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Genetic Diversity of Siamese Rosewood *Dalbergia cochinchinensis* Pierre in Thailand: Insights from Chloroplast and Nuclear DNA Analysis

Benjarat Prompen [1,2], Weerachai Saijuntha [3,4], Warayutt Pilap [2,4] and Sudarat Thanonkeo [2,4,*]

[1] Graduate School, Mahasarakham University, Maha Sarakham 44150, Thailand; BenjaPrompen@hotmail.com
[2] Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham 44150, Thailand; warayutt@msu.ac.th (W.P.); sudarat.t@msu.ac.th (S.T.)
[3] Faculty of Medicine, Mahasarakham University, Maha Sarakham 44000, Thailand; weerachai.s@msu.ac.th
[4] Center of Excellence in Biodiversity Research, Mahasarakham University, Maha Sarakham 44150, Thailand
* Correspondence: sudarat.t@msu.ac.th; Tel.: +66-86-2268160

Abstract: Siamese rosewood (*Dalbergia cochinchinensis* Pierre) is a highly valuable economic tree species in Thailand. This study investigated genetic diversity across 72 tree samples from 17 Thai locations using two genetic markers: the chloroplast maturase K (*matK*) gene and the nuclear internal transcribed spacer (ITS) region, which includes ITS1, 5.8S ribosomal RNA gene, and ITS2. Analysis of 48 *matK* and 65 ITS sequences revealed 34 (DcM1–DcM34) and 31 (DcI1–DcI31) haplotypes, respectively. The Saraburi province haplotype DcM10 exhibited the highest genetic divergence, differing by 25 mutational steps from other haplotypes. Phylogenetic analysis incorporating GenBank sequences from other *Dalbergia* species showed that *matK* sequences separated *D. cochinchinensis* into two distinct clades: Clade A comprising most Thai samples, and Clade B containing Saraburi samples grouped with sequences from Laos and Cambodia. In contrast, ITS analysis revealed a single monophyletic group. These findings have important applications for conservation strategies, particularly in prioritizing the genetically distinct Saraburi population for protection, and can inform breeding programs to maintain genetic diversity. Additionally, the identified genetic markers could be developed into tools for timber authentication to combat illegal logging, while the revealed population structure can guide reforestation efforts and international conservation collaboration with Laos and Cambodia.

Keywords: conservation; genetic variation; haplotype network; phylogenetic tree; Siamese rosewood

1. Introduction

Siamese rosewood (*Dalbergia cochinchinensis* Pierre) is one of approximately 250 species in the genus *Dalbergia* (family Fabaceae) found across pan-tropical regions [1]. While its genus has a broad distribution, *D. cochinchinensis* is endemic to Southeast Asia, specifically Thailand, Myanmar, Cambodia, Lao PDR, and Vietnam. In Thailand, where 26 *Dalbergia* species have been documented [2], Siamese rosewood populations primarily occur in the northeastern, eastern, and central regions. The species' highly prized heartwood makes it one of Thailand's most economically valuable plants, with strong demand in both domestic and international markets. However, this commercial value has led to extensive exploitation, threatening its survival and natural distribution patterns across Thailand.

The threat to Siamese rosewood has intensified significantly in recent years. In 2018 alone, Thai authorities recorded 576 cases of illegal harvesting in natural forests, predominantly in the eastern and northeastern regions, with damages valued at 103 million baht [3]. The species' critical status has prompted multiple protective measures: it was classified as restricted timber under the Royal Decree BE 2530 and the Forest Act BE 2484, and in 2013, Thailand successfully advocated for its inclusion in

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CITES List 2 for controlled international trade [4]. Further protection came through National Announcement No. 106/2014 [5], and the species is currently listed as critically endangered on the IUCN Red List.

Conservation of genetic resources is crucial for preserving endangered species with economic significance and maintaining genetic diversity for future breeding programs. Two main conservation approaches are employed: in situ conservation (preservation in natural forest conditions) and ex situ conservation (establishment of plantations). Genetic studies using DNA analysis have revealed that Siamese rosewood is an outcrossing species with high genetic diversity both within and between populations. Research has shown genetic differences between populations of approximately 0.127 [6,7]. Population structure studies have employed various genetic markers, including RAPD and ISSR [8,9], microsatellite [10], chloroplast maturase K (matK), and nuclear internal transcribed spacer (ITS) regions [11]. Among these markers, the matK and ITS regions have emerged as particularly valuable tools for genetic analysis, despite some limitations in discrimination power. These markers are part of the plant DNA barcode system and offer complementary insights: matK is maternally inherited and helps track seed dispersal patterns, while ITS provides information about both parental lineages and can reveal recent evolutionary events [11].

While both government agencies and farmers have begun establishing economic forest plantations for Siamese rosewood, a significant challenge remains: the shortage of high-quality seedlings from superior mother plants. To address this issue, molecular approaches using chloroplast and nuclear genome markers are essential for studying genetic diversity. The present research aims to assess the genetic diversity of *D. cochinchinensis* using matK and ITS regions as genetic markers. This genetic database will support future propagation efforts, breeding development, and conservation programs for sustainable utilization.

2. Materials and Methods

2.1. Sample Collection

The Siamese rosewood specimens in this study were initially identified based on their distinctive morphological characteristics (Figure 1) using the guideline described by Niyomdham [2]. Voucher specimens from this study were deposited in the Forest Herbarium-BKF under the following codes: "Prompen, B. No. 1 BKF NO. 224371" and "Prompen, B. No. 2 BKF NO. 224372". To preserve and propagate the genetic material, grafting branches from the original trees were collected from natural forests [12] and established at the Royal Forest Department's collection within the Northeastern Forest Tree Seed Center, Khon Kaen Province, Thailand.

For molecular analysis, this study examined 72 *D. cochinchinensis* samples collected from 17 distinct geographical locations (Table 1 and Figure 2). Young leaves were harvested using sterile scissors, preserved in silica gel, and transported to the Walai Rukhavej Botanical Research Institute laboratory for further analysis.

Table 1. List of samples and molecular markers examined in this study.

Sample no.	Sample code	Molecular marker		Sample collection site
		*matK**	ITS**	
1	TRT-M1	DcM1	DcI23	Mueang District, Trat Province
2	TRT-M2	DcM2	DcI24	
3	TRT-M3	DcM2	DcI24	
4	TRT-M4	DcM1	n/a	
5	TRT-KY1	DcM4	DcI21	Khlong Yai District, Trat Province
6	TRT-KY2	DcM3	DcI21	
7	TRT-KY3	DcM4	DcI22	
8	TRT-KY4	DcM3	n/a	
9	SSK-PS1	DcM6	n/a	Phu Sing District, Sisaket Province

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10	SSK-PS2	DcM6	DcI19	
11	SSK-PS3	DcM5	DcI19	
12	SSK-PS4	n/a	DcI19	
13	SSK-PS5	DcM5	DcI18	
14	SSK-KL1	DcM7	DcI20	Kantaralak District, Sisaket Province
15	SSK-KL2	DcM7	DcI14	
16	SSK-KL3	DcM7	DcI20	
17	SSK-KL4	n/a	DcI14	
18	SRN-KC1	DcM9	DcI18	Kap Choeng District, Surin Province
19	SRN-KC2	DcM8	DcI19	
20	SRN-KC3	DcM9	DcI11	
21	SRN-KC4	DcM8	n/a	
22	SRI76	DcM10	n/a	Muak Lek District, Saraburi Province
23	SRI80	DcM10	n/a	
24	SRI	DcM10	n/a	
25	SRI6	n/a	DcI28	
26	SRI27	n/a	DcI29	
27	SRI34	n/a	DcI11	
28	SNK-PP1	DcM15	DcI15	Phu Phan District, Sakon Nakhon Province
29	SNK-PP2	DcM14	DcI16	
30	SNK-PP3	DcM13	DcI5	
31	SNK-PP4	DcM12	DcI17	
32	SNK-PP5	DcM11	DcI5	
33	SNK-M1	DcM20	DcI12	Mueang District, Sakon Nakhon Province
34	SNK-M2	DcM19	DcI13	
35	SNK-M3	DcM18	DcI5	
36	SNK-M4	DcM17	DcI14	
37	SNK-M5	DcM16	DcI15	
38	SKW-WY1	DcM23	DcI1	Wang Nam Yen District, Sa Kaeo Province
29	SKW-WY2	DcM22	DcI1	
40	SKW-WY3	DcM21	DcI11	
41	NMA-KB1	DcM26	DcI1	Khonburi District, Nakhon Ratchasima Province
42	NMA-KB2	DcM25	DcI9	
43	NMA-KB3	DcM24	DcI10	
44	KKN-WK1	DcM32	DcI2	Wiang Kao District, Khon Kaen Province
45	KKN-WK2	DcM31	DcI3	
46	KKN-WK3	DcM30	DcI4	
47	KKN-WK4	DcM29	DcI5	
48	KKN-WK5	DcM28	DcI6	
49	KKN-WK6	DcM27	DcI7	
50	KKN-WK7	n/a	DcI8	
51	CCO-TT1	DcM34	DcI1	Tha Takiap District, Chachoengsao Province
52	CCO-TT2	DcM33	DcI1	
53	CCO-TT3	DcM34	DcI1	
54	CCO-TT4	DcM33	DcI1	
55	UBN-NC1	n/a	DcI24	Na Chaluai District, Ubon Ratchathani Province
56	UBN-NC2	n/a	DcI21	
57	UBN-NC4	n/a	DcI21	
58	UBN-NY1	n/a	DcI22	Nam Yuen District, Ubon Ratchathani Province
59	UBN-NY2	n/a	DcI22	
60	UBN-NY3	n/a	DcI22	
61	MDH8	n/a	DcI25	Kham Chai District, Mukdahan Province
62	MDH12	n/a	DcI13	
63	MDH41	n/a	DcI25	

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64	MDH67	n/a	DcI17	
65	UDN36	n/a	DcI30	Nong Wua So District, Udon Thani Province
66	UDN37	n/a	DcI2	
67	UDN43	n/a	DcI2	
68	UDN45	n/a	DcI31	
69	PLK5	n/a	DcI16	Nakhon Thai District, Phisanulok Province
70	PLK9	n/a	DcI26	
71	PLK21	n/a	DcI27	
72	PLK32	n/a	DcI13	

* *matK* haplotype delection, ** ITS haplotype detection.

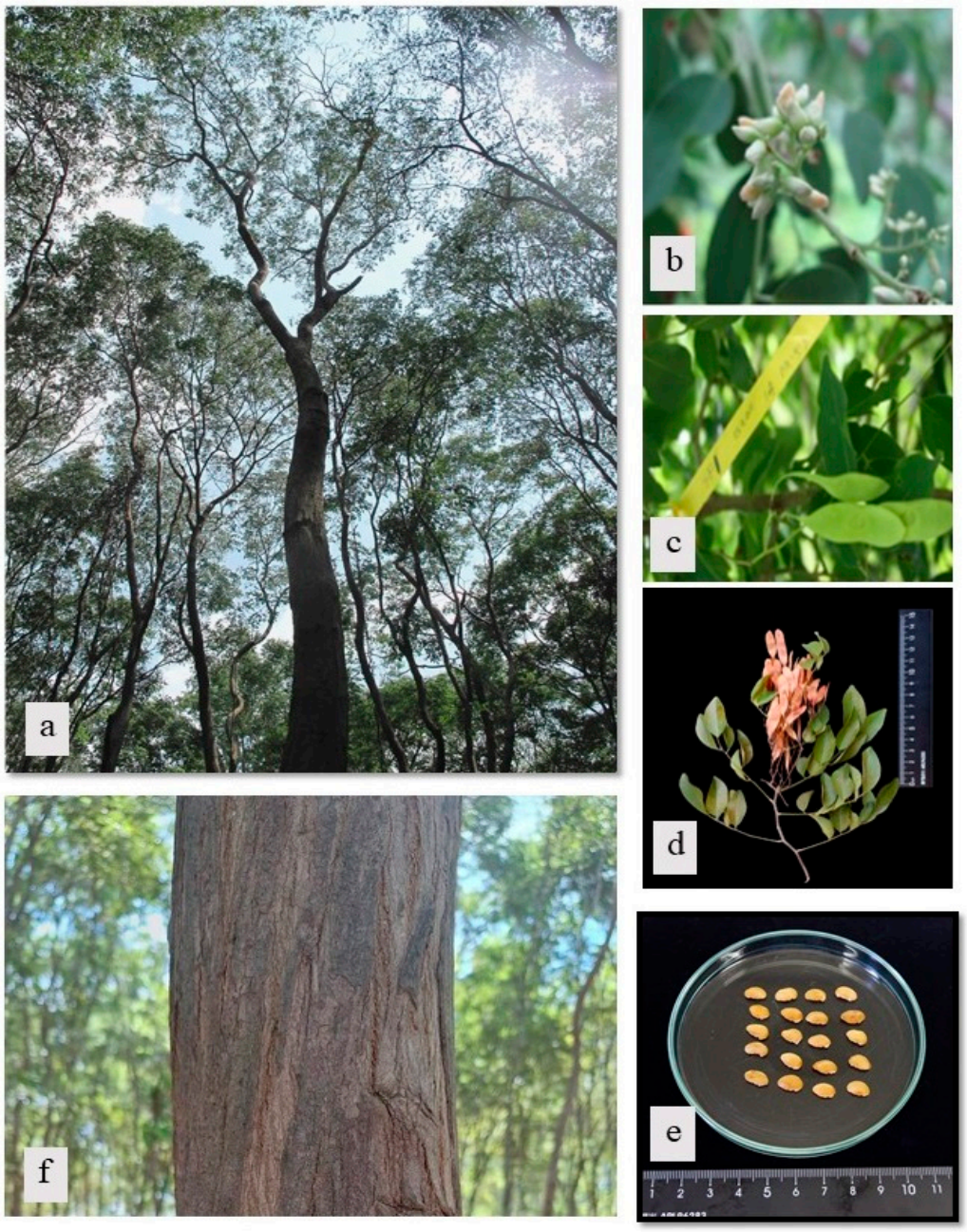

Figure 1. Morphological characteristics of *D. cochinchinensis* used for species identification: (a) Siamese rosewood tree, (b) inflorescences, (c) young leaves, (d) pods, (e) seeds, and (f) stem.

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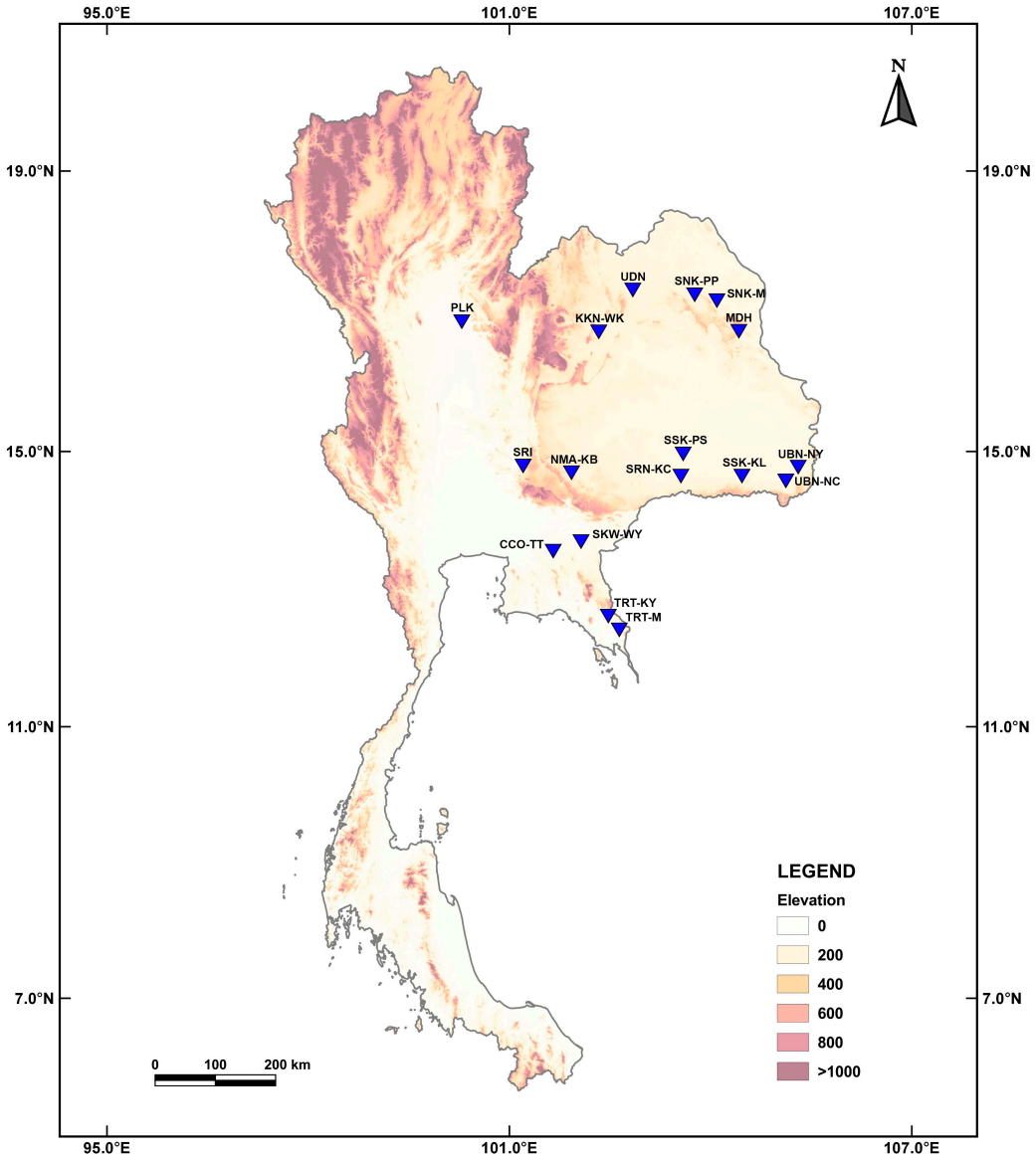

Figure 2. Geographic distribution of *D. cochinchinensis* sampling locations in this study.

2.2. Molecular Analysis

Genomic DNA was extracted from young leaves using the DNeasy® Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol, with samples stored at –20 °C until analysis. Two genomic regions were targeted for amplification: the chloroplast maturase K (*matK*) and the nuclear internal transcribed spacer (ITS). For *matK* amplification, the forward primer matK-3F_IH (5'-CGT ATA GTA CTC TTG TGT TTA CGA G-3') and reverse primer matK-3R (5'-ATC CTA TTC ATC TGG AAA TCT TGG TTC-3') were used [13], while ITS amplification employed forward primer ITS-F (5'-GGA AGG AGA AGT CGT AAC AAG G-3') and reverse primer ITS5-R (5'-TCC TCC GCT TAT TGA TAT GC-3') [14]. PCR amplification was performed using the HotStar*Taq* Master Mix Kit (Qiagen, Germany) in 25 µl reaction volumes. Both genomic regions were amplified using a thermal cycler (Applied Biosystems, USA) under identical conditions consisting of initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. The amplified products were visualized on 1% agarose gels in 0.5x TBE buffer using GelRed™ Nucleic Acid Gel Stain (Biotium, Inc., Hayward, CA), after which PCR products were excised, purified using the E.Z.N.A.® Gel Extraction Kit (Omega Biotek, USA), and sequenced using Sanger sequencing (ATGC Co., Ltd., Thailand).

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2.3. Data Analysis

Sequence alignment and analysis were performed using the ClustalW program [15], with manual editing conducted in BioEdit [16]. Molecular diversity indices and haplotype data were generated using DnaSp v5 [17]. Genetic differentiation between populations was assessed using both *p*-distance and Kimura 2-parameters (K2P) [18] in MEGA XI [19]. To visualize haplotype relationships, a minimum-spanning network was constructed using Network version 10.2, employing the median-joining algorithm [20].

Phylogenetic analyses were conducted separately for *matK* and ITS sequences using haplotypes identified from all *D. cochinchinensis* populations in this study. Additional *Dalbergia* species sequences were retrieved from GenBank for comparative analysis. *Tectona grandis* and *Pterocarpus indicus* were selected as outgroups for *matK* and ITS trees, respectively. Phylogenetic trees were constructed using two methods in MEGA XI [21]: Maximum likelihood (ML) with the general time reversible model incorporating gamma distribution and invariant sites (GTR+G+I) [22], and neighbor-joining [23]. Node support was assessed using 1,000 bootstrap replicates.

3. Results

3.1. Genetic Diversity of D. cochinchinensis

We analyzed genetic variation in *D. cochinchinensis* populations using two genetic markers: *matK* and ITS sequences. For the *matK* gene, we successfully amplified and sequenced an 821 bp fragment from 48 samples collected across 12 localities. These sequences were deposited in GenBank (accession numbers PQ317478 – PQ317748). Analysis revealed 66 variable nucleotide sites (8% of total sites), comprising 5 singletons and 61 parsimony informative sites. We identified 34 distinct haplotypes (DcM1 – DcM34), each exclusively found in a specific geographical area (Figure 3). The *matK* sequences showed high genetic diversity, with haplotype diversity of 0.986±0.007 and nucleotide diversity of 0.0161±0.0022 (Table 2).

Table 2. Molecular diversity indices of the 17 populations of *D. cochinchinensis* based on ITS and *matK* sequences analyses.

Populations	ITS						*matK*					
	n	**S**	**H**	**Uh**	**Hd±SD**	**Nd±SD**	**n**	**S**	**H**	**Uh**	**Hd±SD**	**Nd±SD**
TRT-M	3	1	2	1	0.667±0.314	0.0010±0.0005	4	5	2	2	0.667±0.204	0.0041±0.0012
TRT-KY	3	1	2	0	0.667±0.314	0.0010±0.0005	4	4	2	2	0.667±0.204	0.0033±0.0001
SSK-KL	4	3	2	1	0.667±0.204	0.0030±0.0009	3	0	1	1	0.000±0.000	0.0000±0.0000
SSK-PS	4	1	2	0	0.500±0.265	0.0008±0.0004	4	11	2	2	0.667±0.204	0.0089±0.0027
SRN-KC	3	3	3	0	1.000±0.272	0.0035±0.0012	4	10	2	2	0.667±0.204	0.0081±0.0025
SRI	3	2	3	2	1.000±0.272	0.0020±0.0007	3	0	1	1	0.000±0.000	0.0000±0.0000
SNK-M	5	5	5	1	1.000±0.126	0.0042±0.0009	5	12	5	5	1.000±0.126	0.0073±0.0011
SNK-PP	5	4	4	0	0.900±0.161	0.0036±0.0007	5	22	5	5	1.000±0.126	0.0122±0.0032
SKW-WY	3	2	2	0	0.667±0.314	0.0020±0.0009	3	10	3	3	1.000±0.272	0.0081±0.0023
NMA-KB	3	2	3	2	1.000±0.272	0.0020±0.0007	3	10	3	3	1.000±0.272	0.0081±0.0025
KKN-WK	7	11	7	5	1.000±0.076	0.0078±0.0012	6	28	6	6	1.000±0.096	0.0138±0.0034
CCO-TT	4	1	1	0	0.000±0.000	0.0000±0.0000	4	12	2	2	0.667±0.204	0.0097±0.0030
UBN-NY	3	1	1	0	0.000±0.000	0.0000±0.0000	n/a	n/a	n/a	n/a	n/a	n/a
UBN-NC	3	1	2	0	0.667±0.314	0.0010±0.0005	n/a	n/a	n/a	n/a	n/a	n/a
UDN	4	5	3	2	0.833±0.222	0.0040±0.0013	n/a	n/a	n/a	n/a	n/a	n/a
MDH	4	4	3	1	0.833±0.222	0.0038±0.0011	n/a	n/a	n/a	n/a	n/a	n/a
PLK	4	6	4	2	1.000±0.177	0.0045±0.0013	n/a	n/a	n/a	n/a	n/a	n/a
Total	**65**	**17**	**31**	**17**	**0.968±0.008**	**0.0069±0.0003**	**48**	**66**	**34**	**25**	**0.986±0.007**	**0.0161±0.0022**

* n/a, not available.

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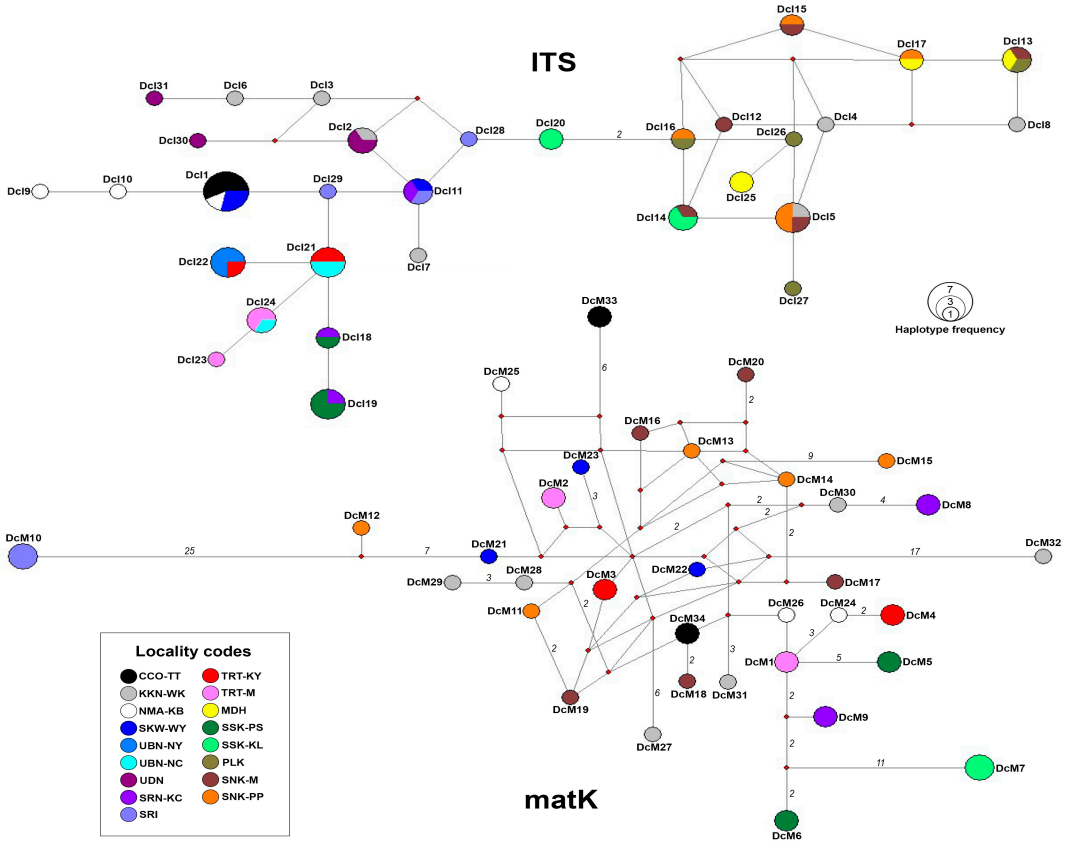

Figure 3. Median-joining haplotype network of the *matK* and ITS depicts the relationships among haplotypes from examined populations of *D. cochinchinensis*. The area of the circles represents the proportion of specimen numbers found in each haplotype. The length of each branch demonstrates the number of mutational steps (ms).

For the ITS region, we obtained sequences from 65 samples representing 17 localities (GenBank accession numbers PQ317478 – PQ317748). The analysis identified 19 variable sites, including 1 singleton and 18 parsimony informative sites. These variations defined 31 haplotypes (DcI1 – DcI34), with 18 haplotypes being location-specific and 13 shared across multiple localities (Figure 3). The ITS sequences also showed substantial genetic diversity, with haplotype diversity of 0.968±0.008 and nucleotide diversity of 0.0069±0.0003 (Table 2).

Genetic differentiation among populations was assessed using both *p*-distance and K2P methods. For *matK*, *p*-distance values ranged from 0.0061 to 0.0572, while K2P distances ranged from 0.0061 to 0.0597 (Table 3). The ITS sequences showed lower genetic differentiation, with *p*-distance ranging from 0.0010 to 0.0127 and K2P from 0.0010 to 0.0129 (Table 4). Notably, populations from SRI showed remarkably high genetic differentiation based on *matK* analysis, with *p*-distance (0.0398-0.0572) and K2P values (0.0411-0.0597) consistently higher than other populations.

Table 3. Pairwise genetic distances between *D. cochinchinensis* populations based on *matK* sequences, showing *p*-distance (lower triangle) and Kimura 2-parameter (K2P) distance (upper triangle).

Populations	TRT-M	TRT-KY	SSK-KL	SSK-PS	SRN-KC	SNK-M	SNK-PP	SKW-WY	NMA-KB	KKN-WK	CCO-TT	SRI
TRT-M	–	0.0061	0.0204	0.0086	0.0086	0.0072	0.0114	0.0071	0.0063	0.0111	0.0092	0.0448
TRT-KY	0.0061	–	0.0223	0.0117	0.0098	0.0074	0.0113	0.0065	0.0065	0.0123	0.0098	0.0429
SSK-KL	0.0201	0.0219	–	0.0179	0.0210	0.0225	0.0260	0.0227	0.0223	0.0235	0.0235	0.0597
SSK-PS	0.0085	0.0116	0.0177	–	0.0117	0.0132	0.0169	0.0137	0.0104	0.0146	0.0135	0.0513
SRN-KC	0.0085	0.0097	0.0207	0.0116	–	0.0108	0.0140	0.0094	0.0094	0.0127	0.0117	0.0467

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SNK-M	0.0072	0.0073	0.0222	0.0130	0.0107	–	0.0095	0.0080	0.0091	0.0119	0.0093	0.0444
SNK-PP	0.0113	0.0112	0.0256	0.0167	0.0139	0.0095	–	0.0116	0.0122	0.0151	0.0126	0.0429
SKW-WY	0.0071	0.0065	0.0223	0.0136	0.0093	0.0080	0.0115	–	0.0094	0.0124	0.0111	0.0411
NMA-KB	0.0063	0.0065	0.0219	0.0104	0.0093	0.0090	0.0121	0.0093	–	0.0118	0.0102	0.0476
KKN-WK	0.0110	0.0122	0.0231	0.0144	0.0126	0.0118	0.0149	0.0123	0.0116	–	0.0129	0.0493
CCO-TT	0.0091	0.0097	0.0231	0.0134	0.0116	0.0093	0.0124	0.0110	0.0102	0.0128	–	0.0467
SRI	0.0432	0.0414	0.0572	0.0493	0.0451	0.0429	0.0414	0.0398	0.0459	0.0475	0.0451	–

Table 4. Pairwise genetic distances between *D. cochinchinensis* populations based on ITS sequences, showing *p*-distance (lower triangle) and Kimura 2-parameter (K2P) distance (upper triangle).

Populations	**TRT-M**	**TRT-KY**	**SSK-KL**	**SSK-PS**	**SRN-KC**	**SNK-M**	**SNK-PP**	**SKW-WY**	**NMA-KB**	**KKN-WK**	**CCO-TT**	**UBN-NY**	**UBN-NC**	**MDH**	**UDN**	**PLK**	**SRI**
TRT-M	–	0.0025	0.0088	0.0046	0.0045	0.0114	0.0108	0.0050	0.0065	0.0104	0.0050	0.0035	0.0015	0.0122	0.0080	0.0103	0.0050
TRT-KY	0.0025	–	0.0073	0.0031	0.0030	0.0099	0.0093	0.0035	0.0043	0.0088	0.0035	0.0010	0.0010	0.0107	0.0063	0.0088	0.0035
SSK-KL	0.0087	0.0072	–	0.0089	0.0075	0.0047	0.0044	0.0063	0.0078	0.0060	0.0068	0.0083	0.0073	0.0064	0.0072	0.0049	0.0048
SSK-PS	0.0046	0.0031	0.0088	–	0.0020	0.0120	0.0114	0.0053	0.0068	0.0104	0.0056	0.0041	0.0031	0.0129	0.0078	0.0110	0.0049
SRN-KC	0.0045	0.0030	0.0075	0.0020	–	0.0109	0.0103	0.0040	0.0055	0.0091	0.0045	0.0040	0.0030	0.0117	0.0064	0.0098	0.0035
SNK-M	0.0113	0.0098	0.0046	0.0119	0.0108	–	0.0034	0.0094	0.0109	0.0069	0.0094	0.0109	0.0099	0.0046	0.0098	0.0041	0.0083
SNK-PP	0.0107	0.0092	0.0043	0.0113	0.0102	0.0034	–	0.0088	0.0103	0.0065	0.0088	0.0103	0.0093	0.0038	0.0091	0.0035	0.0077
SKW-WY	0.0050	0.0035	0.0062	0.0052	0.0040	0.0093	0.0087	–	0.0022	0.0078	0.0010	0.0045	0.0035	0.0102	0.0053	0.0083	0.0023
NMA-KB	0.0065	0.0043	0.0077	0.0067	0.0055	0.0108	0.0102	0.0022	–	0.0091	0.0015	0.0040	0.0050	0.0117	0.0063	0.0098	0.0038
KKN-WK	0.0104	0.0087	0.0060	0.0103	0.0090	0.0068	0.0065	0.0078	0.0090	–	0.0084	0.0095	0.0089	0.0077	0.0067	0.0065	0.0065
CCO-TT	0.0050	0.0035	0.0067	0.0056	0.0045	0.0093	0.0087	0.0010	0.0015	0.0084	–	0.0045	0.0035	0.0102	0.0060	0.0083	0.0030
UBN-NY	0.0035	0.0010	0.0082	0.0041	0.0040	0.0108	0.0102	0.0045	0.0040	0.0094	0.0045	–	0.0020	0.0117	0.0068	0.0098	0.0045
UBN-NC	0.0015	0.0010	0.0072	0.0031	0.0030	0.0098	0.0092	0.0035	0.0050	0.0089	0.0035	0.0020	–	0.0107	0.0065	0.0088	0.0035
MDH	0.0121	0.0106	0.0064	0.0127	0.0116	0.0046	0.0038	0.0101	0.0116	0.0077	0.0101	0.0116	0.0106	–	0.0102	0.0039	0.0092
UDN	0.0080	0.0062	0.0071	0.0078	0.0064	0.0097	0.0090	0.0052	0.0062	0.0066	0.0060	0.0067	0.0065	0.0101	–	0.0085	0.0043
PLK	0.0102	0.0087	0.0049	0.0109	0.0097	0.0040	0.0034	0.0082	0.0097	0.0064	0.0082	0.0097	0.0087	0.0039	0.0084	–	0.0073
SRI	0.0050	0.0035	0.0047	0.0049	0.0035	0.0083	0.0077	0.0023	0.0038	0.0065	0.0030	0.0045	0.0035	0.0091	0.0042	0.0072	–

3.2. Haplotype Network

Haplotype networks were constructed separately for 34 *matK* (DcM1 – DcM34) and 31 ITS (DcI1 – DcI34) sequences. The *matK* analysis revealed distinct geographical patterns with no shared haplotypes between localities. Three haplotypes showed notable genetic distances from the others: DcM10 from Muak Lek District, Saraburi Province (25 mutational steps), DcM32 from Wiang Kao District, Khon Kaen Province (17 mutational steps), and DcM7 from Kantaralak District, Sisaket Province (11 mutational steps) (Figure 3).

In contrast, the ITS sequence analysis showed minimal genetic differentiation, with a maximum of two mutational steps between haplotypes. Unlike *matK*, the ITS analysis identified 14 shared haplotypes across different localities: DcI1, DcI5, DcI11, DcI12, DcI13, DcI14, DcI15, DcI16, DcI17, DcI18, DcI19, DcI21, DcI22, and DcI24 (Figure 3).

3.3. Phylogenetic Tree

Phylogenetic analyses were conducted separately for *matK* and ITS sequences, incorporating both our sequences and previously published sequences of *D. cochinchinensis* and other *Dalbergia* species from GenBank. The ITS-based phylogenetic tree demonstrated that all *D. cochinchinensis* samples formed a well-supported monophyletic group, distinct from other *Dalbergia* species. Within the genus, *D. cochinchinensis* showed the closest phylogenetic affinity to *D. sericea* from Bhutan (Figure 4).

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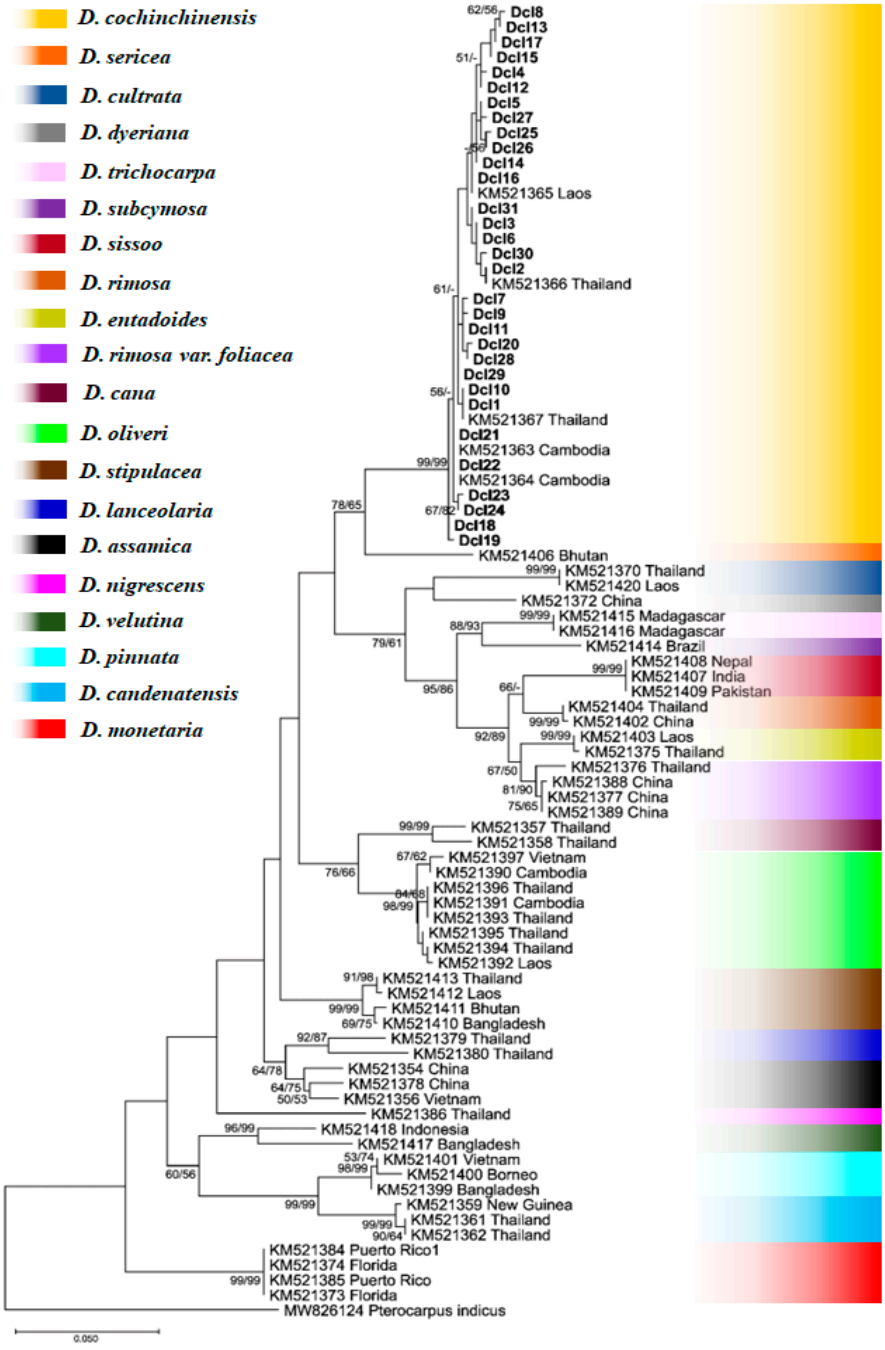

Figure 4. Phylogenetic tree based on ITS sequences showing relationships among *D. cochinchinensis* samples from this study and previously published sequences of *D. cochinchinensis* and other *Dalbergia* species from GenBank. Numbers at nodes indicate bootstrap support values from maximum likelihood/maximum parsimony analyses. *Pterocarpus indicus* was used as an outgroup.

The *matK*-based phylogenetic analysis revealed two distinct clades within *D. cochinchinensis* (Figure 5). Clade A comprised all *matK* haplotypes generated in this study except DcM10, while Clade B contained haplotype DcM10 from Saraburi Province together with previously published *D. cochinchinensis* sequences from Thailand, Laos, and Cambodia. Notably, both clades showed close phylogenetic relationships with *D. ovata* sequences from Thailand and Myanmar, suggesting potential evolutionary connections between these species within the region.

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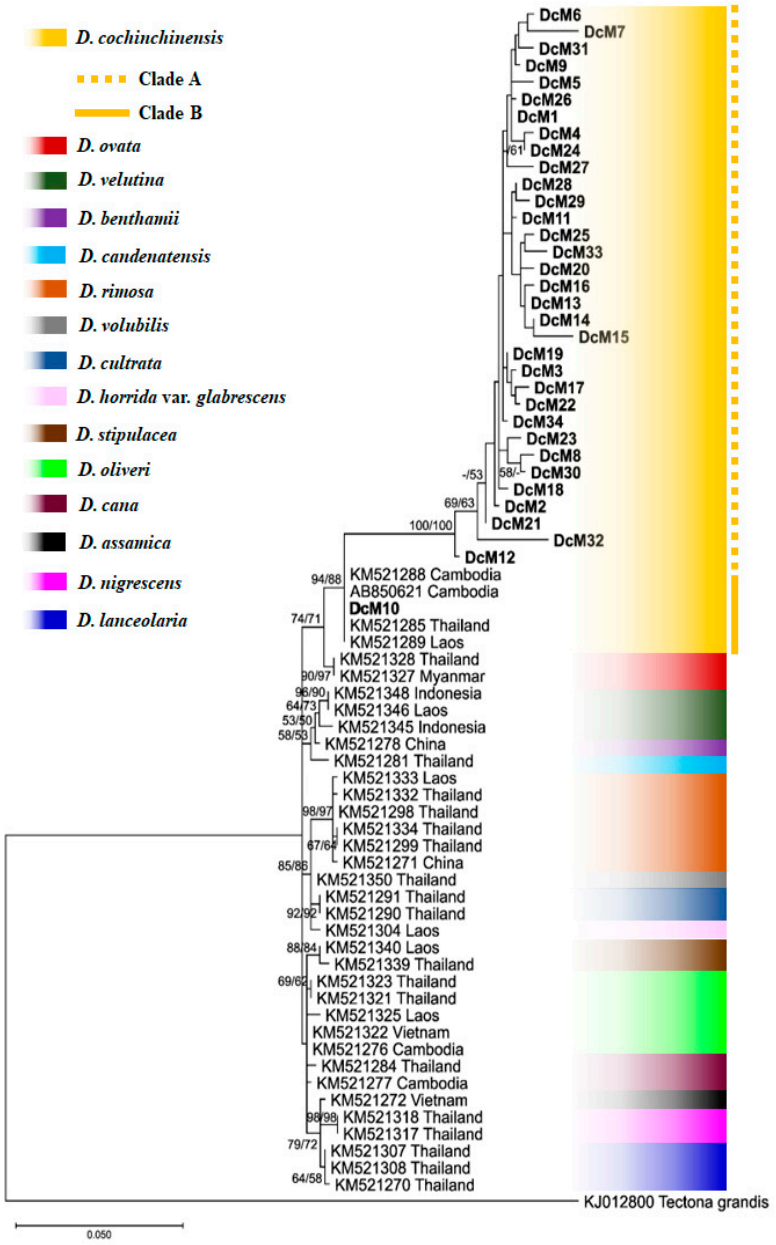

Figure 5. Phylogenetic tree based on *matK* sequences showing relationships among *D. cochinchinensis* samples from this study and previously published sequences of *D. cochinchinensis* and other *Dalbergia* species from GenBank. Two distinct clades (A and B) were identified within *D. cochinchinensis*. Numbers at nodes indicate bootstrap support values from maximum likelihood/maximum parsimony analyses. *Pterocarpus indicus* was used as an outgroup.

4. Discussion

Our analysis revealed significant genetic diversity patterns in *D. cochinchinensis* populations across Thailand. The *matK* sequences showed high overall genetic diversity (haplotype diversity = 0.986±0.007 and nucleotide diversity = 0.0161±0.0022), with 34 distinct haplotypes exhibiting strong geographical structuring. Population-level diversity varied considerably, with the highest haplotype diversity (1.000) observed in populations from Khon Kaen (DC-KKN-WK), Nakhon Ratchasima (DC-NMA-KB), Sa Kaeo (DC-SKW-WY), and Sakon Nakhon provinces (DC-SNK-M), while the lowest diversity (0.000) was found in Saraburi (DC-SRI). Similarly, ITS sequences revealed 31 haplotypes

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with substantial overall diversity (haplotype diversity = 0.968±0.008 and nucleotide diversity = 0.0069±0.0003), though with less pronounced geographical structuring.

The relatively low nucleotide diversity observed in both markers aligns with previous findings. Yooyuen [7] reported low genetic diversity (haplotype diversity = 0.608 and nucleotide diversity = 0.00033) in northeastern and central Thai populations, identifying only 11 haplotypes across populations. This consistently low genetic diversity may reflect the species' historically narrow distribution and specific ecological requirements, as *D. cochinchinensis* occupies a more restricted ecological niche compared to related species like *D. oliveri* [2,24,25].

Our phylogenetic analyses revealed distinct patterns between markers. The ITS-based phylogeny showed *D. cochinchinensis* as a well-supported monophyletic group, while *matK* analysis identified two distinct clades with differential geographic distribution. These patterns suggest that landscape features have played a crucial role in shaping the species' genetic structure, consistent with recent findings by Hartvig et al. [10] and Huang et al. [26], who demonstrated that drainage systems significantly influence phylogeographic patterns of *Dalbergia* species in Indochina.

The genetic patterns observed in this study have important implications for conservation and breeding strategies. The selected specimens exhibited desirable traits for breeding programs, including high growth rates, straight trunks, quality wood characteristics, and disease resistance. The distinct genetic lineages identified through both markers suggest the importance of maintaining population-level diversity in conservation efforts. Moreover, the strong geographic structuring observed particularly in *matK* sequences indicates that conservation strategies should consider preserving populations across different regions to maintain the full range of genetic diversity.

Several limitations should be considered when interpreting our results. Our study focused primarily on Thai populations, potentially missing important genetic variations in neighboring countries. The use of only chloroplast and nuclear markers may not fully capture the genetic complexity of the species, and the lack of temporal genetic data limits our understanding of how diversity patterns have changed over time in response to anthropogenic pressures.

Future research directions should focus on expanding the geographical scope to include populations from neighboring countries, which would provide a more comprehensive understanding of regional genetic patterns. The incorporation of additional genetic markers, particularly those related to adaptive traits, would offer deeper insights into the species' evolutionary potential. Studies investigating the impact of recent habitat fragmentation on genetic diversity, the relationship between genetic diversity and phenotypic variation in important timber quality traits, and the effects of current conservation practices on genetic diversity maintenance would be valuable. Furthermore, the examination of potential hybridization patterns with closely related *Dalbergia* species and the development of more comprehensive landscape genetic models incorporating contemporary environmental changes would enhance our understanding of this economically important species and inform more effective conservation strategies.

5. Conclusions

This study reveals significant insights into the genetic diversity of *D. cochinchinensis* through analysis of *matK* and ITS markers. Our findings demonstrate high genetic diversity within the species, evidenced by numerous haplotypes (34 *matK* and 31 ITS) and high haplotype diversity indices (*matK*: 0.986±0.007; ITS: 0.968±0.008). The markers showed contrasting patterns: *matK* sequences exhibited strong geographical structuring with location-specific haplotypes, while ITS sequences showed more limited differentiation with shared haplotypes across localities. Notably, phylogenetic analyses identified two distinct evolutionary lineages, with the Saraburi Province population showing remarkable genetic divergence. This population, along with samples from neighboring countries, forms a separate clade that may represent a unique evolutionary line requiring special conservation attention.

These findings suggest that despite recent population declines, *D. cochinchinensis* maintains substantial genetic diversity. For conservation, this implies the need to protect multiple populations

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across its range, with a particular focus on preserving distinct evolutionary lineages and maintaining connectivity between populations to ensure the species' long-term survival.

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References

1. Vatanparast, M.; Klitgård, B.B.; Adema, F.A.; Pennington, R.T.; Yahara, T.; Kajita, T. First molecular phylogeny of the pantropical genus *Dalbergia*: implications for infrageneric circumscription and biogeography. *South Afr. J. Bot*. **2013**, *89*, 143–149.
2. Niyomdham, C. An accont of *Dalbergia* (Leguminosae-Papilionoideae) in Thailand. *Thai For. Bull (Bot.).* **2002**, *30*, 124–166.
3. Department of National Parks, Wildlife and Plant Conservation. Report summarizing statistics of illegal cases related to forestry. 2018. Available online: https://portal.dnp.go.th/Content?contentId=2134 (accessed on 20 December 2023).
4. CITES. Appendices I, II and III: valid from 4 October 2017. Convention on International Trade in Endangered Species of Wild Fauna and Flora. 2018.
5. National Council for Peace and Order. Announcement of the National Council for Peace and Order No. 106/2014 regarding amendments to the forest law. 2014.
6. Soonhuae, P.; Piewluang, C.; Boyle, T. Population genetics of *Dalbergia cochinchinensis* Pierre and implications for genetic conservation. Technical Publication No. 18, ASEAN Forest Tree Seed Centre Project, Muak-Lek, Saraburi, Thailand. (1994).
7. Yooyuen, R.; Duangjai, S.; Changtragoon, S. Chloroplast DNA variation of *Dalbergia cochinchinensis* Pierre in Thailand and Laos. In IUFRO World Series Volume 30: Asia and the Pacific Workshop Multinational and Transboundary Conservation of Valuable and Endangered Forest Tree Species, Guangzhou, China, 5–7 December 2011.
8. Hien, V.T.T.; Phong, D.T. Genetic diversity among endangered rare *Dalbergia cochinchinensis* (Fabaceae) genotypes in Vietnam revealed by random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. *Afr. J. Biotechnol*. **2012**, *11*, 8632–8644.
9. Bal, P.; Panda, P.C. Molecular characterization and phylogenetic relationships of *Dalbergia* species of eastern India based on RAPD and ISSR analysis. *Int. J. Innov. Science Res. Technol*. **2018**, *3*, 417–422.
10. Hartvig, I.; So, T.; Changtragoon, S.; Tran, H.T.; Bouamanivong, S.; Theilade, I.; Kjaer, E.D.; Nielsen, L.R. Population genetic structure of the endemic rosewoods *Dalbergia cochinchinensis* and *D. oliveri* at a regional scale reflects the Indochinese landscape and life-history traits. *Ecol. Evol*. **2018**, *8*, 530–545.
11. Hartvig, I.; Czako, M.; Kjaer, E.D.; Nielsen, L.R.; Theilade, I. The use of DNA barcoding in identification and conservation of rosewood (*Dalbergia* spp.). *PLoS ONE*. **2015**, *10*, e0138231.
12. Pérez-Luna, A.; Hernández-Díaz, J.C.; Wehenkel, C.; Simental-Rodríguez, S.L.; Hernández-Velasco, J.; Prieto-Ruíz, J.A. Graft survival of *Pinus engelmannii* Carr. in relation to two grafting techniques with dormant and sprouting buds. *PeerJ*. **2021**, *9*, e12182.

 doi:10.20944/preprints202501.0556.v1

13. Dunning, L.T.; Savolainen, V. Broad-scale amplification of *matK* for DNA barcoding plants a technical note. *Bot. J. Linn. Soc*. **2010**, *164*, 1–9.
14. Sotuyo, S.; Pedraza-Ortega, E.; Martinez-Salas, E.; Linares, J.; Cabrera, L. Insights into phylogenetic divergence of *Dalbergia* (Leguminosae: Dalbergiae) from Mexico and Central America. *Front. Ecol. Evol*. **2022**, *10*, 910250.
15. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; McGettigan, P.A.; McWilliam, H.; et al. Clustal W and clustal X version 2.0. *Bioinform*. **2007**, *23*, 2947–2948.
16. Hall, T.A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acid S*. **1999**, *41*, 95–98.
17. Librado, P.; Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinform.* **2009**, *25*, 1451–1452.
18. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120.
19. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol*. **2021**, *18*, 3022–3027.
20. Bandelt, H.J.; Forster, P.; Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48.
21. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. **2018**, *35*, 1547–1549.
22. Nei, M.; Kumar, S. Molecular evolution and phylogenetics. Oxford University Press, New York, 2000
23. Saitou, N.; Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **1987**, *4*, 406–425.
24. Niyomdham, C.; Hô, P.H.; Dy Phon, P.; Vidal, .E. Leguminoseae-Papilionoideae Dalbergieae. In: Morat, P. (ed), Flore du Cambodge du Laos et du Viêtnam. Muséum National d'Histoire Naturelle, Paris. 1997.
25. CTSP. Cambodian tree species. Monographs. Cambodia tree seed project, FA, Cambodia and DANIDA, Denmark. 2004.
26. Huang, J.F.; Li, S.Q.; Xu, R.; Peng, Y.Q. East–West genetic differentiation across the Indo-Burma hotspot: evidence from two closely related dioecious figs. *BMC Plant Biol.* **2023**, *23*, 321.