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Genetic diversity and population structure of two threatened ginseng species in Vietnam

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Abstract

Two ginseng species *Panax vietnamensis* and *Panax stipuleanatus* are precious medicinal plants restricted in several Vietnam provinces. They are very limited and endangered due to degraded habitats and over-harvesting. To preserve these two species, we used eight nuclear microsatellite markers to investigate genetic variability from the nine populations with 246 individuals for these two ginseng species. Our findings showed a moderate genetic heterozygosity in two species, *P. vietnamensis* (H_E = 0.386) and *P. stipuleanatus* (H_E = 0.342). Deficiency of heterozygosity was observed in all the studied populations of *P. vietnamensis* and three populations of *P. stipuleanatus*. Some populations had high allelic richness for both species. Private alleles were determined in all the studied populations of *P. vietnamensis* and two *P. stipuleanatus* populations. Genetic differentiation was low in two ginseng species. However, habitat loss, over-utilization and over-harvesting can be the main causes of reduced genetic heterozygosity. Neighbor-joining tree and discriminant analysis of principal components detected three major genetic groups. Finally, based on our findings, we propose *in situ* conservation of populations with high expected heterozygosity, allelic richness, and private alleles. Seed collection should be performed for *ex-situ* conservation as genetic pools in the future.

Keywords: degraded habitats, gene exchange, ginseng, medicinal plants, overharvesting, species conservation.

Introduction

The *Panax* (*Araliaceae*) is a ginseng genus with six species distributed in North America and mainly in Southeast and East Asia (Xiang and Lowry 2007). Some species are commonly used as a crucial source of human medicines because of their abundant content of saponins with ginsenosides (Hostettmann and Marston 1995, Konoshima *et al.* 1998). Two ginseng species, *Panax vietnamensis* and *P. stipuleanatus* are grown in Vietnam where they are the most important medicinal plants.

The distributional range of *P. stipuleanatus* is restricted to northwestern Vietnam. It was found in the four provinces of Yen Bai, Lai Chau, Lao Cai, and Son La

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Abbreviations: AFLP - amplified fragment length polymorphism; *AMOVA* - variance components molecular variance; CR - critically endangered; DAPC - discriminant analysis of principal components; F_{IS} - fixation index; F_{IS}IIM - corrected inbreeding coefficient for null alleles; H_E - expected heterozygosity; H_O - observed heterozygosity; H_T - total expected heterozygosity; HWE - Hardy-Weinberg equilibrium; ISSR - inter simple sequence repeat markers; IUCN - international union for conservation of nature; N_A - alleles per locus; N_E - effective alleles; NJ - neighbor-joining; N_P - private alleles; N_R - allelic richness; PcoA - principal coordinate analysis; PCR - polymerase chain reaction; PPL - proportion of polymorphic loci; RAPD - random amplified polymorphic DNA; SMM - the stepwise mutation model; SSR - microsatellite; T_m - PCR annealing temperature; TPM - the two-phase model.
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in subtropical montane forests on sandy and shale soils at altitudes between 1 300 and 2 400 m. *Panax vietnamensis* was only found in two provinces of Kon Tum and Quang Nam. These ginsengs grow slowly, taking about 7 - 8 years to reach maturity and form small, scattered populations in the herbaceous understory of montane forests. Human activities in the last few decades have greatly reduced their habitats. Their natural distributional area has declined. The habitats have been disturbed and degraded. Demographically, the remnant populations remain in small and isolated fragments. As a result, these species are listed as critically endangered (CR) based on the IUCN criteria (Hammer and Khoshbakht 2005) and the national categories (Vietnam Red Data Book 2007). Therefore, establishing conservation strategies is needed to provide a sustainable environment for these two species. Genetic diversity plays a highly important role in the ability to adapt to change in the environment (Laurance 2004, Sebbenn *et al.* 2017, Flower *et al.* 2018). Genetic variation of a species is associated with factors such as its distributional range, life cycle, mating system, and evolutionary history (Hamrick *et al.* 1992, Frankham *et al.* 2002, Flower *et al.* 2018). Isolated populations with small sizes become inbred and susceptible to genetic drift, causing a dramatic increase in stress sensitivity (Bijlsma *et al.* 1997). Thus, knowledge is necessary about genetic variability, gene flow, and genetic relationships between studied populations for each endangered species *Panax vietnamensis* and *P. stipuleanatus*.

In the last decades, various molecular markers have been used to investigate the genetic diversity of threatened plant species to improve their conservation, management, and restoration (Hamrick and Godt 1996, Brütting *et al.* 2012, Bruford *et al.* 2017). In previous reports, Kim *et al.* (2007) and Ahn *et al.* (2009) developed a set of genomic microsatellite markers from Korean wild *Panax ginseng*. Zhuravlev *et al.* (2008) compared different methods and showed that the genetic diversity of *P. ginseng* was low with allozymes and high with AFLP (amplified fragment length polymorphism) and SSR (microsatellite) markers. Trieu *et al.* (2016) used the ISSR (inter simple sequence repeat markers). They were used to investigate the genetic diversity in *P. stipuleanatus* in Vietnam. Similarly, Zhuravlev *et al.* (2004) reported the genetic diversity of *P. ginseng* in Russia using RAPDs (random amplified polymorphic DNA). Among used markers, microsatellites have been successfully used in the investigation of the genetic diversity of rare plants (Kalia *et al.* 2011). Studies on the genetic diversity of *Panax* species using SSR markers were performed (Park *et al.* 2009, Jo *et al.* 2009, Van Dan *et al.* 2010, Liu *et al.* 2011, Reunova *et al.* 2014). Vu *et al.* (2020) developed a set of EST-SSRs (expressed sequence tags microsatellite) markers from *P. vietnamensis* and revealed a moderate genetic diversity of the three populations of *P. vietnamensis* in Vietnam.

In this study, we applied nuclear microsatellites to evaluate the genetic variation within and among populations of two endangered ginsengs, *Panax vietnamensis* and *P. stipuleanatus*, using clear microsatellites developed from *P. ginseng* in Korea (Ahn *et al.* 2009), and establish conservation strategies for providing a sustainable environment for these species in Vietnam.

Materials and methods

Plants and DNA isolation: This study selected two ginseng species, *Panax vietnamensis* Duy and *P. stipuleanatus* Tsai&Feng. *Panax vietnamensis* is very limited in the Tay Nguyen Highlands area, whereas *P. stipuleanatus* is in the Northwest area (Ho 2002, Tap 2005). Targeted locations in two provinces Quang Nam and Kon Tum for *P. vietnamensis* and five provinces of Yen Bai, Lai Chau, Son La, and Lao Cai for *P. stipuleanatus* represented the natural distribution ranges per species (Fig. 1, Table 1). A total of 246 plant individuals were randomly sampled from nine populations, *i.e.*, four populations in *P. vietnamensis* and five populations in *P. stipuleanatus*. Genomic DNA was isolated from leaf tissues using the CTAB method (Doyle and Doyle 1990). DNA amount was tested by a *NanoDrop 2000C* (*Thermo Scientific*, Waltham, Massachusetts, USA). DNA concentration was diluted to 10 ng μL^{-1}.

Microsatellite amplification: We tested a total of 21 primer pairs of microsatellite markers from *Panax ginseng* (Ahn *et al.* 2009). Twelve SSRs were determined for *P. vietnamensis* and the nine SSRs for *P. stipuleanatus* based polymorphism reproduced in the tests. However, the eight SSRs of these primers for both two species were used to analyze genetic diversity in this study (Table 1S). The polymerase chain reaction (PCR) was implemented in 0.2 mL microtubes with a 20 µL of reaction mixture including 12 µL *Dream Taq Green PCR Master Mix*, 2.5 µL of pure water, 10 pmol of each primer, and 10 ng of genomic DNA. The PCR protocol was performed in the *GeneAmp PCR System 9700* (*Thermo Scientific*, Waltham, Massachusetts, USA) as follows: 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C - 57°C according to primer and 1 min at 72°C, with 10 min at 72°C for a final extension. The products were electrophoresed in a 7% polyacrylamide gel and then visualized with a *GelRedTM* nucleic acid gel stain (*Biotium*, Fremont, California, USA). The PCR product sizes were identified using the *GenoSens1850 Gel-Analyzer* software (*Clinx Science Instruments*, Shanghai, China) with a 50 bp DNA ladder.

Data analysis: Null allele was checked in the software *Micro-checker* (Van Oosterhout *et al.* 2004) using 1 000 bootstrap iterations. The genetic diversities including observed and expected heterozygosity (H_O and H_E, respectively), allele (N_A) and effective allele (N_E), the fixation index (F_{IS} *i.e.*, the inbreeding value), the difference among populations [the Weir and Cockerham F_{ST} index (Weir and Cockerham 1984) and Hedrick G'_{ST} index (Hedrick 2005)] and total expected heterozygosity (H_T) were determined in *GenAlEx* (Peakall and Smouse

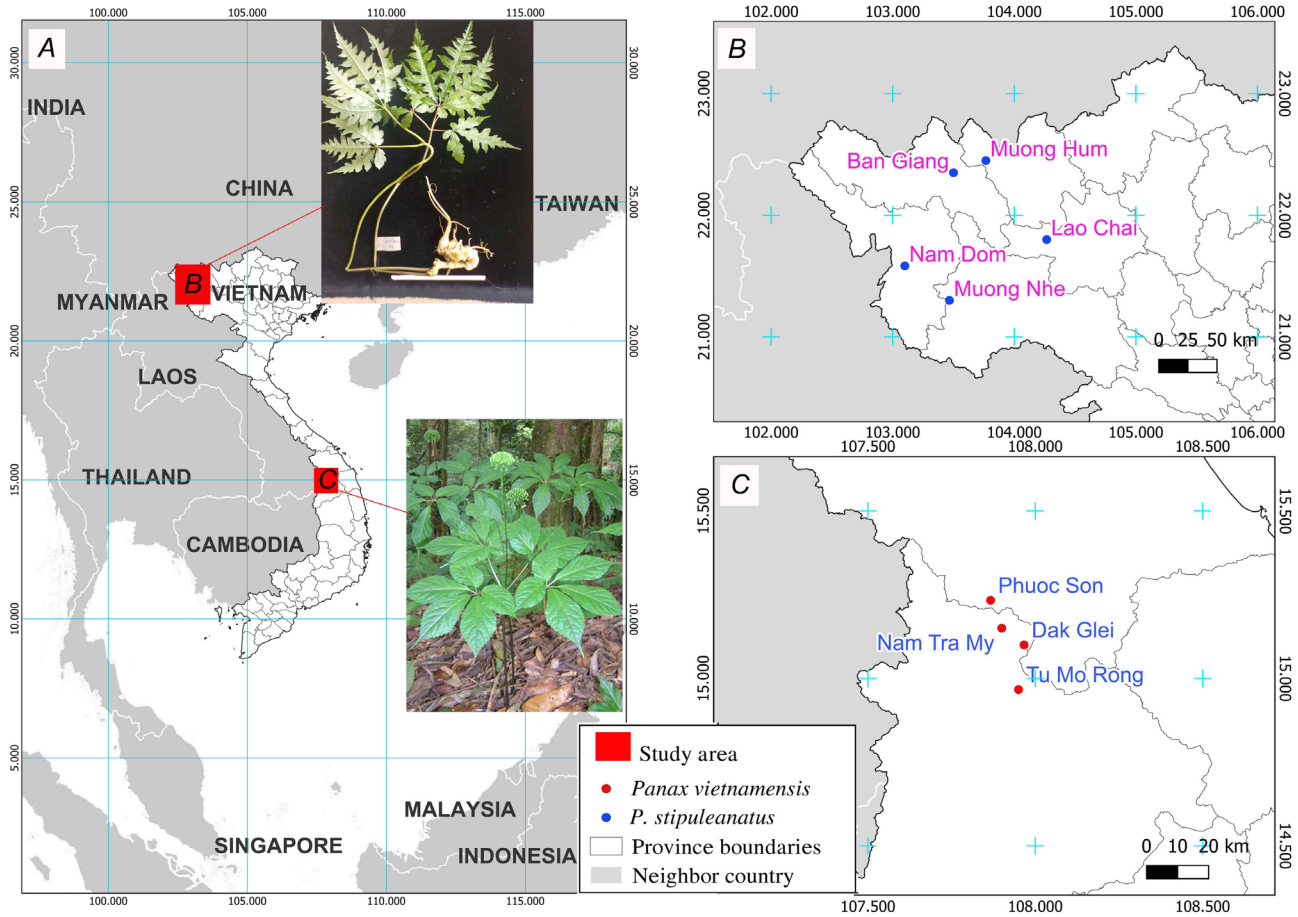

Fig. 1. Map showing studying sites of two ginseng species.

Table 1. Collection localities of two ginseng species.

	Population	Sampling size	Collection locality	Altitude [m]	Latitude (N)	Longitude (E)
Panax vietnamensis	Nam Tra My	28	Nam Tra My, Quang Nam	1 875	15°09'	107°54'
	Phuoc Loc	27	Phuoc Son, Quang Nam	1 679	15°14'	107°52'
	Dak Glei	30	Dak Glei, Kon Tum	1 847	15°06'	107°58'
	Tu Mo Rong	27	Tu Mo Rong, Kon Tum	1 789	14°58'	107°57'
Panax stipuleanatus	Lao Chai	26	Mu Cang Chai, Yen Bai	1 267	21°48'	104°16'
	Ban Giang	26	Ho Chau, Lai Chau	1 812	22°21'	103°30'
	Muong Nhe	25	Muong Nhe, Lai Chau	1 137	21°18'	102°28'
	Muong Hum	28	Bat Xat, Lao Cai	1 788	22°27'	103°46'
	Nam Dom	29	Muong La, Son La	1 376	21°35'	103°06'

2012). Allelic richness (N_R) and allelic private (N_P) per locus and per population were conducted in *FSTAT* (Goudet 2002) and *GenAlEx*. The individual inbreeding model was performed to evaluate the F_{IS} index for null allele frequency (F_{IS}IIM) using *INEst* (Chybicki and Burczyk 2009). Deviations from Hardy-Weinberg equilibrium (HWE) for loci within populations were tested in *Cercus* (Kalinowski *et al*. 2007) based on 1 000 permutations of alleles among individuals. The *Bottleneck* (Piry *et al.* 1999) was used to detect bottleneck events. These tests were evaluated through the one-tailed *Wilcoxon signed-rank* test. The variance components molecular variance (*AMOVA*) analysis were tested in *Arlequin 3.0* (Excoffier *et al*. 2005). *Poptree2* (Takezaki *et al.* 2010) was used to create a neighbor-joining (NJ) diagram to detect the genetic association between populations based on F_{ST} values. A principal coordinate analysis (PCoA) was identified by *GenAlEx v.6.5*. Discriminant analysis of principal

components (DAPC) was conducted according to Jombart *et al.* (2010) using the *R package adegenet*. The number of clusters (*K*) of genetically related individuals was run from 1 to 10 to identify the optimal *K* value.

Results

The frequency of null alleles was determined in four loci for each species, *Panax vietnamensis* and *P. stipuleanatus*, at the significance level of 0.05. Deviation from HWE was significant at two loci PG281 and PG352, in *P. vietnamensis* and at two loci of PG167 and PG424, in *P. stipuleanatus* (Table 2S). In total, 31 alleles across 112 *P. vietnamensis* plants and 28 alleles across 134 *P. stipuleanatus* plants were produced from the eight polymorphic microsatellite markers. The percentage of polymorphic loci was high (100%) in all *P. vietnamensis* populations. This value averaged 97.5%, ranging from 87.5% in the Muong Nhe population to 100% in the remaining four populations of *P. stipuleanatus*. The parameters of genetic diversity for each species were presented in Table 2. The highest number of alleles (23) was detected in Tu Mo Rong and the lowest (19) in Nam Tra My for *P. vietnamensis*. Similarly, the highest value of alleles (24) was found in the two populations Muong Hum and Nam Dom, and the lowest value (18) in Muong Nhe for *P. stipuleanatus*. The observed mean alleles per locus (N_A) was 2.6, ranging from 2.4 in Nam Tra My to 2.9 in Tu Mo Rong for *P. vietnamensis*, and 2.7, ranging from 2.2 in Muong Nhe to 3 in two populations of Muong Hum and Nam Dom for *P. stipuleanatus*. The highest allelic richness per locus was observed in Tu Mo Rong (N_R = 2.9) for *P. vietnamensis* and Muong Hum (N_R = 3) for P. *stipuleanatus*. The lowest value of effective alleles was observed in Phuoc Loc (N_E = 1.4) and in Lao Chai (N_E = 1.4), whereas this value was the highest in Tu Mo Rong (N_E = 2) and in Muong Hum (N_E = 3) for *P. vietnamensis* and *P. stipuleanatus*, respectively. The observed mean heterozygosity (H_O) and mean expected heterozygosity (H_E) were 0.345 and 0.386 for *P. vietnamensis*, and 0.315 and 0.342 for *P. stipuleanatus*. The lowest values of H_O and H_E were detected in Phuoc Loc with 0.204 and 0.255, respectively, for *P. vietnamensis*, and in Lao Chai with 0.25 and 0.243, respectively, for *P. stipuleanatus*. The fixation value ranged from 0.061 (Nam Tra My) to 0.219 (Phuoc Loc), with an average of 0.132 for *P. vietnamensis*, and from -0.071 (Muong Nhe) to 0.245 (Ban Giang), with a mean value of 0.09 for *P. stipuleanatus*. Significantly positive F_{IS} values were observed in two populations, Phuoc Loc and Dak Glei for *P. vietnamensis*, and three populations, Ban Giang, Muong Hum, and Nam Dom, for *P. stipuleanatus*, showing a deficiency of heterozygotes. The inbreeding value based on the individual inbreeding model (F_{IS}IIM) showed the F_{IS}IIM values varied from 0.1 (Nam Tra My) to 0.019 (Phuoc Loc), with a mean value of 0.014 for *P. vietnamensis*, and from 0.008 (Nam Dom) to 0.012 (Muong Nhe), with an average of 0.01 for *P. stipuleanatus*. In all studied populations for both ginseng species, there are no bottleneck events (Table 2). Private alleles were observed in all studied populations, ranging from one in Nam Tra My to five in Phuoc Loc for *P. vietnamensis*, while this value was only observed in the two populations of Nam Dom and Muong Hum for *P. stipuleanatus*. At the locus level, alleles for each locus (N_A) averaged 3.9 (3 at three loci PG186, HPG132, and HPG126 to 5 at two loci PG167 and PG352) and 3.5 (3 at five loci PG281, PG424, PG450, HPG126, and HPG132 to 5 at locus PG352) for *P. vietnamensis* and *P. stipuleanatus*, respectively

Table 2. Genetic diversity values and results of bottleneck tests for two ginseng species. N - sample size; PPL - proportion of polymorphic loci; N_A - alleles per locus; N_E - effective alleles; N_R - allelic richness; N_P - number of private alleles; H_O and H_E - observed and expected heterozygosity; F_{IS} - fixation index; F_{IS}IIM - corrected inbreeding coefficient for null alleles; SMM - the stepwise mutation model; TPM - the two-phase model; ns - not significant; * - $P < 0.05$.

Populations	N	PPL [%]	Alleles	N_A	N_E	N_R	N_P	H_O	H_E	F_{IS}	F_{IS}IIM	*P* value of bottleneck	
												SMM	TPM
Panax vietnamensis													
Nam Tra My	28	100	19	2.4	1.7	2.4	1	0.379	0.396	0.061	0.010	ns	0.01*
Phuoc Loc	27	100	21	2.6	1.4	2.6	5	0.204	0.255	0.219	0.019	ns	ns
Dak Glei	30	100	22	2.7	1.8	2.7	2	0.367	0.427	0.157	0.014	ns	ns
Tu Mo Rong	27	100	23	2.9	2.0	2.9	2	0.431	0.465	0.093	0.013	ns	ns
Mean				2.6	1.7	2.6		0.345	0.386	0.132	0.014		
Panax stipuleanatus													
Lao Chai	26	100	21	2.6	1.4	2.6	-	0.250	0.243	-0.009	0.010	ns	ns
Ban Giang	26	100	23	2.9	1.9	2.9	-	0.293	0.379	0.245	0.011	ns	ns
Muong Nhe	25	87.5	18	2.2	1.7	2.2	-	0.375	0.344	-0.071	0.012	ns	ns
Muong Hum	28	100	24	3.0	1.8	3.0	2	0.344	0.390	0.136	0.009	ns	ns
Nam Dom	29	100	24	3.0	1.6	2.9	1	0.306	0.353	0.150	0.008	ns	ns
Mean		97.5		2.7	1.7			0.314	0.342	0.090	0.010		

(Table 2S). The number of allelic richness (N_R) varied from 2.5 at HPG126 to 4.1 at PG167, with an average of 3.1 for *P. vietnamensis*, whereas the lowest value was found at locus PG186 (N_R = 2.5) and the highest at PG352 (N_R = 3.9) for *P. stipuleanatus*. Private alleles (N_P) were detected at five loci in *P. vietnamensis* and three loci in *P. stipuleanatus*. The H_O value differenced from 0.188 at HPG126 to 0.533 at PG450, and the H_E value differenced from 0.276 to 0.51 for HPG126 and PG450, respectively, for *P. vietnamensis*. Similarly, the H_O and H_E values varied between 0.141 and 0.14, and 0.454 and 0.518 for PG186 and PG167, respectively, in *P. stipuleanatus*. The highest value of total expected heterozygosity (H_T) was found at PG450 (0.544) and PG167 (0.622) for *P. vietnamensis* and *P. stipuleanatus*, respectively, and the lowest at PG186 for the two ginseng species (Table 2S). A positive fixation index (F_{IS}) was observed at five loci for both ginseng species, showing an excess of homozygosity and inbreeding.

The genetic difference (F_{ST}) among loci averaged 0.062 (0.011 - 0.114) and 0.097 (0.032 - 0.166) for *P. vietnamensis* and *P. stipuleanatus*, respectively (Table 2S), showing that 6.6 and 11.25% of the genetic variation existed between populations for *P. vietnamensis* and *P. stipuleanatus*, respectively (Table 3). The F_{ST} value among populations varied from 0.007 (Dak Glei *vs*. Tu Mo Rong) to 0.082 (Phuoc Loc *vs*. Nam Tra My) for *P. vietnamensis*, and from 0.028 (Nam Dom *vs*. Ban Giang) to 0.147 (Muong Nhe *vs*. Lao Chai) for *P. stipuleanatus*. Significant differentiation was observed for pairwise F_{ST} values for both the ginseng species ($P < 0.05$, 0.01, and 0.001), except for the pair of Dak Glei and Tu Mo Rong in *P. vietnamensis* (Table 3S). Two clustering approaches were used to detect the genetic groups for the two ginseng species. The NJ diagram revealed three genetic clusters for both ginseng species (Fig. 2). The two *P. vietnamensis* populations of Dak Glei and Tu Mo Rong were grouped with a bootstrap value of 64%, whereas Nam Tra My separated from these two populations to form a single cluster. The two *P. stipuleanatus* populations of Lao Chai and Nam Dom were clustered in one group. Of the remaining populations, Ban Giang and Muong Nhe clustered together with a bootstrap of 66% to form another group. Muong Hum separated from these two populations and formed one group. The DAPC analysis without prior information on population origin also revealed three genetic groups (Fig. 3*B*,*D*). Group 1 included most individuals from Phuoc Loc for *P. vietnamensis* (Table 4S, Fig. 1S). Several individuals from the remaining four populations were also assigned to this group. Most individuals from the two populations of Dak Glei and Tu Mo Rong were assigned to group 2. In cluster 2, there were also nine individuals in Nam Tra My and two in Phuoc Loc. Group 3 included most individuals in Nam Tra My, eleven in Tu Mo Rong, thirteen in Dak Glei, and four in Phuoc Loc. All individuals of *P. stipuleanatus* from Lao Chai, except for three individuals, and those from Nam Dom, except for ten individuals assigned to cluster 3. This cluster also included eleven individuals in Ban Giang, two in Muong Nhe, and four in Muong Hum. Cluster 2 included thirteen individuals from each population Ban Giang and Muong Nhe, ten in Mong Hum, and seven in Nam Dom. No individuals from Lao Chai were included in cluster 2. Most individuals from Muong Hum were assigned to cluster 1. The ten individuals in Muong Nhe, three from each population Nam Dom and Lao Chai, and two in Ban Giang were assigned to cluster 1. The DAPC, with prior information, showed individuals within and between populations (Fig. 3*A*,*C*). The high overlap between populations indicated a low genetic difference. Our findings showed high overlaps between Dak Glei and Tu Mo Rong, with the F_{ST} value of 0.007 for *P. vietnamensis*, and between Ban Giang and Nam Dom, with the F_{ST} value of 0.028 for *P. stipuleanatus*. Similarly, low overlaps were found between Phuoc Loc and Nam Tra My, with the F_{ST} value of 0.082 for *P. vietnamensis*, and Lao Chai

Table 3. Analysis of molecular variance from natural populations for two ginseng species. df - degree of freedom; F_{IS} - fixation index; F_{IT} - coefficient of total inbreeding; F_{ST} - genetic differentiation index; *** - $P < 0.001$.

	df	Sum of squares	Variance components	Total variation [%]	Fixation indices
Panax vietnamensis					
Among populations	3	24.065	0.112	6.6	$F_{IS} = 0.124^{***}$ $F_{ST} = 0.066^{***}$ $F_{IT} = 0.182^{***}$
Among individuals within populations	108	191.739	0.196	11.57	
Within inviduals	112	155	1.384	81.83	
Total	223	370.804	1.691		
Panax stipuleanatus					
Among populations	4	44.212	0.177	11.25	$F_{IS} = 0.104^{***}$ $F_{ST} = 0.112^{***}$ $F_{IT} = 0.205^{***}$
Among individuals within populations	129	199.452	0.146	9.27	
Within inviduals	134	168	1.254	79.48	
Total	267	411.664	1.577		

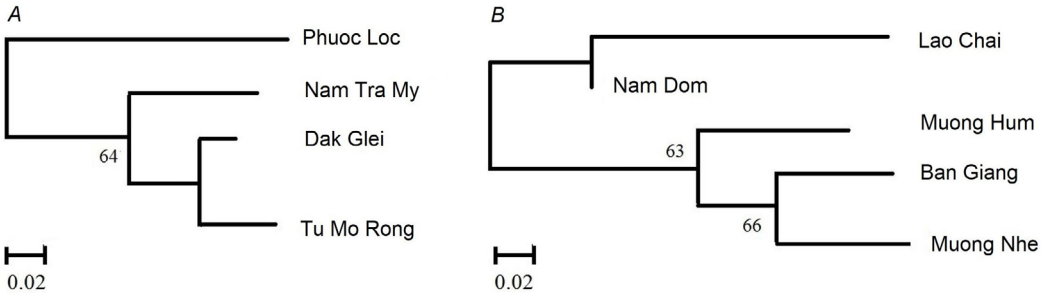

Fig. 2. Genetic relationships among populations based on neighbor-joining (NJ) using the F_{ST} values produced from *Poptree2*. *A* - *Panax vietnamensis*; *B* - *Panax stipuleanatus*.

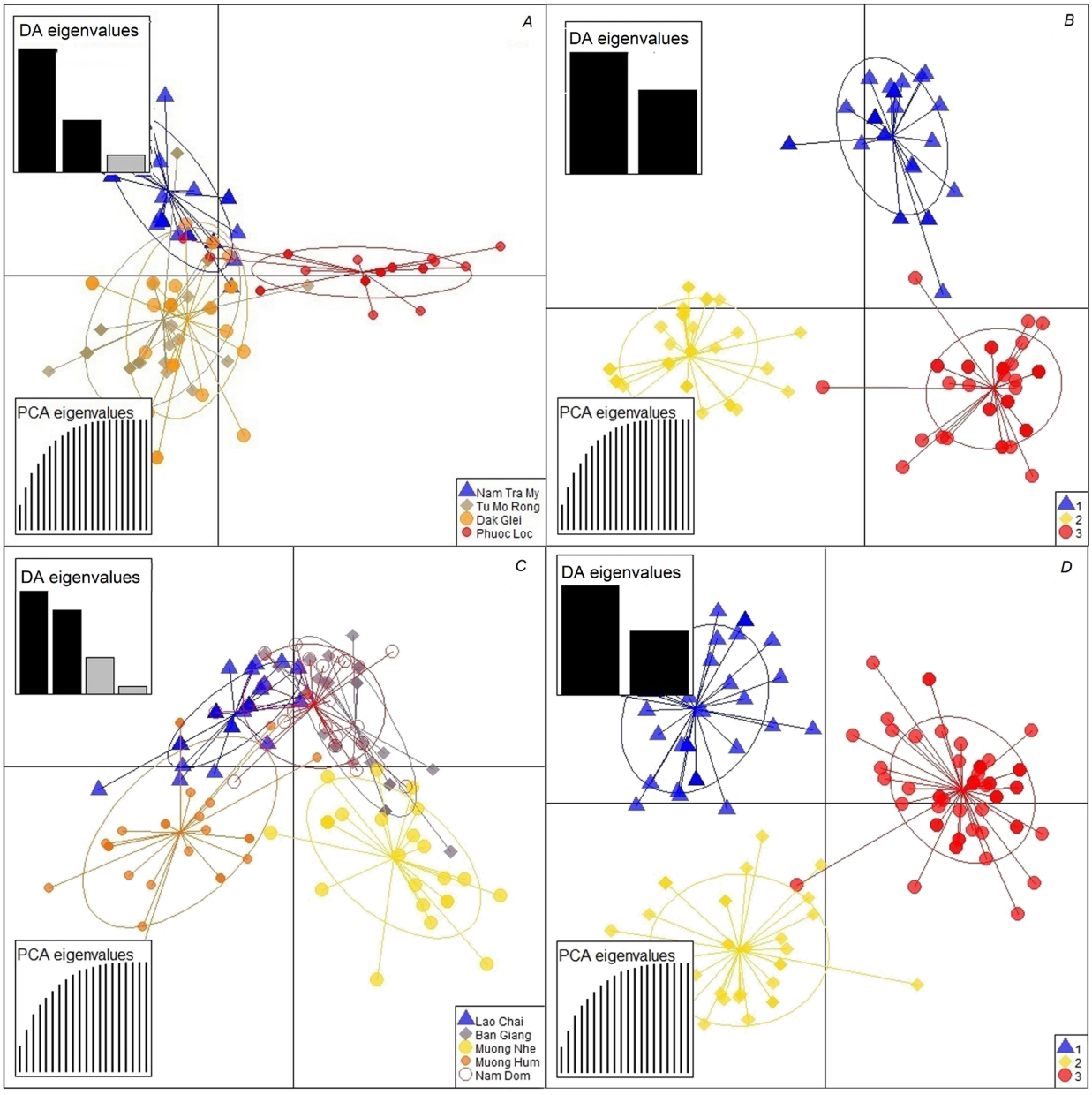

Fig. 3. Analysis of population structure using DAPC. *A* and *C* - Scatterplot of the DAPC with prior information. *B* and *D* - Scatterplot of the DAPC without prior information. *A* and *B* - for *Panax vietnamensis* and *C* and *D* - for *P. stipuleanatus*; #1,2,3 is genetic cluster.

and Muong Nhe, with the F_{ST} value of 0.147, and Ban Giang and Lao Chai, with the F_{ST} value of 0.124 for *P. stipuleanatus*.

Discussion

Two ginseng species, *P. vietnamensis* and *P. stipuleanatus* are very limited in the tropical forests, the two provinces of southern Vietnam for *P. vietnamensis* and some provinces of northern Vietnam for *P. stipuleanatus*. They are dioecious species with insect pollination (Tap 2005). Outcrossing species will have a high genetic diversity compared with other species (Hamrick and Godt 1990, Nybom 2004). As the expectation, these ginseng species will maintain high genetic diversity and low genetic difference. Previous studies showed a moderate genetic diversity for some ginseng species, such as *P. notoginseng* in China using genomic SSRs (H_O = 0.291, H_E = 0.35, Liu *et al.* 2011), *P. ginseng* in Russia (N_R = 2.5, H_O = 0.453, H_E = 0.393) using eleven polymorphic nuclear SSRs (Reunova *et al.* 2014), *P. vietnamensis* in Vietnam (H_O = 0.422, H_E = 0.479, Vu *et al.* 2020; N_R = 2.2, H_O = 0.367, H_E = 0.437, Vu *et al.* 2022) using EST-SSRs. In the present study, our findings showed a relatively moderate genetic diversity with N_R = 2.6, H_O = 0.345, H_E = 0.386 for *P. vietnamensis* and N_R = 2.7, H_O = 0.314, H_E = 0.342 for *P. stipuleanatus*. These results were lower than those reported, such as *P. ginseng* (H_E = 0.502, Ma *et al.* 2007) and *P. vietnamensis* (H_E = 0.55, Reunova *et al.* 2011) using genomic SSRs. However, low genetic diversity was observed, such as *P. stipuleanatus* in Vietnam (H_E = 0.254, Trieu *et al.* 2016) using ISSR markers, *P. ginseng* in Russia using allozymes (H_O = 0.018, H_E = 0.023), AFLP [Nei's gene diversity (H) = 0.255] and SSRs (H = 0.259) (Zhuravlev *et al.* 2008). Similarly, Reunova *et al.* (2010) showed low diversity for *P. ginseng* in Russia using RAPD (H_E = 0.013) and ISSRs (H_E = 0.014).

Genetic diversity is related to habitat disturbance and a reduction in population size, which can be decreased through high homozygosity for common alleles due to the loss of rare alleles (Honnay and Jacquemyn 2007, Gijbels *et al.* 2015). In this study, habitats are restricted to small areas for both the two ginseng species of *P. vietnamensis* and *P. stipuleanatus* and can affect genetic diversity of these two species. Habitat loss and excessive harvesting of both these ginseng species led to low genetic heterozygosity. These can be the major causes contributing to the decrease in genetic diversity. Small and isolated populations are vulnerable and subsequently at high risk of extinction (Falk and Holsinger 1991, Honnay and Jacquemyn 2007, Gijbels *et al.* 2015). Of all studied populations, lower genetic diversity was observed in Phuoc Loc for *P. vietnamensis* and Lao Chai for *P. stipuleanatus*. These findings suggest that these populations may be suffering from anthropogenic disturbance and overharvesting. Low genetic diversity may be associated with a high degree of habitat disturbance and small population size. High genetic diversity was identified in Tu Mo Rong (H_E = 465) and Dak Glei (H_E = 0.427) for *P. vietnamensis*, and Muong Hum (H_E = 0.39) and Ban Giang (H_E = 0.379) for *P. stipuleanatus*. Our study determined high allelic richness in the three *P. vietnamensis* populations of Tu Mo Rong (NR = 2.9) and Dak Glei (2.7), and the three *P. stipuleanatus* populations of Muong Hum (3.0), Ban Giang (2.9) and Nam Dom (2.9). Private alleles were also detected in all the *P. vietnamensis* populations and the two *P. stipuleanatus* populations of Muong Hum and Nam Dom. These populations might prioritize genetic conservation activities (Petit *et al.* 1998). Heterozygosity deficits in all the populations, except for the two *P. stipuleanatus* populations of Lao Chai and Muong Nhe, suggest the existence of inbreeding in both *P. vietnamensis* and *P. stipuleanatus*, although these ginsengs are the predominantly outcrossing species.

Previously, some studies revealed the low genetic difference between populations, such as *P. ginseng* in Russia using genomic SSRs [G_{ST} = 0.261, Zhuravlev *et al.* 2008; F_{ST} = 0.115, F_{ST} = 0.09 (0.001 - 0.211), Reunova *et al.* 2014] and using AFLP (F_{ST} = 0.148, Zhuravlev *et al.* 2008), *P. stipuleanatus* in Vietnam using ISSRs (G_{ST} = 0.03, Trieu *et al.* 2016), *P. vietnamensis* in Vietnam using EST-SSRs (F_{ST} = 0.133, F_{ST} = 0.172 (0.033 - 0.232), Vu *et al.* 2020, 2022). Our study detected low genetic differentiation among populations for both the two ginseng species, *P. vietnamensis* (F_{ST} = 0.011) and *P. stipuleanatus* (0.097), indicating high gene flow for both the two ginseng species (N_m > 1). High gene flow for two ginsengs was confirmed by high numbers of migrants, which reduced genetic differentiation between populations for each ginseng species.

The *AMOVA* detected a low molecular variance among populations per ginseng species (P < 0.001). This may be a consequence of the reduction in genetic differentiation between populations. The two ginsengs *P. vietnamensis* and *P. stipuleanatus* are pollinated *via* insects. Pollen dispersal may contribute significantly to both ginseng species's gene exchange and genetic structure.

The clustering methods detected the genetic structure of two ginsengs *P. vietnamensis* and *P. stipuleanatus*. The NJ diagram showed three genetic groups associated with population differentiation. Populations with high gene exchange or low genetic differences frequently clustered into distinct groups. The two populations of Dak Glei and Tu Mo Rong in *P. vietnamensis* clustered together and formed one group (F_{ST} = 0.007), while Phuoc Loc was separated from the remaining three populations (F_{ST} > 0.07). Similar results showed in the relationships between *P. stipuleanatus* population. The DAPC analysis without prior information on origin populations showed three groups. This result was consistent with the NJ analysis. The mixture of all three clusters was detected in four populations per ginseng species, except for the Lao Chai population of *P. stipuleanatus*. Several individuals in Nam Tra My, Dak Glei, and Tu Mo Rong were found in the first cluster. Similarly, a few individuals of Phuoc Loc were observed in both the remaining two clusters. Similar results were detected in *P. stipuleanatus*. Some individuals in Lao Chai, Ban Giang, and Nam Dom were observed in the first cluster, and several individuals in Muong Nhe and

Muong Hum in the third cluster. Therefore, the isolated populations can be related to anthropogenic disturbance, leading to a decrease in gene change between populations through the dispersal of pollen grains. However, the DAPC, with prior information, showed genetic relationships among populations per ginseng species. High overlaps were found between populations per ginseng species and were consistent with low genetic differentiation.

Conclusions

In this study, we determined moderate genetic diversity and low genetic differentiation among populations for the two ginseng species *Panax vietnamensis* and *P. stipuleanatus*. Without prior information, the NJ diagram and the DAPC identified three main genetic groups. Besides high genetic diversity in some populations for both ginseng species, the number of allelic richness, and private alleles were also found. These alleles are important gene pools for adapting to altered environmental pressure. Our findings indicated the high allelic richness in two *P. vietnamensis* populations of Tu Mo Rong and Dak Glei, and three *P. stipuleanatus* populations of Muong Hum, Ban Giang, and Nam Dom. Private alleles were identified in all the *P. vietnamensis* populations and two *P. stipuleanatus* populations of Nam Dom and Muong Hum. These populations could prioritize *in situ* conservation. From all the studied populations could be collected seeds for *ex-situ* conservation of these two ginseng species.

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