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Molecular Diversity And Identification Of Native *Cymbidium* Orchids Species In Vietnam Based On Maturase K (*Mat*k) Sequencing

Tong Van Giang[1], Tran Thi Huyen[2], Hoang Thi Loan[2], Tran Duy Duong[3]
[1]Hong Duc University
[2]Hung Yen University of Technology and Education
[3]Agricultural Genetics Institute
***Corresponding author E-mail:** giangvantong201@gmail.com

A*bstract*
The orchid genus Cymbidium has promptly diversified and has significant value in ornamental horticulture, the economy, ecology, and culture; however, its morphological classification remains debated. However, the actual number may be higher due to natural hybridization facilitated by pollinating insects and the existence of undiscovered wild orchid species in nature. To date, there have been limited studies on the taxonomic classification of Cymbidium orchids, making it difficult to categorize traditional Vietnamese Cymbidium species within scientific classification systems. Only a few species with clearly defined morphological characteristics such as stems, roots, leaves, and flowers have been classified based on published global data. In this study, 16 Vietnamese Native Cymbidium Orchid were identified by matK sequencing. Based on phylogenetic trees, we identified several species within the Cymbidium genus, including Cymbidium sinense (Tran Mong, Dai Mac La Dung, and Hoang Diem Vang), Cymbidium haematodes (Cam To) and Cymbidium aloifolium (Kiem Lo Hoi). However, the matK gene alone has some limitations in accurately identifying orchid varieties.
Keywords: *Cymbidium; Orchids; Phylogenetics trees;* Maturase K *(matK).*

INTRODUCTION

Cymbidium Swartz genus is a member of the Orchidaceae family, which includes approximately 48–55 species and is primarily available in subtropical and tropical Asia[1].*Cymbidium* orchids are impressive in worldwide horticulture due to their fragrant and attractive flowers, and variegated leaves, and they have been grown for over ten centuries[2-3]. In Vietnam, there are 24 species, including 13 that grow on trees, 5 that grow in soil or rock crevices, 5 that cling to rocks, and 1 species that lacks leaves and survives in humus thanks to a highly developed root system. Vietnam, with its tropical and subtropical climate and numerous high-altitude mountainous areas, provides ideal conditions for the growth of many *Cymbidium* species. These orchids are widely distributed across the country, particularly in northern provinces such as Hanoi, Hung Yen, Ninh Binh, Sapa (Lao Cai), Vinh Phuc, Hoa Binh, Yen Tu, Lang Son, Cao Bang, and the Hoang Lien Son Mountain range. In the south, they are mainly found in the Central Highlands, especially in Da Lat (Lam Dong) and along the Truong Son Mountain range[4].

Since the concept of DNA barcoding emerged and its applications, many countries worldwide focused on building DNA databases for native organisms. Orchids quickly became a subject of great interest among scientists due to their unique biological characteristics and economic and medicinal value[5-6]. The Orchidaceae family is considered one of the most challenging plant families to identify and classify, especially during the non-flowering stage. This presents significant difficulties in species identification, conservation, and evolutionary studies. The application of DNA barcoding has helped address these challenges, providing a more accurate method of species recognition compared to traditional morphology-based approaches[7]. Many species differ from their closely related counterparts by only very subtle and minor morphological characteristics, making it difficult to identify using conventional morphology-based methods. Furthermore, because Orchidaceae species can easily hybridize both in the wild and under cultivation, numerous intermediate forms and variations arise, making their classification at the genus and species levels extremely challenging[8-10].

DNA barcoding has been extensively applied in various studies to identify numerous taxa within the Orchidaceae family such as *Dendrobium*[11-15], *Phalaenopsis*[16], *Cypripedium*[3], *Grammatophyllum*[17], *Cymbidium*[18], *Vanda*[19-20], *Spathoglottis*[21], and *Holcoglossum*[22]. With the advent of DNA barcoding technology, it quickly became a powerful tool for the identification of species, particularly in orchids[8,23]. DNA barcoding has been successfully applied in the classification and identification of orchid species. The psbA-trnH intergenic spacer in the chloroplast genome, along with the *matK* and rbcL gene sequences, have been successfully used to classify many plant species. Specifically, DNA barcoding has been successfully applied to several *Dendrobium* species[24-25]. For instance, the *mat*K gene has been particularly effective in distinguishing medicinal species such as *D. pulchellum*, *D. fimbriatum*, *D. nobile*, *D. moniliforme*, and *D. tosaense*[25]. In the case of *Cymbidium* species from Thailand[18], four barcode regions—rpoC1, rpoB, trnH-psbA, and matK—were analyzed to facilitate species identification. These genetic markers were used to assess nucleotide variation and calculate genetic distances, which ranged from 0.026 to 0.528 for *trn*H-*psb*A, 0.012 to 0.546 for *rpo*B, 0.052 to 0.385 for *mat*K, and 0.018 to 0.546 for *rpo*C1 across 19 *Cymbidium* species. Phylogenetic relationships within the genus have also been explored using a variety of molecular markers, including RAPD[26], AFLP[27], ISSR[28-29], and SSR[30]. Additionally, the *mat*K and *ITS* regions have been employed to investigate evolutionary relationships among commonly cultivated *Cymbidium* species[29,31].

The present study focused on testing the applicability of specific DNA barcoding regions in distinguishing species within the genus Cymbidium and inferring their phylogenetic relationships. The matK gene served as a molecular marker for identifying 16 native *Cymbidium* species in Vietnam and exploring their evolutionary affiliations.

MATERIALS AND METHODS

Plant materials

Sixteen samples of Vietnamese native *Cymbidium sp* orchids (aged about 2-4 years) species were collected in Quang Ninh, Lao Cai, Hoa Binh, Ha Noi, Lao Cai, and Da Lat provinces. The information on sample collection including locality of collection, geographic area distribution, and the common name in Vietnamese was done following the previous protocols described by Huong et al (2022) and Trung et al (2013). After sampling, all samples were grown at the net house of Agricultural Genetics Institute, Hanoi, Vietnam.

DNA extraction and PCR performance

Genomic DNA extraction was made from the young and fresh leaves or flowers using the DNeasy Plant Mini Kit (QIAGEN, Hamburg, Germany), according to the manufacturer's guidelines. Genomic DNA was isolated from fresh leaf tissue with the DNA Plant Mini Kit (Qiagen). Briefly, the PCR reaction mixture (15µl) contained 1.5µl of 10x PCR buffer (with MgCl2), 0.2µl of dNTPs (10mM/µl), 0.1µl of Taq polymerase (5U/µl), and 1.5µl of forward and reverse primers (10pmol/µl). The primers used to amplify the *mat*K region included *mat*K2.1F forward primer (5'-CCTATCCATCTGGAAATCTTAG-3') and *mat*K5R reverse primer (5'-TCTAGCACAAGAAAGTCG-3'). A template of approximately 50-100 ng of genomic DNA was used for the reaction.

PCR amplification was carried out using a reaction mixture containing 2.5 units of Taq DNA polymerase (Promega, Madison, WI) and 200 ng of each primer. The thermal cycling was performed in two phases. The initial phase consisted of 10 cycles with denaturation at 94°C for 30 seconds, annealing at 63°C for 20 seconds, and extension at 72°C for 60 seconds. This was followed by 30 additional cycles with denaturation at 94°C for 30 seconds, annealing at 57°C for 20 seconds, and extension at 72°C for 60 seconds. A final extension was carried out at 72°C for 7 minutes. The PCR products were separated by electrophoresis (120 V) for 40 min on zed by 1.0 % agarose gel and visualized using ethidium bromide staining under UV light (Geldoc Bio-Rad). DNA purification was carried out using the DNA Purification System (Qiagen Kit)[32].

Sequencing

In this study. PCR products were sequenced directly using the ABI PRISMTM 310 Genetic Analyzer (Applied Biosystems), employing *mat*K2.1F and *mat*K5R primers. To ensure accuracy and reduce the

likelihood of polymerase-induced errors, each sample was sequenced at least twice in both the 5′ and 3′ directions. The start and end points of the *mat*K region were identified through alignment with reference sequences from Orchidaceae species in GenBank.

Statistical analysis

The obtained sequences were aligned using the ClustalW program, and all comparative and phylogenetic analyses were conducted using MEGA software version 5.2.1.

RESULTS AND DISCUSSION

Molecular markers to identify *Vietnamese native Cymbidium* based on *matK* region sequences

The *mat*K region was successfully amplified using the primer pair 2.1F and 5R, respectively. PCR results revealed high-quality amplicons, each displaying a single, distinct band of the expected size—approximately 800 to 900 bp (Fig. 1). The clarity and accuracy of the band size indicate that the products are suitable for downstream sequencing.

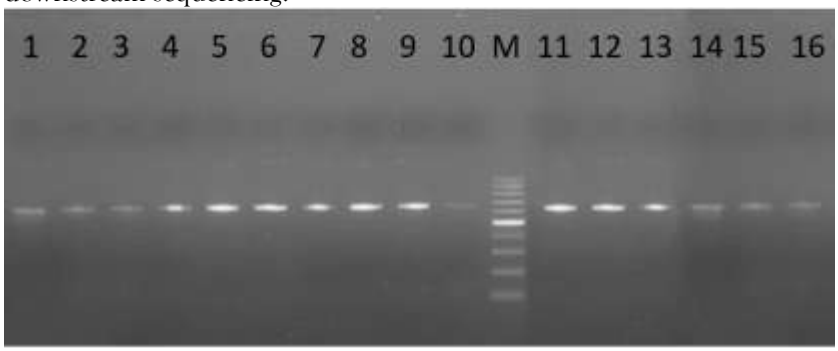

Figure 1. Electrophoresis of amplification *matK* segment on 16 *Vietnamese native Cymbidium* species samples by PCR.*Lanes: 1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 12, 14, 15, 16*: 16 *Vietnamese native Cymbidium samples; M: 100bp ladder*

Analysis of Vietnamese native *Cymbidium* species samples based on *matK* sequences

Nucleotide Composition and DNA Sequence Length Vietnamese Native *Cymbidium species*

Sequencing results indicated successful amplification of the *mat*K region in all 16 native *Cymbidium* species from Vietnam, with sequence lengths ranging from 485 to 936 nucleotides, and an average length of 774.1 nucleotides, as summarized in Table 1.

Table 1. Nucleotide Composition and DNA Sequence Length Vietnamese Native *Cymbidium species*

Names of samples	T(U)	C	A	G	Length of sequencing (bp)
Thanh Truong	39.3	16.4	29.9	14.5	512.0
Kiem lo hoi	37.8	17.2	30.2	14.8	913.0
Mac rung yen tu	37.9	17.4	29.5	15.2	914.0
Bach Ngoc Tieu Kieu	39.6	16.6	29.8	14.1	604.0
Bach Ngoc Dai Kieu	38.9	16.8	30.3	13.9	719.0
Bach Ngoc Xuan	38.7	16.2	30.4	14.6	917.0
Hong Hoang Sapa	38.4	16.5	29.9	15.2	916.0
Dai Mac la dung	39.0	16.5	30.3	14.2	485.0
Hoang Diem Vang	38.9	16.6	29.5	15.0	519.0
Dai Mac la cong	33.0	25.6	22.8	18.6	848.0
Thanh Ngoc	38.5	15.4	29.5	16.7	936.0
Dao Co	37.6	14.9	30.0	17.4	919.0
Mac Bien	39.5	16.5	29.5	14.4	478.0
Cam To	37.5	16.9	29.8	15.7	915.0
Tu Thoi	38.6	16.7	29.4	15.2	914.0
Tran Mong	39.2	15.8	30.4	14.5	877.0
Total	38.3	17.0	29.5	15.3	774.1

The percentage of nucleotide as T (U) =38.3%; C = 17%; A =29,5%; and G = 15.3%. The nucleotide composition analysis reveals that thymine (T) and adenine (A) constitute approximately 67.8% of the total bases, while guanine (G) and cytosine (C) make up about 32.3%. This indicates a high AT content in the sequences, which is characteristic of the *matK* gene, known for its higher A-T content compared to G-C, facilitating genetic identification within the Orchidaceae family (TLTK). Comparing sequence lengths among the samples, the longest sequences are found in *Bach Ngoc Xuan* (917 bp), *Hong Hoang Sapa* (916 bp), and *Dao Co* (919 bp). The shortest sequences are observed in *Dai Mac la dung* (485 bp) and *Mac Bien* species (478 bp), with an average sequence length of approximately 774 bp across the samples. Samples with longer sequences (~914-919 bp) are likely species or varieties that are genetically stable and less affected by mutations. In contrast, samples with shorter sequences (~478-485 bp) may have undergone genetic variations or gene segmentations due to environmental factors or evolutionary differences.

Alignment of Vietnamese native *Cymbidium* species and *Cymbidium* speciesin the world

In order to compare Vietnamese native Cymbidium species with international species, we analyzed sequence data from both groups. This comparison was based on aligned nucleotide positions across all samples. In this study, 16 Vietnamese native *Cymbidium* species were alighted with 24 cymbidiums in GenBank. By the analysis of aligns upcoming column (alignments), The "Dai mac la cong" sample exhibits many unique SNPs that have not appeared in the other orchid samples, indicating that it has accumulated numerous distinct mutations during its evolution. This suggests that this sample may have diverged from other orchid lines very early, or it may carry characteristic mutations that set it apart evolutionarily from the others. Therefore, "Dai mac la cong" represents a valuable genetic resource, important for evolutionary and conservation studies, and it holds potential for use in breeding programs to enhance desirable traits in orchids (Fig 2). Our results were in line with the previous findings to accurately identify the genus of rosa species[32].

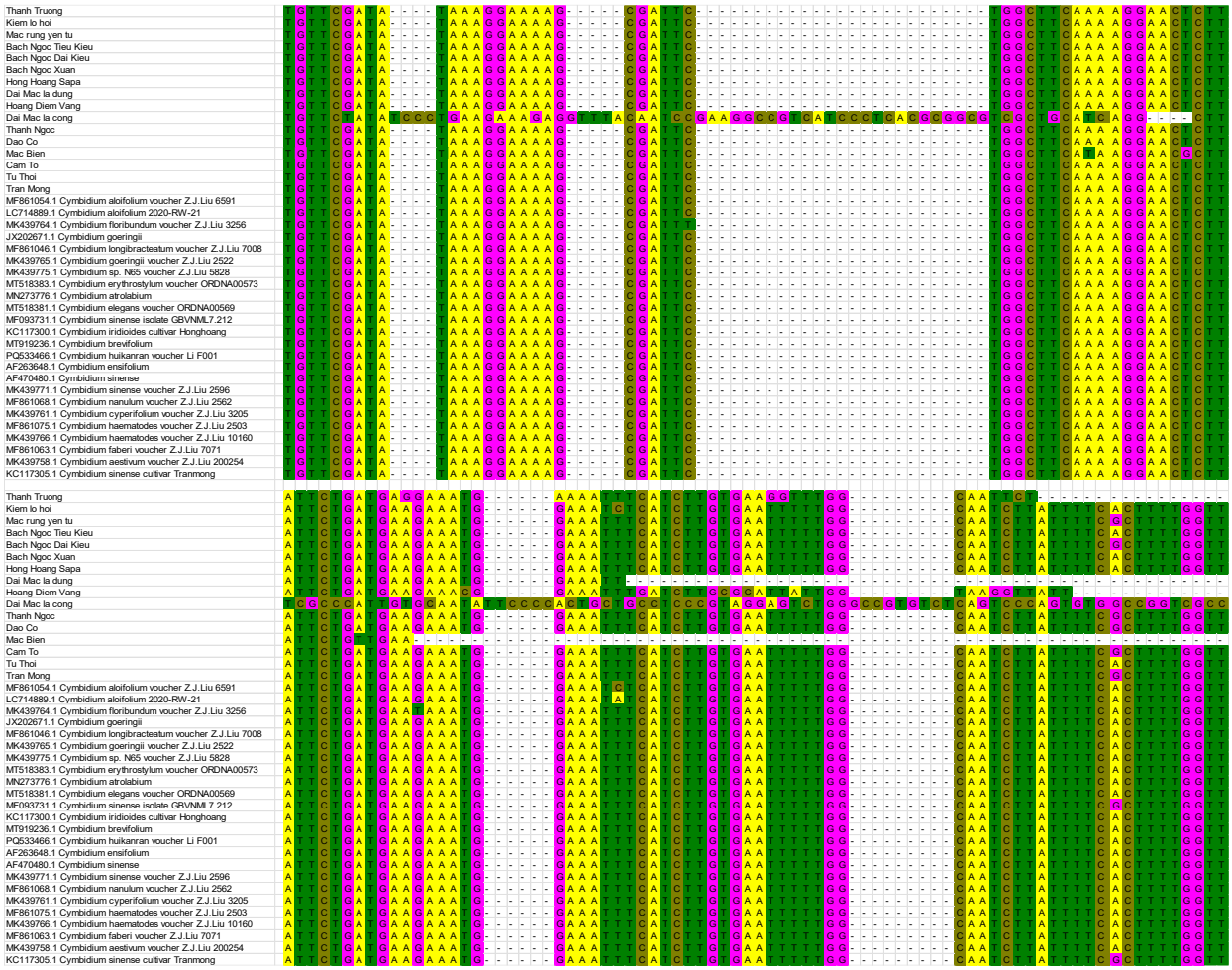

Figure 2. Alignment of Vietnamese native *Cymbidium* species and *Cymbidium* species in the world

Phylogenetic trees constructed using aligned *mat*K gene sequences

As a preliminary step before generating the database, we curated a dataset comprising the 16 Vietnamese *Cymbidium* species examined in this study. To ensure robust phylogenetic comparison, 24 reference *mat*K sequences closely related to Cymbidium species were retrieved and included. Sequence alignment was carried out using the MEGA 5.2.1 software. The final alignment included 40 sequences in total, and 24 reference sequences and 16 which were from the target Vietnamese samples.According to the phylogenetic tree, the 16 Vietnamese native Cymbidium species were categorized into three separate groups (Fig. 3). Group I contained 12 Vietnamese native species along with 21 Cymbidium species from around the world, which were further subdivided into three distinct subgroups.

- **Subgroup I.1:** This subgroup includes 11 samples, consisting of five Vietnamese Cymbidium samples and six international Cymbidium varieties. The samples in this subgroup were included: Mac rung Yen tu, Dao Co, Bach Ngoc Dai kieu, JX202671.1 (*Cymbidium goeringii*) KC117305.1 (*Cymbidium sinense* cultivar), MT518383.1 (*Cymbidium erythrostylum* voucher ORDNA00573,)MK439758.1 (*Cymbidium aestivum* voucher Z.J.Liu 200254), AF470480.1 (*Cymbidium sinense*), Tran Mong, Dai Mac La Dung, Hoang Diem Vang, MF093731.1 (*Cymbidium sinense*), MF861046.1 (*Cymbidium longibracteatum* voucher Z.J.Liu 7008), MK439765.1 (*Cymbidium goeringii* voucher Z.J.Liu 2522), MK439775.1 (*Cymbidium sp. N65* voucher Z.J.Liu 5828)This group consists of closely related Cymbidium orchids, including common species such as *Cymbidium sinense* and *Cymbidium goeringii*, along with some local varieties. This branch may represent an ancient lineage of Cymbidium orchids, with some samples exhibiting significant evolutionary divergence. Mac rung Yen tu, Dao Co, Bach Ngoc Dai Kieu may form a localized group with close evolutionary relationships. These samples are positioned very closely together in the phylogenetic tree, indicating a strong genetic connection. This could be due to a shared origin or natural hybridization within a geographically close area. They may represent different variants of the same species, with minor morphological or ecological differences. These three samples are closely related to JX202671.1 (*Cymbidium goeringii*), suggesting a connection between the local samples and the widespread species *Cymbidium goeringii*.Three samples such as Tran Mong, Dai Mac la dung, and Hoang Diem Vang are clustered within the same major branch as *Cymbidium sinense* and *Cymbidium goeringii*, indicating a close evolutionary relationship. These samples might belong to local lineages or hybrid varieties with some genetic differentiation. The bootstrap value of this branch is 5, indicating that the genetic differences among the samples are not very significant, possibly originating from a common recent ancestor. Trần Mộng is closely related to *Cymbidium sinense* within the group, potentially representing a variant or hybrid between *C. sinense* and another species in the same genus. It may have unique floral traits, although specific mutation characteristics are not well documented. Dai Mac La Dung is positioned near Tran Mong, suggesting a similar origin. It could be a form of *Cymbidium sinense* or a hybrid with *Cymbidium goeringii*. Hoang Điem Vang is in the same group as Dai Mac La Dung and Tran Mong but may have distinct genetic characteristics, possibly belonging to a local lineage of *Cymbidium sinense* with slightly different evolutionary traits. In summary, Tran Mong, Dai Mac La Dung, and Hoang Diem Vang have a close evolutionary relationship and may be variants or hybrids of *Cymbidium sinense*.

The Thanh Ngoc sample is evolutionarily related to species in the *Cymbidium* genus, particularly those connected to *Cymbidium sinense* and *Cymbidium atropalabium*. Thanh Ngọc has a moderate level of genetic differentiation compared to other samples and is grouped with MN273776.1 (*Cymbidium atropalabium*), AF263648.1 (*Cymbidium ensifolium*) and MT919236.1 (*Cymbidium brevifolium*). This group has a bootstrap value of 5, indicating a moderate level of phylogenetic support, suggesting that some differentiation still exists within the group. Evolutionarily, Thanh Ngọc is closely related to *Cymbidium atropalabium*, a species with distinct floral morphology, including long and narrow lips adapted to semi-arid environments. If Thanh Ngoc shares a close relationship with this species, it may exhibit similar adaptive traits. Furthermore, Thanh Ngọc is also associated with *Cymbidium ensifolium*, a widely cultivated *Cymbidium* species with compact foliage and fragrant flowers. Its proximity to this group suggests that it may share some genetic traits that help it adapt to similar habitats.

- **Subgroup I.2:** This subgroup includes the following samples: Bach Ngoc Xuan, Tu Thoi, Bach Ngoc Tieu Kieu, Hoang Hoang Sa Pa, MT518381.1 (*Cymbidium elegans* voucher ORDNA00569) and

KC117300.1 (*Cymbidium iridioides* cultivar Honghoang). In this group, the bootstrap values for this group range from 8 to 42, indicating varying levels of phylogenetic support. The relationship between Bach Ngoc Xuan, Tu Thoi suggests a close connection, possibly belonging to the same *Cymbidium lineage* or sharing a common origin. Their relatively low genetic divergence suggests they may be variants of the same species or have recently diverged. Bach Ngoc Tieu Kieu and Hoang Hoang Sa Pa showed greater differences, as indicated by a higher bootstrap value (42). This group represents an intermediate branch in *Cymbidium* evolution, with moderate genetic divergence but still maintaining close relationships. The samples include both wild species (*C. elegans*, *C. iridioides*) and cultivated varieties (Hong Hoang Sa pa), making this group significant for understanding the evolutionary history of *Cymbidium* orchids. They have high potential for conservation and breeding, particularly in developing commercially valuable *Cymbidium* hybrids.

- Subgroup I.3 were included MF861075.1 (*Cymbidium haematodes* voucher Z.J.Liu 2503), MK439766.1 (*Cymbidium haematodes* voucher Z.J.Liu 10160), Cam To, K439771.1 (*Cymbidium sinense* voucher Z.J.Liu 2596), and MF861068.1 (*Cymbidium nanulum* voucher Z.J.Liu 2562) and MK439761.1 (*Cymbidium cyperifolium* voucher Z.J.Liu 3205) samples. Cam To species related closely to *Cymbidium haematodes*, a species with small, brightly colored flowers found across Asia. This suggests that Cam To may share genetic material with *C. haematodes* or have a common evolutionary ancestor. It is also closely related to *Cymbidium sinense*, indicating potential evolutionary overlap or shared ancestry.

Group I.4 included Thanh Truong, Mac Bien and MK439764.1 (*Cymbidium goeringii*) species based on their positions in the phylogenetic tree, the Thanh Truong species is located within a branch close to a species belonging to the genus *Cymbidium*, particularly *Cymbidium sinense* and *Cymbidium goeringii*. This indicates that Thanh Truong has an evolutionary relationship closely related to the common Asian *Cymbidium* species. Its proximity to *C. sinense* and *C. goeringii* suggests that Thanh Truong may share many genetic traits with these species. It may exhibit similar morphological and biological characteristics, such as flower structure, leaf shape, and habitat preferences.

Mac Bien species is positioned within a branch closely related to *Cymbidium ensifolium* and *Cymbidium kanran*. This suggests that Mạc Mac Biên has a close evolutionary relationship with widely distributed *Cymbidium* species in East Asia. In terms of genetic characteristics, Mạc Biên's similarity to *C. ensifolium* and *C. kanran* suggests that it may possess similar genetic traits, such as cold tolerance or distinct floral morphology. It may also exhibit specific genetic variations, reflecting adaptations to environmental conditions. In summary, the three species as Thanh Truong, Mac Bien and, and MK439764.1 belong to the *Cymbidium* genus and have close evolutionary relationships with common Asian *Cymbidium* species. Their proximity suggests they share many genetic and morphological characteristics.

Group II consists of three samples including Kiem Lo Hoi (*Cymbidium aloifolium*), MF861054.1 (*Cymbidium aloifolium* voucher Z.J.Liu 6591) and LC714889.1 (*Cymbidium aloifolium* 2020-RW-21) samples. Based on the phylogenetic tree, Kiem Lo Hoi (*Cymbidium aloifolium*) forms a distinct branch along with the two samples LC714889.1 and MF861054.1. This has significant implications for the genetic and evolutionary understanding of this sample. Kiem Lo Hoi is most closely related to MF861054.1 (*Cymbidium aloifolium* voucher Z.J.Liu 6591), indicating that they belong to the same species (*Cymbidium aloifolium*), but they may represent different strains or originate from different geographical regions. The sample LC714889.1 (*Cymbidium aloifolium* 2020-RW-21) is also part of this group, forming a branch with a high bootstrap value of 99, which strongly supports the genetic relationships among these samples.

Kiem Lo Hoi and its related samples are obviously distinct from other species within the *Cymbidium* genus, particularly from the Mac Bien and Thanh Truong groups. This suggests that *Cymbidium aloifolium* may have diverged early in the evolutionary historical profile of the *Cymbidium* genus. The branch containing Kiem Lo Hoi has a relatively long length compared to other groups, which may reflect a high mutation rate or accumulated genetic differences. In summary, Kiem Lo Hoi belongs to the *Cymbidium aloifolium* group, closely related to samples MF861054.1 and LC714889.1, forming a branch with a high bootstrap value (99). This indicates stable genetic relationships within this group. Further research on the genetic differences among strains within *Cymbidium aloifolium* could provide valuable information for conservation and hybridization efforts.

Group III:

Group III consists of the Dai Mac La cong sample. Dai Mac La cong is positioned at the farthest end of the phylogenetic tree, forming an entirely separate and distinct branch. It has a very large genetic distance from all other samples in the tree, as evidenced by the longest branch length compared to any other sample. The genetic uniqueness of Dai Mac La cong is reflected in the fact that it has no close genetic relatives, indicating that it may have evolved along an entirely independent evolutionary pathway.

Over the course of evolution, Dai Mac La cong may have accumulated numerous unique mutations, making it significantly different from other groups within the *Cymbidium* genus. Compared to other groups, the closest group to Dai Mac La cong is the *Cymbidium aloifolium* group (including Kiem Lo Hoi, LC714889.1, and MF861054.1), but there remains a significant genetic distance between them. When compared to groups such as *Cymbidium sinense*, *Cymbidium goeringii*, and *Cymbidium ensifolium*, Dai Mac La cong shows an even greater genetic divergence, suggesting that it may represent a distinct species or a highly mutated lineage within the evolutionary process. Due to its highly distinct genetic characteristics, Dai Mac La cong may contain a unique gene pool, making it crucial for conservation to prevent the loss of rare genetic traits. The significant genetic distance indicates that it could be an ancient lineage or have originated from a separate evolutionary branch within *Cymbidium*. This could provide valuable insights into the evolutionary historical profile of the genus. Because of its considerable genetic differences, Dai Mac La cong could serve as an important genetic resource for breeding programs to enhance specific traits in other orchid varieties, particularly in terms of adaptability and floral morphology.

Overall, Dai Mac La cong is the most genetically distinct sample in the phylogenetic tree, located on an entirely separate branch. It may have diverged early in the evolutionary history of *Cymbidium* or carried unique mutations that differentiate it from all other species.

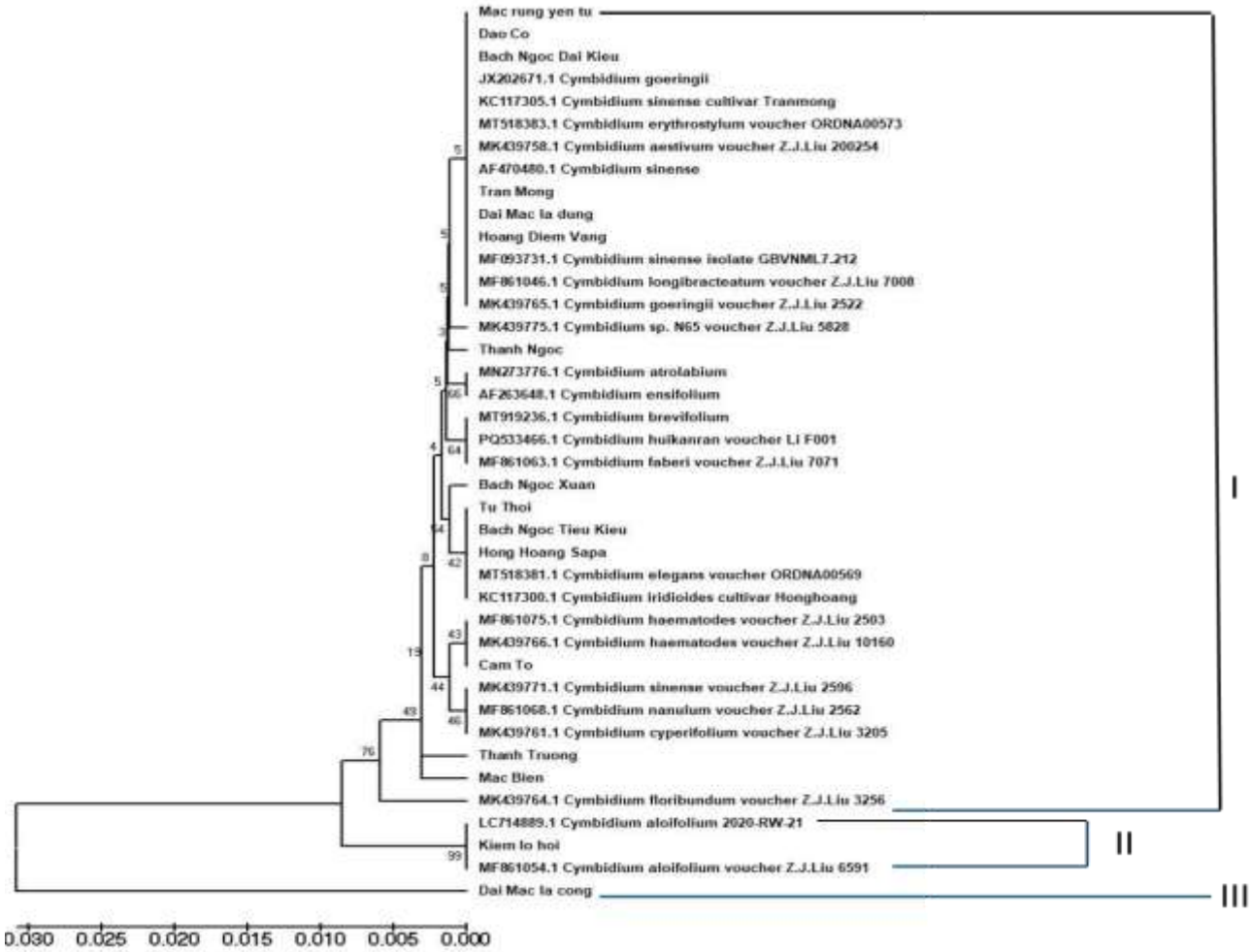

Figure 3. Phylogenetic trees based on *matK* sequences

Morphology-based methods generally take more time- and cost-effective than molecular identification techniques. Nevertheless, to *Cymbidium* and some land plants, These techniques primarily rely on reproductive structures that are often difficult to distinguish, which limits their accuracy. In the case of leaves and roots, there are few morphological traits that are unique to specific species, making it challenging to differentiate between similar organisms based on these parts alone, often resulting in misidentification. Most *Cymbidium* orchid species in Vietnam are now endangered or threatened to extinction due high demand for ornamental trade of these beautiful species. However, it is difficult to distinguish them without their flowers (Fig 5). For example (Dai mac la cong and Dai Bien have similar leaves and some different *Cymbidium* species). Therefore, it is needed to develop molecular markers to accurately identify these species.

Figure 4: Dai mac la cong and Mac Bien species

CONCLUSION

Our present study aims to set the first steps in constructing a molecular database of *Cymbidium* orchids for further assessment of *matK* gene of *Cymbidium* species from this country, to contribute to the conservation of these valuable orchids. These results of the *matK* gene regions in 16 *Cymbidium* orchid samples showed a successful amplification rate of 70 - 100%. Based on phylogenetic trees, we identified several species within the *Cymbidium* genus, including *Cymbidium sinense* (*Tran Mong, Dai Mac La Dung, and Hoang Diem Vang*), *Cymbidium haematodes* (*Cam To*) and *Cymbidium aloifolium* (*Kiem Lo Hoi*). However, the *matK* gene alone has limitations in accurately identifying orchid varieties. Therefore, combining multiple gene regions is recommended, such as *ITS* + *rbc*L + *matK* or ITS + *trn*H-*psb*A. Therefore, it should be developed molecular markers as well as established multi-gene approaches will enhance the accuracy of species identification and genetic relationship analysis within *Cymbidium*.

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Conflict of interest The authors would like to declare that there are no conflicts of interest in this work

REFERENCES

[1] Yang, J.B.; Tang, M.; Li, H.T.; Zhang, Z.R.; Li, D.Z. Complete chloroplast genome of the genus *Cymbidium*: Lights into the species identification, phylogenetic implications and population genetic analyses. *BMC Evol. Biol.*, 2013, 13, 84.

[2] Yang, F.X.; Gao, J.; Wei, Y.L.; Ren, R.; Zhang, G.Q.; Lu, C.Q.; Jin, J.P.; Ai, Y.; Wang, Y.Q.; Chen, L.J. The genome of *Cymbidium sinense* revealed the evolution of orchid traits. *Plant Biotechnol. J.* 2021, 19, 2501–2516.

[3] Kim, J.S.; Kim, H.T.; Son, S.-W.; Kim, J.-H. Molecular identification of endangered Korean lady's slipper orchids (Cypripedium, Orchidaceae) and related taxa. *Botany*, 2015, 93, 603–610.
[4] Averyanov, L.; and Averyanova, A.L. *Updated checklist of the Orchids of Vietnam*. Vietnam National University Publishing House, Hanoi, 2003.
[5] Li Y.L., Tong Y., Xing F.W. DNA barcoding evaluation and its taxonomic implications in the recently evolved genus Oberonia Lindl. (Orchidaceae) in China. *Front. Plant Sci*. 2016, 7, 1791. doi: 10.3389/fpls.2016.01791.
[6] Hoang, N.; Nhung, N.T.; Trang, V.T.H.; Khanh, T.D.; Hung, H.V.Molecular Analysis of Genetic Diversity and Genetic Relationship of Polygonatum kingianum Samples Collected in Northern Mountainous Regions in Vietnam. *Eur Chem Bull.*, 2023, 12(8), 9296-9304.
[7] Li, X.W.; Yang, Y.; Henry, R.J.; Rossetto, M.; Wang, Y.T.; Chen, S.L. Plant DNA barcoding: From gene to genome. *Biol. Rev. Camb. Philos. Soc*. 2015, 90, 157–166
[8] Hebert P.D.N.; Cywinska A.; Ball S.L.; DeWaard J.R. Biological identifications through DNA barcodes. *Proc. R. Soc. B Biol. Sci*., 2003, 270, 313–321. doi: 10.1098/rspb.2002.2218.
[9] Kress W.J., Erickson D.L. DNA barcodes: Genes, genomics, and bioinformatics. *Proc. Natl. Acad. Sci. USA*., 2008, 105, 2761–2762. doi: 10.1073/pnas.0800476105.
[10] Trung, K.H.; Khanh, T.D.; Ham, L.H.; Duong, T.D.; Khoa, N.T. Molecular phylogeny of the endangered Vietnamese Paphiopedilum species based on the internal transcribed spacer of the nuclear ribosomal DNA. *Adv Stu Biol*, 2013, 5(7), 337-346. http://dx.doi.org/10.12988/asb.2013.3315.
[11] Xu, S.; Li, D.; Li, J.; Xiang, X.; Jin, W.; Huang, W.; Jin, X.; Huang, L. Evaluation of the DNA Barcodes in Dendrobium (Orchidaceae) from Mainland Asia. *PLoS ONE* .,2015, 10, e0115168.
[12] Singh, H.K.; Parveen, I.; Raghuvanshi, S.; Babbar, S.B. The loci recommended as universal barcodes for plants based on floristic studies may not work with congeneric species as exemplified by DNA barcoding of Dendrobium species. *BMC Res*., 2012, 5, 42.
[13] Wu, C.-T.; Gupta, S.K.; Wang, A.Z.-M.; Lo, S.-F.; Kuo, C.; Ko, Y.-J.; Chen, C.; Hsieh, C.-C.; Tsay, H.-S. Internal Transcribed Spacer Sequence Based Identification and Phylogenic Relationship of Herba Dendrobii. *J. Food Drug Anal*., 2012, 20, 143–151.
[14] Xu, Q.; Zhang, G.-Q.; Liu, Z.-J.; Luo, Y.-B. Two new species of Dendrobium (Orchidaceae: Epidendroideae) from China: Evidence from morphology and DNA. *Phytotaxa*., 2014, 174, 15.
[15] Duong, T.D.; Trung, K.H.; Nghia, L.T.; Thuy, N.T.T.; Hen, N.B.; Khoa, N.T.; Dung, T.H.; Trung, D.M.; Khanh, T.D. Identification of Vietnamese native Dendrobium species based on ribosomal DNA internal transcribed spacer sequence, *Adv Stud Biol*., 2018, 10(1), 1-12; https://doi.org/10.12988/asb.2018.7823
[16] Lin, J.-Y.; Lin, B.-Y.; Chang, C.-D.; Liao, S.-C.; Liu, Y.-C.; Wu, W.; Chang, C.-C. Evaluation of chloroplast DNA markers for distinguishing Phalaenopsis species. *Sci. Hortic*. 2015, 192, 302–310.
[17] Yukawa, T.; Kinoshita1, A.; Tanaka, N. Molecular Identification Resolves Taxonomic Confusion in *Grammatophyllum speciosum* Complex (Orchidaceae). *Bull. Natl. Mus. Nat. Sci. Ser. B*., 2013, 39, 137–145.
[18] Siripiyasing, P.; Kaenratana, K.; Mokkamuk, P.; Tanne, T.; Sudoon, R.; Chaveerach, A. DNA barcoding of the *Cymbidium* species (Orchidaceae) in Thailand. *Afr. J. Agric. Res*., 2012, 7, 393–404.https://doi.org/10.5897/AJAR11.1434.
[19] Khew, G.S.-W.; Chia, T.F. Parentage determination of Vanda Miss Joaquim (Orchidaceae) through two chloroplast genes rbcL and matK. *AoB Plants*., 2011, plr018.
[20] Senthilkumar, S.; Xavier, T.F.; Gomathi, G. DNA Barcoding of Vanda Species from the Regions of Shevaroy and Kolli Hills using *rbc*L gene. *Biotechnol. Res*. 2017, 3, 126–128.
[21] Ginibun, F.C.; Saad, M.R.M.; Hong, T.L.; Othman, R.Y.; Khalid, N.; Bhassu, S. Chloroplast DNA Barcoding of Spathoglottis Species for Genetic Conservation. *Acta Hortuc*. 2010, 878, 453–460.
[22] Xiang, X.G.; Hu, H., Wang,.; DNA barcoding of the recently evolved genus Holcoglossum (Orchidaceae: Aeridinae): a test of DNA barcode candidates. *Mol Ecol Res*., 2011, 11(6), 1012-1021.https://doi.org/10.1111/j.1755-0998.2011.03044.x.
[23] De Vere, N.; Rich, T.; Trinder, S.; Long, C. DNA barcoding for plants. *Med Mol Biol*, 2015, 1245, 101-118. Doi:10.1007/978-1-4939-1966-6_8.
[24] Yao, H.; Song, J.Y.; Ma, X.Y.; Liu, C.; Li, Y.; Xu, H.X.; Han, J.P.; Duan, L.S.; Chen, S.L. Identification of Dendrobium species by a candidate DNA barcode sequence *psb*A–*trn*H intergenic region. *Planta Med*, 2009, 75 (6),667–669.
[25] Asahina, H., Shinozaki, J., Masuda, K., Morimitsu, Y., Satake, M. Identification of medicinal Dendrobium species by phylogenetic analyses using *mat*K and *rbc*L sequences. *J. Nat. Med.,* 2010, 64 (2), 133–138.
[26] Obara-Okeyo, P.; Kako, S. Genetic diversity and identification of *Cymbidium* cultivars as measured by random amplified polymorphic DNA (RAPD) markers. *Euphytica*.,1998, 99, 95–101.
[27] Wang, H.Z.; Wang, Y.D.; Zhou, X.Y.; Ying, Q.C.; Zheng, K.L. Analysis of genetic diversity of 14 species of *Cymbidium* based on RAPDs and AFLPs. *Shi Yan Sheng Wu Xue Bao*., 2004, 37, 482–486.
[28] Wu, Z.X.; Wang, H.Z.; Shi, N.N.; Zhao, Y. The genetic diversity of *Cymbidium* by ISSR. *Yi Chuan*., 2008, 30, 627–632.
[29] Sharma, S.K.; Dkhar, J.; Kumaria, S.; Tandon, P.; Rao, S.R. Assessment of phylogenetic inter-relationships in the genus *Cymbidium* (Orchidaceae) based on internal transcribed spacer region of rDNA. *Gene*., 2012, 495, 10–15.
[30] Moe, K.T.; Zhao, W.; Song, H.S.; Kim, Y.H.; Chung, J.W.; Cho, Y.I.; Park, P.H.; Park, H.S.; Chae, S.C.; Park, Y.J. Development of SSR markers to study diversity in the genus *Cymbidium*. *Biochem. Syst. Ecol.,* 2010, 38, 585–594.

[31] Van den Berg, C.; Ryan, A.; Cribb, P.; Chase, M. Molecular phylogenetics of *Cymbidium* (Orchidaceae: Maxillarieae) sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA and plastid matK. *Lindleyana*., 2002, 17, 102–111.
[32] Huong, B.T., Anh, D.X., Cuong, N.H., An, N.T., Gioi, D.H., Tuong, H.M., Ha, H.C., Ha, T.T.T., Trung, K.H., Khanh, T.D. Morphological characteristics and DNA barcoding in bach hop (*Lilium poilanei* Gagnep) in Vietnam. *Aust J Crop Sci*., 2022, 16(4), 471-478.
[33] Trung, K.H., Khanh, T.D., Ham, L.H., Duong, T.D., Khoa, N.T. Molecular phylogeny of the endangered Vietnamese *Paphiopedilum* species based on the Internal Transcribed Spacer of the Nuclear Ribosomal DNA. *Adv Stu Biol*., 2013, 5(7), 337-346.
[34] Tuong, L.Q.; Tam, T.V.; Dong, D.V.; Duong, T.D.; Cuc, D.T.K.; Thu, P.T.L.; Luong, D.T.; Tuyen, V.T.M.; Giang, N.V.; Tuan, N.T.; Trung, N.T.; Khanh, T.D.; Trung, K.H. Identification of Vietnamese native rose species by using internal transcribed spacer (ITS) sequencing. *Plant Cell Biotech Mol Biol*., 2020, 11&12, 1-10.