# MINISTRY OF EDUCATION VIETNAM ACADEMY OF SCIENCE AND TRAINING AND TECHNOLOGY

### GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY



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# STUDY ON THE ECOLOGY, GENETIC DIVERSITY, AND SPECIES DISTRIBUTION MODELING OF Cinnamomum parthenoxylon (Jack) Meisn. FOR THE CONSERVATION OF GENETIC RESOURCES IN VIETNAM

### SUMMARY OF DISSERTATION ON BIOLOGY

Major: Ecology Code: 9.42.01.20

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### INTRODUCTION

### 1. Rationale of the study

Cinnamomum parthenoxylon (Jack) Meisn. is a rare plant species of high medicinal and economic value, currently facing the risk of extinction due to habitat degradation, overexploitation, and limited natural regeneration capacity [6–14]. In response to this situation, the application of modern, interdisciplinary research methods is necessary to identify ecologically suitable areas, combined with genetic diversity analyses to clarify relationships among populations and the adaptive potential of the species. On this basis, the doctoral candidate conducted the dissertation entitled: "study on the ecology, genetic diversity of populations, and species distribution modeling of Cinnamomum parthenoxylon (Jack) Meisn. for conservation of genetic resources in vietnam", with the objectives of assessing the current distribution, constructing species distribution models, and analyzing the genetic structure of C. parthenoxylon populations in Vietnam. The research results will provide a foundation for breeding programs, genetic resource management, and the development of conservation strategies for the species under natural conditions.

# 2. Research Objectives

### 2.1. General Objective

This study aims to assess and update the current distribution and genetic diversity at both the population and species levels of *C. parthenoxylon* in representative tropical forest ecosystems, in order to identify priority conservation areas through projections of population dynamics across forest ecological zones under climate change scenarios in Vietnam.

### 2.2. Specific Objectives

- Investigations, evaluations, and updates on the current distribution of the endangered species *C. parthenoxylon* in forest ecological regions of Vietnam.
- Construction of species distribution models based on geospatial data to predict shifts in suitable habitats for *C. parthenoxylon* across historical,

present, and future periods.

- Evaluation of genetic diversity at both population and species levels for application in breeding programs and genetic resource management, and proposition of conservation measures for *C. parthenoxylon* under natural conditions.

### 3. Scope of the Study

### 3.1. Study Sites and Research Areas

Study sites: For the investigation of ecological characteristics and genetic diversity of *C. parthenoxylon*, the study was conducted in natural forest ecosystems representing different Forest Ecological Zones. Specifically, 9 populations were sampled across 5 regions: Hoa Bình (HB) in the Northwestern region; Vinh Phuc (VP), Phu Tho (PT), and Quang Ninh (QN) in the Northeastern region; Thanh Hoa (TH) in the North Central region; Phu Yên (PY) and Quang Nam (QNm) in the South Central region; and Gia Lai (GL) and Lam Dong (LĐ) in the Central Highlands region.

Research areas: For species distribution modeling, geospatial data (climate, soil, topography, vegetation) were collected from available datasets. Certain regions where the species has not been recorded at present were still considered within the spatial extent of the study, in order to predict potential suitable habitats that might have been ecologically suitable for the species in the past or in the future.

*3.2. Research time:* This study was conducted from November/2022 to May/2025, with a total of 15 field surveys.

### 4. Research Contents

**Content 1:** Assessment of the current distribution of *C. parthenoxylon* across 5 Forest Ecological Zones in Vietnam.

**Content 2:** Development of species distribution models for *C. parthenoxylon* across past, present, and future periods under climate scenarios.

**Content 3:** Evaluation of genetic diversity and population structure of *C. parthenoxylon* across 5 Forest Ecological Zones using SSR molecular markers.

**Content 4:** Proposal of conservation strategies for *C. parthenoxylon* within 5 Forest Ecological Zones of Vietnam.

### CHAPTER 1. OVERVIEW OF RESEARCH

#### 1.1. State of research worldwide

Scientific basis for studies on endangered and rare plant species: The IUCN Red List evaluates species extinction risk based on population size. The CITES Convention regulates trade of endangered organisms [26]. Habitat loss caused by agriculture, industry, and unsustainable exploitation has led to population declines. Climate change affects the distribution of many species. Global conservation policies, such as the Convention on Biological Diversity, have contributed to the sustainable protection of biological resources.

Studies on morphological and ecological characteristics of C. parthenoxylon and other species in the genus Cinnamomum: The genus Cinnamomum was named in 1760 by J.C. Schaeffer and belongs to the Lauraceae family. The species C. parthenoxylon (Cinnamomum parthenoxylon) has a complex taxonomic history, with numerous synonyms recorded by botanists [39]. Kameyama & Nakajima (2018) and Zhou et al. (2021) evaluated the influence of environmental factors on the growth and distribution of these species in Japan and China [45, 49].

Studies on population-level and species-level genetic diversity of C. parthenoxylon and other Cinnamomum species: Han et al. (2024) reported high genetic diversity in C. cassia [60]. Zhang et al. (2021) found low genetic diversity and evidence of inbreeding in C. chago [61]. Li et al. (2023) and Zhong et al. (2019) analyzed C. camphora at large scales, showing genetic structure associated with geography and the influence of climate change on species distribution. Kameyama et al. (2017) clarified differentiation among old-growth populations in Japan, China, and Taiwan. Joy & Maridass (2008) laid the foundation for genetic taxonomy in this genus [62–65].

Studies on species distribution modeling (SDM) for conservation purposes: Getzin et al. (2008) emphasized the role of both intrinsic and extrinsic factors in population spatial structure [69]. The Maxent model—

developed from Jaynes' (1957) principle of maximum entropy [77] and formally applied to species distribution modeling by Phillips et al. (2006)—has become a key tool due to its strong performance with presence-only data [78]. Maxent, along with other machine learning algorithms such as Random Forest (RF), has been widely applied, with RF reported to outperform Maxent in certain cases [92].

### 1.2. Research status in Vietnam

Scientific basis for research on endangered and rare plants: The Government has issued important policies such as Directive 194-CT (1986) on the system of Special-Use Forests, the Forestry Law (2017), and decrees such as Decree 32 to protect endangered species [7]. The Vietnam Red Data Book (2024) published a list of 847 species belonging to 201 families requiring protection, demonstrating a strong commitment to biodiversity conservation [6].

Research on morphological and ecological characteristics of C. parthenoxylon and other species in the genus Cinnamomum: Pham Hoang Ho (1991, 2003) recorded 43 species [107], while Nguyen Kim Dao (2017) increased the total to 49 species and 2 varieties, accounting for approximately 16% of the global Cinnamomum diversity [109]. Nguyen Van Ly (2016) provided detailed morphological descriptions of 22 species in the genus [108]. Tran Hop (2002), Le Thi Dien et al. (2010) reported that the species are distributed sparsely across regions such as the Northeast, North Central, and Central Highlands, contributing to the biodiversity of the upper canopy in natural forests [15, 111]. C. parthenoxylon contains essential oils with high concentrations of Safrole and Methyleugenol [114].

Research on genetic diversity of populations and species of C. parthenoxylon and other species in the genus Cinnamomum: Hoang Van Sam (2023) and Ha Bich Hong et al. (2021) employed chloroplast DNA sequences (matK, rbcL, trnH-psbA) to identify C. parthenoxylon in five northern provinces, revealing high accuracy and the existence of distinct

genetic lineages [17, 19]. Ha Van Huan (2015) and Ha Thi Phuc (2015) used RAPD markers to assess genetic diversity in *C. camphora* and *C. cassia* [128]. Vu Dinh Duy et al. (2022) provided evidence of high genetic diversity within natural populations of *C. balansae* [132].

Research on species distribution modeling (SDM) for conservation purposes: Studies have demonstrated the effectiveness of models in identifying spatial segregation and potential evolutionary divergence among populations. Research on *C. balansae* by Dinh Tien Tai et al. (2022) highlighted the potential of species distribution models in evaluating the impacts of climate change on forest plant species in Vietnam [140].

#### CHAPTER 2. OBJECTS AND RESEARCH METHODS

### 2.1. Research Object

Cinnamomum parthenoxylon belonging to the genus Cinnamomum. It was classified as Critically Endangered (CR A1a, c, d) according to the Vietnam Red Data Book (2024) [6], and IIA in the Appendix of Decree 84/2021/NĐ-CP [7].

#### 2.2. Research methods

**2.2.1.** Collection of primary data: Collection of documents related to natural conditions, socio-economic aspects, and relevant scientific studies.

#### 2.2.2. Field methods

- a) Morphological description: Observation of bud, bark, and stem characteristics; photographic documentation for description and comparison with specialized references. Species identification and morphological classification were conducted by leading taxonomic experts.
- b) Study on species distribution characteristics: Total 110 sample plots (OTCs) were established.
- c) Study on silvicultural characteristics: In each standard plot (OTC, 1000 m<sup>2</sup>), all trees with DBH (D<sub>1.3</sub>)  $\geq$  6 cm were measured: species name, DBH, total height (H<sub>vn</sub>), crown diameter (D<sub>t</sub>), and growth quality. In addition, five regeneration

subplots (OTS, 25 m²) were established within each OTC to investigate natural regeneration.

- *d) Six-tree sample plots:* In each plot, one central *C. parthenoxylon* tree was selected, and growth parameters of the six nearest neighboring trees were recorded.
- *e)* Sampling for molecular analysis: 208 leaf/bark samples were collected from 09 populations and preserved at –80°C and –30°C for further analysis.

### 2.2.3. Laboratory methods

- *a) Forest type classification:* Following the Forest Management Planning Standards (QPN 6-84), forest habitat types were classified.
  - b) Species identification: Based on references by Nguyen Kim Dao (2017).
- c) Determination of stand density, basal area, dominant species, and species composition: Stand density was calculated as the number of trees per hectare, while basal area reflected stem size through diameter at breast height (DBH). Dominant species in the canopy layer were determined using the Importance Value Index (IVI%), and neighboring trees were classified based on occurrence frequency.

### 2.2.4. Methods for constructing the species distribution model

The Species Distribution Model (SDM) was developed using species occurrence data from field surveys and geospatial data, including topography, climate, soil, and vegetation. Algorithms (MaxEnt, Random Forest (RF), Support vector machine (SVM), Classification and regression tree (CART) and Gradient Boosting (Boosting) were implemented.

The Last Glacial Maximum (LGM) and Mid-Holocene (MH) scenarios from CHELSA version 1.2 were applied; the present-time (HT) climate scenario was obtained from WorldClim 2.1. Future climate scenarios included ACCESS-CM2, MIROC6, EC-Earth3-Veg, and MRI-ESM2-0. Four emission pathways were considered corresponding to low, medium, high, and very high emission scenarios (SSP126, SSP245, SSP370, and SSP585) for the periods 2061–2080 and 2081–2100.

### 2.2.5. Genetic diversity analysis

Extraction of total RNA/DNA was performed. Design of SSR primers was carried out after primer search, with subsequent PCR-SSR, polyacrylamide gel electrophoresis, allele size analysis using a Fragment Analyzer.

### 2.2.6. Transcriptome sequencing data analysis

Assessment of sequence quality was conducted with FastQC, while assembly and SSR primer screening were carried out using Trimmomatic, Trinity, MISA, and Primer. Analysis of population diversity and genetic structure was performed with Arlequin, Genepop, STRUCTURE, BOTTLENECK, DAPC, and UPGMA, along with tests for HWE, Fst, and Mantel test.

# 2.2.7. Integration of genetic diversity assessment and ecological modeling for conservation

Application of the overlay method in QGIS was employed to identify priority conservation areas.

### **CHAPTER 3. RESULTS AND DISCUSSION**

# 3.1. Characteristics of the current populations of *C. parthenoxylon* distributed across 5 Forest Ecological Zones

### 3.1.1. Morphological and ecological characteristics of C. parthenoxylon

C. parthenoxylon is a large timber tree with a straight cylindrical trunk reaching 20–28 m in height and up to 106 cm in diameter. The trunk is straight and cylindrical, with the lower portion usually branchless; the bark is gray to dark gray-brown with longitudinal fissures. Leaves are alternate, obovate or narrowly elliptic; flowers are bisexual and yellowish-white (Fig. 3.1). Flowering occurs from May to July, fruit set from July to September, and fruit maturation from September to December. A total of 357 mature individuals and 373 regenerating seedlings were recorded.

### 3.1.2. Forest structure in habitats with C. parthenoxylon

# 3.1.2.1. Species composition formula of the canopy layer

The species composition formula (SCF) revealed variation from 20 species (HB, VP, and QN) to 88 species (GL)... (Table 3.1).

**Table 3.1.** Species composition formula (SCF) of the canopy layer in forest habitats where *C. parthenoxylon* was distributed

Region	Site	Number of OTC	Number of species	Number of species in SCF	Species composition formula (SCF)
Northwest	НВ	12	20	4	24,51M+19,91Dg+ 16,11Tmq + 5,02Pm+ 34,45Lk
	PT	13	38	4	23,79M + 16,69Dg + 11,59Tmq + 5,74Pm + 42,19Lk
Northeast	VP	14	20	3	26,05M + 18,33Dg + 13,51Tmq + 42,11Lk
	QN	10	20	3	26,49M + 20,54Dg + 12,16Tmq + 40,81Lk
North Central	TH	15	84	3	13,76M + 13,61Dg + 12,47Tmq + 60,16Lk
South	PY	10	66	6	13,97Sm+12,72Chx+8,36Trv + 7,01P + 5,43X + 5,13Du + 47,38Lk
Central	QNm	10	50	4	27,6Pm + 15,4Ht + 10,3Ddđ + 5,3Gt + 41,4Lk
Central Highlands	LĐ	12	88	7	11,1 K + 10,4 Tđl + 8,9 Dg + 8,4 Côm + 7.1 Gx + 5.6 Tđld + 5,01X + 43,49 Lk
	GL	15	92	7	15,1 Ht + 8,5 Ct + 5,5 Chx + 6,3 Bt + 5,6 Td + 5,2X + 5,0 K + 48,8Lk

Notes: Du: Symplocos atriolivacea; Dg: Castanopsis aff. boisii; Ddđ: Baccaurea ramiflora; Chx: Schima wallichii; Ct: Calophyllum saigonense; Gt: Neolamarckia cadamba; Gx: Michelia mediocris; Ht: Dacrydium elatum; K: Machilus parviflora; M: Manglietia conifera; P: Duabanga sonneratioides; Pm: Fokienia hodginsii; Sm: Madhuca pasquieri; Tmq: Hopea mollissima; Tđl: Pinus dalatensis; Tđld: Taxus wallichiana; Tđ: Syzygium wightianum; Trv: Syzygium cumini; X: C. parthenoxylon; Lk: Others).

### 3.1.2.2. Relationship between C. parthenoxylon and dominant tree species

The results revealed the relationship between *C. parthenoxylon* and associated tree species (Table 3.2).



**Fig. 3.1.** *C. parthenoxylon* tree in Tam Dao National Park, Vinh Phuc province (bark, regenerating individuals, branches bearing inflorescences and fruits)

**Table 3.2.** Associated tree species with *C. parthenoxylon* 

Name	WN	NE	NC	SN	СН	Total
C. parthenoxylon (Jack) Meisn.	X	X	X	X	X	5
Fokienia hodginsii (Dunn) A. Henry & Thomas	X	X	X	X	X	5
Syzygium wightianum Wight et Arn.	X	X	X	X	X	4
Carallia brachiata (Lour.) Merr.	X	X	X		X	4
Choerospondias axillaris (Roxb.) Burtt et Hill.	X	X	X		X	4
Hopea odorata Roxb.	X	X	X		X	4
Alangium spp.	X	X	X		X	4
Manglietia spp.	X	X	X		X	4

Notes: X: associated; NW: Northwest; NE: Northeast; NC: North Central; SC: South Central, CH: Central Highlands.

### 3.1.2.3. Vertical structure of C. parthenoxylon habitats

The dominant vegetation is secondary forest, where upper-canopy trees occur at low density and belong mainly to the families Fagaceae, Magnoliaceae, Theaceae, Mimosaceae, and Fabaceae. In the Central Highlands, the mean diameter of the forest reaches up to 36.61 cm (Table 3.3).

**Table 3.3**. Basic characteristics of *C. parthenoxylon* and associated tree species in its forest habitat

		(tre	N ee/ha)	G (	m²/ha)	D 1.3(cm)			
Region Population		C. parthenoxylon	In the study area	C. parthenoxylon	In the study area	C. parthenoxylon	In the study area		
	PT	22	531	0,93	25,69	22,07±6,79	23,54±7,88		
Northwest	VP	23	573	1,51	35,51	28,29±6,71	26,89±8,13		
	QN	19	476	0,78	23,02	21,98±6,60	23,54±7,84		
Northeast	HB	20	548	1,03	30,64	24,79±6,31	25,6±7,48		
North Central	ТН	19	518	1,67	27,61	27,85±18,23	25,91±9,63		
South	PY	42	594	1,09	25,65	18,2±6,05	24,25±7,12		
Central	QNm	40	578	0,97	23,90	17,6±5,59	23,74±6,15		
Central	LĐ	52	605	3,39	35,74	28,9±6,45	30,97±7,55		
Highlands	GL	53	715	5,34	36,05	35,70±6,94	36,61±8,94		

# 3.1.3. Natural regeneration characteristics of *C. parthenoxylon* and forest stands where the species occurs

A total of 373 regenerating individuals were recorded. Seed regeneration capacity is relatively strong, with Quang Nam showing higher regeneration compared to other regions (Table 3.4).

# 3.2. SPECIES DISTRIBUTION MODELS of C. parthenoxylon FOR THE PAST, PRESENT, AND FUTURE PERIODS UNDER CLIMATE SCENARIOS

### 3.2.1. Algorithms and environmental factors for model optimization

### 3.2.1.1. Environmental factors for model

Four groups of environmental and climatic parameters (F1–F4) were incorporated into the model (F1: 19 bioclimatic variables; F2: soil type and silt content; F3: vegetation; F4: elevation, slope, and aspect) (Table 3.5).

Table 3.4. Results of regenerating C. parthenoxylon

Region	Population	Number of OTCs	Regenerating trees		generatio ality (%)			eration n (%)	Proportion (%)		
Region	Pop	Pop	Pop	Numbe	Total	Great	Medium	Not good	Seed	Shoot	Pro
	QN	10	55	74,17	25,83	0	95,42	4,58	80,07		
Northwest	VP	14	47	29,74	70,26	0	96,54	3,46	9,23		
	PT	13	32	60,15	39,85	0	94,55	5,45	52,58		
Northeast	HB	12	56	22,08	77,92	0	79,31	20,69	49,17		
North Central	ТН	15	33	59,24	40,76	0	81,52	18,48	67,73		
South	PY	10	22	62,73	37,27	0	94,1	5,9	40,91		
Central	QNm	10	23	22,81	77,19	0	100	0	26,09		
Central	LĐ	12	41	73,17	26,83	0	90,9	9,1	65,85		
Highlands GL		15	64	81,88	18,12	0	96,2	3,8	65,63		
Sum		111	373								

### 3.2.1.2. Algorithm prioritization

Eleven models were implemented using five machine learning algorithms. The Random Forest algorithm achieved the highest AUC (0.92).

### 3.2.2. Suitable habitats of C. parthenoxylon in the present period

The results indicated that *C. parthenoxylon* naturally occurs mainly in the forest ecological regions: Northeast (Zone II), Northwest (Zone III), North Central (Zone IV), South Central (Zone V), and the Central Highlands (Zone VI).

**Table 3.5.** The models were tested using different algorithms and sets of environmental parameters

Model	Algorithms	Algorithms Parameters		AUC
Model-1	Random Forest	F1+F2+F3+F4	Presence-Absence	0,89
Model-2	Random Forest	F1+F2	Presence-Absence	0,86
Model-3	Random Forest	F1	Presence-Absence	0,92
Model-4	Boosting	F1	Presence-Absence	0,86
Model-5	CART	F1	Presence-Absence	0,82
Model-6	SVM	F1	Presence-Absence	0,68
Model-7	Maxent	F1	Presence-Absence	0,64
Model-8	Maxent	F1	Presence	0,74
Model-9	Maxent	F1 + F2	Presence-Absence	0,66
Model-10	Maxent	F1+F2+F3+F4	Presence-Absence	0,63
Model-11	Maxent	F1	Presence Only	0,74

### 3.2.3. Suitable habitats of *C. parthenoxylon* under different climate scenarios

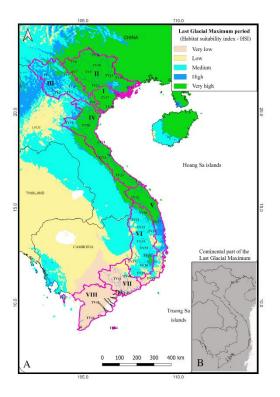
During the Last Glacial Maximum (LGM), the species' distribution was primarily concentrated in Northern (Fig. 3.2). In the present period, suitable ecological areas also appeared in the Central Highlands, which are predicted to have emerged during the transition from LGM to Mid-Holocene (MH) (Fig. 3.3-3.5). Climate change scenarios indicate a clear trend of decreasing distribution area with increasing emission levels (Fig. 3.4 and 3.6).

# 3.3. GENETIC DIVERSITY AND POPULATION STRUCTURE OF *C. parthenoxylon* IN FIVE FOREST ECOLOGICAL REGIONS

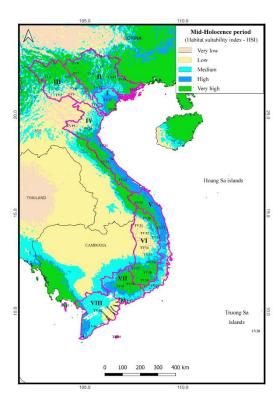
### 3.3.1. Development of microsatellite (SSR) markers for C. parthenoxylon

### 3.3.1.1. Characteristics of the transcriptome of C. parthenoxylon

The assembly results indicated that most unigenes and transcripts were concentrated in the 200–400 bp range, reflecting the fragmentation level of the assembled data. The N50 of the transcripts (1,682 bp) was substantially higher than that of unigenes and contigs, suggesting a higher quality of transcript assembly.



**Fig. 3.2**. Habitat suitability map of *C. parthenoxylon* during the Last Glacial Maximum (LGM)



**Fig. 3.3**. Habitat suitability map of *C. parthenoxylon* during the Mid-Holocence (MH)

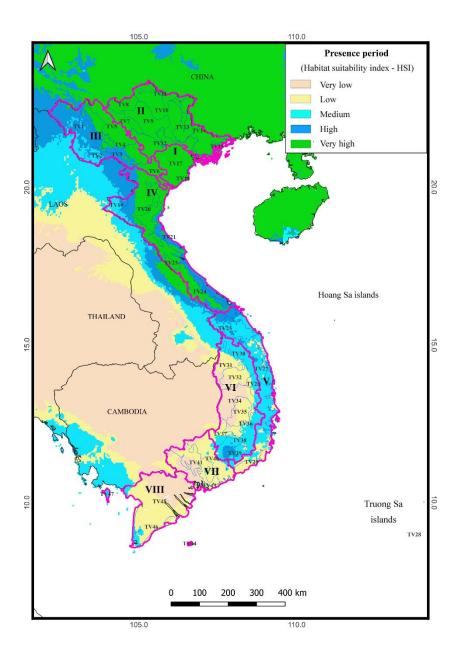
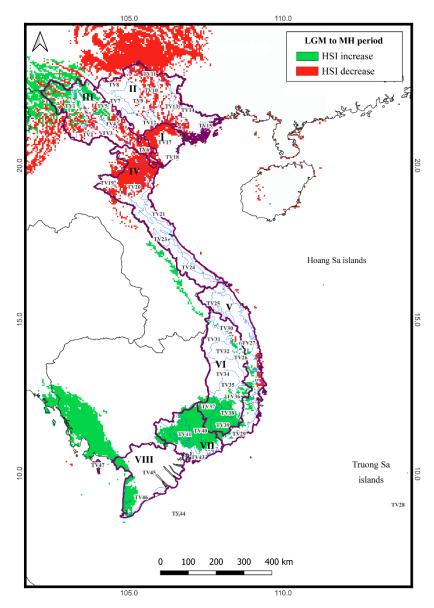
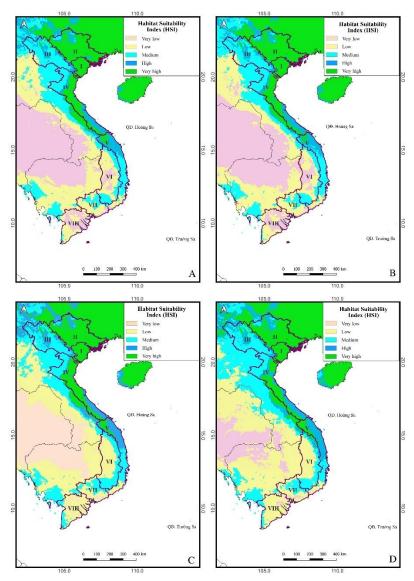


Fig. 3.4. Habitat suitability map of C. parthenoxylon in presence



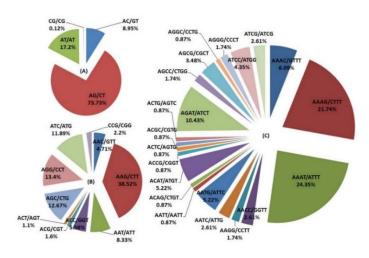
**Fig. 3.5.** Changes in suitable ecological areas for *C. parthenoxylon* from the Last Glacial Maximum (LGM) to the Mid-Holocene (MH)



**Fig. 3.6.** Predicted ecological suitability maps of C. parthenoxylon under medium emission scenarios (ACCESS and EC). (**A**: ACCESS 245 scenario, 2061–2080; **B**: ACCESS 245 scenario, 2081–2100; **C**: EC 245 scenario, 2061–2080; **D**: EC 245 scenario, 2081–2100).

### 3.3.1.2 Microsatellite (SSR) characteristics of C. parthenoxylon

Using SSRIT, 12,849 SSR markers were identified from 18,246 unigenes. Compound SSRs accounted for 816 loci; 15 types of pentanucleotide repeats and 22 types of hexanucleotide repeats were detected (Fig. 3.7). From the unigene sequences, 134 optimal SSR-containing sequences were selected and registered in GenBank (OR536813 to OR536946).



**Fig. 3.7.** Distribution of microsatellite repeat types in the genome of *C. parthenoxylon* 

# **3.3.1.3 Polymorphic SSR marker characteristics for** *C. parthenoxylon* Fifteen SSR primer pairs, out of a total of 134, exhibited clear polymorphism (Table 3.4).

# 3.3.2. Genetic diversity and population structure of *C. parthenoxylon* in five forest ecological regions

### 3.3.2.1. Genetic diversity of C. parthenoxylon

Populations of *C. parthenoxylon* in the Northeast, Northwest, and North Central regions showed signs of inbreeding. In contrast, populations in the South Central and Central Highlands regions had Fis values <0, indicating heterozygote excess and high levels of outcrossing in these populations (Table 3.5).

Table 3.4. Nucleotide sequences of 15 polymorphic SSR primer pairs for C. parthenoxylon

Name of SSR	Nucleotide sequences (5'-3')	Repeat nucleotide	Size (bp)	Ta (°C)	GenBank accession number
PMP01	F: ACAGAAGTGAACATTCCCGC R: TGCTGAAATGGGTGCTTGTA	(TCACGA)6	120-150	55	OR536945
PMP02	F: CATCCACGTCAACTCCATTG R: CATAGCAAGCCTTCCGAGTC	(AGG)7	105-133	55	OR536868
PMP03	F: CGCGAGTCTGGGTAATAAGC R: GAGAAAATGGTGCAGGCAAT	(CTT)8	128-232	55	OR536874
PMP04	F: CAAAGTTACGGGCATGCTTT R: AGTCTCCAAACTCAAGGCCA	(CAG)7	245-259	55	OR536886
PMP05	F: TGCTGTGGCTGATACGAGTC R: AGCACACGAACACACAGC	(ATCCG)5	230-260	55	OR536940
PMP06	F: GCAGCCAGAAGTGGAACATT R: ATTTCCCAATTCCCACACAA	(GTC)7	147-175	55	OR536854
PMP07	F: ATTGTGGGATTGCCTACTGC R: TCCATCTCTGCCTTTCAAGC	(AT)11	198-220	55	OR536843
PMP08	F: AACAGAATCCAGCCATGGAG R: TGTTGGACTTGGGGGAGATA	(GGA)7	105-133	55	OR536901
PMP09	F: TGCAACCACCACAACTTTCT R: ACTGCCCAATCAAGCACTCT	(ATA)7	126-154	55	OR536877
PMP10	F: AAGGGAGTGGAGACAAGGGT R: CAATAGAACTGCACTCGGCA	(AGT)8	192-208	55	OR536855
PMP11	F: AAACTCACAAGCATCATCA R: AGAGCTGGATTTGGGGTCTT	(ACCAC)9	207-234	55	OR536941
PMP12	F: TTGGATTGGAAGTCGGAGAC R: TACGACCTCAACATGTCCCA	(GTAT)6	240-258	55	OR536945
PMP13	F: AAAGTCCAGCACAAACACCC R: GGGTGCAGGAATAAGTTCCA	(ACT)7	112-133	55	OR536933
PMP14	F: ATCGAAATGGCGTATCGAAG R: GAGAGAGGAATCGCGATGAG	(TCC)7	168-182	55	OR536882
PMP15	F: TGAGTGCACACCATTCTCTTCT R: AACGTTTATTGGCTGTTGGC	(CTAGCC)5	180-195	55	OR536946

*Notes:* F denotes the forward primer; R denotes the reverse primer

**Table 3.5.** Genetic diversity and bottleneck assessment of 09 populations of *C. parthenoxylon* across 05 Forest Ecological Zones in Vietnam

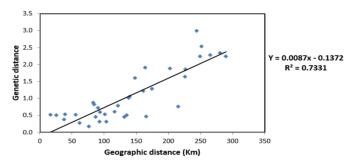
Regions	Population	N	Na	Ne	P%	Ho He H	Fis	F <sub>is</sub> IIM	P value of bottleneck		
										TPM	SMM
Central	GL	40	4,13	2,91	93,33	0,58	0,55	-0,14	0,027	ns	ns
Highlands	LĐ	31	4,40	2,71	100	0,55	0,54	-0,09	0,010	**	ns
South Central	QNm	20	3,80	2,21	100	0,50	0,49	-0,03	0,017	ns	ns
Region	PY	22	2,67	1,85	100	0,68	0,41	-0,64	0,015	ns	ns
North Central Region	ТН	20	3,93	2,40	100	0,52	0,54	0,01	0,018	ns	ns
	VP	19	2,40	1,85	80,00	0,40	0,71	0,71	0,022	*	*
Northwest	PT	19	3,13	2,00	100	0,45	0,69	0,40	0,019	ns	ns
	QN	19	2,73	1,84	86,67	0,39	0,50	0,19	0,018	ns	ns
Northeast	НВ	18	2,40	1,84	86,67	0,39	0,39	0,01	0,038	ns	ns
Mea	n		3,29	2,18	94,07	0,56	0,47	-0,19	0,02	· ·	

**Notes:** Sample size (N); Population (Pop); Number of alleles per locus (Na); Effective number of alleles (Ne); Percentage of polymorphic loci (P%); Observed heterozygosity (Ho); Expected heterozygosity (He); FisIIM: inbreeding coefficient adjusted for null alleles; Infinite Allele Model (IAM); Two-Phase Mutation Model (TPM); not significant (ns); significance levels: \*P<0.05; \*\*P<0.01.

The Two-Phase Mutation Model (TPM) and the Stepwise Mutation Model (SMM) indicated a bottleneck signature in the VP population (p < 0.05).

# 3.3.2.2. Population genetic structure and mantel test

The correlation between genetic distance and geographic distance was r = 0.856, indicating a significant positive correlation (Fig. 3.8). STRUCTURE analysis identified two genetic clusters: Cluster 1 comprising the Central Highlands and South Central regions, and Cluster 2 comprising the North Central, Northwest, and Northeast regions (Fig. 3.9).



**Fig. 3.8.** Mantel test for genetic distance versus geographic distance among nine populations

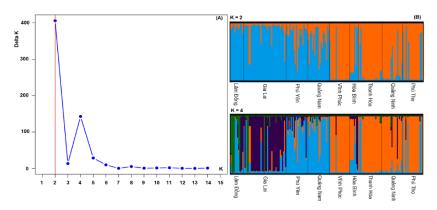
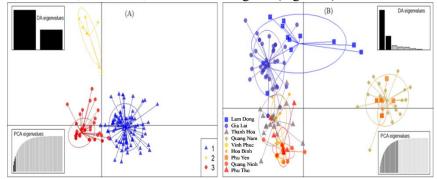


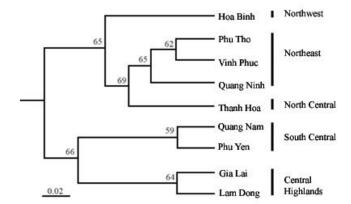
Fig. 3.9. Genetic structure of *C. parthenoxylon* (highest  $\Delta K$  at K = 2) based on Bayesian analysis

### 3.3.2.3. Genetic distances among populations

UPGMA clustering (Fig. 3.10) confirmed the existence of three groups corresponding to three major geographic regions. Group 1 comprised the two Central Highlands populations, an area known for relatively stable ecological conditions and low fragmentation; Group 2 included the two South Central populations; and Group 3 consisted of five populations distributed across the North Central, Northwest, and Northeast regions (Fig. 3.11).



**Fig. 3.10.** Discriminant analysis of principal components (DAPC) of *C. Parthenoxylon;* DAPC scatterplot without prior information (A) and DAPC scatterplot with prior information (B)



**Fig. 3.11.** Genetic relationships among *C. parthenoxylon* populations across five forest ecological zones inferred by UPGMA

# 3.3.3. General remarks on the dynamics of suitable ecological zones and genetic hotspots

The results of SDM and genetic diversity analyses support the formation of distinct genetic clusters in the South Central and Central Highlands.

# 3.4. CONSERVATION STRATEGIES FOR *C. parthenoxylon* IN FOREST ECOLOGICAL ZONES

### 3.4.1. Forest area dynamics

According to annual statistics from the Department of Forestry (2003–2023), Gia Lai and Lam Dong recorded a decline in natural forest area.

# 3.4.2. Drivers of forest area dynamics and genetic resource degradation

The main drivers include: (1) Timber harvesting; (2) Environmental pollution and climate change; (3) Illegal exploitation of forest products; (4) Harvesting for ornamental purposes and essential oil extraction; (5) Road construction; (6) Tourism development; (7) Livestock grazing; (8) Agricultural land expansion; (9) Forest fires.

### 3.4.3. Proposed strategies for conservation, restoration

Conservation strategies must be aligned with the requirements of the 2017 Forestry Law: (1) Establish high-quality seed orchards in areas of high genetic diversity (Central Highlands), while collecting and preserving germplasm from areas of low genetic diversity (HB, VP, PY). To improve genetic quality, selective breeding and propagation of superior trees are proposed, along with maintaining geographic separation to enhance genetic diversity and resilience to climate change; (2) The SSR molecular markers can be used for assessing genetic diversity and verifying the origin of seed sources. The integration of species distribution models provides a spatial framework for identifying priority conservation areas. In the Central Highlands, a combination of flexible in situ conservation and proactive ex situ conservation is recommended. In low-diversity regions (HB, PT, VP, QN, PY), conservation should focus on ex situ approaches. These measures should be coupled with community outreach and education to raise awareness of the ecological role and economic value of the species.

#### CONCLUSIONS AND RECOMMENDATIONS

#### Conclusions

1. Current distribution of C. parthenoxylon:

The species occurs sporadically from northern to Central Highlands provinces, with 357 mature individuals recorded (average height >20 m, DBH >100 cm). It is mainly found in tropical and subtropical evergreen seasonal forests that have been selectively logged.

Neighboring trees were dominated by *Fokienia hodginsii* (Dunn) A. Henry & Thomas, *Syzygium wightianum* Wight et Arn., and *Carallia brachiata* (Lour.) Merr.; the South Central and Central Highlands exhibit higher species diversity.

Natural regeneration of *C. parthenoxylon* was less successful through sprouting than by seed, with seed-derived individuals comprising the majority of regeneration across all study areas.

- 2. Species distribution modeling (historical and future projections): Random Forest (RF) on Google Earth Engine outperformed Maxent, Boosting, SVM, and CART. Populations in the Central Highlands and South Central regions expanded from the LGM to MH. Future projections (2061–2100) predict a decline in suitable habitats, with the Central Highlands and South Central regions most affected.
  - 3. Genetic diversity and population structure:

A total of 12,849 SSR nucleotide sequences were identified, and 134 SSR loci were submitted to GenBank (OR536813–OR536946). Fifteen novel polymorphic SSR markers were developed.

The present study showed that *C. parthenoxylon* maintains a moderate level of genetic diversity and low differentiation among populations. The HB population exhibited the lowest diversity, whereas GL and LĐ populations showed the highest.

Two genetic groups were identified: Group 1 (Northwest, Northeast, North Central) and Group 2 (Central Highlands and South Central).

### 4. Conservation of C. parthenoxylon:

Strengthened protection of forest habitats, especially in the Central Highlands. Seed banks should be established from superior trees in northern populations to ensure long-term conservation and sustainable use.

### Recommendations

- 1. *Conservation priority:* Central Highlands populations should be prioritized for in situ conservation, with nurseries established for seedling production. A combination of dynamic in situ and proactive ex situ strategies is recommended.
- 2. *Modeling approaches:* Use of Google Earth Engine improved efficiency by leveraging global cloud-based GIS datasets.
- 3. *Knowledge natural history:* Population size is shaped by anthropogenic activities, geographic barriers, and biotic interactions.
- 4. Future research: Improve model accuracy and comprehensiveness; conduct genomic studies to explore genetic variation and evolutionary relationships; establish long-term monitoring of population dynamics under climate change.

### NOVEL CONTRIBUTIONS OF THE DISSERTATION

- + This study updated morphological, ecological, distributional, and genetic diversity data for the threatened *C. parthenoxylon* across five forest ecological regions in Vietnam.
- + This study provided the first spatial prioritization for conservation and genetic resource management by integrating species distribution modeling with genetic diversity analysis.
- + The first developed species-specific microsatellite (SSR) markers for assessing population genetic diversity, with applications in breeding programs and genetic resource management of *C. parthenoxylon* in natural forests of Vietnam.

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### LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

### A. INTERNATIONAL JOURNAL ARTICLES

- 1. Pham Mai Phuong, Bui Thi Tuyet Xuan, Vu Dinh Giap, Nguyen Van Sinh, Nguyen Minh Duc, Vu Dinh Duy, 2024, Microsatellite markers reveal genetic diversity and population genetic structure of the threatened Martaban camphor [*Cinnamomum parthenoxylon* (Jack) Meisn], Biologia plantarum, 68, pp. 87-96. (SCIE, IF=1.74, Q2).
- **2.** Pham Mai Phuong, Vu Dinh Duy, Bei Cui, Bui Thi Tuyet Xuan, Vu Dinh Giap, Syed Noor Muhammad Shah, 2024, Characterisation of the *Cinnamomum parthenoxylon* (Jack) Meisn (Lauraceae) transcriptome using Illumina paired-end sequencing and EST-SSR markers development for population genetics, Biodiversity Data Journal, 12, e123405. (SCIE, IF=1.2, Q2).
- **3**. Pham Mai Phuong, Vu Dinh Duy, Nguyen Thanh Tuan, Nguyen Van Sinh, 2024, Predictive ecological niche model for *Cinnamomum parthenoxylon* (Jack) Meisn.(Lauraceae) from last glacial maximum to future in Vietnam, Biodiversity Data Journal, 12, e122325. (SCIE, IF=1.2, Q2).
- **4.** Vu Dinh Duy, Pham Mai Phuong, Dang Ngoc Huyen, Le Xuan Dac, Dang Hung Cuong, Nguyen Huu Thuc, Nguyen Dang Hoi, 2024, Identification and genetic diversity analysis of *Cinnamomum parthenoxylon* (Jack) Meisn species in Song Hinh protection forest, Vietnam based on three chloroplast gene regions, Biomed Biotechnol Resources Journal, 8, pp. 415-21. (ESCI, Scopus, Q3).

### **B. NATIONAL JOURNAL ARTICLES**

- 1. Vu Dinh Duy, Pham Mai Phuong, Bui Thi Tuyet Xuan, Hoang Thi Tham, 2025, Molecular characterization of *Cinnamomum parthenoxylon* (Jack) Meisn. in Northern Vietnam based on ITS-rDNA and ycfl gene sequences, Vietnam Journal of Biotechnology. Vietnam Journal of Biotechnology, 23(3): 361-382.
- **2.** Pham Mai Phuong, Nguyen Thi Thu Nga, Nguyen Quoc Khanh, Nguyen Duy Liem, Vu Dinh Duy, 2025. Cloud-Based Habitat Suitability Mapping for Climate change adaptation of *Cinnamomum parthenoxylon* (Jack) Meisn. in Central Highlands, Vietnam. Journal of Agriculture and Environment, 507, pp. 88–100.