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Phylogenetic Analysis of Selected Chrysanthemum Species Using matK Gene Sequences: An In Silico Approach

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ABSTRACT

This study evaluated the effectiveness of the matK gene as a molecular marker for identifying and establishing phylogenetic relationships among species within the genus *Chrysanthemum*. DNA sequence data were retrieved from the NCBI database and analysed using MEGA X software with the UPGMA method and 1000 bootstrap replicates. The resulting phylogenetic tree showed clear species differentiation, with strong clustering between *C. indicum* and *C.* × *morifolium*, and distinct divergence among other species such as *C. zawadskii*, *C. chanetii*, and *C. lucidum*. These findings confirm that matK is a reliable DNA barcoding marker for systematic and taxonomic studies of Chrysanthemum, particularly in addressing the limitations of morphological identification caused by phenotypic variation and hybridisation. However, this study was limited to a single marker, and future research should incorporate additional loci or genomic approaches to obtain a more comprehensive understanding of Chrysanthemum phylogeny.

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1. Introduction

Chrysanthemum, a genus within the family Asteraceae, is widely distributed in East Asia and Northern Europe, with China recognized as the center of its species diversity. These perennial herbs are cultivated primarily for ornamental purposes due to their vibrant floral display and diverse morphological characteristics. Flower colour ranges from white, yellow, and orange to lavender, purple, and red, often with bicolour variations. The inflorescence typically features a central disc floret surrounded by ray florets, with structural variations ranging from daisy-like to pompon or button forms, and leaves that are alternate, lobed, or serrated, enhancing their visual appeal [1].

This genus plays a significant role economically and ecologically, particularly in the global horticultural industry [1]. However, accurate taxonomic identification of *Chrysanthemum* species remains challenging due to high levels of morphological variation and overlapping characteristics among cultivars. The complexity is further intensified by polyploidy, interspecific hybridisation, and phenotypic plasticity, which complicate species delimitation when relying solely on morphological features [2],[3].

Traditional taxonomy based on morphology often fails to resolve species boundaries within *Chrysanthemum*, especially among closely related or hybrid species. These limitations underscore the necessity for more objective and robust tools to investigate phylogenetic relationships and resolve taxonomic ambiguities.

Molecular techniques, particularly DNA barcoding, have emerged as reliable methods in systematic botany. DNA barcoding employs short, standardised DNA sequences to facilitate species identification with high precision and reproducibility [4]–[6]. Among the commonly used plastid markers, the *matK* (maturase K) gene is frequently selected due to its relatively fast evolutionary rate and broad taxonomic utility across angiosperms [7],[8].

Despite the widespread application of *matK* in various plant families, its specific utility in resolving phylogenetic relationships within *Chrysanthemum* remains underexplored [9]–[12]. This presents an opportunity to employ computational approaches to evaluate the effectiveness of *matK* in species discrimination and evolutionary inference in this genus.

This study is designed to evaluate the applicability of the *matK* gene as a DNA barcode in the genus *Chrysanthemum*. It involves sequence alignment and phylogenetic reconstruction using in silico methods to assess genetic divergence and species clustering. The anticipated outcome is to enhance understanding of molecular systematics in *Chrysanthemum*, and to support biodiversity conservation and breeding efforts through accurate phylogenetic frameworks.

2. Methods

Data Retrieval

The genetic data used in this study consisted of matK gene sequences from species classified under the genus Chrysanthemum. A total of 12 sequences were retrieved from the NCBI GenBank database, comprising 10 Chrysanthemum species and 2 outgroup taxa (*Ismelia carinata* and *Glebionis coronaria*). Only complete sequences with accurate and validated taxonomic annotations were included in the analysis, while entries that were partial, ambiguous, or lacked taxonomic clarity were excluded to ensure data reliability.

Sequence Alignment

All sequences were aligned using the ClustalW algorithm, implemented in MEGA X software under default parameters. This step ensured positional homology among nucleotides across species, a prerequisite for accurate phylogenetic inference.

Phylogenetic Tree Construction

Phylogenetic analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), which constructs a tree based on pairwise genetic distances under the assumption of a constant molecular clock. Bootstrap analysis with 1000 replicates was applied to assess the statistical support of the clades. Bootstrap values ≥70% were considered indicative of strong phylogenetic support, as commonly accepted in molecular systematics [6],[10].

Outgroup Selection

To root the phylogenetic tree and provide an external reference point, two taxa were included as outgroups: *Ismelia carinata* and *Glebionis coronaria* (Asteraceae). These species were selected because they are closely related to *Chrysanthemum* within the Asteraceae family and provide an appropriate evolutionary context for interpreting the tree topology and rooting the analysis [7],[12].

Analytical Justification

Although UPGMA is relatively simple compared to other phylogenetic methods, its use in this study is justified by its ability to generate a basic representation of species clustering when supported by robust bootstrap analysis. Limitations of this method, particularly its assumption of equal evolutionary rates across lineages, were acknowledged. As a result, the interpretation of tree topology was supported with statistical confidence derived from bootstrap replicates. The study also recognises that future research should incorporate more advanced methods such as Maximum Likelihood or Bayesian Inference for improved resolution and accuracy [11],[13].

Software and Visualisation

All sequence alignment, tree construction, and result visualisation were performed using MEGA X software. The resulting phylogenetic tree was interpreted to assess species clustering, lineage divergence, and potential evolutionary relationships among *Chrysanthemum* species.

3. Results and Discussion

Phylogenetic analysis based on matK sequences successfully resolved the evolutionary relationships among the analysed Chrysanthemum species (**Table 1**). Most major nodes exhibited strong bootstrap support (≥70%), which is generally considered a threshold for reliable clustering in molecular systematics. The clade comprising *C. indicum* and *C.* × *morifolium* showed particularly high bootstrap support (>90%), indicating a robust and recent common ancestry likely associated with domestication and hybridisation events. This observation is consistent with earlier findings that *C. morifolium* cultivars are products of complex breeding histories, with hybridisation shaping both morphological diversity and metabolic traits, including anthocyanin biosynthesis [1].

Table 1. List of *Chrysanthemum* Species and *matK* Gene Access Numbers from NCBI Database

SPECIES	ACCESS NUMBER
	matK
Chrysanthemum indicum	JN867589.1
Chrysanthemum x morifolium	JQ362483.1
Chrysanthemum vestitum	MH165288.1
Chrysanthemum lavandulifolium	MH165287.1
Chrysanthemum zawadskii	MG799556.1
Chrysanthemum lucidum	MH028788.1
Chrysanthemum boreale	MG913594.1
Chrysanthemum przewalskii	OP723183.1
Chrysanthemum dichroum	MT919689.1
Chrysanthemum chanetii	MT919688.1
Ismelia carinata	MG710387.1
Glebionis coronaria	MW874476.1

Other Chrysanthemum species, such as *C. zawadskii*, *C. chanetii*, and *C. lucidum*, formed distinct clades that reflect substantial genetic divergence. These divergences may be explained by geographic isolation and ecological specialization, processes that frequently contribute to speciation in the Asteraceae. Previous studies on viroid infections have also demonstrated that such genetic differences are correlated with

variable susceptibility among Chrysanthemum species, further supporting the role of genomic divergence in shaping biological traits [2]. The detection of well-supported clades in the present study reinforces the value of matK as a marker capable of distinguishing closely related taxa, an ability that has been confirmed in various other plant groups [6],[10].

The phylogenetic tree reconstructed from the matK dataset (**Figure 1**) clearly illustrates these relationships, with distinct clustering and reliable bootstrap support at most nodes. The inclusion of *Glebionis coronaria* and *Ismelia carinata* as outgroups provided a robust external reference, confirming the placement of Chrysanthemum within the Asteraceae family. Outgroup choice is crucial in phylogenetic inference, as inappropriate outgroups can bias tree rooting and misrepresent evolutionary history [Edwards, 2019]. The congruence of these results with previous Asteraceae studies highlights the utility of plastid genes for species-level resolution in complex genera.

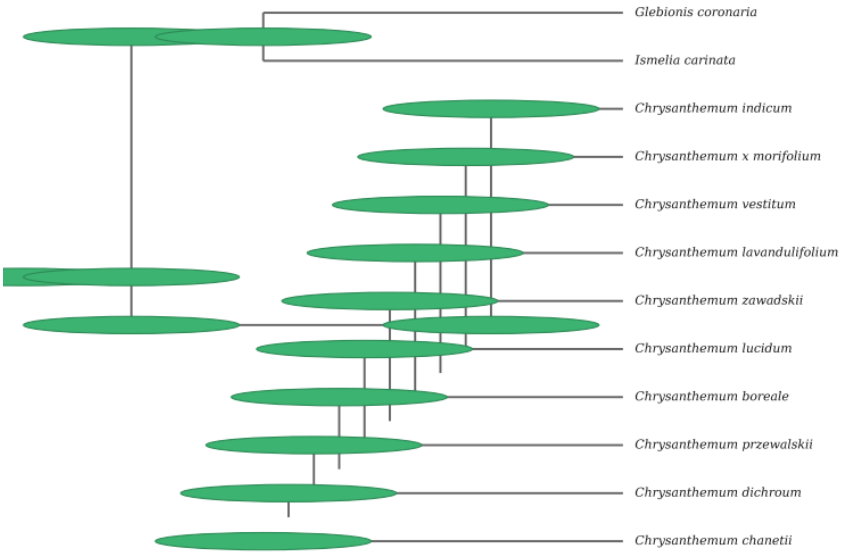

Figure 1. List of *Chrysanthemum* Species and *matK* Gene Access Phylogenetic tree

While matK has demonstrated considerable discriminatory power, comparative analyses indicate that additional loci such as rbcL and ITS should be incorporated to improve resolution [7],[13],[14]. Indeed, multilocus approaches are now widely recommended to overcome the limitations of single-gene barcoding [11],[15]. The broader consensus is that DNA barcoding provides a reproducible and standardised framework for species identification [8],[9],[12], but its accuracy is enhanced when multiple markers are combined. Thus, although this study validates matK as a suitable marker for Chrysanthemum, it also underscores the importance of adopting a multilocus strategy in future research.

Methodologically, the use of UPGMA clustering, while relatively simple, yielded a meaningful representation of species relationships when supported by bootstrap replication. The strong support values observed here demonstrate that even basic algorithms can produce reliable phylogenies when combined with robust statistical validation. Nevertheless, UPGMA assumes a constant molecular clock, which may not always be biologically realistic. More advanced approaches such as Maximum Likelihood and Bayesian Inference provide greater flexibility in modelling evolutionary

processes and are therefore recommended for future studies to confirm the topology generated in this work [11],[13].

Beyond its implications for taxonomy, these findings hold relevance for conservation and breeding programs. Accurate knowledge of genetic relationships is fundamental for selecting parental lines in breeding initiatives aimed at improving ornamental traits and stress tolerance. For example, the clustering of *C. indicum* and *C.* × *morifolium* may inform targeted breeding strategies to enhance desirable floral characteristics [1]. Similarly, recognising genetic divergence among species such as *C. zawadskii* and *C. lucidum* can help maintain genetic diversity, which is crucial for resilience against pathogens and environmental stressors [2]. Furthermore, the phylogenetic framework established here provides a baseline for conserving wild relatives of cultivated chrysanthemums, which are valuable reservoirs of genetic variation.

This study, however, is subject to certain limitations. The analysis was restricted to 12 sequences available in the NCBI database, which represent only a fraction of the genus's extensive diversity. The reliance on a single chloroplast marker, matK, provides useful but incomplete information, as nuclear and mitochondrial genomes were not considered. Additionally, the assumption of equal evolutionary rates inherent to UPGMA may oversimplify the dynamics of speciation in Chrysanthemum. Therefore, future research should expand sequence sampling, incorporate multilocus or genomic datasets, and employ advanced phylogenetic methods to achieve a more comprehensive and accurate understanding of Chrysanthemum evolution.

4. Conclusion

This study confirmed that the matK gene is a reliable DNA barcoding marker for distinguishing species in the genus *Chrysanthemum*. Phylogenetic trees constructed from matK sequence data revealed clear evolutionary relationships and identified species groups with strong genetic affinities, particularly between *C. indicum* and *C.* × *morifolium*. The consistently high bootstrap values across most clades validate the robustness of the inferred tree topology. These findings highlight the strategic role of matK in modern plant systematics, especially for taxa with high morphological complexity and hybridisation histories. They also emphasise the importance of molecular approaches in supporting biodiversity conservation and guiding ornamental plant breeding programmes. Future studies should combine matK with other barcoding loci such as rbcL or ITS to improve taxonomic resolution, apply advanced phylogenetic methods such as Maximum Likelihood or Bayesian Inference to confirm tree topology, and expand data coverage to include wild and endemic species from diverse geographical regions. Integrating phylogenetic data into conservation planning and breeding strategies will enhance the development of new varieties that are both genetically diverse and adaptive to environmental change.

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Conflicts of Interest:

The authors declare that there are no conflicts of interest associated with this research or its publication.

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