repeats is commonly found while comparing cp genomes of different plant species. However, this software obtained slight variation in the number and types of long repeat elements among four cp genomes within one species (Table 1). Most of these motifs are in two types consisting of "forward" and "palindromic"; only the MN317571.1 sequence shows the presence of motifs in "complement" and "reverse" regions. This data is in line with previous studies when almost long repeats are forward and palindromic repeats in *Paphiopedilum delenatii* (Vu et al. 2020), *Corydalis temulifolia* and *C. adunca* (Huang et al. 2022), *Dipterygium glaucum*, *Cleome chrysantha*, *Cleomella lutea*, and *Tarenaya hassleriana* (Alzahrani et al. 2021). The abundance of repeats in forward regions was also reported in *Cypripedium* species (Zhang et al. 2022). The different numbers and locations of these long repeats could come from the architecture of studied cp genomes and display the influence of evolution on cp genomes. Identifying long repeats is important in understanding plant genomes' reorganization, rearrangement, and phylogeny (Song et al. 2022).

The outcomes of the phylogenetic analysis on the four chloroplast (cp) genomes revealed a strong relationship among four *L. discolor* accessions. Three *L. discolor* accessions from China were grouped into a single cluster, whereas *L. discolor* accession from Vietnam was separated into another branch (Figure 2). The clustering pattern of this phylogenetic tree is reliable due to the high bootstrap value indicated on the tree. The phylogenetic tree is considered stable and reliable when this bootstrap value is greater than 75, and the higher the value, the more consistent the relationship in evolutionary analysis (Xue et al. 2019). Specifically, the three accessions from China, MN317571.1, MN880628.1, and NC_030540.1, exhibited a closer relationship and clustered within a monophyletic group. On the other hand, the accession from Vietnam formed a relatively distinct branch, indicating a separation from the Chinese accessions.

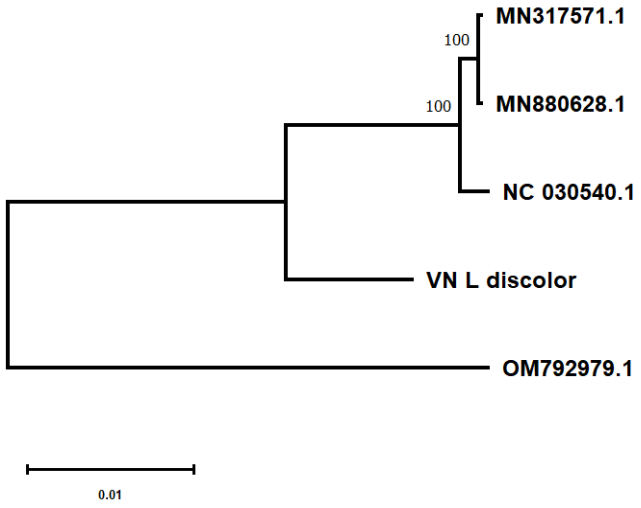

Figure 2. Phylogenetic tree of four cp genomes of *Ludisia discolor* orchid accessions. The chloroplast sequences of *Dendrobium sinense* (OM792979.1) were used as an outgroup. Numbers near branches are bootstrap values.

Table 1. The different repeat motifs and location of long repeat types in the chloroplast genomes of Ludisia *discolor* accessions

Accession	Country of origin	Repeat motifs					Total	Location of long repeat types				Total
		A	T	C	AT	TA		Forward	Complement	Reverse	Palindromic	
VN_L_discolor	Vietnam	15	37	0	0	1	53	21	0	0	27	48
MN317571.1	China	22	38	0	1	1	62	22	1	2	24	47
MN880628.1	China	23	38	0	1	1	63	24	0	0	26	50
NC_030540.1	China	22	30	1	1	1	55	22	0	0	27	49

Phylogenetic analysis based on complete cp genomes has increased power in plant taxonomy. After characterizing two cp genomes consisting of one common accession of *Goodyera schlechtendaliana* collected from China and another *G. schlechtendaliana* accession collected from Korea, both of them a highly similar in morphology and just different in the lateral appendages of the column in their flowers in the later. Oh et al. (2019b) found that the Korean accession is a sister of China's accession and could be a new species. Seventeen accessions from *Aegilops tauschii* were relatively divided into three main clusters corresponding to collection sites (Su et al. 2020). Also applying NGS method, the oolong tea (*Phoenix dancong*) from China was detected with a complicated genetic relationship with different tea species in the genus *Camellia*. Among 75 complete cp genomes, cp genomes of five Fujian oolong tea species are most similar to that of *Phoenix dancong* and form a monophyletic cluster (Liu et al. 2022). These results prove invaluable effectiveness of NGS in determining the origin and classification of plants which could facilitate the effective utilization and management of plant's genetic resources.

In conclusion, our study involved the sequencing and characterization of the complete chloroplast (cp) sequence of *L. discolor*, a jewel orchid species collected from Vietnam. By comparing the newly sequenced cp genome with existing sequences, we identified distinct features such as variations in genome size and sequence repeat motifs among different cp genomes. These findings contribute to understanding cp genomes' typical structure and content, specifically in Vietnam's *L. discolor*. The observed differences among cp genomes also provide insights into the genetic structure within the genus *Ludisia*. Furthermore, the unique TA repeat motifs and highly divergent regions identified in the Vietnam *L. discolor* cp genome have the potential for developing molecular markers, which could be utilized in future investigations related to this valuable herb's taxonomy and conservation efforts in Vietnam.

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REFERENCES

Alzahrani D, Albokhari E, Yaradua S, Abba A. 2021. Complete chloroplast genome sequences of *Dipterygium glaucum* and *Cleome chrysantha* and other Cleomaceae species, comparative analysis and phylogenetic relationships. Saudi J Biol Sci 28 (4): 247602490. DOI: 10.1016/j.sjbs.2021.01.049.

Amar MH, Magdy M, Wang L, Zhou H, Zheng B, Jiang X, Atta AH, Han Y. 2019. Peach chloroplast genome variation architecture and phylogenomic signatures of cpDNA introgression in *Prunus*. Can J Plant Sci 99 (6): 885-896. DOI: 10.1139/cjps-2019-0129.

Beier S, Thiel T, Münch T, Scholz U, Mascher M. 2017. MISA-web: a web server for microsatellite prediction. Bioinformatics 33: 2583-2585. DOI: 10.1093/bioinformatics/btx198.

Burkhan H, Rajan KS, Appalasamy S, Poobathy R, Chew BL, Mariappan V, Subramaniam S. 2022. Effect of cryopreservation method supported with biochemical analyses in the axillary bud of jewel orchid, *Ludisia discolor*. Plants 11 (7): 879. DOI: 10.3390/plants11070879.

Chai Q, Zeng J, Zhang X. 2022. Identification of fake *Anoectochilus roxburghii* based on Bayesian optimized convolutional neural network. Acta Agr Zhe 34 (2): 391-396. DOI: 10.3969/j.issn.1004-1524.2022.02.21.

Chen JR, Shiau YJ. 2015. Application of internal transcribed spacers and maturase K markers for identifying *Anoectochilus*, *Ludisia*, and *Ludochilus*. Biol Plant 59 (3): 485-490. DOI: 10.1007/s10535-015-0520-3.

Daniell H, Lin CS, Yu M, Chang WJ. 2016. Chloroplast genomes: diversity, evolution, and applications in genetic engineering. Genome Biol 17:134. DOI: 10.1186/s13059-016-1004-2.

Dong S, Zhou M, Xhu J, Wang Q, Ge Y, Cheng R. 2022. The complete chloroplast genomes of *Tetrastigma hemsleyanum* (Vitaceae) from different regions of China: molecular structure, comparative analysis and development of DNA barcodes for its geographical origin discrimination. BMC Genom 23: 620. DOI: 10.1186/s12864-022-08755-7.

Gao C, Zhang F, Zhang J, Guo S, Shao H. 2009. Identification of *Anoectochilus* based on rDNA ITS sequences alignment and SELDI-TOF-MS. Int J Biol Sci 5 (7): 727-735. DOI: 10.7150/ijbs.5.727.

Ho VT, Tran TKP, Vu TTT, Widiarsih S. 2021. Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. J Genet Eng Biotechnol 19: 93. DOI: 10.1186/s43141-021-00188-1.

Huang X, Xu X, Wang D. 2022. Insight from newly sequenced chloroplast genome challenges the primitive position of *Corydalis temulifolia* (Papaveraceae). Phytotaxa 548 (2): 223-239. DOI: 10.11646/phytotaxa.548.2.6.

Huynh HD, Nguyen TG, Duong HX, Ha TL, Phan DY, Tran TT, Do DG. 2019. Using some DNA barcode for the genetic analysis and identifying some species of *Anoectochilus* spp. Can Tho Univ J Sci 55 (1): 14-23. DOI: 10.22144/ctu.jsi.2019.002.

Kang Y, Deng Z, Zang R, Long W. 2017. DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. Sci Rep 7: 12564. DOI: 10.1038/s41598-017-13057-0.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20 (4): 1160-1166. DOI: 10.1093/bib/bbx108.

Kim TH, Kim JH. 2022. Molecular phylogeny and historical biogeography of *Goodyera* R.Br. (Orchidaceae): A case of the vicariance between East Asia and North America. Front Plant Sci 13: 850170. DOI: 10.3389/fpls.2022.850170.

Konhar R, Debnath M, Vishwakarma S, Bhattacharjee A, Sundar D, Tandon P, Dash D, Bishwal DK. 2019. The complete chloroplast genome of *Dendrobium nobile*, an endangered medicinal orchid from north-east India and its comparison with related *Dendrobium* species. PeerJ 7: e7756. DOI: 10.7717/peerj.7756.

Kurtz S, Choudhuri JV, Ohlebusch E, Schleiermacher C, Stoye J, Giegerich R. 2001. REPuter: The manifold applications of repeat analysis on a genomic scale. Nucleic Acids Res 29 (22): 4633-4642. DOI: 10.1093/nar/29.22.4633.

Lei W, Ni D, Wang Y, Shao J, Wang X, Yang D, Wang J, Chen H, Liu C. 2016. Intraspecific and heteroplasmic variations, gene losses and inversions in the chloroplast genome of *Astragalus membranaceus*. Sci Rep 6: 21669. DOI: 10.1038/srep21669.

Li B, Lin F, Huang P, Guo W, Zheng Y. 2020. Development of nuclear SSR and chloroplast genome markers in diverse *Liriodendron chinense* germplasm based on low-coverage whole genome sequencing. Biol Res 53: 21. DOI: 10.1186/s40659-020-00289-0.

Liu Y, Li X, Chen J, Xue Y, Zhu Y. 2021. Effects of different factors on adventitious bud induction from stem explants of *Ludisia discolor*. Web Conf 245: 03024. DOI: 10.1051/e3sconf/202124503024.

Liu Y, Lin L, Yang D, Zou X, Zhang Z, Liu M, Lin M, Zheng Y. 2022. Comparative phylogenetic analysis of oolong tea (*Phoenix Dancong* tea) using complete chloroplast genome sequences. Heliyon 8: e12557. DOI: 10.1016/j.heliyon.2022.e12557.

Nguyen TT, Phung VP. 2023. Flora diversity in the proposed delacour's langur species and habitat conservation area, Ha Nam province. Res Man Env 15: 139-147. DOI: 10.55250/jo.vnuf.2023.15.139-147.

Oh SH, Suh HJ, Park J, Kim Y, Kim S. 2019a. The complete chloroplast genome sequence of *Goodyera schlechtendalianain* in Korea (Orchidaceae). Mitochondrial DNA B 4 (2): 2692-2693. DOI: 10.1080/23802359.2019.1641439.

Oh SH, Suh HJ, Park J, Kim Y, Kim S. 2019b. The complete chloroplast genome sequence ofa morphotype of *Goodyera schlechtendaliana* (Orchidaceae) with the column appendages. Mitochondrial DNA Part B 4 (1): 626-627. DOI: 10.1080/23802359.2018.1564390.

Park J, Xi H, Oh S. 2020. Comparative chloroplast genomics and phylogenetic analysis of the *Viburnum dilatatum* complex (Adoxaceae) in Korea. Korean J Pl Taxon 50 (1): 8-16. DOI: 10.11110/kjpt.2020.50.1.8.

Poobathy R, Zakaria R, Murugainyah V, Subramaniam S. 2019. Surface sterilization and micropropagation of *Ludisia discolor*. Biocatal Agric Biotechnol 22: 101380. DOI: 10.1016/j.bcab.2019.101380.

Raskoti BB, Ale R. 2021. DNA barcoding of medicinal orchids in Asia. Sci Rep 11: 23651. DOI: 10.1038/s41598-021-03025-0.

Song W, Ji C, Chen Z, Cai H, Wu X, Shi C, Wang S. 2022. Comparative analysis the complete chloroplast genomes of nine *Musa* species: Genomic features, comparative analysis, and phylogenetic implications. Front Plant Sci 13: 832884. DOI: 10.3389/fpls.2022.832884.

Su JM, Zhu H, Huang ZY, Mo ZZ, Chen RX, Dong YP, Zheng CX. 2017. Study on the proliferation of axillary buds and rooting of *Ludisia discolor*. J Yulin Normal Univ 38: 90-92.

Su Q, Liu L, Zhao M, Zhang C, Zhang D, Li Y, Li S. 2020. The complete chloroplast genomes of seventeen *Aegilops tauschii*: genome comparative analysis and phylogenetic inference. PeerJ 8: e8678. DOI: 10.7717/peerj.8678.

Tillich M, Lehwark P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organelle genomes. Nucleic Acids Res 45: W6-W11. DOI: 10.1093/nar/gkx391.

Vu HT, Tran N, Nguyen TD, Vu QL, Bui MH, Le MT, Le L. 2020. Complete chloroplast genome of *Paphiopedilum delenatii* and phylogenetic relationships among Orchidaceae. Plants 9 (1): 61. DOI: 10.3390/plants9010061.

Wang X, Lu D, Zhu W, Zheng S, Yu X, Ai Y, Zhang Q. 2020. The complete chloroplast genome of an ornamental orchid, *Vanda coerulescens* (Orchidaceae). Mitochondrial DNA Part B 5 (1): 465-467. DOI: 10.1080/23802359.2019.1704195.

Xue S, Shi T, Luo W, Ni X, Iqbal S, Ni Z, Huang X, Yao D, Shen Z, Gao Z. 2019. Comparative analysis of the complete chloroplast genome among *Prunus mume*, *P. armeniaca*, and *P. salicina*. Hortic Res 6: 89. DOI: 10.1038/s41438-019-0171-1.

Yu X, Xie TX, Liu B, Zhang DY, Liu XD, Ai Y, Liu ZJ. 2019. The complete chloroplast genome sequence of *Ludisia discolor* from Hainan of China. Mitochondrial DNA B 4 (2): 3663-3664. DOI: 10.1080/23802359.2019.1678438.

Zhang JY, Liao M, Cheng YH, Feng Y, Ju WB, Deng HN, Li X, Moraj AP, Xu B. 2022. Comparative chloroplast genomics of seven endangered *Cypripedium* species and phylogenetic relationships of Orchidaceae. Front Plant Sci 13: 911702. DOI: 10.3389/fpls.2022.911702.

Zheng XM, Wang J, Feng L, Liu S, Phang H, Qu L, Li J, Sun Y, Qiao W, Zhang L, Cheng Y, Yang Q. 2017. Inferring the evolutionary mechanism of the chloroplast genome size by comparing whole chloroplast genome sequences in seed plants. Sci Rep 7: 1555. DOI: 10.1038/s41598-017-01518-5.