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Original Research

# Genetic Diversity and Relationships of *Dendrobium* Based on *trn*L-F Markers: *An In Silico Approach*

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**Abstract**

The development of DNA sequencing technologies has greatly enhanced our understanding of genetic variation within *Dendrobium* species and clarified their evolutionary relationships. In silico approaches facilitate efficient phylogenetic reconstruction by leveraging computational tools to process sequence data with high accuracy. This study aims to contribute to a deeper understanding of genetic diversity within the genus *Dendrobium* based on the trnL-F marker, with potential implications for the conservation and management of these plant genetic resources. This study analyzes the genetic diversity of 12 *Dendrobium* species using the chloroplast trnL-F region, with sequences retrieved from the NCBI GenBank database. Phylogenetic relationships among the species were inferred using the Maximum Likelihood (ML) method with 1,000 bootstrap replicates to assess node support. Nucleotide diversity analysis identified a total of 103 polymorphic sites across the dataset, indicating low variability. The phylogenetic reconstruction grouped the species into three distinct clades, with bootstrap support values exceeding 87% for major branches, suggesting a high degree of relatedness within each group. These findings provide valuable insights for the conservation and management of *Dendrobium* genetic resources.



## Introduction

The Orchidaceae family, widely recognized for its extraordinary floral diversity and ecological adaptability, is one of the largest and most diverse families of flowering plants. It comprises approximately 25,000 species across 900 genera, with a global distribution ranging from tropical rainforests to temperate regions (Chase et al., 2015). Orchids exhibit remarkable morphological variability, intricate pollination strategies, and complex mycorrhizal associations, all of which contribute to their evolutionary success and ecological significance (Dressler, 1993; Givnish et al., 2015). Among these, the genus *Dendrobium* holds a particularly prominent position due to its extensive species richness and considerable economic importance.

The name *Dendrobium* originates from the Greek words *dendron* (tree) and *bios* (life), reflecting the predominantly epiphytic nature of these plants, which typically grow attached to tree trunks or branches (Yukawa & Stern, 2002). The genus is estimated to comprise approximately 1,500 species distributed across Southeast Asia, China, Japan, India, the Malacca Peninsula, Indonesia, and Australia (Mahfut et al., 2021). Several species within this genus are widely cultivated as ornamental plants and as sources of medicinal ingredients, further enhancing their economic significance (Feng et al., 2014;

Teixeira da Silva et al., 2016). Bioactive compounds isolated from certain *Dendrobium* species have demonstrated antioxidant, anti-inflammatory, and anticancer activities, underscoring their pharmaceutical and commercial value (Ng et al., 2012; Zhao et al., 2019).

Despite its economic and ecological importance, species identification and classification within *Dendrobium* remain challenging. Traditional morphological approaches are often insufficient due to phenotypic plasticity influenced by environmental factors such as temperature, humidity, and light intensity (Christenson & Wood, 2003; Sharma et al., 2018). Such ambiguity has resulted in taxonomic inconsistencies and frequent misidentification, complicating conservation efforts, breeding programs, and bioprospecting initiatives (Yukawa et al., 2005; Sharma et al., 2018). Consequently, molecular markers have emerged as reliable tools for species delimitation, phylogenetic reconstruction, and genetic diversity assessment.

DNA barcoding, a technique that uses short and standardized DNA sequences for species identification, has been successfully applied to *Dendrobium* and other orchids (Chen et al., 2015; Hollingsworth et al., 2011). In plants, commonly used barcode markers include plastid DNA regions (rbcL, matK, trnL, and trnH–psbA) and nuclear DNA regions (ITS and ITS2) (Cahyaningsih et al., 2022). Among these markers, the trnL-F intergenic spacer region located within the chloroplast genome has shown particular promise due to its high variability and ease of amplification across diverse plant taxa (Taberlet et al., 1991). The non-coding nature of trnL-F allows for a relatively high rate of structural mutations, including insertions and deletions, making it suitable for inferring species relationships and detecting intraspecific genetic diversity (Shaw et al., 2007).

Despite the established utility of trnL-F, its application in *Dendrobium* diversity and phylogenetic studies remains underexplored compared to other molecular markers such as ITS and matK (Wang et al., 2009; Xu et al., 2015). This gap is particularly significant given the need for efficient, cost-effective, and universally applicable markers to assess genetic relationships within the genus. Moreover, previous studies have often relied on a limited number of species or combined multiple markers, making it difficult to evaluate the specific effectiveness of trnL-F as a standalone marker in *Dendrobium* systematics. The urgency of this research is further underscored by increasing conservation concerns regarding wild *Dendrobium* populations, many of which are threatened by habitat loss, overexploitation, and climate change (Seidenfaden, 1985; Subedi et al., 2013). Accurate species identification and a clear understanding of genetic relationships are essential for developing effective conservation strategies, particularly for species with high ornamental or medicinal value (Teoh, 2020).

This study aims to address these gaps by conducting an in silico analysis of genetic diversity and phylogenetic relationships among 12 *Dendrobium* species using trnL-F sequences obtained from GenBank. One species from the genus *Phalaenopsis* will serve as an outgroup to root the phylogenetic analysis. By evaluating the informativeness and discriminatory power of trnL-F in resolving species relationships within *Dendrobium*, this research contributes to improving molecular identification techniques, supporting more effective conservation management, and advancing taxonomic resolution for this economically and ecologically important genus.

## Method

### 2.1 Materials

The trnL-F sequence data used in this study were retrieved from the GenBank database hosted on the NCBI website (https://www.ncbi.nlm.nih.gov). A total of 13 sequences were selected, consisting of 12 sequences from various *Dendrobium* species originating from Indonesia and China, and one sequence from a *Phalaenopsis* species from the USA, which served as the outgroup (Mursyidin et al., 2021). The accession numbers, sequence lengths (in base pairs), and geographic origins of all samples are summarized in Table 1. All sequences were stored in FASTA format for subsequent analyses.

### 2.2 Methods

#### 2.2.1 Data Collection

Thirteen trnL-F sequences were retrieved from NCBI GenBank, comprising 12 *Dendrobium* species and one *Phalaenopsis* species used as the outgroup (Table 1). The sequences ranged in length from 955 to 1,312 base pairs and originated from Indonesia, China, and the USA.

**Table 1**. The *TrnL-F* sequence data usedJPC preparation.

| Species Name | Access | Length (bp) | Sequence origin |
|---|---|---|---|
| *Dendrobium anosmum* | PQ014469 | 955 | Indonesia |
| *Dendrobium fimbriatum* | PQ014474 | 1029 | Indonesia |
| *Dendrobium aphyllum* | PQ014470 | 975 | Indonesia |
| *Dendrobium officinale* | EF397937 | 1278 | China |
| *Dendrobium devonianum* | EF397919 | 1312 | China |
| *Dendrobium heterocarpum* | EF397924 | 1297 | China |
| *Dendrobium lohohense* | EF397926 | 1290 | China |
| *Dendrobium loddigesii* | EF397927 | 1277 | China |
| *Dendrobium williamsonii* | EF397930 | 1312 | China |
| *Dendrobium moniliforme* | EF397935 | 1284 | China |
| *Dendrobium brymerianum* | EF397911 | 1292 | China |
| *Dendrobium primulinum* | EF397929 | 1262 | China |
| *Phalaenopsis deliciosa* | DQ091444 | 1040 | USA |

### 2.2.2 Multiple Alignment

The 13 selected trnL-F sequences were aligned using MEGA XII with the ClustalW algorithm under default parameters. This multiple sequence alignment facilitated the identification of mutation sites and polymorphic regions, which are essential for assessing genetic diversity and inferring phylogenetic relationships among the species (Mursyidin et al., 2021).

### 2.2.3 Analysis of genetic kinship and relationships between species

Kinship analysis was performed using DNAsp, which included the assessment of polymorphic sites, insertion–deletion (InDel) polymorphisms, Tajima's test, conserved DNA regions, and nucleotide diversity. The nucleotide diversity index was categorized into three levels: low (0.1–0.4), moderate (0.5–0.7), and high (0.8–2.0). Additional parameters analyzed included the estimated transition/transversion bias. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method with 1,000 bootstrap replicates, conducted in MEGA XII (Mursyidin et al., 2021).

## Results and Discussion

In this study, sequence data for the trnL-F region were retrieved from the GenBank database available on the NCBI website. Sequence selection was based on several criteria: (1) the sequences had to represent species from the genus *Dendrobium*; (2) each sequence required clear species identification and sufficient metadata, such as collection locality or voucher information; (3) the sequences needed to cover the complete trnL-F region or at least the targeted conserved portion; and (4) the sequences had to be free from ambiguous base calls or evident sequencing errors. In addition, priority was given to sequences derived from species distributed in Southeast Asia, particularly Indonesia, due to the region's high *Dendrobium* species richness. The genetic diversity parameters of the trnL-F sequence are presented in the following table:

**Table 2.** Sequence genetic information.

| Parameter | Value |
|---|---|
| Sequence Length Range (bp) | 955-1312 |
| *Haplotype number* (h) | 13 |
| *Haplotype diversity* (hd) | 1,000 |
| Number of Polymorphism Points (S) | 103 |

| | |
|---|---|
| Conservation Sequence (C) | 0,843 |
| Transition Bias (R) | 0,48 |
| Nucleotide Diversity (π) | 0,02948 |
| Nilai Neutrality Tajima's (D) | -1,89590 |

A total of 13 sequences were selected and stored in FASTA format for further analysis. Of these, 12 sequences represented *Dendrobium* species, while one sequence from *Phalaenopsis* was included as an outgroup to root the phylogenetic tree and enhance the robustness of the analysis (Mursyidin et al., 2021). Notably, only three of the twelve *Dendrobium* sequences originated from Indonesia. This relatively low representation raises concerns about whether the selected sequences adequately capture the genetic diversity of *Dendrobium* within Indonesia, a recognized biodiversity hotspot for the genus. The small sample size (only 12 *Dendrobium* sequences) may also limit the overall resolution of the phylogenetic relationships. Future studies should therefore consider incorporating a larger number of sequences from diverse geographic regions, particularly from underrepresented areas such as Indonesia. Despite these limitations, the selected sequences were considered sufficient to provide preliminary insights into the genetic relationships among *Dendrobium* species and to demonstrate the feasibility of using the trnL-F marker for phylogenetic inference in this genus. More comprehensive sampling and data integration are recommended for future research to improve the representativeness and robustness of the analysis.

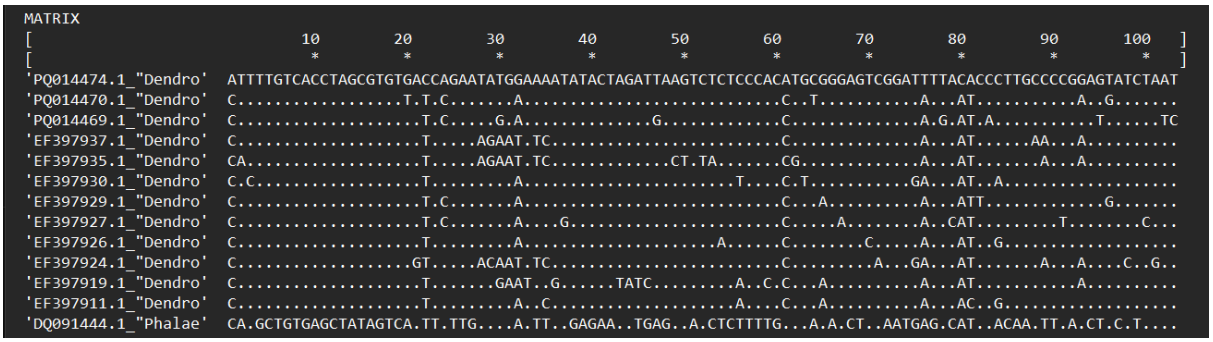
Genetic diversity is determined by variations in the arrangement of nitrogenous bases within a DNA sequence. Changes in the molecular structure and sequence of these bases can drive organisms to evolve and adapt to unfavorable environmental conditions. Naturally, DNA can change through two primary mechanisms. The first is mutation, which involves substitutions, deletions, or insertions of one or more nitrogenous bases. The second involves the exchange of genetic information between organisms during reproductive processes (Morihito et al., 2017). A deletion is the loss of one or more nitrogenous bases from a sequence relative to the ancestral sequence. An insertion refers to the addition of one or more bases into a sequence. A substitution, meanwhile, occurs when one nitrogenous base is replaced with another. These mutation events can alter the translated sequence and change the total number of bases within a DNA sequence.

Substitution mutations are classified based on the type of nitrogenous base that is replaced. These mutations are categorized into transitions and transversions. A transition refers to the replacement of adenine (A) with guanine (G), or thymine (T) with cytosine (C). In contrast, a transversion involves the replacement of a purine (adenine or guanine) with a pyrimidine (cytosine or thymine), or vice versa (Morihito et al., 2017). The estimated transition bias value (R) in this study is 0.48, indicating a preferential occurrence of certain types of nucleotide substitutions. This value suggests that transition mutations—substitutions occurring between two purines (A ↔ G) or two pyrimidines (C ↔ T)—occur more frequently than transversion mutations, which involve substitutions between purines and pyrimidines. The observed transition bias implies that evolutionary mechanisms, such as natural selection or DNA repair processes, tend to favor transitions over transversions. This bias plays an important role in shaping genetic variation and evolutionary pathways, as it influences mutation rates and substitution patterns across species and populations (Stoltzfus & McCandlish, 2017).

According to the findings of Chatterjee & Walker (2017), mutations in DNA sequences can result in either an increase or a decrease in the number of nitrogenous bases. In this study, the level of genetic diversity was assessed based on the sequence length, which ranged from 955 to 1,312 bp. This range reflects variation among the individuals or species analyzed, where differences in nucleotide composition may indicate evolutionary adaptations or responses to environmental pressures.

A total of 103 polymorphic sites were identified, representing nucleotide variations within the dataset (Figure 1). The nucleotide diversity value of 0.02948 indicates a low level of genetic variation in the population. This suggests that the analyzed sequences display minimal genetic divergence, implying a high degree of similarity among individuals. As shown in the figure, the close evolutionary

relationships among the TrnL-F sequences further support the conclusion that these sequences share a common ancestry with limited genetic differentiation.



```
MATRIX
[                        10        20        30        40        50        60        70        80        90       100   ]
[                         *         *         *         *         *         *         *         *         *         *   ]
'PQ014474.1_"Dendro'  ATTTTGTCACCTAGCGTGTGACCAGAATATGGAAAATATACTAGATTAAGTCTCTCCCACATGCGGGAGTCGGATTTTACACCCTTGCCCCGGAGTATCTAAT
'PQ014470.1_"Dendro'  C.................T.T.C......A.............................C..T...........A...AT...........A..G.......
'PQ014469.1_"Dendro'  C...................T.C.....G.A.............G.............C..............A.G.AT.A...........T......TC
'EF397937.1_"Dendro'  C...................T.....AGAAT.TC.........................C..............A...AT......AA...A..........
'EF397935.1_"Dendro'  CA..................T.....AGAAT.TC.............CT.TA.......CG.............A...AT.......A...A..........
'EF397930.1_"Dendro'  C.C.................T.........A.....................T...C.T...........GA...AT..A...................
'EF397929.1_"Dendro'  C...................T.C.......A.............................C...A..........A...ATT.............G.......
'EF397927.1_"Dendro'  C...................T.C.......A....G.....................C.....A........A..CAT.........T........C...
'EF397926.1_"Dendro'  C...................T.........A.....................A......C........C.....A...AT..G...................
'EF397924.1_"Dendro'  C...................GT.....ACAAT.TC.....................C.........A...GA...AT.......A...A....C..G..
'EF397919.1_"Dendro'  C...................T.......GAAT..G......TATC.........A..C.C...A..........A...AT...........A..........
'EF397911.1_"Dendro'  C...................T.........A..C.....................A....C...A..........A...AC..G...................
'DQ091444.1_"Phalae'  CA.GCTGTGAGCTATAGTCA.TT.TTG....A.TT..GAGAA..TGAG..A.CTCTTTTG...A.A.CT..AATGAG.CAT..ACAA.TT.A.CT.C.T....
```

Figure 1. Polymorphic Sites Identified in the TrnL-F Sequence Alignment

Tajima's D is a statistical test used in population genetics to evaluate whether a DNA sequence is evolving neutrally or under the influence of selection. Developed by Fumio Tajima in 1989, the test compares two measures of genetic diversity: the average number of pairwise nucleotide differences among sequences and the number of segregating (polymorphic) sites in the sample. In this study, Tajima's D was applied to detect deviations from the neutral evolutionary model in *Dendrobium* and to assess potential selective pressures. The negative Tajima's D value obtained (-1.89590) suggests an excess of low-frequency polymorphisms, which may indicate positive selection or a recent population expansion (Ponnikas et al., 2022).

A positive Tajima's D value indicates lower genetic variation than expected under a neutral model and is often associated with purifying or balancing selection, which maintains specific genetic variants within a population. In contrast, a negative Tajima's D value—as observed in this study—reflects an excess of low-frequency alleles. This pattern can be interpreted as evidence of positive selection, in which a newly advantageous allele rapidly increases in frequency, or as a signature of recent population expansion. Therefore, the negative Tajima's D value obtained for *Dendrobium* not only suggests the potential influence of positive selection but also provides insight into the evolutionary dynamics shaping the species (Ponnikas et al., 2022).

Additionally, a G+C content value of 0.314 was obtained from approximately 754 data points. G+C content refers to the proportion of guanine (G) and cytosine (C) bases within the analyzed sequences. The GC content of 31.4% indicates a predominance of adenine (A) and thymine (T) bases in the genetic sequences of the *Dendrobium* and *Phalaenopsis* species examined. As shown in Table 3, the average proportion of adenine (A) is 36.86%, while thymine (T) accounts for 34.17%, resulting in a total AT content of 71.03%. In contrast, the GC content is considerably lower at 28.97%, reflecting a typical genomic pattern characterized by AT richness.

*Phalaenopsis deliciosa* exhibits an AT content of 69.72%, which is slightly lower than the average observed in *Dendrobium*. This relatively low GC content indicates that the DNA sequences analyzed are predominantly composed of AT bases. A low GC content and AT dominance are typical characteristics of non-coding DNA regions such as TrnL-F. This pattern arises because AT base pairs form weaker and less thermally stable bonds compared to GC base pairs. Furthermore, coding regions—which are responsible for protein synthesis—are subject to stronger selective pressure to preserve essential genetic information, resulting in distinct nucleotide composition patterns between coding and non-coding regions (Liu et al., 2023).

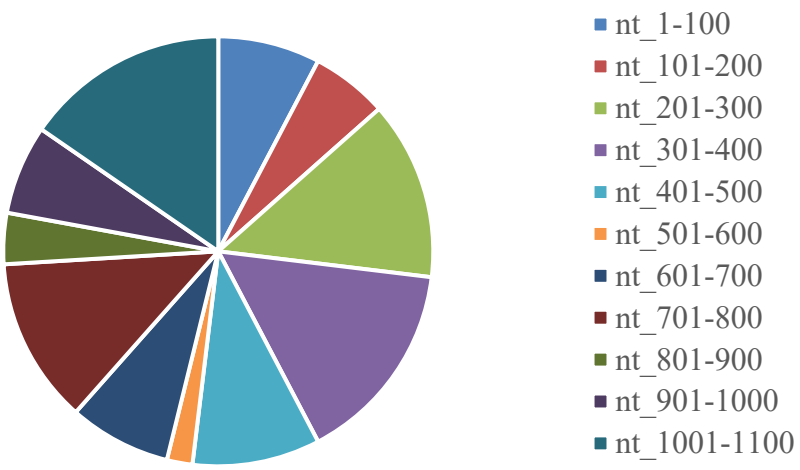



Figure 2. Distribution of Polymorphic Sites Across the Sequences

Analysis of the polymorphism data revealed a clear distinction between the *Dendrobium* group and *Phalaenopsis*, with the latter serving as the outgroup. The *Phalaenopsis deliciosa* sample (DQ091444) exhibited greater nucleotide variation than the *Dendrobium* group, as indicated by numerous nucleotide positions that differed substantially from the reference sequence, *Dendrobium fimbriatum* (PQ014474). This greater variation suggests that *Phalaenopsis deliciosa* is more genetically divergent from *Dendrobium*, reflecting a greater evolutionary distance between the two genera. These differences likely result from distinct ecological adaptations or independent evolutionary histories.

In contrast, the *Dendrobium* group displayed considerably lower levels of genetic variation among its samples. Most sequences within *Dendrobium* contained numerous periods (.) in the alignment, indicating high nucleotide similarity with the reference sequence. This pattern reflects greater genetic homogeneity, which implies a closer evolutionary relationship among the analyzed *Dendrobium* species—possibly due to shared habitats or a more recent divergence. Based on Figure 1 and Figure 2, the distribution of polymorphic sites shows that the highest concentrations of variation occur within the nucleotide position ranges of 201–300, 301–400, and 1001–1100. When comparing the nucleotide sequences of various *Dendrobium* species to the reference sequence *Dendrobium fimbriatum* (PQ014474), similarity can be assessed by counting the number of dots (".") representing identical nucleotides and the number of polymorphic substitutions. The species most similar to the reference appears to be *Dendrobium aphyllum* (PQ014470) or *Dendrobium* anosmum (PQ014469), as they show the fewest differences, whereas *Dendrobium brymerianum* (EF397911) appears to be the most divergent among the *Dendrobium* samples.

```
MATRIX
[                          10        20        30        40        50        60 ]
[                           *         *         *         *         *         * ]
'PQ014474.1_"Dendro'   AGAGAGAAAAAGAGAAAAAGAAAGAAAAAAAAAAGAAAAAAAAAGGAAAGGAAGGAAAGAA
'PQ014470.1_"Dendro'   ...A.....G.A.A..G..A.G.A.....G.G....G....G..A...........GA..
'PQ014469.1_"Dendro'   .AGA.....G.A.A..G..A.G.A...G........G.......AAG.........G.A..
'EF397937.1_"Dendro'   ..........AG.....GA...AG..G..........GG....AAG..A........A.G
'EF397935.1_"Dendro'   GA.........AG.G........A.G.G.....GA...GG.G..AAG..A.....G..A.G
'EF397930.1_"Dendro'   ..........A.A.....A...A...G.............G.A......GGA..G.A..
'EF397929.1_"Dendro'   .........G.A.A..G..A.G.A...G........G.......AA...........GA..
'EF397927.1_"Dendro'   ..........A....G..A.G.A...G........G......GA..............
'EF397926.1_"Dendro'   ..........AG...G..A..GA...G..............AAG.........G.A..
'EF397924.1_"Dendro'   ..........AG..........A.G.G..........GG....AAG..A........A.G
'EF397919.1_"Dendro'   .......G...A...G..........G...G......G......AA.G.........GA..
'EF397911.1_"Dendro'   .A........GA....G..A..GA...G..............AAG.G....A....A..
'DQ091444.1_"Phalae'   .A.AGAGGG..A.A...G.AG..A....G...G.AG....G...AA...AA...A...AG.
```

Figure 3. Distribution of 61 InDel Polymorphism Sites Across All Nucleotide Sequences (Model 4, Multiallelic Option)

**Table 3.** Insertion–Deletion (InDel) Sites and Corresponding Nucleotide Positions

| Sites | Nucleotide Number | Sites | Nucleotide Number | Sites | Nucleotide Number | Sites | Nucleotide Number | Sites | Nucleotide Number |
|---|---|---|---|---|---|---|---|---|---|
| [1] | nt_56 | [2] | nt_57 | [3] | nt_57 | [4] | nt_69 | [5] | nt_69 |
| [6] | nt_97 | [7] | nt_194 | [8] | nt_300 | [9] | nt_311 | [10] | nt_319 |
| [11] | nt_321 | [12] | nt_322 | [13] | nt_322 | [14] | nt_329 | [15] | nt_349 |
| [16] | nt_351 | [17] | nt_365 | [18] | nt_366 | [19] | nt_369 | [20] | nt_371 |
| [21] | nt_381 | [22] | nt_383 | [23] | nt_385 | [24] | nt_386 | [25] | nt_386 |
| [26] | nt_391 | [27] | nt_406 | [28] | nt_414 | [29] | nt_414 | [30] | nt_418 |
| [31] | nt_418 | [32] | nt_422 | [33] | nt_448 | [34] | nt_469 | [35] | nt_486 |
| [36] | nt_486 | [37] | nt_493 | [38] | nt_495 | [39] | nt_496 | [40] | nt_513 |
| [41] | nt_527 | [42] | nt_528 | [43] | nt_528 | [44] | nt_528 | [45] | nt_530 |
| [46] | nt_545 | [47] | nt_546 | [48] | nt_554 | [49] | nt_566 | [50] | nt_588 |
| [51] | nt_652 | [52] | nt_670 | [53] | nt_675 | [54] | nt_701 | [55] | nt_763 |
| [56] | nt_960 | [57] | nt_968 | [58] | nt_969 | [59] | nt_970 | [60] | nt_971 |
| [61] | nt_972 | | | | | | | | |

In Figure 3 and Table 3, a total of 61 insertion and deletion (InDel) sites are identified. In the alignment, (G) represents a gap (deletion), (A) represents a non-gap nucleotide, and (.) indicates similarity to the reference sequence *Dendrobium fimbriatum* (PQ014474). A greater number of insertions or deletions within a sequence reflects more substantial structural changes, which may indicate specific genetic or evolutionary adaptations (Choate et al., 2023). Based on the alignment, *Phalaenopsis deliciosa* (DQ091444) displays the highest number of differences relative to the reference sequence, as evidenced by the numerous insertions (additional nucleotides) and deletions (gaps) present. Excluding *Phalaenopsis deliciosa*, the sequence *Dendrobium moniliforme* (EF397935) shows the greatest variation within the *Dendrobium* group, suggesting that this species—or its close relatives—has undergone more mutations or structural genomic changes compared to other *Dendrobium* species. In contrast, *Dendrobium aphyllum* (PQ014470) exhibits the highest similarity to the reference sequence in terms of insertion and deletion patterns. This indicates a more conserved structural arrangement, with fewer InDel events. The minimal variation supports a close evolutionary relationship between *Dendrobium aphyllum* (PQ014470) and *Dendrobium fimbriatum* (PQ014474).

**Table 4**. Information on Conserved DNA Regions

| Region | Start-End | Conservation | Homozygosity | P-value |
|---|---|---|---|---|
| Region_1: | 1-55 | 0,927 | 0,986 | 0,0178 |
| AATTCAGAGAAACCCTGGAAMTAAAAAWGGGCAATCCTGAGCCAAATCTYTKTTT | | | | |
| Region_2: | 63-196 | 0,938 | 0,992 | 0,0000 |
| RAAAAAAAAYKATGGAAAAKGAGAATAAAAAGGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACG | | | | |
| AATGAAATTGAYTACGTTACGTTAGTAGCTAAAARMCTTCTATCGAAATGACAGAAAGGATMAC | | | | |
| Region_3: | 201-268 | 0,912 | 0,986 | 0,0230 |
| TATRYRCCTAAKACGTACGTATACATACTGACATAGCAAACGATTAATCACAACCCAAATCTKATATY | | | | |

| Region | Start-End | Conservation | Homozygosity | P-value |
|---|---|---|---|---|
| Region_4: | 215-272 | 0,931 | 0,987 | 0,0116 |
| GTACGTATACATACTGACATAGCAAACGATTAATCACAACCCAAATCTKATATYDRAT | | | | |
| Region_5: | 638-694 | 0,929 | 0,986 | 0,0155 |
| ATAGAGATCAAAAAGATMTATGAAAAATKKAAGAGTTATKGTGAATCAATTCCAATT | | | | |
| Region_6: | 760-959 | 0,939 | 0,986 | 0,0000 |
| TTTTTGATAGATCTTTTGAARWTKAATCGGACGAGAATAWAGAGAGAGTCCCATTTTACATGTCAATACCG | | | | |
| ACAACAATGAAATTTATAGTAAKAGGAAAATCCGTCGAATTTTKMMATNCGTGAGGGTTCAAGTCCCTCTA | | | | |
| TCCCCAATAAAAAGCCCATTHTACTTCYTCGCTCTTTATTTATCCTCATMCTCTTTVT | | | | |
| Region_7: | 963-1040 | 0,933 | 0,985 | 0,0031 |
| TTTTTTTTTTCATCAGTGRCTCAGTTTAAACAAAATGAAATATMTTTHTCATTTYATTCACTYTGTTCTTTCACA | | | | |
| AAT | | | | |

The conservation analysis results presented in Table 4 show that 7 DNA regions from the 13 sequences analyzed using DNAsp6 exhibit exceptionally high levels of conservation and homozygosity, indicating substantial genetic stability within the population. Among these regions, Region_6 displayed the highest conservation value at 0.939, followed closely by Region_2 (0.938), Region_7 (0.933), and Region_4 (0.931). The lowest conservation value was observed in Region_3 (0.912), suggesting a slightly higher degree of genetic variation compared to the other regions.

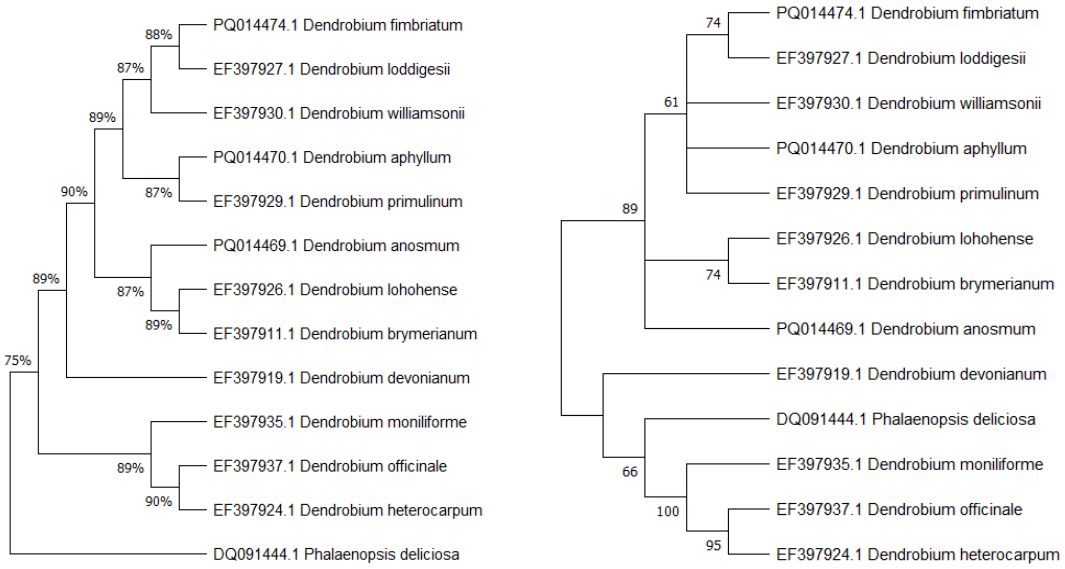
Regarding homozygosity, Region_2 exhibited the highest value (0.992), indicating an exceptionally high level of genetic stability, whereas Region_7 showed the lowest homozygosity value (0.985). This interpretation is further supported by the highly significant p-values obtained for all regions, with Region_6 and Region_2 demonstrating the strongest statistical reliability, each with a p-value of 0.0000. Overall, the analyzed DNA regions display very high levels of genetic conservation, with only minimal variation detected among the sequences (Nagar et al., 2013). Regions with the greatest conservation and homozygosity values, such as Region_6 and Region_2, are likely to be involved in essential biological functions. Consequently, these regions may serve as important focal points for studies on molecular evolution, genetic adaptation, and species conservation.

**Table 5**. Genetic Distances Among Species

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PQ014474 | | | | | | | | | | | | | |
| PQ014470 | 0.029 | | | | | | | | | | | | |
| PQ014469 | 0.036 | 0.024 | | | | | | | | | | | |
| EF397937 | 0.060 | 0.044 | 0.039 | | | | | | | | | | |
| EF397935 | **0.066** | 0.051 | 0.046 | 0.018 | | | | | | | | | |
| EF397930 | 0.026 | 0.017 | 0.027 | 0.047 | 0.058 | | | | | | | | |
| EF397929 | 0.026 | 0.012 | 0.018 | 0.043 | 0.050 | 0.017 | | | | | | | |
| EF397927 | 0.023 | 0.017 | 0.023 | 0.052 | 0.060 | 0.021 | 0.013 | | | | | | |
| EF397926 | 0.032 | 0.020 | 0.024 | 0.046 | 0.053 | 0.025 | 0.018 | 0.020 | | | | | |
| EF397924 | 0.065 | 0.050 | 0.044 | **0.010** | 0.022 | 0.053 | 0.048 | 0.058 | 0.052 | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EF397919 | 0.055 | 0.039 | 0.046 | 0.051 | 0.056 | 0.039 | 0.036 | 0.047 | 0.044 | 0.054 | |
| EF397911 | 0.032 | 0.022 | 0.020 | 0.038 | 0.046 | 0.026 | 0.016 | 0.025 | 0.014 | 0.043 | 0.040 |
| DQ091444 | **0.100** | 0.097 | 0.096 | 0.104 | 0.103 | 0.094 | 0.099 | 0.100 | 0.095 | **0.106** | **0.103** | 0.097 |

The genetic distance matrix quantifies the evolutionary divergence among the analyzed sequences, with values ranging from 0.0104 to 0.1057. Lower genetic distance values, such as 0.0104 (between *Dendrobium heterocarpum* (EF397924) and *Dendrobium loddigesii* (EF397937)), indicate a high degree of sequence conservation and therefore a close evolutionary relationship. In contrast, higher values, such as 0.1057 (between *Phalaenopsis deliciosa* (DQ091444) and *Dendrobium heterocarpum* (EF397924)), reflect substantial genetic divergence, suggesting long-term evolutionary separation. *Phalaenopsis deliciosa* (DQ091444), functioning as an outgroup, exhibits the highest genetic distances overall. Excluding the outgroup, the greatest genetic distance among the *Dendrobium* species is 0.0661 (between *Dendrobium fimbriatum* (PQ014474) and *Dendrobium moniliforme* (EF397935)). These variations in genetic distance indicate a complex evolutionary history that can be further clarified through phylogenetic analyses to visualize the relationships among these sequences.




**Original tree** **Bootsrap tree**

**Figure 4.** *Dendrobium* Phylogeny Inferred Using the Bootstrap Method with 1,000 Replications.

In Figure 4, two types of phylogenetic trees are presented: the Original Tree and the Bootstrap Tree. These trees illustrate the evolutionary relationships inferred from the same dataset but constructed using different analytical approaches. The Original Tree represents a direct reconstruction of the data without additional statistical validation, providing an initial visualization of species relationships; however, it does not indicate the reliability of these inferred relationships. In contrast, the Bootstrap Tree incorporates a bootstrap resampling technique to evaluate the robustness of each branch. The bootstrap values reflect how frequently a particular branching pattern appears across multiple resampled datasets. High bootstrap values (>70%) indicate strong support, making the Bootstrap Tree more conservative and reliable because it accounts for uncertainty within the dataset (Kallner, 2017; Haldar, 2019). This analysis revealed that the genus *Dendrobium* forms a monophyletic group, indicating that all species within the genus share a common ancestor. Despite the low level of nucleotide diversity, the species are divided into several major clusters based on genetic similarity. *Phalaenopsis deliciosa*, used as the outgroup, occupies the most basal branch, confirming its distant evolutionary relationship to the *Dendrobium* species. The bootstrap values assigned to the tree

branches indicate the level of confidence in these relationships, with higher values reflecting stronger support (Kallner, 2017).

Several major clades can be identified, including groups such as *D. fimbriatum*, *D. loddigesii*, *D. williamsonii*, and *D. aphyllum*, which exhibit close evolutionary relationships supported by bootstrap values ranging from 88% to 89%. Other clusters, including *D. primulinum*, *D.* anosmum, *D. lohohense*, and *D. brymerianum*, also show close relationships but with slightly greater genetic divergence. Additionally, *D. devonianum*, *D. moniliforme*, *D. officinale*, and *D. heterocarpum* form a highly cohesive cluster, with *D. officinale* and *D. heterocarpum* displaying an exceptionally strong relationship, supported by a bootstrap value of 100%. This phylogenetic tree reflects a generally low level of genetic diversity while providing valuable insights into the evolutionary relationships within the *Dendrobium* genus. The observed patterns suggest potential adaptations to environmental stresses and highlight significant phylogenetic connections that contribute to a deeper understanding of the evolutionary history of this group.

*Dendrobium* species from Indonesia, such as *Dendrobium* anosmum, *Dendrobium fimbriatum*, and *Dendrobium aphyllum*, are positioned relatively close to one another in the phylogenetic tree, indicating a shared ancestral origin and lower genetic divergence. In contrast, species from China—including *Dendrobium officinale*, *Dendrobium devonianum*, *Dendrobium heterocarpum*, *Dendrobium loddigesii*, and others—display greater genetic diversity and form a larger, more complex cluster. This pattern suggests that the Chinese region facilitates broader evolutionary differentiation, likely driven by its diverse environmental conditions and extensive geographic range. Meanwhile, *Phalaenopsis deliciosa* from the USA serves as the outgroup, emphasizing its clear genetic distinction from the *Dendrobium* species.

## Conclusions

Based on the genetic diversity analysis and phylogenetic relationships of the *TrnL-F* sequences, the level of genetic diversity among *Dendrobium* species is low. This indicates a high degree of genetic similarity among the analyzed sequences. The phylogenetic analysis revealed that the species are grouped into three main clusters. The first group consists of *D. fimbriatum*, *D. loddigesii*, *D. williamsonii*, and *D. aphyllum*. The second group includes *D. primulinum*, *D.* anosmum, *D. lohohense*, and *D. brymerianum*. The final group comprises *D. devonianum*, *D. moniliforme*, *D. officinale*, and *D. heterocarpum*, with *P. deliciosa* serving as the outgroup.

## Author contribution

Mahfut conceived and designed the study. Ikhwan Ismail collected and curated the *TrnL-F* sequence data from GenBank, performed the sequence alignment and genetic analyses using MEGA XII and DnaSP software, and conducted the phylogenetic analysis and interpretation of the results. Mahfut and Ikhwan Ismail drafted the manuscript. All authors reviewed, revised, and approved the final version of the manuscript.

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## Conflict of Interest

The authors declare no conflicts of interest related to this article.

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