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EFFECTS OF CLIMATE CHANGE ON THE DEVELOPMENT OF *Hopea ferrea* IN TROPICAL FORESTS

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ABSTRACT

Hopea ferrea (Dipterocarpaceae) is a valuable timber tree distributed in the rainforests of Vietnam. This species is threatened due to its overexploitation and destruction of habitats. To determine the effects of global climate change, we analyzed 225 *H. ferrea* individuals from eight natural populations covering the distribution range in Vietnam using eight polymorphic microsatellite markers. We detected low genetic distances for population pairs in the same area (average of 0.045) and high genetic distances for population pairs between different areas (average of 0.213). Similarly, high genetic identity for population pairs in the same areas (average of 0.956) and low genetic identity for population pairs between different areas (average of 0.811). We detected the genetic associations between eight populations using two different methods: a neighbor-joining tree and principal coordinates. The clustering analysis showed that all populations in the same area were clustered together to form a cluster. We detected the two or three genetic groups based on the genetic differentiation between populations. With two clusters, the first cluster included the three populations in the Highlands, and the second was composed of five populations in the two areas of southeast and center. With the three genetic clusters, the first cluster included the three populations in Highlands; the second included three populations in the southeast area;

and the third included two populations in the central area. The approximate Bayesian computation showed that *H. ferrea* populations diverged during the last glacial maximum.

Keywords: Demographic history, genetic relationships, *Hopea ferrea*, microsatellites, tropical forests.

INTRODUCTION

Historical climate change during the glacial periods has profoundly impacted species' distribution range, demographic fluctuations, and genetic structure (Hewitt, 2004; Stewart *et al*., 2010). During these periods, temperatures were reduced, with the climate cooler and drier than present, resulting in lower sea levels and expanded coastal areas. Thus, plants have changed to adapt to the changing climate. In interglacial periods, tropical climate was characterized by increased rainfall and a higher temperature, leading to increased sea levels. Therefore, species often find higher temperatures. Some studies showed that glacials are considered major factors promoting the divergence of plant populations (Soltis *et al*., 2006; Provan and Bennett, 2008). Tropical refugia on population dynamics in the Last Glacial Maximum (LGM) were suggested by Mourguiart and Ledru (2003). Due to widespread aridity during glacials, forests declined into forest patches (Carnaval and Moritz, 2008). Glacial periods affected the genetic structure and have been studied for some species, such as *Shorea parvifolia* in Malaysia, Sumatra, and Borneo (Iwanaga *et al*., 2012), *Shorea leprosula* (Ohtani *et al*., 2013), *Cryptomeria japonica* (Kimura *et al*., 2014), *Pinus koraiensis* in northeast Asia (Bao *et al*., 2015), *S. macrophylla* in the Kalimantan rainforest (Utomo *et al.,* 2018), *Dipterocarpus turbinatus* (Duc *et al*., 2023), and *Anisoptera costata* (Duc *et al.,* 2023) in Vietnam forests. The divergence of populations for these species was also reported in these studies.

The dipterocarp species (Dipterocarpacae) are dominant in tropical forests, with approximately 50 species from six genera in Vietnam (Nguyen Hoang Nghia, 2005). Most dipterocarps are endemic and native. Moreover, the dipterocarp species are of considerable importance as timber trees and play a dominant role in the ecology of tropical rainforests. The dipterocarp wood is utilized for purposes such as houses, boats, and plywood. Dipterocarps also provide other products such as resins for paint, varnish and lacquer, aromatic oils, and illipe nuts. In the past few decades, due to human activities, many dipterocarp trees have been severely threatened. Dipterocarp forests have been heavily degraded or destroyed. Additionally, dipterocarps are also overexploited. These threaten the long-term survival of dipterocarps. Thus, these species are recorded in patches of secondary forests. Fragmented habitats and overexploitation increase the risk of extinction through genetic drift and inbreeding (Falk and Holsinger, 1991; Keller and Waller, 2002). In Vietnam, about 33 dipterocarp species are listed as threatened (BKHCN, 2007). *Hopea ferrea* is the dominant dipterocarp species that forms the canopy in deciduous, evergreen forests (Thai Van Trung, 1978). Further, this species is also the habitat of many animals. Therefore, the influence of climate change during glacial periods has the potential to lead to a divergence in the ecological characteristics of this species.

Hopea ferrea, a valuable woody tree, commonly occurs in the lowland forests of Vietnam (Nghia Hoang Nguyen, 2005; Pham Ngoc Nam, 2010). This species occurs in ecological regions with a mean annual temperature of 25-28ºC, humidity of 78-84%, precipitation of 1400-3024 mm, and a dry season lasting 4-6 months. Due to anthropogenic disturbances such as deforestation and overexploitation, together with the change in climate in the last few decades as major causes to threaten the species, the species is listed as threatened in the IUCN Red List Categories (Ly *et al*., 2017) and the Red Data Book (BKHCN, 2007). However, in recent years, this species has still been threatened due to a lack of effective conservation and restoration policies combined with climate risks. In the present study, we determined the genetic association of recent populations of *H. ferrea* and estimated the divergence of these populations during glacial periods. In addition, we used the results to guide the protection and management of the species.

MATERIALS AND METHODS

Plant materials and DNA extraction

To determine the genetic relationships of current populations of *Hopea ferrea*, we collected from 225 trees in eight natural populations, including three populations, CMR, CP, and YD in the Highlands; two populations, NC and TK in the coastal area; and three populations, MD, TP, and LG-XM in the southeast area (Table 1). Inner bark was sampled from these trees. Genomic DNA was extracted from the samples using a plant genomic DNA kit. The 1.2% agarose gel and NanoDrop^{TM} 2000 were used to test the quality and quantity of genomic DNA.

PCR amplification

We selected SSR markers based on consistency and polymorphism, developing from *Hopea bilitonensis* (Lee *et al*., 2004) (Table 2). Reaction mixtures of 25 volumes included 3 µL of template DNA, 12 µL of 2X Taq Master mix, 1 µL of each SSR primer, and 8 µL of deionized water. PCR (polymerase chain reaction) amplifications were implemented using the GeneAmp^{TM} PCR System 9700 (Applied Biosystems, Foster City, CA, USA) as follows: initial denaturation at 94ºC for 4 minutes; 35 cycles of 1 minute at 94ºC, 30 s at a 55-56ºC annealing temperature for each primer pair, and 1 minute of extension at 72ºC; and the cycle of final extension for 10 minutes at 72ºC. The products were detected by the Sequi-Gen GT DNA system of 7% polyacrylamide gel, visualized by GelRed Nucleic Acid Gel Stain, and sized by the Gel-Analyzer software of GenoSens 1850 with a 25 bp DNA ladder.

Table 1. Information on the collected location of the *Hopea ferrea* populations.

Code	Study sites	Latitudes	Longitudes	Elevation (m)	Habitat	Sample sizes
CMR	Sa Loong, Ngoc Hoi, Kon Tum	14.467	107.6	428	Secondary forest	28
CP	Chu Prong, Gia Lai	13.433	107.733	132	Secondary forest	29

YD	Ban Don, Cu Jut, Dak Lak	13.083	107.6	136	Secondary forest	30
MD	Ma Da, Vinh Cuu, Dong Nai	11.2	107.167	178	Secondary forest	28
TP	Phu Ngoc, Dong Nai	11.35	107.45	112	Secondary forest	28
LG-XM	Tan Bien, Tay Ninh	11.467	105.817	34	Secondary forest	26
TK	Ta Kou, Ham Thuan Nam, Binh Thuan	10.75	107.433	74	Secondary forest	29
NC	Nui Chua, Ninh Hai, Ninh Thuan	11.633	103.117	218-239	Secondary forest	27

Table 2. Nucleotide sequences of SSRs for *H. ferrea* according to Lee *et al*. (2004).

Primer	Primer sequences	Repeat motif	Annealing temperature (°C)
Hbi1	F: 5'-AAGACCATCAGGAGGTATAG-3' R: 5'-ATTAACACTCACAACATTCA-3'	$(CT)_{30}$	51
Hbi2	F: 5'-TTCCATCCATTACCCAAAAG-3' R: 5'-TAGACAAAACATCCCACATC -3'	$(GA)_{17}$	52
Hbi3	F: 5'-GCCGAGAGATTTTTGTTTCC-3' R: 5'-TCCGCACCTACCTCCACC -3'	$(GA)_{11}$	52
Hbi4	F: 5'-GTTAGGATTTTGTGGTTGGT-3' R: 5'-ATTCCAGAGTTGGTAATAGTTG-3'	$(CT)_6T_9$	52
Hbi5	F: 5'-ATGAGTGATGCTGTTGAAGG-3' R: 5'-TCGCTTACACTGTGTTCGTC-3'	$(GGA)_7$	52
Hbi6	F: 5'-CTTTTTACGCTTTCAGTTCC-3' R: 5'-TGTCCTCGCACCCATCA -3'	$(CT)_9$	52
Hbi7	F: 5'-TTCTACTATTGCTTTTACAGGGA-3' R: 5'-TAACTTTAGACGACGCCATT -3'	$(GA)_{10}$	53
Hbi8	F: 5'-CAAAATCACAATCAAATAAAC-3' R: 5'-CGAGTTCCTGGCGGTTC-3'	$(GA)_{17}$	52

Data analyses

We used Poptree2 (Takezaki *et al*., 2010) to determine the genetic association among *H. ferrea* populations based on the matrix of the F_{ST} values. We also used the method of principal coordinates (PCoA) based on the matrix of the G'_{ST} values by GeneALEx (Peakall and Smouse, 2012). To determine genetic distance and genetic identity for population pairs, we performed GeneALEx. To reveal the population history of the species, we used the method of approximate Bayesian computation (ABC) and performed DIYABC (Cornuet *et al*., 2014). Based on genetic groups obtained from the neighbor-joining dendrogram (NJ) and PCoA, we simulated the following four scenarios with $K = 2$ (Figure 1): Population 1 was composed of the three populations of CMR, CP, and YD; population 2 was composed of the remaining five populations. We used 300,000 simulated data sets to identify the best scenario and predict divergence times. The prior values were obtained with a uniform distribution for all parameters. The best scenario was identified by the posterior probabilities based on logistic regression analysis. Divergence times were calculated using the posterior distribution parameters of the best scenario. We also simulated the following seven scenarios with $K = 3$ (Figure 1): Population 1 was composed of the three populations in the Highlands as mentioned above; Population 2 included the two populations of TK and NC in the coastal area; and population 3 was composed of the remaining three populations of TP, MD, and LG-XM in the southeast area.

RESULTS AND DISCUSSION

Genetic relationships among populations

The genetic identities and genetic distances obtained from all pairwise comparisons of populations are presented in Table 3. Mean genetic distance among the studied populations was 0.174, ranging from 0.016 between TK and NC to 0.38 between TP and CP. Low distances were detected between populations in the same area. The distance among populations in the same area averaged 0.045, ranging from 0.016 between TK and NC in the coastal area to 0.073 between populations in the southeast area. High distances were detected between populations in different areas. High distances were found between TP in the southeast area and CP in the Highlands (0.38), and between MD and CMR (0.249). Similarly, the genetic identity among populations was also detected (Table 3). The genetic identity among the studied populations averaged 0.846, ranging from 0.684 between CP and TP to 0.984 between TK and NC. The high genetic identity was detected among populations in the same area, averaging 0.956. The highest value was found between TK and NC (0.984). Low genetic identity was recorded between populations in different areas, with an average of 0.811 (0.684 between CP in the Highlands and TP in the southeast area to 0.952 between LG-XM in the southeast area and TK in the coastal area).

The NJ tree using the genetic differentiation matrix (F_{ST} values) among the studied populations showed different genetic clusters in Figure 2A. Our results indicated two or three genetic clusters were detected. With three clusters, cluster I included three populations: CMR, CP, and YD in the Highlands, while the two populations of CMR and CP were separated into a separate cluster with a bootstrap value of 61%. Cluster II included TK and NC in the coastal

area, with a bootstrap value of 64%. Cluster III included TP, MD, and LG-XM in the southeast area. Similar results also showed three genetic groups, based on the F'_{ST} values (Figure 2B). With two genetic clusters, cluster I included the three populations of CMR, CP, and YD. Cluster II included the remaining five populations.

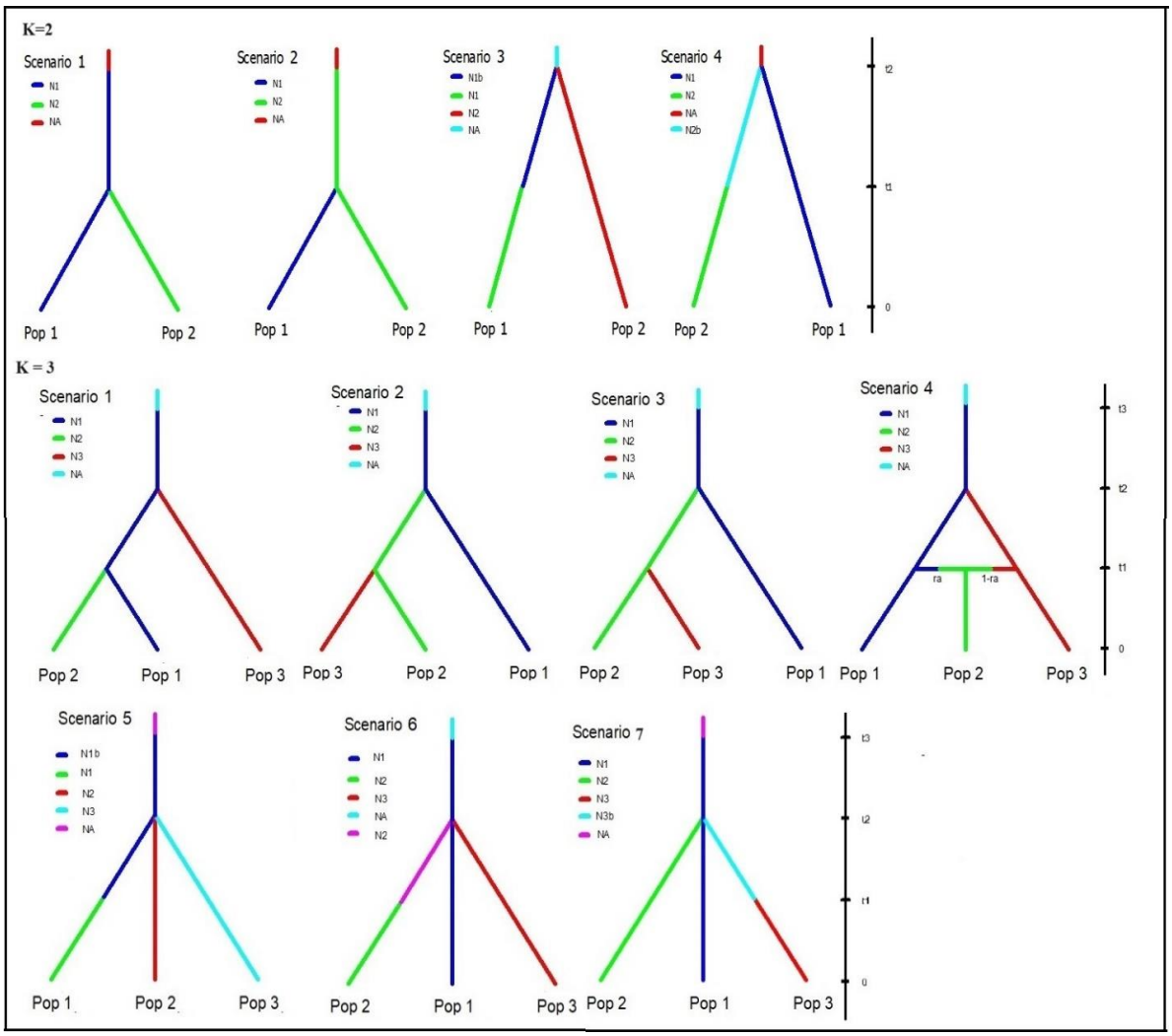

Figure 1. Scenarios of the *H. ferrea* demography history, *t#*: generations. *N*1-3 and *NA*: effective population size for pop 1, pop 2, pop 3 and the ancestral population, respectively.

Our results were consistent with previous reports (Tam *et al*., 2021; Thanh *et al*., 2022; Duc *et al*., 2022 and 2023; Vu *et al*., 2023). Based on the genetic differentiation among *A. costata* populations, Tam *et al*. (2021) showed two genetic groups, one including TK and NC in the coastal area and the other group including seven populations in the southeast area. Similarly, Duc *et al*. (2022) analyzed the eight *Dipterocarpus condorensis* populations using both Bayesian analysis and discriminant analysis of principal components and determined three genetic clusters. Thanh *et al*. (2022) investigated the eight *Amentotaxus argotaenia* conifer populations in Vietnam

using the neighbor-joining tree and detected three genetic groups; one group including three populations in the northeast area, a second group including three populations in the northwest area, and a third group including two populations in the central area. The neighbor-joining tree, a principal coordinate analysis, and the admixture model-based method for seven *Panax vietnamensis* populations showed distinct genetic groups. Genetic group I included four Quang Nam populations, and group II included three Kon Tum populations (Vu *et al*., 2024).

Table 3. Nei's (1972) genetic identity (above diagonal) and genetic distance (below diagonal).

	TK	NC	LG-XM	CMR	YD	CP	TP	MD
TK		0.984	0.952	0.814	0.823	0.762	0.886	0.926
NC	0.016		0.931	0.800	0.805	0.747	0.895	0.914
LG-XM	0.049	0.072		0.763	0.778	0.730	0.932	0.939
CMR	0.206	0.223	0.271		0.977	0.955	0.762	0.780
YD	0.195	0.217	0.252	0.023		0.930	0.788	0.789
CP	0.272	0.291	0.315	0.046	0.072		0.684	0.709
TP	0.121	0.111	0.071	0.272	0.238	0.380		0.919
MD	0.077	0.090	0.063	0.249	0.237	0.344	0.085	

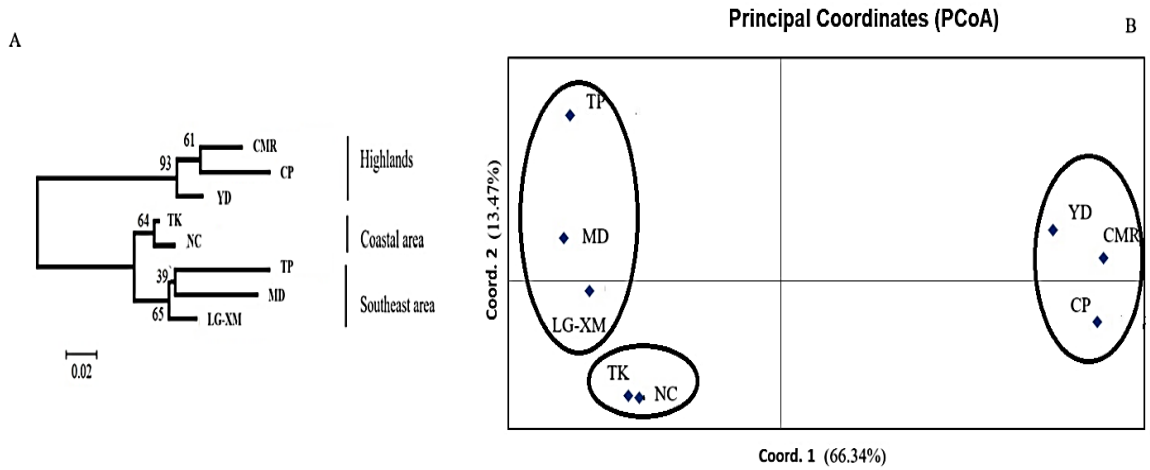

Figure 2. Relationships among the eight *H. ferrea* populations using the Neighbor-joining tree (A), and the PCoA method (B).

Population divergence history

Firstly, we simulated the *Hopea ferrea* demographic history based on two genetic groups (K = 2) as implemented by the neighbor-joining analysis. Table 4 showed that scenario 1 had the highest posterior probability (p = 0.48, Cis = 0.445-0.516). In this scenario, population 2, including two populations of TK and NC in the coastal area and three populations of TP, MD, and LG-XM in the southeast area, diverged from population 1 (three populations of CMR, CP, and YD) in the Highlands.

Table 4. Posterior probabilities of the *H. ferrea* scenarios.

K = *2*

Scenario	1	2	3	4
Posterior probabilities	0.480	0.472	0.019	0.028
Confidence intervals (CIs)	0.445 - 0.516	0.438 - 0.507	0.000 - 0.051	0.000-0.060

K = 3

Scenario	1	2	3	4	5	6	7
Posterior probabilities	0.141	0.051	0.060	0.121	0.189	0.216	0.223
Confidence intervals (CIs)	0.105 - 0.176	0.028 - 0.074	0.038 - 0.082	0.098 - 0.143	0.189 - 0.215	0.186 - 0.246	0.193 - 0.253

The posterior distribution of effective population size was detected per population, population 1 and population 2, with median values of 1880 and 2430, respectively. The ancestral population size (*NA*) averaged 5870. The *t*1 divergence and time of population size change *t*2 averaged 366 and 5050 generations ago, respectively. Assuming that the generation time was 35 years (observed data), the divergence of two populations might have occurred 12,810 years ago, and the ancestral population size change might have occurred 176,750 years ago.

Three genetic groups (K = 3) showed that scenario 7 (Table 4) was the likeliest, with the highest probability (p = 0.223, CIs = 0.193-0.253). In scenario 7, two populations, population 2 (the coastal populations) and population 3 (the southeast populations), diverged simultaneously from population 1 (the Highlands populations) at time *t*2. The effective population sizes for population 1, population 2, and population 3 had median values of 3600, 3660, and 3520, respectively. The *NA* value was 5870. The *t*1, *t*2, and *t*3 values diverged 339, 606, and 6030 generations ago. Thus, the divergence of three populations occurred 21,210 years ago, and the *NA* value change occurred 211,050 years ago. Thus, the divergence time for two simulations of *H. ferrea* occurred during the Pleistocene ice age. The difference in divergence time between scenario 1 in K = 2 and scenario 7 in K = 3 can be related to different scenarios and insufficient data to accurately estimate the divergence time of this species. However, previous studies also showed *Shorea macrophylla* populations diverged between 118,800 and 280,800 years ago (Utomo *et al*., 2018) and *D. turbinatus* populations diverged from 12,215-225,400 years ago (Duc *et al*., 2023). During the Pleistocene, the global climate changed through multiple main temperature and precipitation fluctuations. A large area of Vietnam was covered by savannah in glaciations due to declines in precipitation and sea level. Therefore, *H. ferrea* has a

limited distribution in tropical forest refugia in the Highlands. The species depends on high precipitation and proximity to rivers and streams. The distribution range of the rainforests was extended. The warming and moistening climate in the Last Glacial Maximum led to extending the forest distribution (Plenley, 1998).

Table 4. Demographic parameters of *Hopea ferrea*.

K = 2

Parameter	Mean	Median	Mode	Quantile 2.5%	Quantile 5%	Quantile 95%	Quantile 97.5%
*N*1	2380	1880	1030	337	449	6280	7620
*N*2	2910	2430	1450	408	565	7220	8330
*t*1	550	366	146	42.2	63.5	1650	2230
NA	5600	5870	8920	393	779	9650	9820
*t*2	5190	5050	3340	940	1280	9430	9690
μmic	1.94×10^{-4}	1.51×10^{-4}	1.00×10^{-4}	1.01×10^{-4}	1.04×10^{-4}	4.58×10^{-4}	5.74×10^{-4}
pmic	0.175	0.166	0.1	0.102	0.105	0.273	0.283
snimic	6.39×-10^{-8}	1.06×10^{-8}	1.00×10^{-8}	1.04×10^{-8}	1.11×10^{-8}	3.38×10^{-8}	5.1×10^{-8}

K = 3

Parameter	Mean	Median	Mode	Quantile 2.5%	Quantile 5%	Quantile 95%	Quantile 97.5%
*N*1	4010	3600	2910	832	1100	5290	8470
*N*2	4000	3660	2950	967	1250	5200	8120
*N*3	3980	3520	2650	748	1000	5380	8580
*t*1	565	339	19,8	14.4	27.3	713	1840
NA	5240	5330	7300	325	679	7560	9480
*t*2	1020	606	281	102	137	1150	3510
*t*3	5690	6030	9450	411	836	8130	9630
μmic	1.33×10^{-4}	1.08×10^{-4}	1.00×10^{-4}	1.00×10^{-4}	1.00×10^{-4}	1.4×10^{-4}	2.15×10^{-4}
pmic	0.172	0.164	0.1	0.102	0.105	0.209	0.27
snimic	1.92×-10^{-7}	3.22×10^{-8}	1.00×10^{-8}	1.00×10^{-8}	1.00×10^{-8}	1.3×10^{-7}	8.07×10^{-7}

N1, N2, N3 and NA: effective population sizes for populations 1, 2, 3 and the ancestral population; μmic: the mean mutation rate of SSR; pmic: the mean distribution of the number of repeats of microsatellites; snimic: the mean rate of single nucleotide insertions/deletions.

CONCLUSION

To determine the genetic relationships of study populations of *Hopea ferrea* and their history has importance under its genetic improvement and conservation. We sampled eight populations covering the natural distribution of *H. ferrea*, and assessed genetic relationships and demographic

history in tropical forests in Vietnam. In this study, we determined two or three genetic clusters using the neighbor-joining tree based on the genetic differentiation matrix between the populations. We also simulated the five different scenarios with two genetic clusters and the seven scenarios with three genetic clusters and suggested that scenario 1 with $K = 2$ and scenario 7 with $K = 3$ were the likeliest. Our results simulated that the divergence time for *H. ferrea* occurred during the last glacial maximum. The results should also improve understanding of population genetics and the evolution of *H. ferrea*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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