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# THE GENETICS STRUCTURE OF THREE THREATENED *HOPEA* SPECIES (DIPTEROCARPACEAE) IN THE PROTECTED AREAS OF VIETNAM

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## *ABSTRACT*

*A total of 237 samples from ten populations of three threatened species (Hopea chinensis (Merr.) Hand.-Mazz. (native to coastal islands in a Quang Ninh province), H. odorata Roxb. (in lowland forests) and H. hainanensis Merr. et Chun. (in only two provinces (Ninh Binh and Thanh Hoa)) was studied the genetics structure based on ten SSR primers. The results showed that inbreeding was only significant in an island population of H. chinensis, a bottleneck event could be detected in H. odorata and H. hainanensis populations. Allele frequency and genetic diversities were lowest for H. hainanensis. Population inbreeding was only significant in an island population of H. chinensis whereas indications of a bottleneck event could be detected in populations of H. odorata and H. hainanensis. Bayesian analysis and $F_{ST}$ values suggested high genetic divergence between populations in H. hainanensis ($F_{ST}$ = 0.230) and H. odorata ($F_{ST}$ = 0.251) even at about one hundred km distance. This study highlights the importance of conserving the genetic resources of Hopea species in different protected areas and at short geographic distance. It is proposed to search in more detail for potential inbreeding effects of the endangered H. chinensis and for bottleneck events in natural and planted stands of the other species.*

***KEYWORDS:*** *Dipterocarp, Hopea, Tropical Tree, Inbreeding, Bottleneck, Microsatellites, Vietnam*

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## INTRODUCTION

The family of Dipterocarpaceae has been known widely distributed in tropical regions, with about 17 genera and 550 species. Shorea is the largest genera in Dipterocarpaceae (about 250 species), followed by *Hopea* (105 species), *Dipterocarpus* (70 species) and *Vatica* (65 species) (Nghia 2005). Many species of the family Dipterocarpaceae have predominated in the international tropical timber market, and play an important role in the economy of many Southeast Asian countries (Aguda 2002). The dipterocarps also constitute important timber for domestic needs in the seasonal evergreen forests of Asia. In addition, these forests are sources of a variety of non-timber forest products, which many forest dwellers directly depend for their survival (Banin *et al.* 2014).

Vietnam is also known to harbor rich diversity of dipterocarp species. It has about 40 species from six genera (*Anisoptera, Hopea, Shorea, Parashorea, Vatica, Dipterocarpus*), all native and endemic to defined locality (Ashton 2004). Most dipterocarps are widely distributed in Vietnam, and many are threatened due to deforestation, changes in land

use systems and exploitation for timber (Millet and Truong 2011). Of six genera, *Hopea* is recorded with more than 100 species, but mostly listed in the Critically Endangered category by the International Union for Conservation of Nature (IUCN 2014). Three rare species, *Hopea chinensis* (Merr.) Hand-Mazz.), *H. odorata* Roxb. and *H. hainanensis* Merr. et Chun., are native to Southeast Asia. *H. odorata* has a scattered distribution in Vietnam, Laos, Cambodia, Myanmar, India, Thailand, Malaysia whereas *H. hainanensis* is restricted to coastal island in Northeast Vietnam and Hainam (China) and *H. chinensis* restricted to Quang Ninh (Vietnam) and Guangxi (China) (Nghia 2005). Like other dipterocarps, three studied species have been important timbers playing a dominant role in the ecology and economics in Vietnam.

In support of forest conservation, many governments have taken measures to protect these timber trees through *in situ* (in natural stands, national parks or protected landscapes) and *ex situ* (in botanical gardens, old reforestation projects, plantations and hedge gardens) conservation strategies (Fernando 2001; Pollisco 2009). Because of exploitation of *Hopea* species for their valuable timber and resin by local people and forestry enterprises, their habitats are heavily affected by deforestation. Logging resulted intensively fragmented habitats, especially in some localities with low-density populations. In such locality, the distance between individuals is more likely to be wider because of logging, it is may threat long-term survival of the genetic resource of these three species. Loss of genetic diversity has been recognized as a potentially serious problem in commercially managed forest tree species (Lee *et al.* 2002; Heywood & Iriondo 2003; Finkeldey *et al.* 2006). All *Hopea* species are bisexual and insect-pollinated most likely giving local pollination and dispersal within each protected area (Appanah & Turnbull 1998). Conservation and management requires information on the ecological and genetic diversity within and among populations. Such assessment has a high priority in developing successful management guidelines and effective diversity may become one of the most important issues influencing future forestry practices.

Microsatellite markers have been used for dipterocarp studies on gene flow, genetic structure and mating systems (Ujino *et al.* 1998; Iwata *et al.* 2000; Takeuchi *et al.* 2004; Pandey & Geburek 2009; Abasolo *et al.* 2009; Chin Hong Ng *et al.* 2013; Jennifer *et al.* 2014). Several species showed an overall high level of gene diversity but low overall differentiation such as *Dryobalanops aromatica* Gaertn. ($G_{ST}$ = 0.067in Lim *et al.* 2001), *Shorea leprosula* Miq. ($G_{ST}$ = 0.117 in Lee *et al.* 2000) and *Shorea lumutensis* Sym. ($G_{ST}$ = 0.048 in Lee *et al.* 2004).

To help conserving three threatened *Hopea* species, our work is designed to investigate the level of genetic variability within and between remnant populations of *Hopea chinensis*, *H. odorata* and *H. hainanensis* in protected areas of Vietnam, and to test for potential distance-related effects, local inbreeding and potential bottleneck events. Our work provides scientific basis to improve and give guidelines for a sustainable management and better conservation of these species in fragmented and isolated areas.

## Materials and Methods

### Material Sampling

Field sampling was carried out in nine sites, two for *Hopea hongayensis* (Ba Mun and Cai Lim islands (Bai Tu Long National Park, Quang Ninh province)), three for *Hopea odorata* (Bu Gia Map National Park (Binh Phuoc province), Tan Phu secondary forests (Dong Nai province), and Ben En National Park (Thanh Hoa province)), and lastly four for *H. hainanensis* (Xuan Hoa and Xuan Thai at Ben En National Park (Thanh Hoa province) and Xom Bong and Xom Dang at Cuc Phuong National Park (Ninh Binh province)) (Figure. 1 and table 1). In natural forests, the number of *Hopea hainanensis* and *H. odorata* trees were very small. Thus, all *Hopea hainanensis* and *Hopea odorata* trees that could be detected and reached were collected.

In total, 237 samples of 237 trees from ten populations belonging to three target species were collected. The samples were immediately placed into paper envelopes and plastic bags with silica gel, then transferred to Laboratory of Molecular Systematics and Conservation Genetics, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology and stored at - 86$^{o}$C until DNA extraction. The samples were identified on the basis of previous taxonomic treatments of collected specimens from these populations and verified with *RBC*L sequences. Voucher specimens were housed at the Department of Botany, Institute of Ecology and Biological Resources.

## DNA Extraction and SSR Amplification

Total DNA was extracted using the modified CTAB method proposed by Doyle and Doyle (1987). Liquid nitrogen was added to about 100 mg of each sample, which was then ground by hand. Total DNA yield and purity were assessed by spectrophotometer and visualization on 1% agarose gel. Stock DNA was diluted to a concentration of 10 ng/µl.

Fifteen SSR primers developed for related species *Shorea curtisii* Dyer ex King (Ujino *et al.* 1998) and *Neobalanocarpus heimii* (King) Ashton (Iwata *et al.* 2000) were initially tested for cross amplification in twelve samples per species. Based on their amplification, ten primers gave polymorphic PCR products were selected and used for analysis (table 2). PCR was performed in a 25 µl reaction mixture containing 5 µl of total DNA (equivalent 50ng of DNA), 2,5 µl of 10x PCR buffer, 200 nM of each primer, 1U of taq DNA polymerase (Omega), 2.5 mM $MgCl_2$ and 0,2 mM of each dNTP. PCR reactions were performed in a thermal cycler (Bio-Rad Mycycler) using the following conditions: 1 cycle at 95$^{o}$C for 5 mins, followed by 35 cycles at 95$^{o}$C for 1 min, 45$^{o}$C for 1 min, 72$^{o}$C for 1 min and a final extension at 72$^{o}$C for 5 mins. PCR products were separated by capillary electrophoresis on a Qiaxcel system (Qiagen).

## *Data Analysis*

A suite of genetic parameters was calculated using GenAlex (Peakall & Smouse 2006) and FSTAT (Goudet 2001), including the mean number of alleles (A), allelic richness (Ar) per population, observed ($H_o$) and expected ($H_e$) heterozygosity, the coefficient of excesses of homozygotes or heterozygotes compared with panmictic expectations within populations ($F_{IS}$, 1000 permutations) and the genetic differentiation ($F_{ST,}$ 1000 permutations) between populations. F-statistics were determined after Weir & Cockerham (1984) as used in FSTAT software with Jackknifing procedure applied over loci in deriving significance level (Goudet 2001). These parameters of population structure are defined as the correlations between pairs of genes within individual (Cap*F*), between individuals in the same population ($\theta$, theta) and within individuals within population (small*f*) and are analogous to Wright's $F_{IT}$, $F_{ST}$ and $F_{IS}$, respectively. Each locus was checked for evidence of null alleles which are commonly found when microsatellite loci are cross-amplified among far-related species or genera; scoring errors and allele drop out using Micro-checker (Van Oosterhout *et al.* 2004). We tested for recent bottlenecks in each population under the two-phase model (TPM) with 70% single-step mutations and 30% multiple-step mutations using BOTTLENECK 1.2.02 (Pyri *et al.* 1999). To determine the population "genetic reduction signatures", characteristics of recent reductions in effective population size, the Wilcoxon's heterozygosity excess test (Piry *et al.* 1999), standard differential test, sign test and the allele frequency distribution mode shift analysis (Luikart *et al.* 1998) were performed. Exact tests of deviation from the Hardy-Weinberg equilibrium for all loci and among populations were performed at the significance level ($p < 0.05$). Significance testing for variance components in the analysis of molecular variance (AMOVA) was implemented on basis of 1000 permutations. A Neighbor Joining Tree for populations of each species was generated to determine the genetic association among populations by using 1000 permutations in Pop tree 2 (Takezaki 2010). Pairwise $F_{ST}$-values based on $\theta$ were calculated between all pairs of populations and tested for

significant differentiation using 999 permutations. Isolation-by-distance between pairs of populations and their geographical distances was tested with $\theta$ /(1-$\theta$) considering straight flight distances, log transformed (Rousset 1997) between populations in a Mantel test using 1000 randomizations (Mantel 1967). A Bayesian clustering method was carried out using STRUCTURE version 2.3.4 (Pritchard *et al.* 2000). We tested K in ten independent runs from 1 to 5 (10,000 burn-in and 50,000 Markov chain Monte Carlo replicates in each run), without using sampling location as a prior to assess convergence of ln (PD). Runs were carried out assuming admixture and an independent model of allele frequencies. The results were uploaded into Structure Harvester (Earl & vonHoldt 2012), which estimates the most likely K value. The number of clusters was determined from the K with the highest posterior probability and using the second-order rate of change of the likelihood function ΔK, as suggested by Evanno *et al.* (2005).

The samples of three *Hopea* species were verified based on the *rbc*L sequence. All sequences were submitted to the GenBankGenbank with accession numbers, KM267144, KM276147, KM267146 for *H. odorata*, *H. hainanensis* and *H. chinensis*, respectively.

## RESULTS

### Genetic Variation

Fifteen SSR primers of the related species *Shorea curtisii* Dyer ex King (Ujino *et al.* 1998) and *Neobalanocarpus heimii* (King) Ashton (Iwata *et al.* 2000) were initially tested for cross amplification in twelve samples per species. Then, nine SSR loci have polymorphisms with *H. odorata* and *H. hainanensis* and ten SSR loci have polymorphisms with *H. chinensis* were chosen. Totally, ten SSR loci produced 31 alleles in *Hopea chinensis*, whereas only nine SSR loci could be considered (excluding Shc11 in *H. odorata* and Nhe11 in *H. hainanensis*) giving 41 alleles in *H. odorata* and 19 alleles in *H. hainanensis*. The proportion of polymorphic loci was high in all populations for three studied species and averaged 90% in *H. chinensis*, 80% in *H. hainanensis* and 77% in *H. odorata*. Allelic richness ranged from 3.0–3.1 and 2.4–3.2 in *H. chinensis* and *H. odorata*, respectively, whereas a much lower value (1.8–1.9) was obtained for *H. hainanensis*. Similarly, the frequency of observed heterozygotes ($H_o$) ranged from 0.382–0.459 and 0.330–0.472 in *H. chinensis* and *H. odorata*, respectively, whereas a much lower value (0.270–0.390) was observed for *H. hainanensis* (table 3). The mean expected heterozygosity ($H_e$) was higher than the observed one in *H. chinensis* whereas the mean He was comparable for populations of *H. odorata* and *H. hainanensis* (table 3).

Three populations including BM for *H. hongayensis*, TP for *H. odorata* and XT for *H. hainanensis* showed positive inbreeding values with $F_{IS}$ value were 0.143, 0.152 and 0.130, respectively but only the BM population of *H. hongayensis* ($F_{IS}$ = 0.143) significant (table 3).

Micro-checker results indicated that there were no scoring errors associated with null alleles, stuttering bands or large allele dropout in all ten loci screened of *H. odorata*. However, null alleles might be present at locus Nhe11 in *H. hongayensis* and at locus Shc 3 in *H. hainanensis*. These loci were then verified for presence of various heterozygotes and for their inbreeding coefficient. In both loci, heterozygotes were present, but observed heterozygotes were always lower than expected ones giving more evidence for inbreeding events in these populations instead of null alleles.

The population of *H. odorata* in TP and *H. hainanensis* in XT showed an excess of homozygotes with $F_{IS}$ values of 0.152 and 0.130, respectively. The populations of BE (*H. odorata*) and XB (*H. hainanensis*) showed an excess of heterozygotes with $F_{IS}$ value were -0.198 and -0.154, respectively. For both populations, this coincided with an evidence for bottleneck events.

### Genetic Structure

An AMOVA revealed that most of the variation remains within the individual (73-88%) in all three species (table 5). The genetic differentiation ($F_{ST}$) was 0.251 and 0.230 for *H. odorata* and *H. hainanensis*, respectively, where as only 0.036 for *H. chinensis* (table 4). The total fixation for each species was lowest in *H. chinensis* ($F_{IT}$ = 0.115) when compared to *H. hainanensis* ($F_{IT}$ = 0.254) and *H. odorata* ($F_{IT}$ = 0.268). The overall estimation of Cap*F*, theta and Small*f* gave similar levels as for AMOVA- $F_{ST}$ (table 4). A gene diversity analysis at species level showed a moderate differentiation for *H. odorata* ($G_{ST}$ = 0.193) and *H. hainanensis* ($G_{ST}$ = 0.177) but much lower for *H. chinensis* ($G_{ST}$ = 0.025).

At population level, the largest differentiation (0.31) was found between the populations BE and TP for *H. odorata*, and the lowest (0.09) between the populations CL and CQ for *H. chinensis*. The pairwise $F_{ST}$ values were mostly significant (table 6) and ranged from 0.05 to 0.09 for *H. chinensis,* 0.17 to 0.31 for *H. odorata* and 0.05 to 0.36 for *H. hainanensis*. Non-significantly low differentiation was only observed for two pairs of geographically very close populations CL-CQ (*H. chinensis*) and XH-XT (*H. hainanensis*).

Bayesian assignment of individuals showed that most individuals of *H. odorata* were not mixed. We obtained two genetic clusters for *H. odorata* and *H. hainanensis* with the highest values at K=2. Three geographically close populations of *H. hongayensis* resulted after Bayesian assignment in three genetic clusters (K=3), however their individuals were mixed among these sites (Figure. 2). A Neighbor-Joining tree, based on pairwise $F_{ST}$ value (Figure. 3) clearly showed for all three *Hopea* species, that populations in close vicinity also cluster as a single entity with very high bootstrap support (> 99%).

A Mantel test within each species gave no significant IBD although a positive trend was obtained for *H. odorata* and *H. hainanensis*.

## DISCUSSIONS

Three *Hopea* species prefer a humidity of 75%-85%, precipitation levels of more than 1500 mm and a mean annual temperature of 25-27$^{o}$C. The original primary forest vegetation of all visited sites was greatly affected by human activities. Parts of the native vegetation at Bu Gia Map (Binh Phuoc), Ben En (Thanh Hoa), and Tan Phu (Dong Nai) have been destroyed because of agricultural expansion. This has led to an alteration of the spatial distribution and age class structure of trees in these sites. However, vegetation structures were still characterized by three strata. In Ben En National Park (Thanh Hoa province), the big sized trees of *Hopea hainanensis* were cut down long time ago, leaving now a days only small and medium sized trees (dhb≤15cm). There is a stand of this species located not so far from Chang River station, but this stand was planted for conservation. Seeds from mature trees were grown and when the juveniles have reached about 90-120 cm in height in nursery condition, these were transferred to the forest for further establishment in their natural habitat.

Our results showed that most population of three *Hopea* species had only moderate levels of genetic diversity within populations with a mean $H_e$ = 0.448, = 0.356 and = 0.339 for *H. chinensis, H. odorata* and *H. hainanensis*, respectively. This can be explained from their life strategy because these *Hopea* species are regionally or narrowly distributed, have a long-life span, high fecundity, are predominantly outcrossess, pollinated by insects (Appanah & Chan 1981; Chan 1981) and late successional. Seeds are dispersed over short distances.

Three populations including BM for *H. hongayensis*, TP for *H. odorata* and XT for *H. hainanensis* showed positive inbreeding values but only the BM population of *H. hongayensis* significant. Micro-checker results indicated that

there were no scoring errors associated with null alleles, stuttering bands or large allele dropout in all ten loci screened of *H. odorata*. However, null alleles might be present at locus Nhe11 in *H. hongayensis* and at locus Shc 3 in *H. hainanensis*. These loci were then verified for presence of various heterozygotes and for their inbreeding coefficient. In both loci, heterozygotes were present, but observed heterozygotes were always lower than expected ones giving more evidence for inbreeding events in these populations instead of null alleles. This inbreeding might, thus, be due to the natural conditions of *H. hongayensis* on an isolated coastal islands. Although the area of BM Island is about 2000 hectare, the *H. hongayensis* only concentrates in an area of about 5km.

The population of *H. odorata* in TP and *Hopea hainanensis* in XT showed an excess of homozygotes. This might be because the forest in TP and XT area in Ben En national park are the areas that were over exploited for many years. In these areas, the large *Hopea* trees were not retained; only medium and small sized trees could be found. Thus, the populations of *H. odorata* in TP and *H. hainanensis* in XT that showed a lack of heterozygotes might reflect a truly inbred status of *H. odorata* and *H. hainanensis* populations, despite the small sample and population sizes.

The populations of BE (*H. odorata*) and XB (*H. hainanensis*) showed an excess of heterozygotes. For both populations this coincided with an evidence for bottleneck events. A population bottleneck is an event that drastically reduces the size of a population. The population bottleneck produces a decrease in the gene pool of the population because many alleles, or gene variants, that were present in the original population are lost. When a small population showed an excess of heterozygotes, this might be a result of bottleneck because this population might be a large population in the past and after any event such as an environmental disaster, the logging of a species to the point of extinction, or habitat destruction that results in the drastically reduces the size of a population. The populations of BE (*H. odorata*) and XB (*H. hainanensis*) showed an excess of heterozygotes. This result is reasonable because XB is a plantation for *H. hainanensis* in Cuc Phuong national park. Thus, the gene pool of this population may have from many different sources. Whereas Ben En national park is an area have been destroyed because of agricultural expansion and over exploitation by illegal logging. The *H. odorata* population in BE have received *H. odorata* juveniles that were transferred from a nursery garden in Ben En national park.

Three *Hopea* species showed their lowest $F_{ST}$ at closest geographic distance as could be expected. Populations of the endemic *H. hongayensis* were separated for only about 10 km and therefore had lowest genetic differentiation. *H. odorata* populations at about 35 km already showed moderate genetic differentiation ($F_{ST}$ = 0.166 between BGM and TP) whereas at 950 km distance this evidently was high ($F_{ST}$ = 0.308 for BGM and BE). For *H. hainanensis*, the largest $F_{ST}$ = 0.362 was observed between XD and XH but these were only separated for 96 km indicating low historical connectivity over such distance in that area.

The differentiation between populations (pairwise $F_{IS}$ and overall $G_{ST}$) thus can be explained on basis of geographic distances. *H. odorata* and *H. hainanensis* have a fairly high $G_{ST}$ (0.18-0.19) when compared to other dipterocarp species, such as *Dryobalanops aromatica* $G_{ST}$ = 0.067 (Lim et al. 2001), *Shorea leprosula* $G_{ST}$ = 0.117 (Lee et al. 2000) and *Shorea lumutensis* $G_{ST}$ = 0.048 (Lee et al. 2004). The limited gene flow via either pollen or seed dispersal thus could play an important role in *Hopea*. Dipterocarp species are insect-pollinated which occurs over only short distances, typically not further than a few kilometers. Moreover, those dipterocarp species growing in swamps and along riverbanks have their seeds dispersed by water. *H. hongayensis* has winged fruits that can float between coastal islands. Together with the close proximity of the studied populations at less than 20 km distance, it might explain why the $F_{ST}$ of *H. hongayensis* was very low (0.036), despite the distinct and separated island populations and in comparison to *H.*

*hainanensis* (0.287) and *H. odorata* (0.251). Only geographically closest populations such as XH with XT and XB with XD (*H. hainanensis*); CL and CQ (*H. hongayensis)*, showed low and non-significant genetic differentiation ($F_{ST} < 0.1$, $p > 0.05$). The high differentiation values at only 100 km distance, suggest that historical gene exchanges among populations remain limited in relation to the larger distributional ranges of *H. hainanensis* and *H. odorata*. Despite considering populations from Northern and Southern Vietnam (≈1000 km distance), only a low allelic richness in *H. hainanensis* could be observed. Most likely, this is due to bottleneck events in both regions and merits further investigation.

Our results give a better understanding of the genetic implications towards the conservation of three threatened *Hopea* species in Vietnam.

Protection of *Hopea* species necessitates survival of seedlings and juveniles in different protected areas because of the very small population sizes of adult and aged trees at low densities. Only in few areas, we could observe seedlings or regeneration of juveniles.

Small population sizes due to fragmentation therefore maintain low allelic richness (Ar) such as in *H. hainanensis*. Conservation at in-situ within national park or nature reserve areas is necessary for *H. hainanensis*. In addition, *H. hainanensis* already showed strong differentiation between populations at a geographic distance below 100km. So, conservation at ex-situ level in difference national parks and nature reserves are required to maintain and ultimately protect the different germplasm in each area.

*H. odorata* showed higher allelic richness than *H. hainanensis* but the populations of *H. odorata* are fragmented and consist of a low density of few adult trees. *H. odorata* also showed strong differentiation between those few populations. So, similar to for *H. hainanensis*, the conservation at in-situ and ex-situ are required. Each population of a National park is protected and is essential for a germplasm collection.

*H. hongayensis* is a narrow endemic species that only is found in few coastal islands in Quang Ninh. This species showed highest level of genetic diversity among three studied *Hopea* species but this can be explained by the many seedlings and juveniles in the analysis. The *H. hongayensis* populations on islands are developed well and showed re-generation under good condition. Juveniles and seedlings of *H. hongayensis* are well-grown and showed a similar spatial structure as the adult trees. Thus, the forestry protector should conserve and protect the natural spatial structure of *H. hongayensis* on each island as their natural habitats and keep them through natural regeneration. As such, restoration with plantation and nursery garden development is may be not necessary.

The future work can track changes in demography and genetic structure with a genetic survey performed on the young generation in comparison to leftover adult trees. Tests for allelic richness and heterozygotes of those selected juveniles in the nurseries, prior to re-introduction and re-plantation efforts also are recommended as future research need.

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*Notes:* N, population size; A, mean number of alleles per locus; Ar, allelic richness; P, the percentage of polymorphic loci; Ho and He, mean observed and expected heterozygosity, respectively; $F_{IS}$ (with $p < 0.05$).

**Table 1: Location and Characteristics of Ten Populations of Three *Hopea* Species**

| Species | Pop Code | Sample Size | Locality | Altitude | Latitude | Longitude | Characteristics |
|---|---|---|---|---|---|---|---|
| *H. chinensis* | BM | 34 | Ba Mun Island, Quang Ninh | 230m | 21º02'N | 107º35'E | Natural, coastal island |
| | CL | 35 | Cai Lim Island, Quang Ninh | 270m | 21º08'N | 107º40'E | Natural, coastal island |
| | CQ | 27 | Cai Quyt bay, Quang Ninh | 200m | 21º05'N | 107º36'E | Natural, coastal island |
| *H. odorata* | BGM | 23 | Bu Gia Map, Binh Phuoc | 130m | 10º56'N | 106º59'E | Natural, inland |
| | TP | 29 | Tan Phu, Dong Nai | 100m | 11º12'N | 107º09'E | Natural, inland |
| | BE | 18 | Ben En, Thanh Hoa | 100m | 19º35'N | 105º30'E | Natural, inland |
| *H. hainanensis* | XH | 20 | Xuan Hoa, Ben En, Thanh Hoa | 120m | 19º35'N | 105º30'E | Natural, inland |
| | XT | 21 | Xuan Thai, Ben En, Thanh Hoa | 200m | 19º32'N | 105º45'E | Planted (>15 years old) |
| | XB | 16 | Xom Bong, Cuc Phuong, Ninh Binh | 150m | 20º19'N | 105º36'E | Natural, inland |
| | XD | 14 | Xom Dang, Cuc Phuong, Ninh Binh | 350m | 20º26'N | 105º40'E | Natural, inland |
| Total | | 237 | | | | | |

**Table 2: Microsatellite Features with SSR Loci, Repeat Motif, Primer Sequences, Size and Number of Alleles Per Locus in *H. Chinensis, H. Odorata* and *H. Hainanensis***

| Locus Designation | | Primer Sequence (5'---3') | Motif | No of Alleles Per Locus in *H. Chinensis* | No of Alleles Per Locus in *H. Odorata* | No of Alleles Per Locus in *H. Hainanensis* |
|---|---|---|---|---|---|---|
| 1 | **Nhe 4** | F: ACGCAAGCCAACACATCC<br>R: TTTGCCATTTCACAATCATCAC | $(GA)_{19}$ | 2 | 2 | 3 |
| 2 | **Nhe 5** | F: GGAGGTGTAAACAAACTCAGTG<br>R: CTACATAATTGTGCAAACTAGGC | $(CT)_{14}$ | 3 | 2 | 2 |
| 3 | **Nhe 11** | F: CCATCTGAGGGTGTTGAAAG<br>R:GAGTAGAAGAAGGCAGGTGATTA | $(GA)_{19}$ | 4 | - | 5 |
| 4 | Nhe 15 | F: CTGCCACTAATCGACCAG<br>R: TGGGCAAATCTCTTAATGTT | $(TC)_{16}(AC)_{9}$ | - | - | - |
| 5 | Nhe 18 | F: GGTATTCTAATCTTTGCCTATT<br>R: GCCAGTGAAGTATCTATGC | $(CT)_{15}$ | - | - | - |
| 6 | **Nhe 19** | F: ATCAGAGTAGCCATGTTGCTTG<br>R: GGAGAGACTGGGCTTGCTC | $(GA)_{14}$ | 4 | 3 | 4 |
| 7 | **Shc 1** | F:GCTATTGGCAAGGATGTTCA<br>R:CTTATGAGATCAATTTGACAG | (CT)8(CA)10CT(CA)4CTA | 3 | 2 | 2 |
| 8 | **Shc 2** | F: CACGCTTTCCCAATCTG<br>R: TCAAGAGCAGAATCCAG | (CT)2CA(CT)5 | 3 | 2 | 2 |
| 9 | **Shc 3** | F:TTGAAGGGAAGGCTATG<br>R:CTTCTCAACTACCTTACC | (CT)8 | 3 | 2 | 2 |
| 10 | **Shc 4** | F:ATGAGTAACAAGTGATGAG<br>R:TATTGACGTGGAATCTG | (CT)16 | - | - | - |
| 11 | **Shc 7** | F:ATGTCCATGTTTGAGTG<br>R:CATGGACTAAAGTGGAG | (CT)8CA(CT)5CACCC(CTCA)3CT(CA)10 | - | - | - |
| 12 | **Shc 8** | F:GAGTCTGTGGTTGATATG<br>R:TTCTATGCAAGGGCTTTTAG | (CT)16 | - | - | - |
| 13 | **Shc 9** | F:TTTCTGTATCCGTGTGTTG<br>R:GCGATTAAGCGGACCTCAG | (CT)12 | - | 3 | - |
| 14 | **Shc 11** | F:ATCTGTTCTTCTACAAGCC<br>R:TTAGAACTTGAGTCAGATAC | (CT)4TT(CT)5 | 2 | 2 | - |
| 15 | **Shc 17** | F:CTAGAATCCGCCATTTCC<br>R:CACAAATACGTCTCCATATC | (CT)5AT(CT)4 | 1 | 1 | 1 |
| | | | Total | 25 | 19 | 21 |

**Table 3: Genetic Variation of Microsatellite Loci in Populations From Three *Hopea* Species**

| Population | N | A | Ar | P | Ho | He | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| ***Hopea Chinensis*** | | | | | | | |
| BM | 34 | 4.4 | 2.8 | 89 | 0.422 | 0.476 | 0.129* |
| CQ | 27 | 4.3 | 2.8 | 89 | 0.523 | 0.473 | -0.086 |
| CL | 35 | 4.3 | 2.8 | 89 | 0.480 | 0.470 | -0.011 |
| Mean | | 4.3 | 2.8 | 89 | 0.476 | 0.473 | 0.006 |
| SE | | | 0.180 | 0 | 0.057 | 0.046 | 0.088 |
| ***Hopea odorata*** | | | | | | | |
| BGM | 23 | 3.0 | 2.6 | 87.5 | 0.402 | 0.368 | -0.072 |
| TP | 29 | 3.2 | 2.6 | 87.5 | 0.392 | 0.389 | 0.008 |
| BE | 18 | 3.4 | 2.6 | 87.5 | 0.403 | 0.419 | 0.066 |
| Mean | | 3.2 | 2.6 | 87.5 | 0.399 | 0.392 | -0.025 |
| SE | | | 0.254 | 0 | 0.061 | 0.046 | 0.009 |
| ***Hopea Hainanensis*** | | | | | | | |
| XH | 20 | 3.0 | 2.1 | 89 | 0.306 | 0.329 | 0.158 |
| XT | 16 | 3.9 | 2.1 | 89 | 0.434 | 0.373 | -0.110 |
| XD | 14 | 3.8 | 2.0 | 89 | 0.373 | 0.406 | 0.137 |
| XB | 21 | 3.4 | 2.1 | 89 | 0.361 | 0.418 | 0.193 |
| Mean | | 1.8 | 2.08 | 89 | 0.368 | 0.382 | 0.106 |
| SE | | | 0.092 | 0 | 0.056 | 0.031 | 0.167 |

**Table 4: Summary of *Hopea* At Species Level of Genetic Diversity, Estimation of Cap*f*, Theta and Small*f* and Partitioning of Genetic Variation among Populations**

| Statistic | *H. Chinensis* | *H. Odorata* | *H. Hainanensis* |
|---|---|---|---|
| $F_{IS}$ | 0.018 | -0.001 | 0.050 |
| $F_{IT}$ | 0.040 | 0.155 | 0.258 |
| $F_{ST}$ | 0.022 | 0.156** | 0.219* |
| $G_{ST}$ | 0.009 | 0.102 | 0.151 |
| Cap*F* | 0.041 | 0.151 | 0.251 |
| Theta | 0.023 | 0.153 | 0.218 |
| Small*f* | 0.018 | -0.001 | 0.038 |
| AMOVA | | | |
| Variation among populations | 2% | 16% | 22% |
| Variation within populations | 98% | 84% | 78% |
| **: $p < 0.01$, *: $p < 0.05$ | | | |

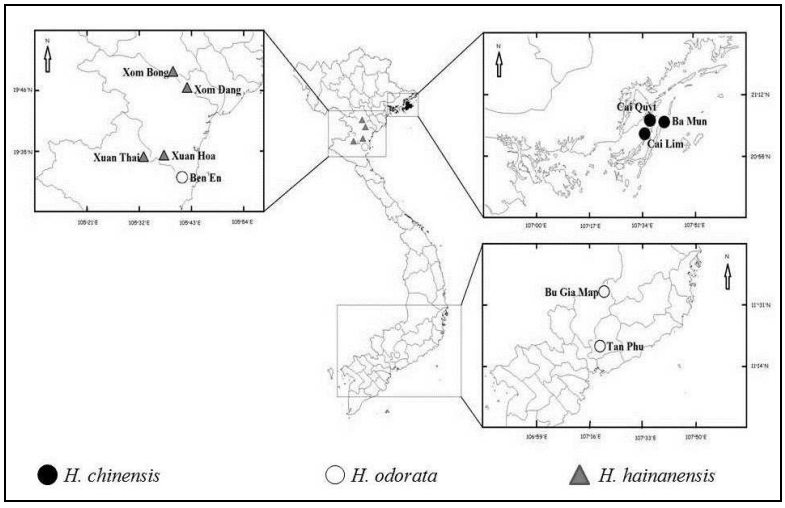


**Figure 1: Sampling Locations of Three *Hopea* Species in Vietnam.**

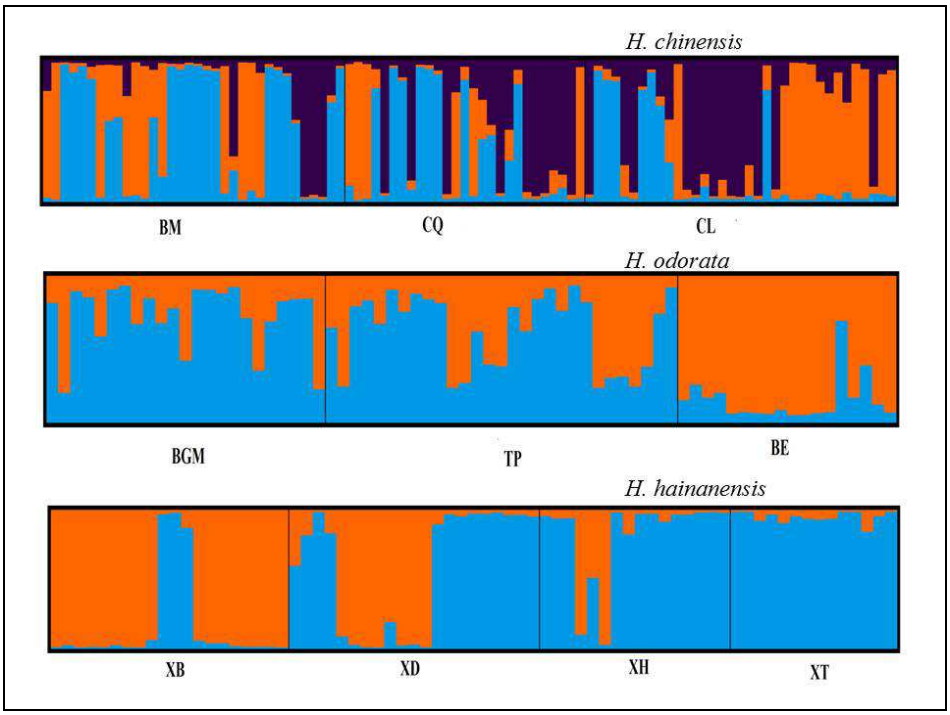


**Figure 2: Structure Analysis of Three Hopea Species With Plot Bar of Clusters At Highest Delta K. A: H. Odorata, B: H. Chinensis and C: H. Hainanensis.**

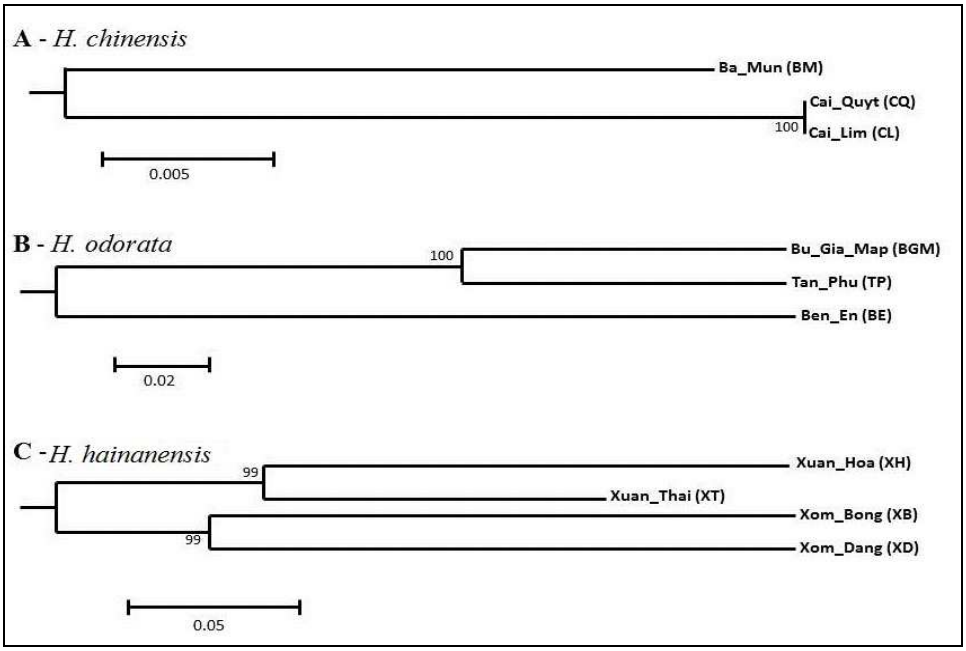


**Figure 3: Neighbor Joining Tree with Bootstrap Value of Populations in Three *Hopea* Species. *H. Odorata* (A), *H. Chinensis* (B) and *H. Hainanensis* (C).**

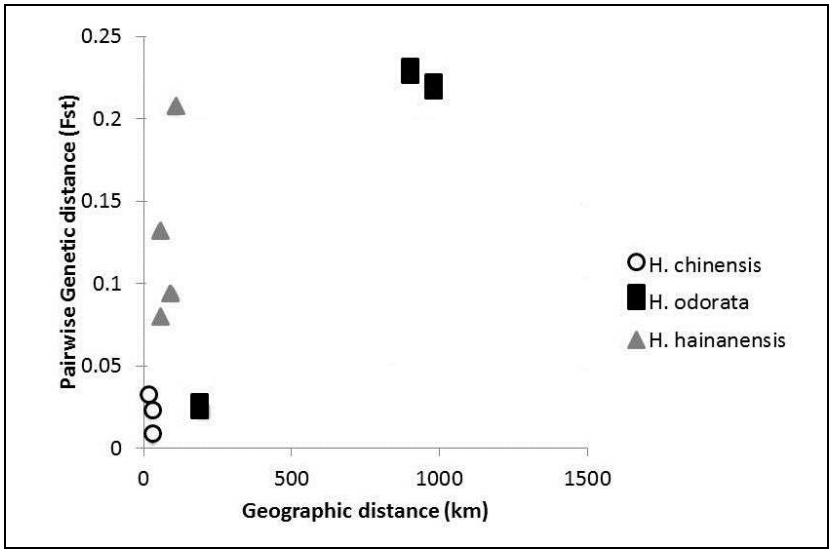


**Figure 4. Diagram Showing the Association Between Geographic Distance (Km) and Pairwise Genetic Distance ($F_{st}$) of Populations for Three *Hopea* Species.**

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