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Research Article
Complete Chloroplast Genome of *Curculigo orchioides* from Vietnam: Structural Features, Repeat Signatures, and High-Resolution Candidate DNA Barcodes

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Abstract

Background and Objective: *Curculigo orchioides* is a valuable medicinal plant that has been overexploited in Vietnam, leading to a significant decline in its genetic resources. The chloroplast genome, characterized by high structural conservation, provides a reliable framework for species identification and phylogenetic inference. This study aimed to characterize the complete chloroplast genome of *C. orchioides* collected in Vietnam and to evaluate its structural features, sequence variation, and phylogenetic position. **Materials and Methods:** Raw sequencing reads were quality-filtered using fastp v1.3.1, assembled with GetOrganelle, and annotated via CPGAVAS2. Repetitive elements were identified using MISA and REPuter/EMBOSS. Codon usage bias was assessed based on GC3s, ENc, CAI, Fop, and CBI indices. Genome alignment and nucleotide diversity (π) analyses were conducted using MAFFT, and phylogenetic relationships were reconstructed with IQ-TREE. **Results:** The chloroplast genome is approximately 157 kb in length and displays a typical quadripartite circular structure, comprising 80 protein-coding genes, 30 tRNA genes, and 4 rRNA genes. A total of 48 simple sequence repeats (SSRs), predominantly A/T mononucleotide motifs, and nine long sequence repeats (LSRs) were detected, indicating overall structural stability. Codon usage analysis revealed a strong A/T bias (low GC3s) and evidence of translational optimization in genes related to the genetic machinery, notably *rps18* with a high CAI value. A hypervariable region (≈118-140 kb) with elevated nucleotide diversity was identified around *rpoC2*, representing a promising candidate for high-resolution DNA barcoding. Phylogenomic analysis confirmed that the Vietnamese accession forms a well-supported monophyletic clade with *C. orchioides* and is closely related to *Hypoxis*. **Conclusion:** These findings provide genomic resources for provenance tracing and phylogenetic studies within Hypoxidaceae.

Key words: *Curculigo orchioides*, chloroplast genome, SSR, LSR, DNA barcoding

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The chloroplast genome is well known for its high degree of structural conservation and has been widely and effectively applied in studies of plant identification, classification, and taxonomy[1]. Owing to its moderate genome size, the chloroplast genome is particularly convenient for whole-genome sequencing and accurate assembly. Nevertheless, despite its overall conserved nature, the chloroplast genome contains locally variable regions, which are highly informative for resolving close phylogenetic relationships, tracing maternal lineages, and developing molecular markers for plant identification.

Structurally, the chloroplast genome of most angiosperms exhibits a typical quadripartite organization consisting of a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat regions (IRa and IRb)[2]. This pattern of selective conservation renders the chloroplast genome an important platform for phylogenetic and phylogenomic studies, comparative genomics, and the identification of highly informative DNA barcoding regions[3].

In the field of medicinal plants, chloroplast genome-based analyses have attracted increasing attention for accurate species identification and authentication of biological origin[4]. During medicinal material processing, raw materials are often subjected to grinding into powders or extraction into concentrates, which largely eliminates diagnostic morphological features traditionally used for identification. This frequently leads to misidentification or confusion among different plant components. Consequently, chloroplast genome-derived molecular markers such as SSRs, nucleotide diversity "hotspot" regions[5], and other highly variable genic or intergenic regions have been proposed as effective and practical solutions, particularly suitable for degraded or processed samples[6].

Curculigo orchioides (Hypoxidaceae) is a valuable medicinal plant widely used across many Asian countries, including Vietnam, but is currently facing genetic erosion due to overexploitation and habitat degradation[7]. From a systematic perspective, Hypoxidaceae belongs to the order Asparagales, a lineage generally characterized by conserved chloroplast genome architecture, yet exhibiting subtle variations at inverted repeat boundaries, intergenic regions, and levels of sequence divergence in certain functional genes[8]. These variations are evolutionarily informative, providing insights into mechanisms of chloroplast genome diversification, and simultaneously offer opportunities for developing high-resolution molecular markers capable of discriminating intra-specific lineages or closely related medicinal sources[9,10].

Despite the availability of plastome data for the Hypoxidaceae family, the plastome of *Curculigo orchioides* from Vietnam remains poorly understood. This study aimed to construct a complete reference plastome and analyze its structural characteristics, repetitive elements, codon usage patterns, nucleotide diversity, and phylogenetic relationships. The findings offer valuable evolutionary perspectives and identify potential genetic markers for precise DNA barcoding and conservation applications.

To address the questions outlined above, this study sequenced and assembled the complete chloroplast genome of *C. orchioides* collected from Than Sa-Phuong Hoang Nature Reserve, Vietnam, and analyzed its structural characteristics while developing molecular markers for the authentication of this medicinal herb.

MATERIALS AND METHODS

Study area and duration: The Than Sa-Phuong Hoang Nature Reserve is located in Vo Nhai District, Thai Nguyen Province, northern Vietnam, approximately 40 km North of Thai Nguyen city. The reserve lies between 21°45 12″ and 21°56 30″ N Latitude, and 105°51 05″ to 106°08 07″ E Longitude. The study area is characterized by a tropical humid evergreen forest ecosystem on both limestone and soil mountains, with high biodiversity and a large number of rare and endemic plant species. The geographical location of the study area is shown in Fig. 1.

This protected area is characterized by tropical humid evergreen forests distributed across both limestone and soil mountainous landscapes, supporting high biodiversity and a large number of rare and endemic plant species. The terrain is rugged, with steep slopes and strongly dissected rock formations resulting from complex geological processes. The Rocky Mountains dominate a large proportion of the area, creating diverse microhabitats associated with variations in elevation, soil depth, and moisture conditions.

The Than Sa-Phuong Hoang Nature Reserve represents a unique forest ecosystem with rich biological resources, including a high diversity of flora and fauna. The area also exhibits a humid tropical monsoon climate, with distinct wet and dry seasons, which plays an important role in shaping vegetation structure and species distribution. Plant materials of *Curculigo orchioides* were collected from natural populations within the reserve from June to September 2024. This sampling period ensured suitable plant conditions for DNA extraction and subsequent chloroplast genome analysis. The overall study was conducted from May 2025 to February 2026.

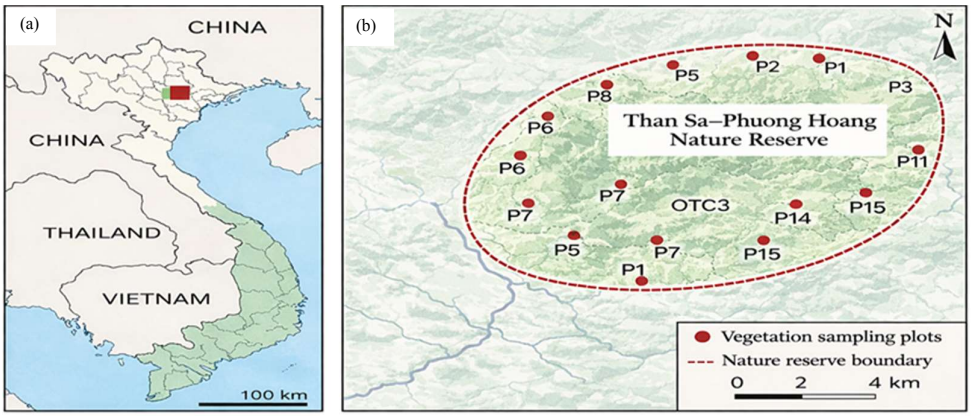

Fig. 1(a-b): Location of the study area in northern Vietnam, (a) Geographic location of Thai Nguyen Province and (b) Location of Than Sa-Phuong Hoang Nature Reserve in Vo Nhai District, Thai Nguyen Province showing the distribution of vegetation sampling plots

Plant material, DNA extraction, and sequencing: Fresh leaves of *C. orchioides* were collected from the Than Sa-Phuong Hoang Nature Reserve, Vietnam (coordinates: 21°84 44″B, 106°01 50″N), and a voucher specimen was prepared and deposited at Thai Nguyen University (voucher No. 20250806). Total genomic DNA was extracted using the STAB method as previously described[11]. Sequencing was performed using a MiSeq platform (Illumina) generating paired-end reads with a total yield of 15 Gb.

Read preprocessing, plastome assembly, and annotation: Raw reads were adapter-trimmed and quality-filtered using fastp v1.3.1. The plastome was de novo assembled using GetOrganelle v1.7.7.1 with the "embplant" database to recruit chloroplast-like reads. Gene annotation (CDS, tRNA, rRNA) was conducted using CPGAVAS2, followed by manual curation of start/stop codons and intron boundaries when necessary. The circular plastome map was generated using a chloroplast genome mapping tool.

SSR and long sequence repeat (LSR) detection: The SSRs (mono- to hexanucleotide motifs) were identified using MISA with default repeat thresholds. Long sequence repeats (forward, palindromic, reverse, and complement) were detected using REPuter/EMBOSS with the minimum repeat length and sequence identity settings specified in the preliminary manuscript configuration.

IR boundary analysis and plastome comparison: The IR expansion/contraction and the positions of LSC/SSC/IR junctions were visualized using CPJSdraw v1.0.0. Whole-plastome sequence similarity among selected species/taxa was assessed using mVISTA (Shuffle-LAGAN).

Codon usage bias (CUB) analysis: Protein-coding genes were extracted and filtered by a minimum length ($\geq$300 bp) and the requirement that the sequence length be divisible by three. Codon usage indices-including GC3s, ENc, CAI, Fop, CBI, and RSCU-were computed using an in-house Python pipeline (BioPython/Pandas). The PR2-bias plots were used to evaluate the relative contributions of mutational pressure versus natural selection.

Nucleotide diversity and identification of candidate DNA barcoding regions: Six plastomes of *C. orchioides* (including the Vietnamese sample and reference accessions from GenBank) were aligned using MAFFT v7.526. SNPs were extracted using SNP-sites, and nucleotide diversity (π) was estimated using VCFtools v0.1.17 with a sliding-window approach (window size: 600 bp; step size: 200 bp). Windows with the highest π values were considered candidate "hotspots" for marker/barcode development.

Phylogenetic inference: A phylogenetic tree based on the whole-plastome alignment was inferred under the maximum likelihood (ML) framework using IQ-TREE 3 v0.23+. ModelFinder was used to select the best-fit substitution model, and branch support was evaluated using 1,000 ultrafast bootstrap (UFBoot) replicates and 1,000 SH-aLRT replicates.

RESULTS

Plastome structure and gene content of *C. orchioides*: The chloroplast genome (plastome) of *C. orchioides* is a circular DNA molecule of approximately 157 kb, exhibiting the typical quadripartite architecture comprising a large single-copy (LSC)

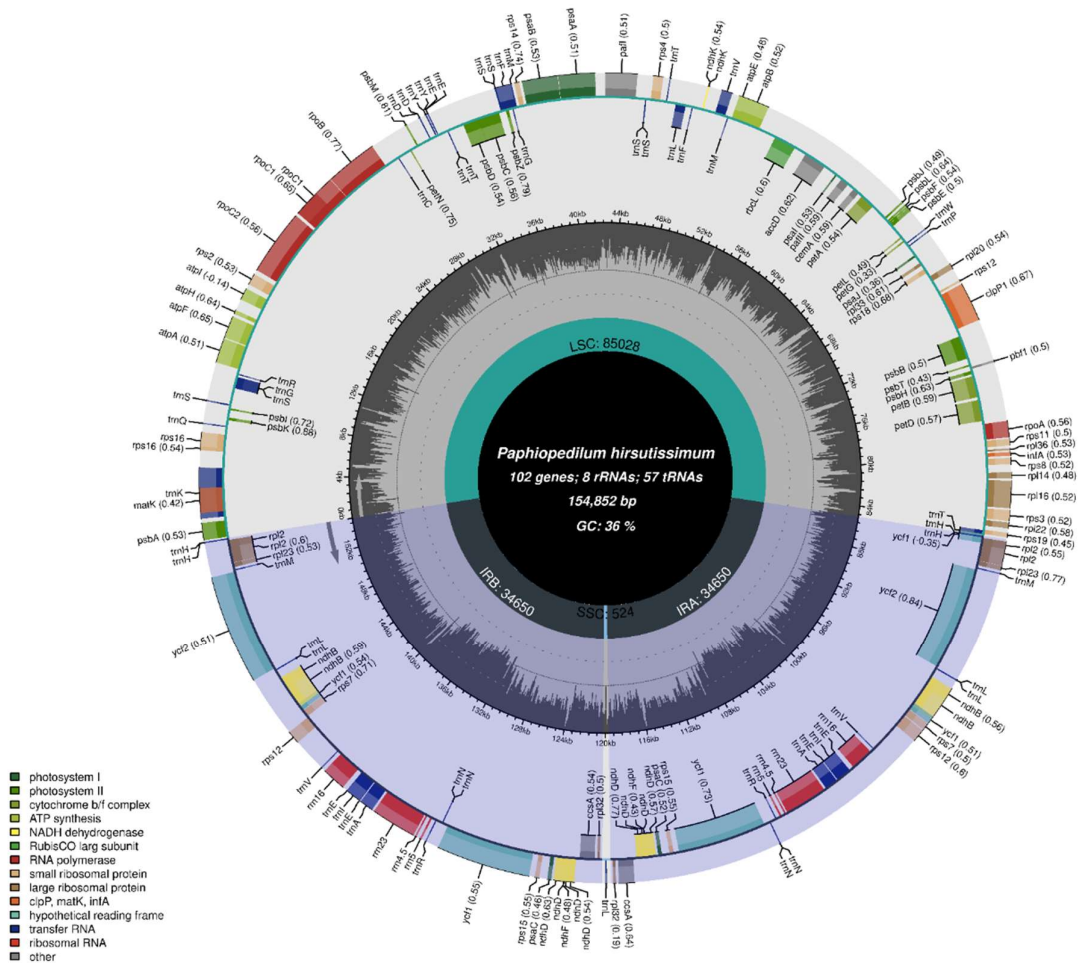

Fig. 2: Chloroplast genome map of Curculigo orchioides from Vietnam
Genes are categorized by functional groups; the two IR regions indicate duplication of the corresponding genes

region, a small single-copy (SSC) region, and a pair of inverted repeats (IRa/IRb). A total of 113 unique genes were annotated, including 80 protein-coding genes, 30 tRNA genes, and 4 rRNA genes, as shown in Fig. 2.

SSR landscape and marker potential: The SSR analysis identified 48 loci across the plastome (Fig. 3). Mononucleotide SSRs were predominant (45/48; 93.75%), mainly poly-A/T repeats, consistent with the A/T-rich nature of chloroplast DNA. Notably, a rare poly-C SSR and three long complex SSRs (~90-117 bp) located in non-coding regions were detected, suggesting strong potential as high-resolution markers for provenance tracing and authentication of medicinal raw materials.

As presented in Fig. 3a, total SSR abundance varied clearly among the examined species. *Cypripedium wardii* displayed the greatest number of SSRs, followed by *Paphiopedilum sangii*, whereas *P. hirsutissimum* and *P. tranlienianum* yielded substantially lower counts. Regarding motif class composition, mono-nucleotide repeats overwhelmingly outnumbered di-, hexa-, and compound SSRs in all taxa. Di-nucleotide motifs occurred only at low frequencies, while hexa-nucleotide and compound microsatellites were exceptional. According to Fig. 3b, raw motif frequencies also differed across species; nevertheless, the A/T motif consistently dominated in every taxon, reflecting the typical AT-richness of plastid genomes. Additional motifs (e.g., AT, CTTCT, GAGAGA) appeared much less frequently and contributed minimally to overall SSR composition. Collectively, these results indicate a relatively conserved SSR distribution pattern, yet interspecific variation remains detectable and potentially useful for taxonomy, phylogenetics, and molecular marker design.

Long sequence repeats (LSRs) and structural stability: Long sequence repeats (LSRs) were identified and classified into three repeat types: Palindromic, reverse, and forward

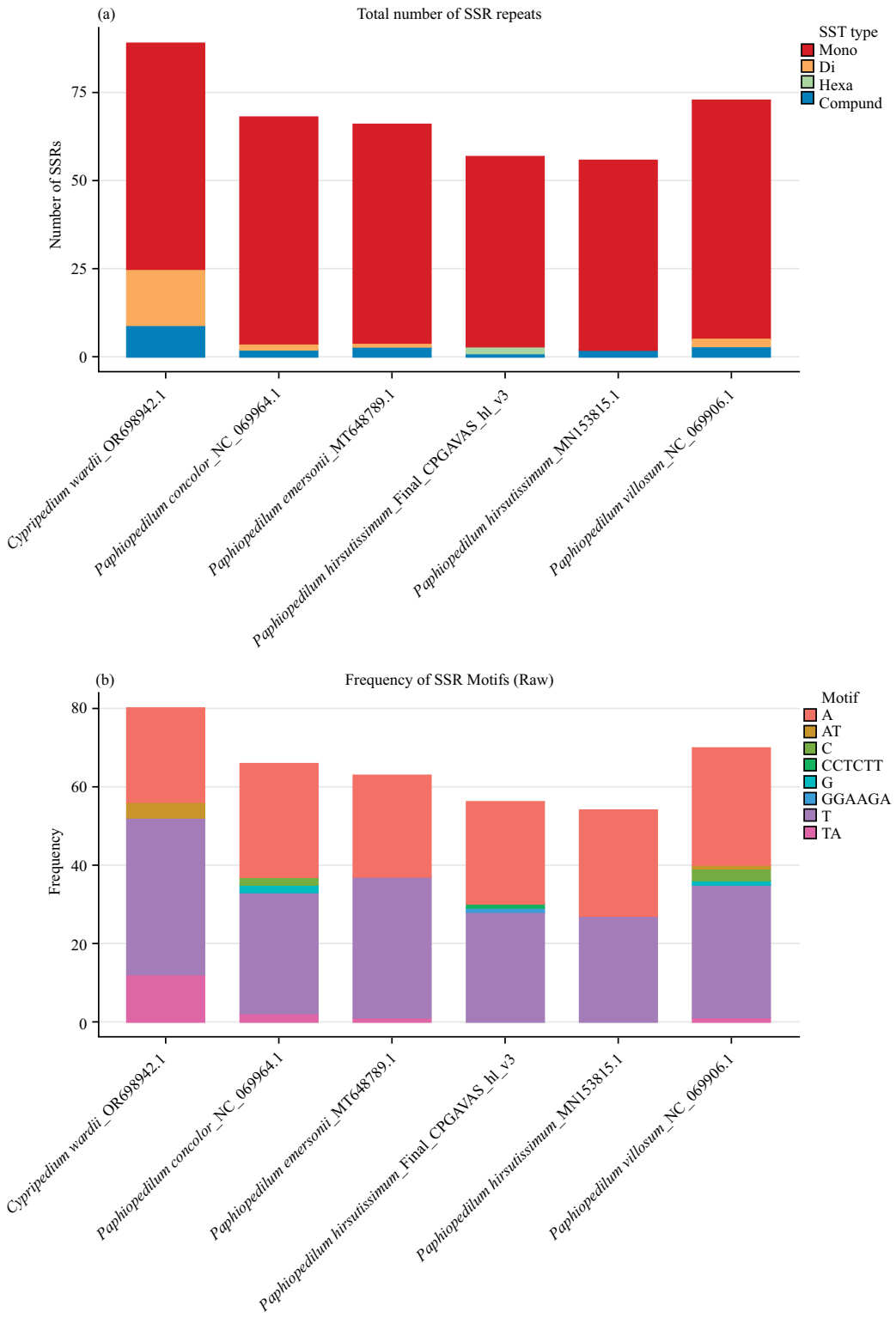

Fig. 3(a-b): Distribution of simple sequence repeats (SSRs) in selected plastomes, (a) Shows the total number of simple sequence repeats (SSRs) classified by repeat type across the selected plastomes, revealing that mononucleotide repeats are predominant in all plastomes and (b) Presents the frequency of individual SSR motifs, showing that A/T mononucleotide motifs are the most abundant, whereas other motifs occur at much lower frequencies, consistent with the AT-rich nature of chloroplast genomes

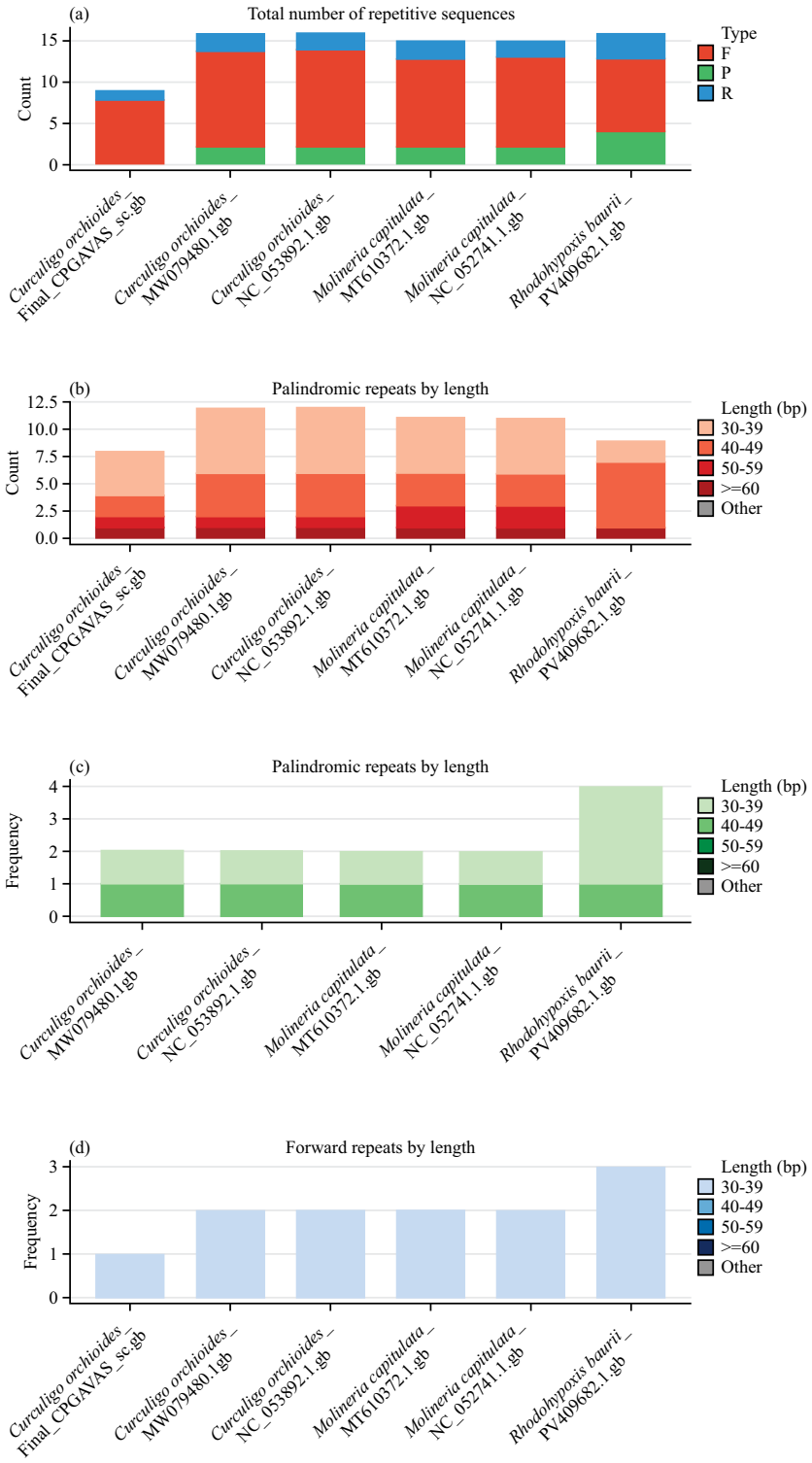

Fig. 4(a-d): Comparative distribution of long sequence repeats (LSRs) in Hypoxidaceae plastomes, (a) Total number of repeats classified by repeat type (palindromic, forward, and reverse) across the analyzed plastomes, (b) Length class distribution of palindromic repeats, demonstrating a high concentration in the 30-49 bp range, (c) Length class distribution of reverse repeats across the selected taxa and (d) Length class distribution of forward repeats, showing low overall abundance across species

repeats (Fig. 4a). Overall, palindromic repeats were the most abundant category across the examined Hypoxidaceae plastomes, whereas forward repeats were comparatively rare and reverse repeats occurred at low to moderate frequencies depending on species. In Fig. 4a, the total number of repeats varied among species, with *Curculigo capitulata* and *Molineria capitulata* showing relatively high repeat counts, largely due to the predominance of palindromic repeats. Figure 4b shows that palindromic repeats were distributed mainly in the 30-39 bp and 40-49 bp length classes, while longer repeats (≥60 bp and other length classes) were less frequent. Figure 4c shows the length distribution of reverse repeats; reverse repeats were detected in fewer plastomes and were concentrated mainly in the 30-39 bp and 40-49 bp classes, with the highest frequency observed in *Hypoxis hemerocallidea*. Figure 4d shows that forward repeats were scarce across the dataset and occurred primarily as single or low-frequency records, with most sequences belonging to short length classes. These results indicate that the repeat profiles of Hypoxidaceae plastomes are dominated by short to intermediate-length palindromic repeats, whereas reverse and forward repeats contribute only a minor fraction of the total repetitive sequences.

IR boundary features and plastome comparison: The IR/SC boundary analysis indicated that gene positions at the LSC/IR and SSC/IR junctions are relatively conserved, implying limited IR expansion/contraction. Comparative mVISTA analysis showed that coding regions are markedly more conserved than intergenic regions; sequence divergence is concentrated in several specific loci, which may be exploited as informative marker candidates.

Codon usage bias: Codon usage bias (CUB) indices indicated low GC3s values (~25.40-33.33%), reflecting an A/T-rich background largely shaped by mutational pressure. However, several genes involved in the genetic machinery, particularly ribosomal protein genes, showed higher CAI, Fop, and CBI values, suggesting translational selection and optimization of translation efficiency in key housekeeping genes.

Nucleotide diversity hotspots and candidate DNA barcodes: Sliding-window estimates of nucleotide diversity (π) revealed a heterogeneous distribution, with variation concentrated in localized regions. A prominent hypervariable interval was detected at ~118-140 kb, where the RNA polymerase gene cluster (rpoC2/rpoC1/rpoB) and several ATP synthase-related genes exhibited elevated π values; the highest peak was observed around rpoC2. These high-π windows represent promising candidates for developing next-generation barcodes to improve discriminatory power in cases of misidentification or adulteration of medicinal plant materials, as shown in Fig 5.

Plastome-based phylogenetic inference: The maximum-likelihood (ML) tree inferred from whole-plastome sequences confirmed that the Vietnamese sample clusters tightly with reference accessions of *C. orchioides* with very strong support, and further indicates a close relationship with Hypoxis within the family Hypoxidaceae in Fig. 6. These results support the accuracy of species identification and the quality of plastome assembly.

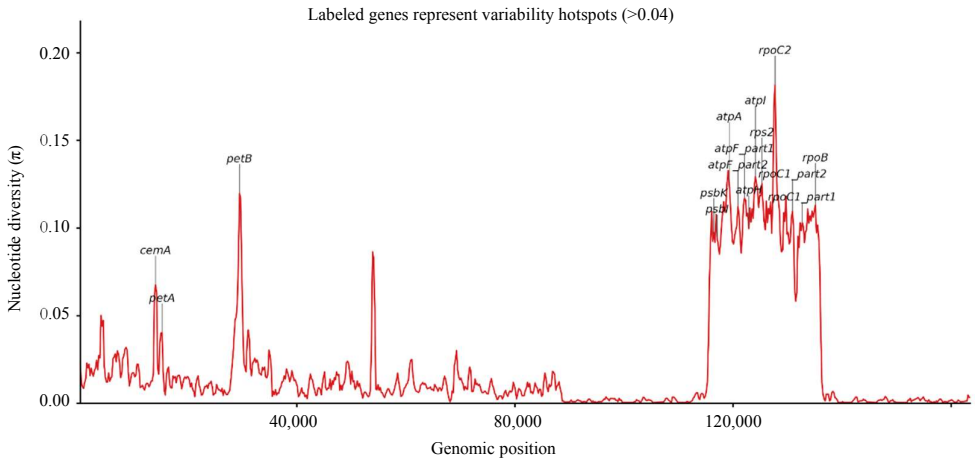

Fig. 5: Nucleotide diversity (π) across the plastome of *Curculigo orchioides* based on sliding-window analysis
Nucleotide diversity (π) represents the average number of nucleotide differences per site between sequences. The analysis was performed using a sliding window (window size: 600 bp; step size: 200 bp). Peaks in π values indicate hypervariable regions, which are considered potential hotspots for the development of molecular markers and DNA barcodes

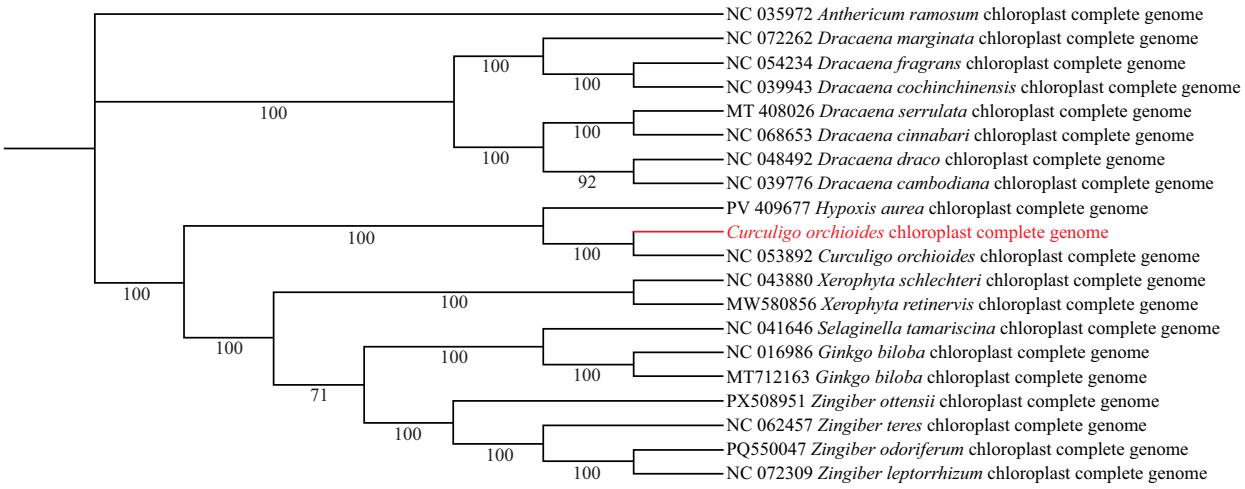

Fig. 6: Maximum-likelihood (ML) phylogenetic tree inferred from whole-plastome sequences (IQ-TREE)
Branch support values are shown as UFBoot and/or SH-aLRT at major nodes

DISCUSSION

This study reports a reference plastome for *Curculigo orchioides* from Vietnam, integrating multiple analytical dimensions, genome structure, repetitive elements, codon usage bias (CUB), nucleotide diversity (π), and phylogenetic reconstruction, to provide enhanced interpretive depth. The assembled plastome is approximately 157 kb in length, exhibits the canonical quadripartite structure (LSC, SSC, IRa, IRb), and harbors a conserved gene set of roughly 113 genes. This architecture aligns with the typical plastome organization documented in monocots and angiosperms[12], thereby validating the assembly and annotation reliability[13]. Beyond serving as a genomic reference, this dataset holds dual significance: It contributes to elucidating plastome evolution within the understudied family Hypoxidaceae (Asparagales)[14], while establishing a foundation for developing molecular tools to authenticate and conserve this valuable medicinal species threatened by overharvesting.

Structural stability is evidenced by the limited number of long sequence repeats (LSRs) and conserved inverted repeat (IR) boundaries. In numerous plant lineages, elevated LSR content, particularly long inverted repeats, can facilitate intramolecular recombination, potentially inducing structural rearrangements, genome size variation, and gene order alterations[15]. The detection of only a few LSRs in *C. orchioides*, predominantly forward repeats, suggests restricted large-scale recombination, consistent with structural stability documented across diverse angiosperms[16]. This stability enhances the plastome's utility for comparative genomics[17], marker development, and phylogenetic inference. Furthermore, the highly conserved IR junctions (IRa/SSC and IRb/SSC boundaries) imply that detectable differences predominantly reflect nucleotide-level variation, typically within intergenic spacers, rather than major structural rearrangements, a pattern similarly observed in other Asparagales plastomes[18].

SSR analysis revealed predominance of A/T mononucleotide repeats, consistent with the A/T-rich composition characteristic of most plastomes[19]. From an applied perspective, the most informative SSR loci combine high polymorphism with robust, reproducible amplification[20]. The presence of long, complex SSRs and a rare poly-C motif merits attention, as such loci may offer enhanced discriminatory power for population genetics and sample differentiation, particularly valuable for processed medicinal materials where DNA is frequently degraded and fragmented[21]. Chloroplast-derived SSRs (cpSSRs) prove especially useful in phylogeographic and population-level analyses due to uniparental inheritance and typically low recombination rates[6]. Practically, cpSSRs can be developed into PCR-based fragment-length assays or multiplex panels[22]. When combined with nucleotide diversity hotspots, they can support multi-tiered authentication frameworks pairing short, easily amplifiable loci with highly variable regions to maximize resolution[23].

CUB analysis provides mechanistic insights into evolutionary pressures shaping the plastome. The low GC content at third codon positions (GC3s) indicates pervasive mutational pressure favoring A/T nucleotides, a common plastome feature[18]. However, elevated values of indices, including the codon adaptation index (CAI), frequency of

optimal codons (Fop), and codon bias index (CBI) in plastid genetic system genes, particularly ribosomal protein and RNA polymerase genes, suggest translational selection optimizing expression of core transcription-translation machinery[24]. Collectively, these findings support an interpretation whereby mutational pressure shapes the overall codon landscape, while natural selection fine-tunes codon usage in genes requiring stable, efficient expression[25]. This also explains why plastid housekeeping genes may exhibit stronger signatures of selective constraint compared to other loci.

A key applied finding concerns the non-uniform distribution of nucleotide diversity (π), with prominent hotspots concentrated within the ~118-140 kb region and peaking near rpoC2. Heterogeneous mutation rates are widely observed across plastomes, with intergenic spacers and introns frequently displaying elevated variability[26]. Such hypervariable regions represent strong candidates for developing high-resolution DNA barcodes for closely related species and population studies[27], particularly when standard barcodes provide insufficient discriminatory power or when supply chain traceability is require[28]. Biologically, the presence of a major hotspot within the rpo gene cluster may reflect heterogeneous functional constraints, with some segments under strong purifying selection and others under relaxed constraint, or broader differences in evolutionary dynamics among plastome regions[29]. For practical marker development, priority should be given to segments of suitable length for amplification from degraded DNA with stable primer-binding sites, ensuring inter-laboratory reproducibility[30].

Phylogenetic inference based on complete plastome sequences strongly supports clustering of the Vietnamese sample with reference *C. orchioides* accessions, confirming robust placement within Hypoxidaceae. Whole-plastome datasets generally provide substantially higher phylogenetic resolution than limited locus set[31]. Here, the phylogenomic result serves both as confirmatory evidence for species identification and as an indicator of assembly quality, mitigating concerns about misassembly or chimeric sequences, important considerations when developing authentication markers[15]. The inferred close relationship with the genus Hypoxis aligns with recent molecular phylogenies of the family[32]. From a conservation perspective, plastomes (typically maternally inherited) can help trace maternal lineages and characterize phylogeographic structure, supporting germplasm selection, assessment of population continuity, and prioritization of genetic resource conservation across ecological regions[27].

Several limitations of this study suggest directions for future research. Although π analysis based on six available plastomes is appropriate for initial hotspot screening, expanded sampling across the species' geographic range is necessary to estimate intraspecific variation better, validate hotspot stability, and assess practical resolution among populations[33]. The proposed SSR loci and hotspot regions require empirical validation through primer design, PCR optimization, and evaluation of specificity, sensitivity, and robustness across diverse sample types, following recommendations for herbal DNA barcoding applications[34].

CONCLUSION

Overall, the *C. orchioides* plastome demonstrates structural conservation while harboring informative regions with strong potential for applied use. The dataset and candidate markers reported here provide a foundation for developing integrated marker systems for herbal authentication and traceability, surveying maternal-lineage diversity across regions to guide conservation prioritization and sustainable harvesting, and expanding phylogenomic analyses within Hypoxidaceae to clarify plastome evolutionary dynamics and identify informative loci for resolving closely related taxa.

SIGNIFICANCE STATEMENT

This study provides the first complete chloroplast genome reference for *Curculigo orchioides* from Vietnam, offering critical insights into Hypoxidaceae evolutionary dynamics. Characterized by high structural conservation and distinct codon optimization, the plastome harbors high-resolution nucleotide diversity hotspots (notably near *rpoC2*) and unique simple sequence repeats. These hypervariable loci serve as powerful molecular barcodes, establishing a robust framework for authenticating processed medicinal materials, tracing provenance, and guiding regional conservation germplasm strategies.

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REFERENCES

1. Dobrogojski, J., M. Adamiec and R. Luciński, 2020. The chloroplast genome: A review. Acta Physiol. Plant., Vol. 42. 10.1007/s11738-020-03089-x.

2. Pan, W., W. Yan, Z. Li, Z. Wu and Q. Tian *et al*., 2026. Analysis of chloroplast genomes and SSR classification and identification of *Agropyron* species and closely related species. Sci. Rep., Vol. 16. 10.1038/s41598-025-33570-x.
3. Cauz-Santos, L.A., 2025. Beyond conservation: The landscape of chloroplast genome rearrangements in angiosperms. New Phytol., 247: 2571-2580.
4. Zhang, N., K. Huang, P. Xie, A. Deng and X. Tang *et al*., 2024. Chloroplast genome analysis and evolutionary insights in the versatile medicinal plant *Calendula officinalis* L. Sci. Rep., Vol. 14. 10.1038/s41598-024-60455-2.
5. Liu, H., Z. Su, S. Yu, J. Liu and X. Yin *et al*., 2019. Genome comparison reveals mutation hotspots in the chloroplast genome and phylogenetic relationships of *Ormosia* species. Biomed Res. Int., Vol. 2019. 10.1155/2019/7265030.
6. Jayaswall, K., H. Sharma, D. Jayaswal, R. Sagar and A. Bhandawat *et al*., 2023. Development of chloroplast derived SSR markers for genus *Allium* and their characterization in the allies for genetic improvement of Alliums. S. Afr. J. Bot., 162: 304-313.
7. Muthiam, B., K. Pathak, H. Pathak, R. Saikia and J.J. Sahariah *et al*., 2025. Medicinal plants of genus *Curculigo*: Exploring traditional applications, phytochemistry, ethnopharmacological perspective. Discover Plants, Vol. 2. 10.1007/s44372-024-00074-3.
8. Ma, D., Q. Tian, Y. Wang, H. Duan, Y. Zhang, Y. Luo and L. Li, 2025. Comparative chloroplast genome of six species in Hypoxidaceae from China: Insights into phylogenetic relationships and molecular marker development. BMC Plant Biol., Vol. 25. 10.1186/s12870-025-07191-5.
9. Jiang, D., X. Cai, M. Gong, M. Xia and H. Xing *et al*., 2023. Complete chloroplast genomes provide insights into evolution and phylogeny of *Zingiber* (Zingiberaceae). BMC Genomics, Vol. 24. 10.1186/s12864-023-09115-9.
10. Song, H., F. Liu, Z. Li, Q. Xu, Y. Chen, Z. Yu and N. Chen, 2020. Development of a high-resolution molecular marker for tracking *Phaeocystis globosa* genetic diversity through comparative analysis of chloroplast genomes. Harmful Algae, Vol. 99. 10.1016/j.hal.2020.101911.
11. Peredo, E.L., 2023. CTAB/chloroform-isoamyl alcohol DNA extraction protocol. Protocols.io, 10.17504/protocols.io.261gednpdv47/v1.
12. Wu, H., J.B. Yang, J.X. Liu, D.Z. Li and P.F. Ma, 2022. Organelle phylogenomics and extensive conflicting phylogenetic signals in the monocot order Poales. Front. Plant Sci., Vol. 12. 10.3389/fpls.2021.824672.
13. Chen, N., L.N. Sha, Y.L. Wang, L.J. Yin and Y. Zhang *et al*., 2021. Variation in plastome sizes accompanied by evolutionary history in monogenomic triticeae (Poaceae: Triticeae). Front. Plant Sci., Vol. 12. 10.3389/fpls.2021.741063.
14. Ji, Y., J.B. Landis, J. Yang, S. Wang, N. Zhou, Y. Luo and H. Liu, 2023. Phylogeny and evolution of Asparagaceae subfamily Nolinoideae: New insights from plastid phylogenomics. Ann. Bot., 131: 301-312.
15. Yin, D., X. Li, Z. Xiao and L. Zhou, 2025. Chloroplast genome features and phylogeny of two nationally protected medicinal plants, *Euchresta tubulosa* and *Euchresta japonica*: Molecular resources for identification and conservation. Genes, Vol. 16. 10.3390/genes16111286.
16. Wei, Y., Z. Lin, J. Jin, W. Zhu and J. Gao *et al*., 2025. Genome-wide identification and functional characterization of LBD gene family in four *Cymbidium* species (Orchidaceae) and potential regulatory role of *CsiLBD27* in floral development of *Cymbidium sinense*. BMC Genomics, Vol. 26. 10.1186/s12864-025-11701-y.
17. Tamboli, A.S., I.S. Choi, J.S. Youn, R.N. Mane and A.R. Gholave *et al*., 2025. Comparative genomics of plastid genome within *Curculigo orchioides* subclade (Hypoxidaceae) reveals evolutionary relationships and document the shortest *ndhA* intron in seed plants. Planta, Vol. 262. 10.1007/s00425-025-04807-w.
18. Liu, H., H. Jacquemyn, Y. Wang, Y. Hu and X. He *et al*., 2025. Comparative analysis of the chloroplast genomes of cypripedium: Assessing the roles of SSRs and TRs in the non-coding regions of LSC in shaping chloroplast genome size. Int. J. Mol. Sci., Vol. 26. 10.3390/ijms26083691.
19. Lin, S., J. Shu, C. Kang, W. Fang, X. Liang, K. Xu and H. Ma, 2026. Integrative chloroplast omics in citrus: Computational insights into genomic structural adaptation and phylogenetic relationships from 35 species. Front. Plant Sci., Vol. 17. 10.3389/fpls.2026.1769665.
20. Zhao, L., J. Zhou, W. Zhao, Y. Wang and P. Wang, 2026. Integrated phenotypic, nutritional, and SSR marker analyses reveal genetic diversity and guide germplasm utilization in bird's-foot trefoil. BMC Plant Biol., Vol. 26. 10.1186/s12870-025-07914-8.
21. Saha, D., R.S. Rana, S. Das, S. Datta, J. Mitra, S.J. Cloutier and F.M. You, 2019. Genome-wide regulatory gene-derived SSRs reveal genetic differentiation and population structure in fiber flax genotypes. J. Appl. Genet., 60: 13-25.
22. Guo, L., Q. Yang, J.W. Yang, N. Zhang and B.S. Liu *et al*., 2020. MultiplexSSR: A pipeline for developing multiplex SSR-PCR assays from resequencing data. Ecol. Evol., 10: 3055-3067.
23. Almeida-Silva, M.A., R.S. Braga-Ferreira, C.P. Targueta, L.C. J. Corvalán and C.M. Silva-Neto *et al*., 2024. Chloroplast genomes of *Simarouba* Aubl., molecular evolution and comparative analyses within Sapindales. Sci. Rep., Vol. 14. 10.1038/s41598-024-71956-5.
24. Mazumdar, P., R.B. Othman, K. Mebus, N. Ramakrishnan and J.A. Harikrishna, 2017. Codon usage and codon pair patterns in non-grass monocot genomes. Ann. Bot., 120: 893-909.
25. Shi, N., Y. Yuan, R. Huang and G. Wen, 2024. Analysis of codon usage patterns in complete plastomes of four medicinal *Polygonatum* species (Asparagaceae). Front. Genet., Vol. 15. 10.3389/fgene.2024.1401013.

26. Ahmad, W., S. Asaf, A. Al-Rawahi, A. Al-Harrasi and A.L. Khan, 2023. Comparative plastome genomics, taxonomic delimitation and evolutionary divergences of *Tetraena hamiensis* var. qatarensis and *Tetraena simplex* (Zygophyllaceae). Sci. Rep., Vol. 15. 10.1038/s41598-023-34477-1.
27. Li, Z., C. Wu, T. Li, Y. Li, J. Ma, L. Gong and L. Tao, 2025. Plastomic insights into the genetic variation and phylogenetic relationships of *Macadamia*. Sci. Rep., Vol. 15. 10.1038/s41598-025-14011-1.
28. Liu, Z.F., H. Ma, X.Y. Zhang, X.Q. Ci and L. Li *et al*., 2022. Do taxon-specific DNA barcodes improve species discrimination relative to universal barcodes in Lauraceae? Bot. J. Linn. Soc., 199: 741-753.
29. Yang, J.Y., M.J. Choi, S.H. Kim, H.J. Choi and S.C. Kim, 2021. Plastome characterization and phylogenomic analysis yield new insights into the evolutionary relationships among the species of the subgenus *Bryocles* (Hosta; Asparagaceae) in East Asia. Plants, Vol. 10. 10.3390/plants10101980.
30. Zhou, Q.R., Y.Y. Ma, H.Q. Lv, Z.C. Lu, L.S. Wang, J.S. Liang and J.J. Li, 2024. Chloroplast mini-barcodes combined with high resolution melting analysis to identify herbal medicine Difengpi (*Illicium difengpi*). Heliyon, Vol. 10. 10.1016/j.heliyon.2024.e38700.
31. Ren, J., J. Tian, H. Jiang, X.X. Zhu and F.M. Mutie *et al*., 2022. Comparative and phylogenetic analysis based on the chloroplast genome of *Coleanthus subtilis* (Tratt.) Seidel, a protected rare species of monotypic genus. Front. Plant Sci., Vol. 13. 10.3389/fpls.2022.828467.
32. Kocyan, A., D.A. Snijman, F. Forest, D.S. Devey and J.V. Freudenstein *et al*., 2011. Molecular phylogenetics of Hypoxidaceae – evidence from plastid DNA data and inferences on morphology and biogeography. Mol. Phylogenet. Evol., 60: 122-136.
33. Lu, R.S., K. Hu, F.J. Zhang, X.Q. Sun, M. Chen and Y.M. Zhang, 2023. Pan-plastome of greater yam (*Dioscorea alata*) in China: Intraspecific genetic variation, comparative genomics, and phylogenetic analyses. Int. J. Mol. Sci., Vol. 25. 10.3390/ijms24043341.
34. Shobhana, V.G. and K. Ashokkumar, 2025. DNA marker development and assay execution: A practice-oriented perspective. J. Curr. Opin. Crop Sci., 6: 146-156.