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In Silico Study of Genetic Diversity in *Goodyera* spp. Using the *mat*K DNA Barcode

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Abstract

The Genus *Goodyera* (Orchidaceae) is a terrestrial orchid, valued for its attractive foliage. However, resolving the taxonomy of *Goodyera* remains difficult based solely on morphology. The *mat*K gene has shown high efficacy in resolving phylogenetic relationships within orchids. This in silico study aims to analyze the genetic diversity and kinship among *Goodyera* species using DNA barcoding based on the *matK* gene region. A total of 11 *mat*K sequences from *Goodyera* species and one outgroup were retrieved from NCBI and analyzed using MEGA 12 and the MULTALIN web-based application. Phylogenetic tree reconstruction based on the *mat*K gene divided *Goodyera* species into two ingroup clades and one outgroup. Genetic distances ranged from 0.000 – 0.138. The *mat*K region indicates a low level of genetic diversity within *Goodyera*, with a genetic variance of 14.22%. Nucleotide composition shows a high content of thymine and adenine. These findings demonstrate the usefulness of *mat*K and support the genetic collection efforts to clarify overlapping morphological characters in *Goodyera*. Further research using additional chloroplast markers is recommended to strengthen species resolution and database completeness.

Keywords: DNA barcoding, Genetic diversity, *Goodyera*, In silico, *mat*K

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Introduction

Orchids are herbaceous plants that belong to the family Orchidaceae, and are one of the largest vascular plant families with the highest species diversity (Christenhusz and Byng, 2016). Approximately 28.000 orchid species are currently classified into 736 recognized genera (Chase *et al*., 2015). Genus *Goodyera* or commonly known as the jewel orchids, based on its smooth brocade leaves with beautiful veins, is classified into the family Orchidaceae, subfamily Orchidoidae, tribes Cranichideae, and subtribes Goodyerinae (Juswara and Ormerod, 2016; Nguyen *et al*., 2023). Goodyera consists of approximately 100 species that are distributed in South Africa, Asia, Northeast Australia, Europe, Madagascar, North America (including Mexico), and the Southwest Pacific islands. It can be found growing on moist ground or mossy rocks of mountains (Goldman, 2010; Kim and Kim, 2022).

Goodyera species generally have a morphological characteristic: terrestrial habit, horizontal creeping stems that are about as thick as the rhizome, and roots with a rather thick pubescence. The leaves are arranged along the stem, either in rosettes or spread

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loosely, evergreen foliage with the upper surface often having white or gold markings veins. The inflorescence is terminal and often pubescent, while the saccate labellum is generally pubescent, often with its internal fleshy hair-like glands, has two sectile pollinia attached to a viscidium and a single stigmatic lobe (Hu *et al*., 2016; Juswara and Ormerod, 2016; Suetsugu *et al*., 2022).

Species of *Goodyera* hold ecological and economic value as ornamental plants due to their attractive foliage, while some are also used in traditional medicine for anti-inflammatory and antimicrobial properties (Teoh, 2016). However, habitat destruction from deforestation, illegal collection for the horticultural trade, and climate change threaten many *Goodyera* species (Liu *et al*., 2010). Several species of *Goodyera* are listed as endangered or vulnerable in regional assessments, yet most lack formal IUCN Red List evaluations (The IUCN Red List of Threatened Species, 2022).

In Indonesia, Goodyera species are not listed as protected plant species under Regulation No. P.106/MENLHK/SETJEN/KUM.1/12/2018 on Protected Plant and Animal Species. Nevertheless, some *Goodyera* species, namely *Goodyera pusilla*, *G. reticulata*, and *G. viridiflora*, are among Indonesia's 50 rare plants (Rugayah *et al*., 2017). This discrepancy highlights a critical gap in *Goodyera* conservation assessment and legal protection in Indonesia.

In addition to ecological threats and lack of legal protection, challenges in taxonomic identification further complicate conservation efforts for *Goodyera* and its relatives. The taxonomy of *Goodyerinae* is notoriously difficult to resolve based solely on morphology due to the uniform vegetative habit and intricate floral structures across genera (Juswara and Ormerod, 2016; Pridgeon *et al*., 2016). For instance, the *Goodyera rubicunda-grandis* complex exhibits significant variation in floral morphology, particularly in labellum structure and basal shape, leading to ambiguities in species delimitation. This complexity prompted a taxonomic revision, culminating in the recognition of *Goodyera rubicunda* var. *australis* from Australia and Papua New Guinea as a distinct variety (Juswara and Ormerod, 2016).

Given these challenges, in silico approaches have gained importance in biological research because they allow rapid, cost-effective identification of genetic characteristics without relying on time-consuming and expensive wet-lab experiments (Sururi *et al*., 2025; Zhang *et al*., 2022). This is particularly relevant for genera like *Goodyera*, where subtle morphological variations often obscure species boundaries. DNA barcoding has emerged as a powerful tool for species identification and genetic classification. For instance, the *mat*K barcode has demonstrated high efficacy in resolving phylogenetic relationships within orchids, even when morphological data from herbarium specimens are incomplete (Ho, 2021). Furthermore, DNA Barcode *mat*K also provides a visual phylogenetic tree to discover taxonomical relationships and can be developed as a primer (Dzikrina *et al*., 2024; Faza *et al*., 2024; Pratiwi *et al*., 2023).

Preceding studies have used *mat*K to reveal its genetic relationships among *Goodyera* species (Ho, 2021; Hu *et al*., 2016). However, more research on the genetic diversity of the genus *Goodyera* through DNA Barcode *mat*K is still needed to support its phylogenetic relationships. Specifically, studies integrating phylogenetic analysis, genetic distance, genetic variation, and nucleotide composition based on matK remain limited. The main aims of this in silico study were to analyze the genetic diversity and kinship of 10 species of *Goodyera* spp. by using DNA barcoding markers *mat*K available on the National Center for Biotechnology Information (NCBI). The obtained result may contribute to *Goodyera* taxonomy, conservation efforts, ornamental plant breeding, and orchid diversity research.

Methods

Data Collection

DNA sequences of *mat*K genes belonging to the family Orchidaceae, genus *Goodyera,* and one outgroup from *Apotasia wallichii* were retrieved from the NCBI nucleotide database (URL: https://www.ncbi.nlm.nih.gov/). The sequences

were selected by entering the keywords "*Goodyera mat*K" and "Apotasia *mat*K", and the first sequence displayed on the web was subjected to the Basic Local Alignment Search Tool (BLAST). About ten target sequences were selected by considering the highest query cover value, e-value, and percent identity value. All data were saved in a Spreadsheet file, and then the selected sequences were downloaded in FASTA Format. All nucleotide base sequences were stored in a Notepad format for further analysis to determine their genetic diversity baes on the DNA marker *mat*K (Chika, 2024; Chika and Zahro, 2024).

Data Analysis

Data analysis was performed using MEGA 12 Software except for the genetic variation analysis. After data collection had been carried out, the sequences were aligned with MUSCLE (Multiple Sequence Comparison by Log-Expectation) in MEGA 12 software. The purpose of sequencing was to obtain genetic data for molecular identification and comparison of species within the genus *Goodyera*. MUSCLE aligns sequences by optimizing the alignment score using a log-expectation model, which balances accuracy and computational efficiency when aligning multiple sequences (Edgar, 2004).

To understand their kinship based on molecular bioinformatic data, a phylogenetic tree was constructed using the Maximum-Likelihood (ML) and the Tamura 3-parameter model with 1000 bootstrap replications (Chika, 2024; Tamura, 1992). The genetic distance analysis was done by using the pairwise deletion method with the maximum composite likelihood model, which accommodates gaps in sequence alignments by deleting them pairwise during the analysis and avoids bias (Özsoy and Tarikahya Hacioğlu, 2025).

Subsequently, the aligned sequences were further analyzed for genetic variation using a separate method. This step involved the use of the MULTALIN web-based application (Multiple Sequence Alignment by Florence Corpet), available at: http://multalin.toulouse.inra.fr/multalin/, to detect and compare variations across the aligned sequences.

Results and Discussion

In this research, a total of 10 *Goodyera* nucleotide sequences and one outgroup based on DNA barcode Maturase-K (*mat*K) from the NCBI GenBank website were selected based on the highest query cover and percent identity (Table 1). The highest query cover was chosen because it indicates the proportion of the query sequences covered by the target sequences. The percent identity shows the similarity of the query sequences to the target sequences (Hovaňáková *et al*., 2024). Query sequences show an evolutionary relationship shared by the lineage and descended from the common ancestor (Horiike, 2016).

Table 1. Selected Species from the genus *Goodyera* based on DNA Barcode *mat*K

No	Scientific	Query Cover	Percent Identity	Accession
1.	*Goodyera procera*	100%	100.00%	MT265041.1
2.	*Goodyera henryi*	100%	97.83%	KT385611.1
3.	*Goodyera fumata*	100%	97.55%	KT385606.1
4.	*Goodyera foliosa*	100%	97.45%	KT385602.1
5.	*Goodyera velutina*	100%	97.39%	KT385648.1
6.	*Goodyera marginata*	100%	97.49%	KT385621.1
7.	*Goodyera repens*	100%	97.37%	KT385636.1
8.	*Goodyera biflora*	100%	97.27%	KT385585.1
9.	*Goodyera nankoensis*	100%	97.32%	KT385624.1
10.	*Goodyera major*	100%	97.17%	MT265040.1
11.	*Apostasia wallichii*	98%	98.64%	AY557212.1

The sequences were then used to reconstruct the phylogenetic tree reconstruction, as illustrated in Figure 1. The phylogenetic tree is the result of phylogenetic analysis, a method to discover evolutionary history and relationships among groups of organisms (Horiike, 2016). A phylogenetic tree is represented in a graphic resembling a tree that illustrates the evolutionary and phylogenetic relationship between biological taxa (Zou *et al*., 2024). Through the phylogenetic tree, character homologies shared by a common ancestor can be inferred and used to reconstruct evolutionary history (Kapli *et al*., 2020).

In this research, phylogenetic tree reconstruction was performed using Maximum Likelihood (ML) and the Tamura 3-parameter model with 1000 bootstrap replications through MEGA 12.0 software (Chika, 2024; Tamura, 1992). ML is a statistical method based on evolutionary models that is suitable for constructing a phylogenetic tree with a relatively small number of taxa, as in this study, which includes 11 sequences. It offers advantages in statistical consistency, robustness, and the ability to select the optimal evolutionary trees (Zou *et al*., 2024). The Tamura 3-parameter model was selected because it accounts for transition and transversion rates, considers G+C content, and corrects multiple substitutions at the same site in sequences. Additionally, to assess the reliability of the inferred tree, 1000 bootstrap replications were conducted for the internal branch support (Rahimi *et al*., 2023).

The reconstructed tree (Figure 1) reveals that the *mat*K region divided *Goodyera* species into two ingroup clades and one outgroup. The first ingroup clade consists of two subclades; the first one includes *G. henryi, G. foliosa, G. biflora, G. fumata,* and *G. velutina,* while the second subclade includes *G. major, G. repens, G. marginata,* and *G. nankoensis*. The second ingroup clade only encompasses *G.* procera. The outgroup only encompasses *Apostasia wallichi* and plays a role as the root of the tree, which determines the starting point of the phylogenetic tree and finds out the primitive characters (plesiomorph) and derived characters (apomorph) of the ingroup (Subari *et al*., 2021). The outgroup species should be chosen based on their close relation to the group under study, but they are less closely related than all the relationships among the species under study (Horiike, 2016). *Apostasia wallichii* (AY557212.1) is a member of the family Orchidaceae, but belong to different subfamily, namely Apostasioideae.

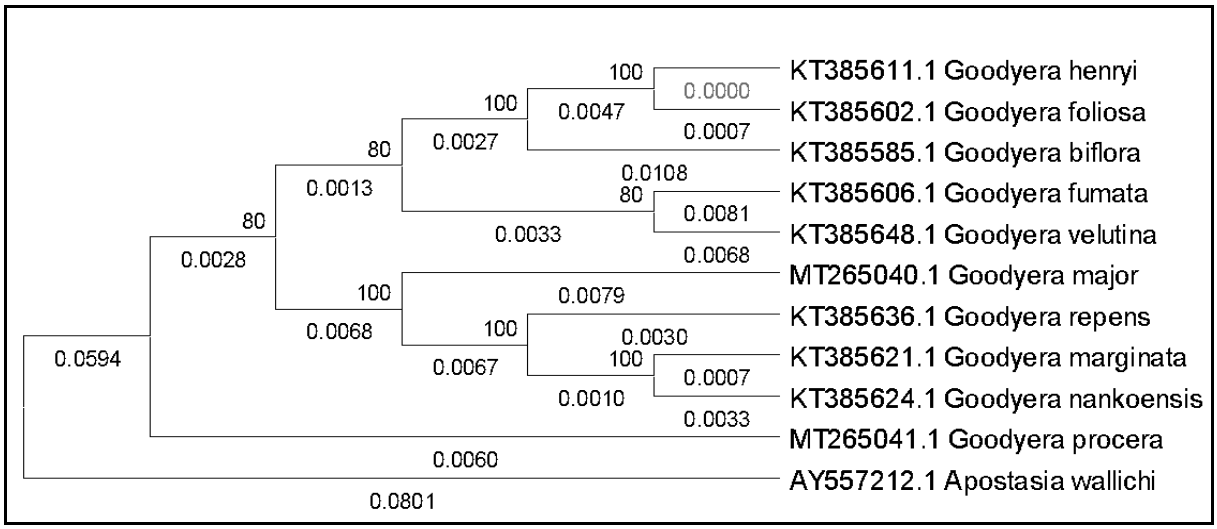


Figure 1. Reconstruction of the phylogenetic tree of the genus *Goodyera* based on DNA Barcode *mat*K using the Maximum Likelihood method and the Tamura 3-parameter model with a bootstrap value of 1000x. The number next to the node shows the bootstrap value, while the number under the branch shows the branch length.

The bootstrap values are shown as numbers on each node of the phylogenetic tree, representing the level of confidence and reliability in each branch. A bootstrap value above 75 and approaching 100 is considered strong support (Chika and Zahro, 2024; Rahimi *et al*., 2023). Based on the *mat*K region, all the branches exhibit bootstrap values ranging from 80-100, indicating a strong phylogenetic support and suggesting a close kinship among the *Goodyera* species. Furthermore, branch length values under the branch represent the number of substitutions per site and indicate the evolutionary distance from a common ancestor (Nei and Kumar, 2000). Longer branches indicate a further evolutionary distance, while shorter branches suggest that the species retains more primitive characteristics and is part of an older lineage. In addition, similar branch lengths in the species imply a close relationship (Wibberg *et al*., 2021).

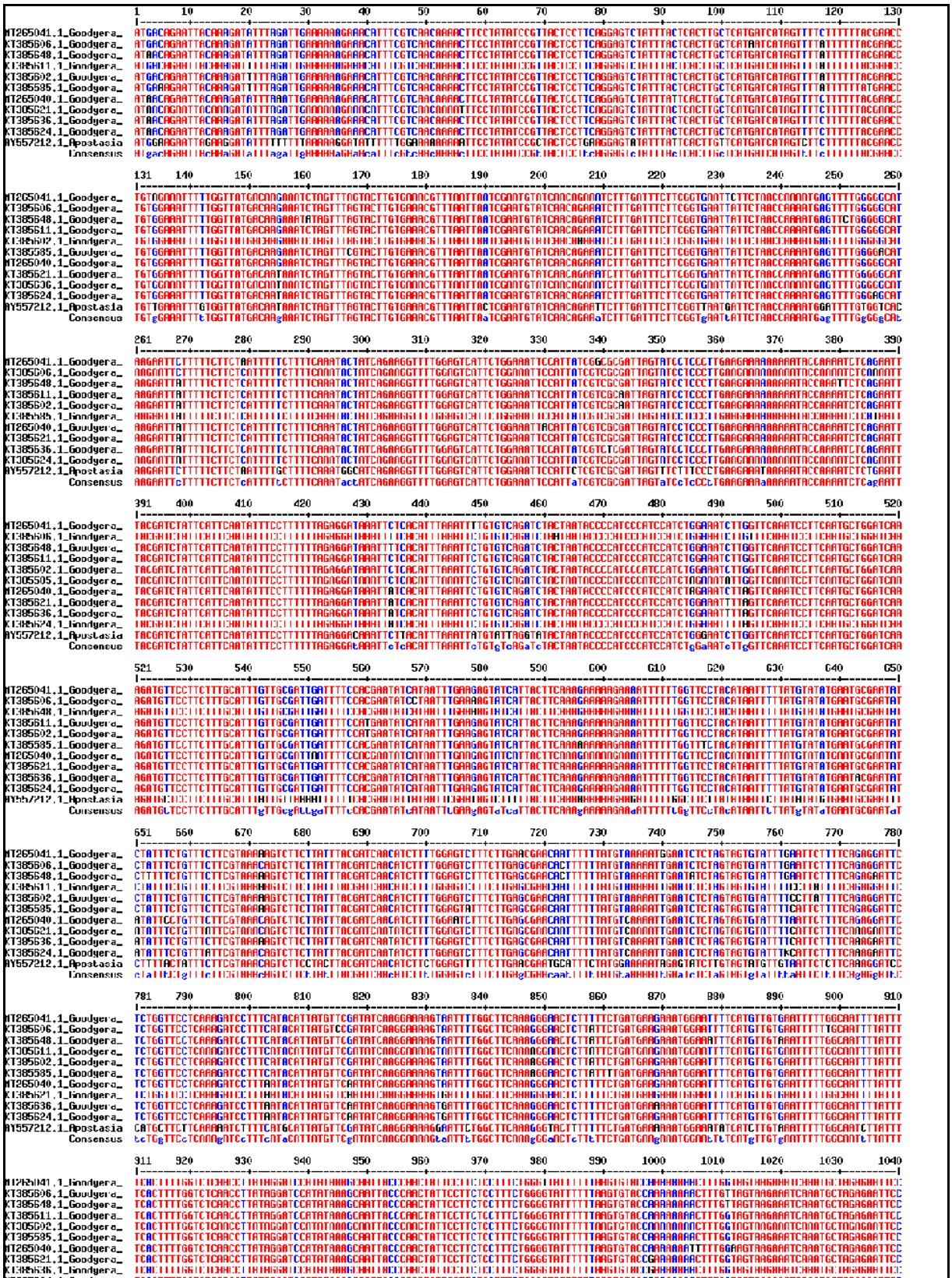
The reconstructed tree suggests the ability of *mat*K to differentiate and identify species in the genus *Goodyera*. Based on the bootstrap and branch location, the closest kinship is owned by *G. henryi* and *G. foliosa,* with a branch length of 0.000 and 0.0007 and supported by a bootstrap value of 100. As the greater the bootstrap value, the level of confidence in the results of the phylogenetic tree reconstruction will be higher (Muthi'ah and Jannah, 2023). In terms of morphology, both species exhibit similar green leaves that lack white markings. Their flowers share a weak-opening characteristic and range in color from white to pale pink.. However, they differ in several key attributes. *G. foliosa* has a larger size, measuring between 15–25 cm, compared to *G. henryi*, which measures between 8–15 cm. The total number of flowers in the rachis also varies; *G. foliosa* typically produces 3 to 15 flowers, while *G. henryi* produces 3 to 9 flowers. Furthermore, the flowers of *G. foliosa* are pubescent and may appear greenish white with ovoid anthers, while those of *G. henryi* are entirely glabrous with lanceolate anthers (Goldman, 2010b).

Table 2. Genetic distance of the genus *Goodyera* and Comparison sequence using DNA Barcode *mat*K

No	Scientific	1	2	3	4	5	6	7	8	9	10	11
1.	*Goodyera procera*		[0.003]	[0.003]	[0.003]	[0.003]	[0.003]	[0.003]	[0.003]	[0.003]	[0.003]	[0.008]
2.	*Goodyera henryi*	0.017		[0.003]	[0.003]	[0.000]	[0.003]	[0.003]	[0.003]	[0.003]	[0.003]	[0.008]
3.	*Goodyera fumata*	0.020	0.018		[0.004]	[0.003]	[0.003]	[0.003]	[0.004]	[0.003]	[0.004]	[0.008]
4.	*Goodyera major*	0.023	0.023	0.025		[0.004]	[0.004]	[0.003]	[0.003]	[0.004]	[0.003]	[0.008]
5.	*Goodyera foliosa*	0.018	0.000	0.019	0.024		[0.003]	[0.003]	[0.004]	[0.003]	[0.004]	[0.008]
6.	*Goodyera velutina*	0.020	0.017	0.014	0.024	0.018		[0.003]	[0.003]	[0.003]	[0.003]	[0.008]
7.	*Goodyera marginata*	0.023	0.022	0.025	0.016	0.022	0.022		[0.001]	[0.004]	[0.001]	[0.008]
8.	*Goodyera repens*	0.024	0.022	0.027	0.016	0.023	0.023	0.004		[0.004]	[0.002]	[0.009]
9.	*Goodyera biflora*	0.022	0.015	0.024	0.028	0.016	0.023	0.027	0.028		[0.004]	[0.008]
10.	*Goodyera nankoensis*	0.025	0.024	0.027	0.018	0.024	0.024	0.004	0.007	0.028		[0.008]
11.	*Apostasia wallichii*	0.129	0.135	0.135	0.134	0.136	0.130	0.136	0.137	0.136	0.138	

The close genetic distance between G. foliosa and G. henryi is presumably because the sequences retrieved from NCBI were obtained from the same study, and both species inhabit similar geographic areas (Hu *et al*., 2016). The genetic distances are known to be related to geographical distance (Nei, 1972). The sequence data from genomic DNA were extracted from similarly collected samples (live or fresh silica gel dried specimens) and the same geographic area (China). According to Plants of the World Online, both G. henryi and G. foliosa can be found in China South-Central, China Southeast, Nepal, and Taiwan. However, their distribution ranges also differ. For example, G. foliosa occurs in Thailand, while G. henryi has not been recorded in that region. Additionally, G. foliosa primarily inhabits the subtropical biome, while G. henryi tends to occur in the temperate biome (POWO, 2025a, 2025b). The reconstructed phylogenetic tree also supported this close relationship by forming a subclade with a bootstrap score of 100 (Figure 1), which was also supported by the previous study, where G. foliosa and G. henryi formed a subclade, namely subsect. Foliosum (Hu *et al*., 2016) .

Genetic variation reflects the differences between individuals, allele presence and frequency within and between populations. Genetic variation might also reveal the degree of relatedness, in which it is beneficial to understand the population characteristics for conservation purposes (Vu *et al*., 2024). Therefore, the analysis of genetic variation in the genus Goodyera is presented in Figure 2. The length of the total Goodyera matK sequence is 1498 bp. Within the genus Goodyera, the matK region contains 1285 conserved sites and 213 variable sites (genetic variations), namely G3A, A4G, C5A, C13G, A16G, A20T, A24T, G25A/T, A26T, G29T, G35A, A38T, C40T, A41T, C45T, T47G, C48A, C51A, C56T/A, T69C, T77G, C78A, C80A, C92T, C100T, T114C, C117A, G134A/T, T142G, G155T, A190C, A209T, G227T, T231G, A247G, G248A, G254T, G257A/T, T260C, C268A, C279A, T285G, A296G, C297G, T298C, A350T, C353T, C355T, T358C, A367T, A385T, G386A/T, T428C, C434T/A, C436T, C448T/A, G452A, C454T, A457G, C459A, G485A, A486G, C491A/T, G494A, T526C, G542A, C546T, G547T, T549A, T550A, G551A, A552T, C557T, C567T, T574C, C577T, A582C, C584T, A585T, G596A, A605G, T612G, G614C, C618T, C621T, T628C, G633A, T637C, A649T, C651A, A653T, T656C/A, G659A, C663A, C672A, T680C, C694T, T700C, C707A, G716A, C721T, A722G, A723C, T724A, T728C, T733G, A734C, T740G/A, A743G, C745A, A749T, G755A, A757G, T760G, T761G/K, A762C, T768C, G773A, G776A, T779C, T781C, C782A, G785C, C789T, C803A, A806G, G817A, T832G, A833G, T837C, G848A, A851T, C855T, T858A, G869A, T878A, T880A, G885C, G890A, T904C, A913G, T929A, C938A, G947A, C954T, C955T, C968T, C971T, C975A, T988C, C997T, A998G, A1000G, A1002T, A1004T, A1005T, G1011T, C1040A, A1046C, A1049G, A1050G, C1054T, C1081T, C1082T, A1089G, T1107A, T1118C, A1131G, C1135T, T1139G/A, A1157G, C1160T, C1166T, A1172T, G1175A, A1188C, C1203A, A1205G, A1207T, T1209A, A1212C, G1213A, A1228T, C1230T, G1234A, C1235T, A1251C, G1253A/T, G1264A, G1271A, G1304A, T1307C, C1331T, T1337A, G1345A, G1349A, T1361C, T1373C, C1393T, C1403A, A1409C, C1410T, G1420T, A1421C, C1428T, A1435G, C1436T, G1449A, C1476T, G1481T, G1493A, T1497C, and T1498C.



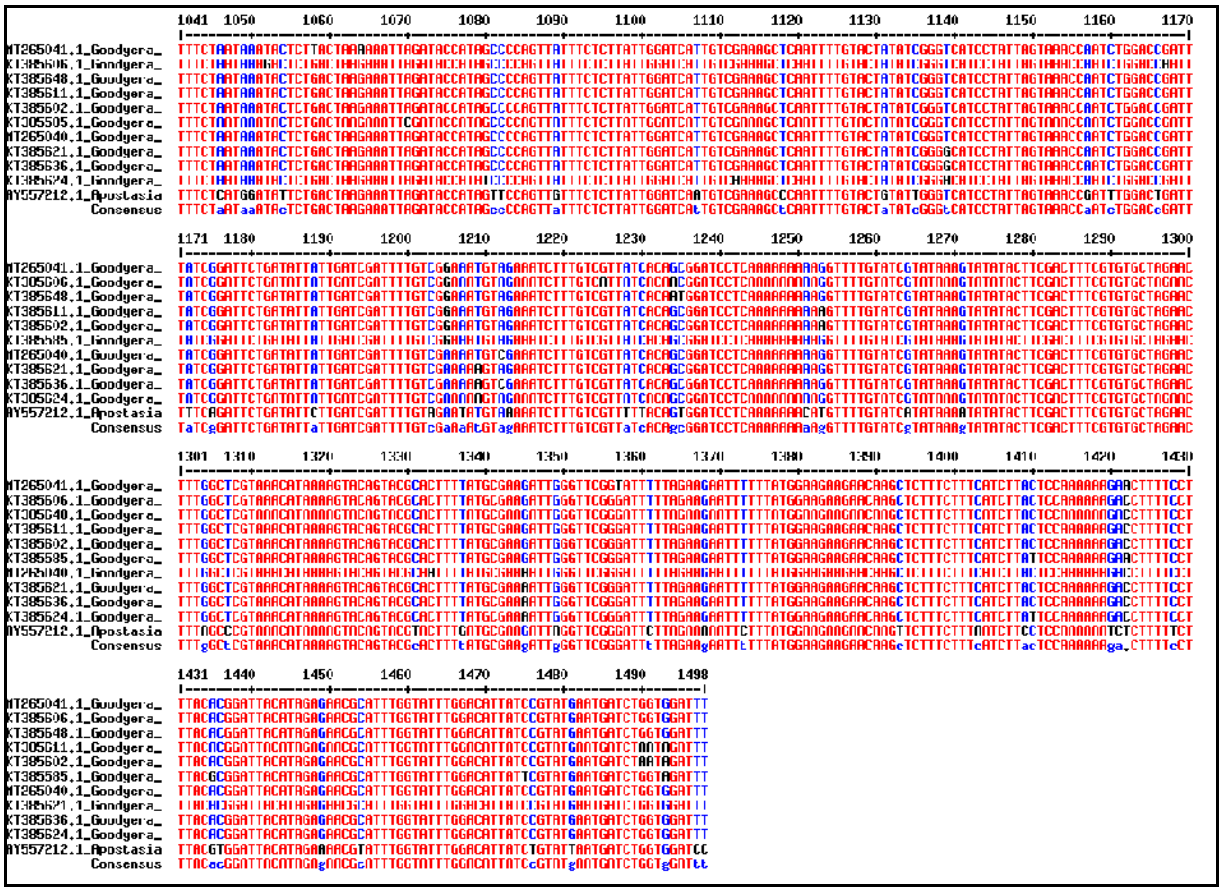


Figure 2. Genetic Variation of the genus *Goodyera* and comparative sequences based on DNA Barcode *mat*K using the MultAlin Website. The square shows a conserved region (red color) and the genetic variation due to a mutational event (column with blue color)

	T(U)	C	A	G	Total
MT265041.1 Goodyera procera	37.5	16.4	30.8	15.2	1498
KT385611.1 Goodyera henryi	37.5	16.4	31.0	15.0	1498
KT385606.1 Goodyera fumata	37.4	16.6	31.0	15.0	1498
MT265040.1 Goodyera major	37.5	16.3	31.4	14.8	1498
KT385602.1 Goodyera foliosa	37.5	16.4	31.1	15.0	1498
KT385648.1 Goodyera velutina	37.5	16.3	31.1	15.1	1498
KT385621.1 Goodyera marginata	37.6	16.2	31.1	15.1	1498
KT385636.1 Goodyera repens	37.6	16.4	31.2	14.9	1498
KT385585.1 Goodyera biflora	37.9	16.0	31.0	15.1	1498
KT385624.1 Goodyera nankoensis	37.6	16.2	31.3	14.8	1497
AY557212.1 Apostasia wallichi	39.3	15.3	30.4	15.1	1498
Avg.	37.7	16.2	31.0	15.0	1497.9

Figure 3. Nucleotide composition of the genus *Goodyera* using DNA Barcode *mat*K in percent

The maturase K gene (*mat*K) region is located inside the intron of the *trnK* gene in the chloroplast genome (Hausner *et al*., 2006). The *mat*K has shown its ability to determine genetic variation in cacao and banana varieties (Immanissa *et al*., 2020; Probojati *et al*., 2021). Genetic variation is driven by mutation events, which reflect the differences and evolutionary events among species (Mursyidin *et al*., 2023). In this study, substitutions (transitions and transversions) are found in the *matK* region of *Goodyera* with no insertion or deletion event.

Overall, *Goodyera* exhibits only 14.22% genetic variety across all the nucleotide positions, suggesting a low level of genetic diversity (Mursyidin *et al*., 2021).

Nucleotides consist of nitrogenous bases, namely thymine/uracil (T/U), cytosine (C), adenine (A), and guanine (G). The composition of percent nucleotides in the sequences is shown in Figure 3.

The overall average nucleotide composition of *Goodyera matK* sequences consists of 37.7% thymine/uracil (T/U), 16.2% cytosine (C), 31.0% adenine (A), and 15.0% guanine (G). Hence, the highest percentages of average nucleotide composition in the genus *Goodyera* and the outgroup *A. wallichi* are thymine and adenine. The high percentage of AT content compared to CG content indicates a substantial AT content in the *mat*K sequence. The low GC content in the *mat*K sequence is also shown by a previous study of *Coelogyne* spp., and *Cinnamomum parthenoxylon* (Pratiwi *et al*., 2023; Vu *et al*., 2024).

Conclusions

An in silico study has analyzed the genetic diversity of the genus *Goodyera* spp. using the DNA barcode *mat*K gene sequence. Phylogenetic tree reconstruction based on the *mat*K divided *Goodyera* species into two ingroup clades and one outgroup, with the closest kinship observed between *G. foliosa* and *G. henryi*. Further analysis suggests a low genetic diversity within the genus *Goodyera*. These findings demonstrate the utility of *matK* and support the genetic collection to clarify overlapping morphological characters in *Goodyera*. Additional research using another gene sequence, such as *psb*A, is recommended to strengthen species resolution and database completeness. In Situ and Ex-situ conservation efforts are also required to support the long-term survival of *Goodyera* spp. in their natural habitats.

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