THE STUDY OF GENETIC DIVERSITY AND STRUCTURE OF EXTREMELY ENDANGERED *MANGLIETIA LONGIPEDUNCULATA* AND ENDANGERED *MANGLIETIA INSIGNIS*

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Abstract. A novel DNA sequencing library construction approach-Hyper-seq was used in this study to develop large-scale genomic single nucleotide polymorphism (SNP) and to evaluate the genetic diversity and population structure of *Manglietia insignis* and *Manglietia longipedunculata*. Genetic diversity analysis demonstrated that the heterozygosity (H_E, 0.1435) and nucleotide diversity (π, 0.1541) of *M. insignis* lower than *M. longipedunculata* (H_E = 0.1793, π = 0.1916). The genetic diversity of the two ex situ conservation populations (H_E = 0.1797, 0.1990, π = 0.1894, 0.2121) was higher than the genetic diversity of wild populations (H_E = 0.1591, π = 0.1733). The mean inbreeding coefficient (F_{IS}) in the *M. longipedunculata* and *M. insignis* populations were -0.0141 and -0.0176, respectively, indicating that the two populations were largely outcrossing in the natural state. The frequency of rare alleles in *M. insignis* (Tajima's D = 0.3436 > 0) and *M. longipedunculata* (Tajima's D = 0.2119 > 0) populations was low, which may be under balanced selection pressure or experienced population shrinkage. A high degree of genetic differentiation between the two species was detected, but moderate among the populations of the same species. AMOVA results indicated that within individuals were the main source of genetic variation for *M. longipedunculata* and *M. insignis*. The results of cluster, principal component and genetic relationship analysis demonstrated a obvious separation between the two species with all individuals clustering according to species differences, but some individuals between the two species have a small amount of the same genetic components.

Keywords: *Manglietia Blume, SNP, Hyper-seq, ex situ conservation, conservation recommendations*

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Abbreviations: AMOVA, analysis of molecular variance; CV, cross-validation; F_{IS}, inbreeding coefficient; F_{ST}, genetic differentiation; H_E, expected heterozygosity; H_O, observed heterozygosity; K, clustering values; PCA, principal component analysis; PCR, polymerase chain reaction; SNPs, single nucleotide polymorphisms; Tajima's D, Tajima's neutrality test; WGS, whole-genome sequencing; π, nucleotide diversity

Introduction

Manglietia insignis (Wall.) Blume is an evergreen tree widely distributed throughout Guizhou, Guangxi, Yunnan, and other provinces in China. This species is commonly found growing in broad-leaved forests in the form of individual plants or groups. However, pure forest or wild community with *M. insignis* as the dominant population has not been found to date (Gao, 2007). *M. insignis* trees are straight, tall, and exhibit rapid growth. The wood has the advantages of fine texture, luster, corrosion resistance, and easy processing. In addition, its flowers have a great aroma and bright color (Li, 2004) and its roots, leaves, flowers, and fruits are commonly used for medicinal purposes (Peng, 2022). *M. insignis* has, therefore, become a popular garden tree species. However, due to its high ornamental and economic value, *M. insignis* has become a major target of deforestation due to its popularity. Combined with the poor regeneration ability of its natural populations, the number of wild populations of *M. insignis* has been decreasing annually. This species has become rare and endangered and has been listed as a national third-class conserved plant in China (Chen et al., 2013). Similar to *M. insignis*, *Manglietia longipedunculata* Q. W. Zeng & Y. W. Law is an evergreen tree in the Family Magnoliaceae. *M. longipedunculata* was first discovered in 2003 by Professor Zeng Qingwen (Chinese Academy of Sciences) and his research team in Nankunshan Nature Reserve, Huizhou City, Guangdong Province, China (Zeng and Law, 2004). To date, only 11 wild plants have been identified in the Nankunshan Nature Reserve. No other populations of *M. longipedunculata* have been found in other parts of the world (Xie, 2009). This species is now listed in the red list of Magnoliaceae plants. Due to the lack of pollinators, the asynchronous development of pistil and stamen, and the extremely short stigma receptivity period, the propagation of this species is extremely challenging. In its natural state, *M. longipedunculata* only blooms and does not bear fruit. Therefore, to conserve this rare species and its population size, researchers have rapidly performed a series of in-situ and ex situ conservation measures including artificial pollination and grafting (Ren et al., 2015). At present, *M. longipedunculata* has been successfully introduced to the Nankunshan Botanical Garden in Huizhou City and Dongguan Botanical Garden in Dongguan City (China).

Due to the limitation of population size, *M. insignis* research has been limited to resource investigation (Zhu, 2021; Zou et al., 2021), seedling breeding (Yu et al., 2013; Guan et al., 2011), and medicinal ingredients (Shang et al., 2012, 2013), all requiring further investigation. In addition to some research on resource investigation (Zhang et al., 2016) and seedling breeding (Zhu, 2021), there are few reports on *M. longipedunculata* at home and abroad. Although these studies have made a positive contribution to species conservation, the evolutionary history of species, the causes of endangerment and future conservation strategies remain unclear. The study of genetic diversity is critical to conservation of endangered plants. It can reveal a species' environmental adaptability and evolutionary potential, provide a scientific basis for conservation measures and germplasm screening, and provide a material basis for

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resource use (Cires et al., 2011). However, research on the genetic diversity and structure of *M. insignis* and *M. longipedunculata* throughout the world is lacking. Genetic diversity of species elucidation began from the initial simple phenotypic identification to the cell identification but now has progressively advanced to molecular markers. Compared with the previous marker techniques, molecular markers are highly polymorphic, fast, highly efficient, and are not affected by the developmental stage of the species or the external environment (Liu et al., 2023). With the continuous progression of molecular biology research, molecular marker technology has been developed to the third generation represented by single nucleotide polymorphisms (SNPs). SNPs are widely distributed within the genome, which commonly exhibits high polymorphisms, high genetic stability, and easy automatic detection, becoming the best option for population genetic analysis and genomics (Wang et al., 2020). SNP-based molecular marker technology has been widely used in genetic diversity studies of many species (Mudaki et al., 2023; Liu et al., 2022; Nagano et al., 2022). In addition, we used Hyper-seq method, which is a novel, effective, and flexible marker-assisted selection and genotyping approach. Compared with the traditional whole-genome sequencing (WGS), Hyper-seq approach has a certain gene region enrichment effect. This approach can flexibly adjust the marker density by using different Hyper-seq primers based on the needs of different species and projects. And without additional enzyme digestion and ligation joints, through a special Polymerase Chain Reaction (PCR) method, a large number of samples can be built at the same time, and a large amount of genotype big data can be produced (Zou and Xia, 2022).

Theoretically, the genetic diversity of widely distributed species is higher than that of small and narrowly distributed species (Kaijund et al., 2010). Due to the high probability of inbreeding, unstable population structure, and genetic drift, small populations of plants will exhibit accelerated loss of genetic diversity. However, some recent studies have identified that the genetic diversity of small-scale populations with fewer individuals is higher than that of large-scale populations (Li et al., 2020; Schou et al., 2017). In addition, *M. insignis* is a relatively primitive species, occupying an important position in the study of the classification, distribution, and flora of the adjacent areas (Wang et al., 2010). Therefore, we used large-scale SNP data to investigate the genetic diversity of the widely distributed species *M. insignis* and the extremely small population of *M. longipedunculata*, and compares the differences in genetic diversity and genetic structure between the two species. Finally, we explores the reasons for the endangerment of two species, reveals their evolutionary history and genetic relationships, analyzes their evolutionary potential, and proposes protection and restoration suggestions tailored to their populations. Simultaneously, we have screened excellent germplasm resources to provide a scientific and material basis for future introduction and cultivation.

Methods

Plant materials

Leaf samples, which are all healthy and mature were collected from 22 *M. insignis* individuals from two populations and 36 *M. longipedunculata* individuals from three populations (*Fig. 1; Table 1*). Five leaves were collected from each plant. Among the three populations of *M. longipedunculata*, both ML-DG and ML-NKC were ex situ

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conservation populations, originating from ML-NKW. Due to the small size of some populations, the total number of samples with the sampling interval was balanced in order to collect as many possible representative samples. The interval between plant individuals sampled was more than ten meters. The samples were all collected from healthy and mature individuals. Fresh leaf samples were collected and quickly dried in discolored silica gel. The materials used in this study were collected and identified by associate researcher Tang Jianmin (Guangxi Botanical Research Institute, China). One sample of each population was preserved in the Herbarium of Guangxi Botanical Research Institute (China). The plant materials collected in this study are in compliance with Chinese and international guidelines and laws, including the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. One plant sample was collected from each population of *M. insignis* and *M. longipedunculata*., and all of them were preserved in the South China Botanical Garden, Guangdong, China. The specimen numbers were MI-CW-20221024, MI-YZL-20221025, ML-DG-20221105, ML-NKC-20221106, ML-NKW-20221107, respectively.

Table 1. *Sampling information of M. insignis and M. longipedunculata*

Population	Location	Type	Latitude	Longitude	No. of samples
MI-CW	Caowang Mountain, Leye County, Guangxi, China	Wild population	24°43’46”N	106°21’21”E	9
MI-YZL	Yinzhulao Mountain, Ziyuan County, Guangxi, China	Wild population	26°15’19”N	110°33’19”E	13
ML-DG	Dongguan Botanical Garden, Guangdong, China	Ex situ population	22°51’16”N	113°47’26”E	14
ML-NKC	Nankunshan Botanical Garden, Huizhou, Guangdong, China	Ex situ population	23°38’40”N	113°52’47”E	12
ML-NKW	Nankun Mountain, Huizhou County, Guangdong, China	Wild population	23°36’60”N	113°50’60”E	10

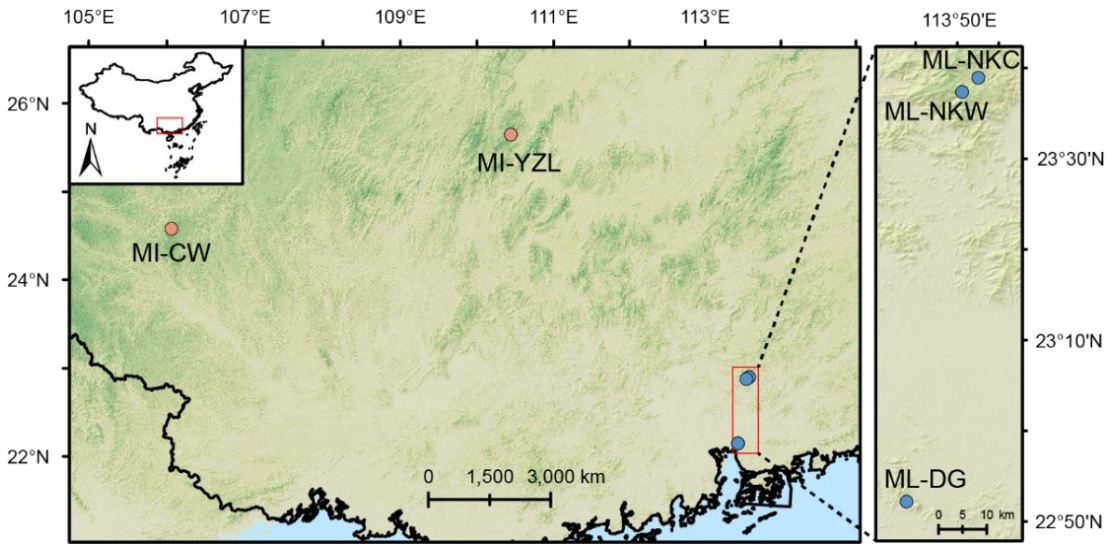

Figure 1. *Sampling sites for M. insignis and M. longipedunculata*

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Hyper-seq library construction and sequencing

E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, USA) was used to extract DNA from 22 *M. insignis* and 36 *M. longipedunculata* leaf samples according to the manufacturer's instructions. The DNA quality was assessed using 1% agarose gel electrophoresis and a Nanodrop 2000 (Thermo Fisher, USA) spectrophotometer. The extracted DNA was quantified using Qubit 3.0 (Thermo Fisher, USA) to ensure that each sample met the following criteria: total mass > 3 μg, concentration > 30 ng/μL, and OD_{260}/OD_{280} = 1.80-1.20. The barcode adaptor was then ligated and the fragment size selection and PCR amplification were performed to obtain a Hyper-seq library (Zou and Xia, 2022). The constructed library was sent for 150-bp paired-end sequencing on the Illumina Novaseq 6000 platform (Illumina, San Diego, USA).

SNP calling

FastQC (Andrews et al., 2010) was used to evaluate the quality of the original data. Trimmomatic v0.36 (set to the default parameters) (Bolger et al., 2014) was used to filter the adaptor sequences, cleavage sites (the first six base pairs of the reads), and low-quality reads (Q20 < 20, length < 36 bp) from the raw sequence data (Bolger et al., 2014). The clean reads were then mapped to the *Magnolia hypoleuca* (*Magnoliaceae*) reference genome (Zhou et al., 2023) using BWA v0.7.12 (Li and Durbin, 2009). The rm-pcr-duplicates parameter of the gstacks module in the Stacks v.2.59 software (Rochette, et al., 2019) was used to remove the PCR repeats and perform SNP calling. Vcftools v0.1.11 (Danecek et al., 2011) was used to filter SNPs, removing sites with a population loss rate of more than 40%, maf > 0.05, and a sequencing depth of less than 3.

Population genetics analysis

Based on the selected high-quality SNP data, Stacks v2.59 was used to calculate genetic diversity parameters, including observed heterozygosity (H_O), expected heterozygosity (H_E), nucleotide diversity (π), and inbreeding coefficient (F_{IS}) (Rochette, et al., 2019). The Tajima's Neutrality Test (Tajima's D) value of the site was calculated using Vcftools v0.1.11 (Danecek et al., 2011), with the sliding window size of the operation set to 500 kb. AMOVA were estimated by Arlequin v.3.5.2.2 (Yun et al., 2020).

Phylogenetic and population structure analyses

A phylogenetic tree between individuals was constructed for 22 *M. insignis* and 36 *M. longipedunculata* using the maximum likelihood (ML) method in IQtree v2.0 (Minh et al., 2020), with *M. insignis* set as the outgroup. Bayesian clustering was performed on all individuals using Admixture v1.3.0 (Alexander et al., 2009) with clustering values (K) set from 1 to 9. The optimal K value was determined based on the minimum cross-validation (CV) error.

Principal component analysis (PCA) and genetic relationship analysis.

PCA and kinship analysis among individuals were performed using MingPCACluster, a new simple and efficient software for population VCF file (Zhu et al., 2023).

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Results

SNP calling based on the reference genome

A total of 430,379 raw reads were generated from 22 *M. insignis* and 36 *M. longipedunculata* samples. After filtering, 6,596 high-quality SNP loci were identified and evenly distributed on the 19 chromosomes (*Fig. 2*).

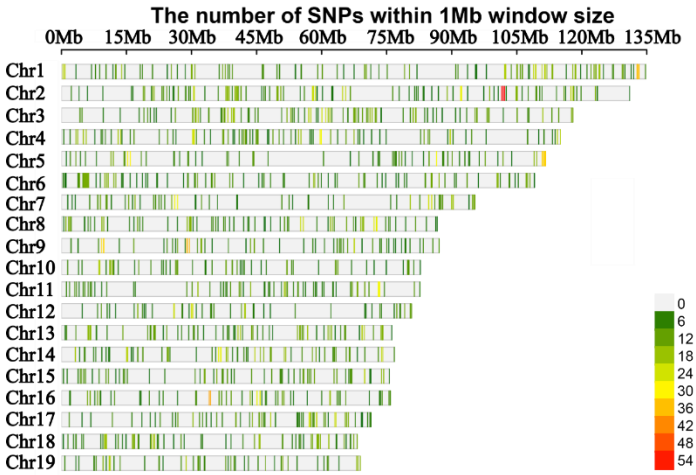

Figure 2. *The distribution of 6,596 SNPs on 19 chromosomes*

Genetic diversity

The genetic diversity of *M. insignis* and *M. longipedunculata* was calculated using 430,379 SNPs (*Table 2; Fig. 3*). Among the five populations, ML-NKC H_O was the largest, followed by ML-DG and ML-NKW, and the smallest was in MI-CW. The H_E of ML-NKC was the largest, followed by ML-DG and ML-NKW, and the smallest was in MI-CW. The ranking of the H_E in each population was consistent with the H_O. The average H_O value of the two populations of *M. insignis* was 0.1649, which was higher than that of H_E. The average H_O value of the three populations of *M. longipedunculata* was 0.2024, which was also higher than that of H_E. The mean value of π (0.1916) in the three populations of *M. longipedunculata* was greater than in *M. insignis*. The genetic diversity was evaluated using H_E and π as follows: *M. longipedunculata* (ML-NKC > ML-DG > ML-NKW) > *M. Insignis* (MI-YZL > MI-CW). Between the three *M. longipedunculata* populations, the genetic diversity of the two ex situ populations was higher than that of the wild population. These results demonstrate the significance of ex situ conservation for maintaining and improving the genetic diversity of *M. longipedunculata*.

The F_{IS} coefficient was used to assess the inbreeding level within the populations. Among the two *M. insignis* populations, the F_{IS} coefficient of one population (MI-CW, F_{IS} = 0.0226) was greater than 0, while the other (MI-YZL, F_{IS} = -0.0507) was less than 0. Among the three *M. longipedunculata* populations, only ML-DG (F_{IS} = 0.0080) F_{IS} was greater than 0, while the other two populations (ML-NKC and ML-NKW) were less than 0. This suggests that selfing or inbreeding between individuals may be occurring in the MI-CW population of *M. insignis* and the ML-DG population of *M. longipedunculata*, demonstrating the loss of heterozygosity phenomenon. The results of

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Tajima's D calculation of *M. insignis* and *M. longipedunculata* were positive at 0.3039-0.3833 and 0.0440-0.3513, respectively (*Fig. 3*). These results suggest that the rare allele frequency in these two populations was low and may be under balanced selection pressure or experiencing population shrinkage.

Table 2. *Genetic diversity of five M. insignis and M. longipedunculata populations*

Pop ID	Private SNP	H_O	H_E	π	F_{IS}	Tajima's D
MI-CW	119	0.1485	0.1421	0.1554	0.0226	0.3039
MI-YZL	365	0.1812	0.1426	0.1528	-0.0507	0.3833
Mean value		0.1649	0.1424	0.1541	-0.0141	0.3436
ML-DG	18	0.1890	0.1797	0.1894	0.0080	0.2404
ML-NKC	30	0.2385	0.1990	0.2121	-0.0495	0.3513
ML-NKW	23	0.1826	0.1591	0.1733	-0.0112	0.0440
Mean value		0.2024	0.1793	0.1916	-0.0176	0.2119

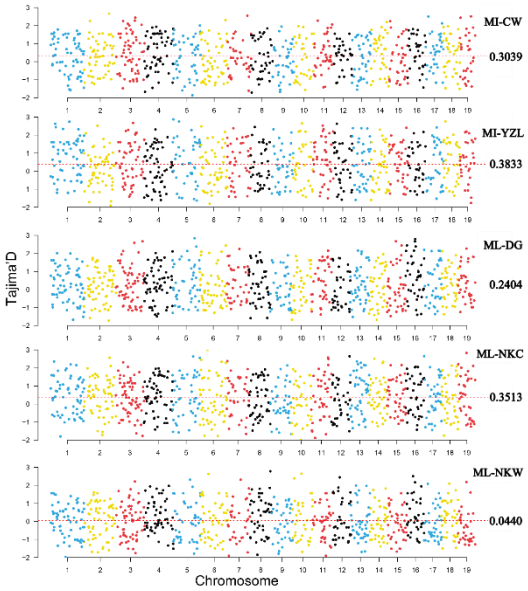

Figure 3. *The distribution of Tajima's D values in five M. insignis and M. longipedunculata populations*

Genetic differentiation and molecular of variance analysis (AMOVA)

The genetic differentiation (F_{ST}) of the population was preliminarily established according to the genetic differentiation coefficient between the populations (*Table 3*). The genetic distance between *M. insignis* and *M. longipedunculata* populations was large, as was the degree of differentiation. The F_{ST} of the MI-CW population and three populations of *M. longipedunculata* were lower than those of the MI-YZL population and three populations of *M. longipedunculata*, suggesting that MI-CW population of *M. insignis* was closer to *M. longipedunculata* than the MI-YZL population. Molecular of variance analysis (AMOVA) showed that 85.08% of genetic variation was from within individuals, 11.43% of genetic variation was from among populations, and the only 3.49% of variation was from among individuals, indicating that the genetic variation

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within individuals was the main source of the variation of *M. longipedunculata* (*Fig. 3a*). Genetic variation within individuals was 50.25%, genetic variation among populations was 42.25%, and genetic variation among individuals was only 7.5%, indicating that genetic variation within individuals was also the main source of variation in *M. insignis* (*Fig. 3b*).

Table 3. *Coefficient of genetic differentiation among M. insignis and M. longipedunculata populations*

	MI-CW	MI-YZL	ML-DG	ML-NKC	ML-NKW
MI-CW		0.141	0.168	0.170	0.189
MI-YZL			0.234	0.236	0.251
ML-DG				0.051	0.065
ML-NKC					0.073

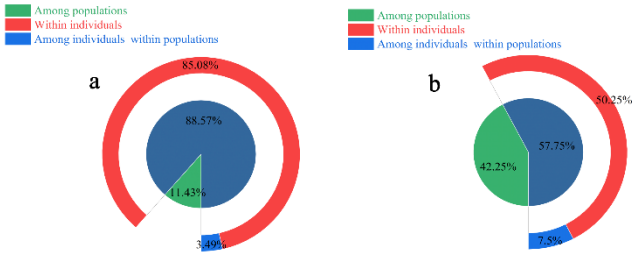

Figure 3. *Analysis of molecular variance (AMOVA) partitioning of molecular variance among populations, within individuals and among individuals within populations of M. longipedunculata (a) and M. insignis populations (b)*

Population structure

The most direct manifestation of genetic diversity is the level of genetic variation. However, a single individual of a species has a limited lifespan and can be susceptible to external factors. A continuous population composed of individuals is therefore a unit of genetic evolution. The specific distribution pattern of these types of populations in nature is the genetic structure of the population. The mixed analysis results demonstrated that K = 2 was the best clustering result among all the samples of *M. insignis* and *M. longipedunculata* (*Fig. 4*). When K = 2, the CV error value was the lowest, and the two species were obviously separated (*Fig. 5*). Since the two *M. longipedunculata* ex situ populations were derived from wild populations (ML-NKW), some individuals were not separated according to population differences but exhibited some individual confounding phenomena. All individuals within the two populations were separated according to population differences. It is worth noting that some individuals (ML-CW-2, 3, 7, 8, 9) in the *M. insignis* ML-CW population were mixed, suggesting that a small number of genetic components of *M. longipedunculata* were mixed in the MI-CW *M. insignis* population. Furthermore, the *M. insignis* ML-CW-7, 8, and 9 individuals were the first to be clustered with *M. longipedunculata* in the phylogenetic tree. This demonstrates that *M. longipedunculata* may have a certain relationship with *M. insignis* in its genetic evolution.

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When K = 3, all *M. longipedunculata* individuals still clustered into one group (*Fig. 5*). While the individuals of the two populations were clustered into one group after the separation of *M. longipedunculata* and *M. insignis*, two of the populations did not show a common genetic component. Two individuals (ML-CW-7 and 8) from the *M. insignis* ML-CW population were also mixed with *M. longipedunculata* genetic components. When K = 4, most *M. longipedunculata* individuals were divided into three categories according to different populations (*Fig. 5*). However, a small number of individuals was separated from the population and clustered with other populations. A few *M. longipedunculata* individuals (ML-NKC-5, 6, and ML-NKW-4) exhibited same genetic components of other *M. longipedunculata* populations, but all same genetic components within the population. All *M. insignis* individuals clustered into one group and individuals (ML-CW-2, 3, 7, 8, and 9) in the ML-CW population were mixed with *M. longipedunculata*. Furthermore, ML-CW-7 and 8 individuals contained mixed genetic information from the three *M. longipedunculata* populations.

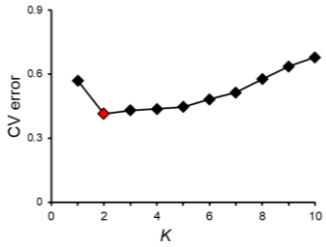

Figure 4. *CV error distribution for K from 1 to 10. K with the smallest CV value is marked in red*

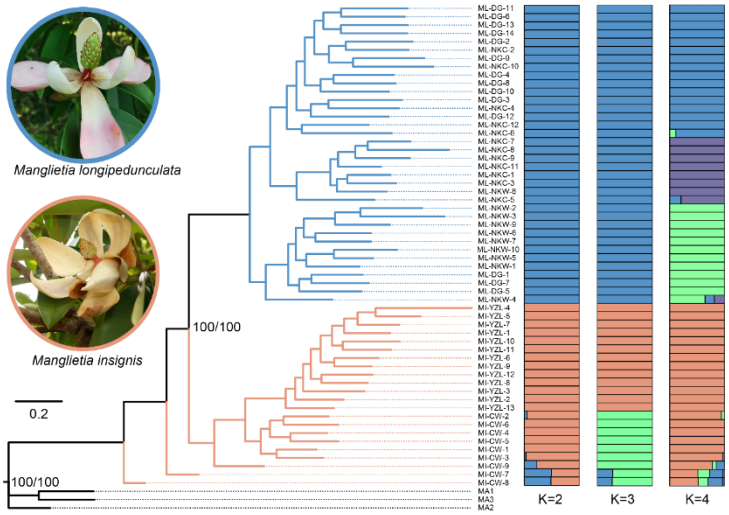

Figure 5. *The maximum likelihood phylogenetic tree for 22 M. insignis and 36 M. longipedunculata*

Principal component analysis (PCA) and genetic relationship analysis

According to the two-dimensional PCA diagram (*Fig. 6*), the contribution rates of the first and the second principal components were 31.15 and 7.34%, respectively. The position distance in the two-dimensional map demonstrated the distance of the genetic

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relationship between the germplasm resources of the *M. insignis* and *M. longipedunculata* populations. The results showed that *M. longipedunculata* and *M. insignis* were obviously separated (*Fig. 6*). The two *M. insignis* populations exhibited considerable separation, with the MI-YZL in the first quadrant and the MI-CW in the third and fourth quadrants, likely due to the geographical distance between the two populations. The three *M. longipedunculata* populations exhibited a small degree of separation and close genetic distance, and all individuals were mixed, which may be related to all individuals originating from the same population. Furthermore, PCA results demonstrated that some ML-CW individuals tended to move closer to *M. longipedunculata*, suggesting the presence of a genetic evolutionary relationship between the two populations, consistent with the genetic structure analysis results.

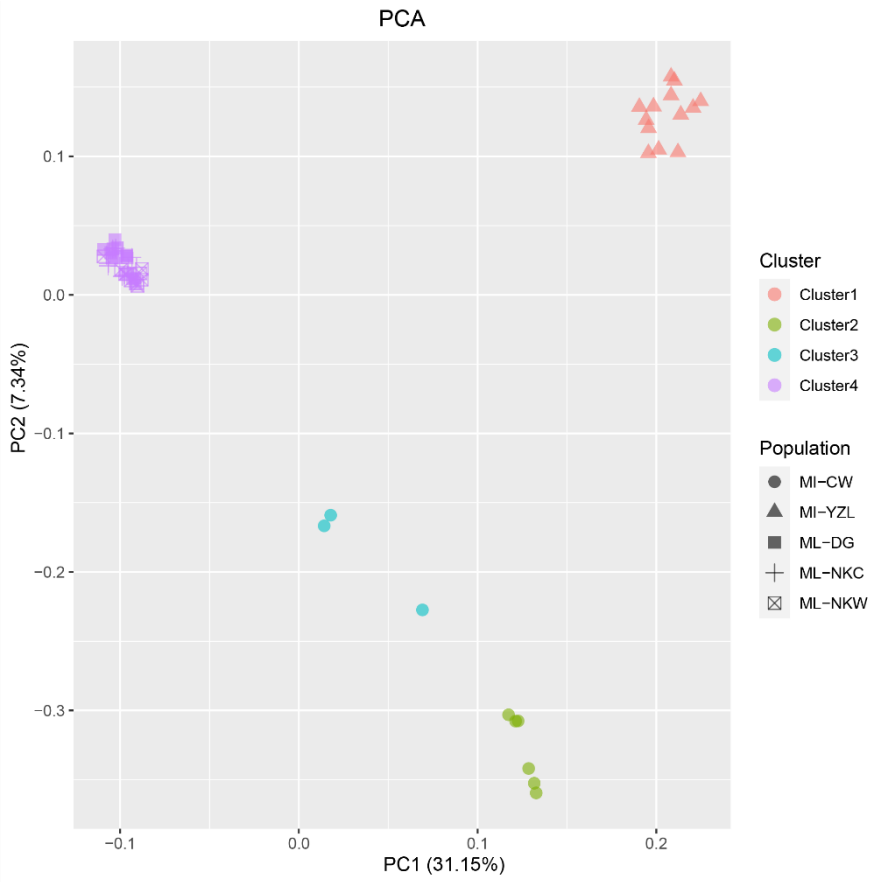

Figure 6. *PCA between M. insignis and M. longipedunculata individuals*

The genetic relationship analysis of all *M. insignis* and *M. longipedunculata* individuals demonstrated that *M. insignis* was largely divided into two categories according to the genetic relationship (*Fig. 7*). Most individuals in the MI-CW population were clustered into one group, as were all individuals in the MI-YZL population. In addition, the MI-CW-7, 8, and 9 individuals in the MI-CW population exhibited considerable separation from most of the MI-CW individuals, suggesting significant differences in the genetic relationship between the two populations. Furthermore, the three MI-CW-7, 8, and 9 individuals demonstrated an evident genetic relationship. In the *M. longipedunculata* genetic relationship analysis, most individuals

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of the three populations were generally divided into three categories according to the differences in populations. Some individuals however were mixed into other populations. Overall, the genetic distance between all individuals of the three *M. longipedunculata* populations was not significantly different. The genetic differentiation was not obvious when compared with *M. insignis*. This was consistent with the PCA results.

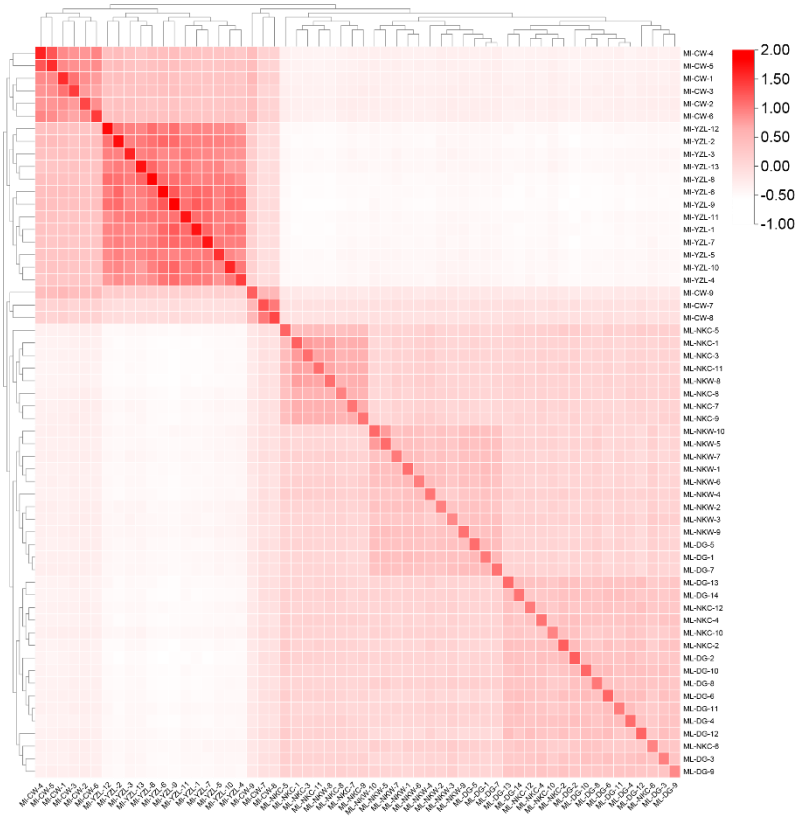

Figure 7. *The genetic relationship between M. insignis and M. longipedunculata individuals*

Discussion

Comparison of genetic diversity between M. insignis and M. longipedunculata

Genetic diversity is the basis of species adaptability and evolutionary potential (Du et al., 2023). Generally, higher genetic diversity of the species results in greater adaptability to the environment, decreased influence of the external environment, and increased evolutionary potential. On the contrary, the evolutionary potential is reduced with lower genetic diversity of the species, increasing the vulnerability to environmental factors (Nybom, 2004). In this study, a large-scale genomic analysis of SNP loci was performed for the first time to evaluate the genetic diversity of *M. insignis* and *M. longipedunculata*. The results showed that the genetic diversity of *M. insignis* ($\pi = 0.1541$) was lower than that of *M. longipedunculata* ($\pi = 0.1916$). On one hand, this phenomenon may be due to *M. insignis* ecology and habitat has undergone great changes in recent years despite having accumulated rich genetic variation over the long-term evolution process. In addition, due to excessive anthropogenic deforestation and natural disasters, its distribution has become fragmented with the individual numbers

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continually decreasing, leading to a decrease in the genetic diversity of its population. On the other hand, due to the continuous habitat fragmentation of *M. insignis*, its original wide and continuous distribution has been broken up. This reduces the species adaptability, causing some populations to completely disappear, increasing the inbreeding rate in the residual population, and resulting in the disappearance of some alleles, which in turn leads to the continuous reduction of *M. insignis* genetic diversity. In addition, this study only collected samples from two wild populations of *M. insignis*. The low sampling rate or one-sidedness may also cause the low results. Although *M. longipedunculata* population was small, high genetic diversity was still maintained. A previous study demonstrated that the high genetic diversity of small populations is dependent on frequent gene flow (Cai et al., 2020). Similar phenomena have been reported in extremely small populations of *Camellia dongxingensis* (Tang et al., 2020), Chinese wild apricot (Wang et al., 2014), and other species.

Many studies have found that the ability of some rare and endemic species to maintain high genetic diversity is closely related to the evolutionary history of species, the maintenance of genetic diversity of glacial refuge population, the regional characteristics of distribution area, and the ecological habits of species including the mating system (Soares et al., 2018). Therefore, the potential effects of these factors on the genetic diversity of *M. insignis* and *M. longipedunculata* were comprehensively analyzed in the current study. The Tajima's D values of the five populations of these two species were positive, suggesting that the frequency of rare alleles in these two species was low and the existing populations may be under balanced selection pressure or have already experienced population shrinkage. The inbreeding ($F_{IS} > 0$) was detected in the *M. insignis* MI-CW population, while in the *M. longipedunculata* population, the wild population (ML-NKW) specifically was dominated by outcrossing ($F_{IS} < 0$). In the case of limited pollinators, selfing is one of the most effective strategies for species to maintain or increase population size (Kalisz, 2004). Although the outcrossing-based mating system of *M. longipedunculata* limits the rapid population expansion, it ensures the high genetic diversity of the population and reduces the risk of species extinction due to any future changes in the habitat. Despite the small size of the wild *M. longipedunculata* population, selfing was not detected. Therefore, it is likely that in natural habitat, there may be self-incompatibility and partial asexual reproduction in *M. longipedunculata*, eventually leading to heterozygous and homozygous deletions within the population (Stoeckel et al., 2006; Wang et al., 2022). However, further research is required via experimental analyses such as artificial pollination.

When the raw habitat of species is destroyed, the measures of ex situ migration and conservation are called 'ex situ conservation' (Cibrian et al., 213). However, due to the lack of complete sampling, unclear resources, and repeated sampling during the implementation of the ex situ conservation plan, the isolated populations established by the ex situ conservation often have the risk of loss or reduction in genetic diversity (Wei and Jiang, 2021). The genetic diversity and population structure information of ex situ populations is one of the most important reference indices to evaluate the success of ex situ conservation. If the genetic diversity of the ex situ population is higher than or generally consistent with the genetic diversity of the wild population, the conservation measures are considered successful (Su et al., 2017). The genetic diversity ($\pi = 0.1894$, 0.2121) in this study of the two ex situ conservation populations of *M. longipedunculata* was higher than that of its wild population ($\pi = 0.1733$). Therefore, the two artificial ex

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situ conservation populations of ML-DG and ML-NKC can effectively conserve the genetic diversity of *M. longipedunculata* species and fully represent the genetic diversity of its wild populations. The ex situ conservation of *M. longipedunculata* individuals largely originated from the seeds collected from the sexual reproduction of wild populations (ML-NKW). In addition, the mating strategy of the wild population based on outcrossing ensures frequent gene exchange. This may be one of the reasons why the genetic diversity of ex situ conservation populations was higher than in the wild populations. Therefore, the ex situ population can be used as the key application material in future genetic breeding work. In this study, the average H_E of *M. insignis* and *M. longipedunculata* was 0.1424 and 0.1793, respectively. This value was significantly lower than in other *Magnoliaceae* species, such as *Manglietia conifera* (H_E = 0.66) (Wen et al., 2017), *Kmeria septentrionalis* (H_E = 0.69) (Lin, 2012), indicating that the genetic diversity of these two *Manglietia* species was low and the genetic basis was narrow, which may be related to the small natural distribution range of these two plant species.

Comparison of genetic structure and differentiation between M. insignis and M. longipedunculata

The investigation of the genetic structure of species is very important for the conservation of species and the formulation of conservation strategies (Pan et al., 2022; Zhu et al., 2023). The *M. longipedunculata* and *M. insignis* clustering map demonstrated a few individual confounding phenomena between the two populations. However, due to the geographical distance between the two populations, there may be two reasons for this phenomenon. On the one hand, a population of *M. insignis* was located near *M. longipedunculata* and there is gene exchange between the two groups. On the other hand, combined with phylogenetic tree clustering analysis, *M. longipedunculata* may be derived from the genetic differentiation of some individuals in *M. insignis*. This hypothesis is also supported by PCA and genetic relationship analysis between individuals. These two analyses demonstrate that some individuals in the MI-CW population of *M. insignis* tend to gradually approach *M. longipedunculata*. Therefore, in future breeding studies, these species with close genetic relationships can be first selected to improve the success rate of the study. Hybridisation experiments are of great significance to the protection of endangered plants. From an evolutionary perspective, such experiments can increase the level of genetic diversity within species, thereby improving their environmental adaptability and survival ability. Furthermore, they can facilitate the recovery of endangered plant populations. From a scientific standpoint, hybridization experiments are instrumental in the study of genetic information, including the evolution and classification of endangered plants, as well as the formulation of scientific protection measures.

In addition, the F_{ST} was also used to measure the distance of genetic relationships or the degree of genetic differentiation (Holsinger and Weir, 2009). The genetic differentiation coefficients of the two populations of *M. insignis* and the three populations of *M. longipedunculata* were different, and the value of MI-CW was lower, suggesting that the population was closely related to *M. longipedunculata*. Previous studies have demonstrated an absence of differentiation among subgroups in populations with F_{ST} values between 0 and 0.05. If the F_{ST} value was 0.05 to 0.15, it was moderately differentiated. High differentiation was considered if the F_{ST} value was between 0.15 and 0.25 (Wright, 1972). The results of this study showed that there was

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moderate differentiation between the two populations (F_{ST} = 0.141), similar to a high degree of differentiation. The AMOVA analysis results indicate that although within individual variation is the main source of genetic variation, 42.25% of genetic variation is still between populations, demonstrating the large genetic differentiation between *M. insignis* populations. *M. insignis* is an insect-pollinated plant that is limited in being able to transfer pollen over long distances. Geographical distance is a significant factor in gene flow between populations, which may be the reason for the considerable genetic differentiation between *M. insignis* populations. The three *M. longipedunculata* populations (F_{ST} = 0.051, 0.065, 0.073) also belonged to moderate differentiation, with almost no differentiation between the two ex situ conservation populations. The degree of genetic differentiation between the wild and ex situ conservation populations was higher than that of the two ex situ conservation populations.

AMOVA analyses indicated that genetic variation of *M. longipedunculata* populations was mainly derived from individuals (85.07%), significantly higher than that among populations (11.43%) and among individuals (3.49%). This could be due to the fact that the individuals of the two ex situ conservation populations (ML-DG, ML-NKC) were derived from the same wild population (ML-NKW). What but is not allow to neglect, there is still 11.43% genetic variation between the populations, indicating that the ex situ conservation populations of *M. longipedunculata* have gradually developed genetic differentiation from the wild populations as they have adapted to the local ecological environment. The degree of differentiation between ex situ conservation and wild populations and between ex situ conservation populations will gradually increase (Cibrian et al., 2013). Furthermore, the adaptability of ex situ conservation species to the native habitat will gradually be lost, eventually delaying the subsequent wild regression experiments of the species (Enßlin et al., 2011). Therefore, to avoid this putative risk, an increase in the gene exchange between the ex situ conservation and the wild populations is necessary via artificial pollination, systematic updates of the ex situ conservation population gene diversity, and finally ensuring that the ex situ population maintains the adaptability to the native environment.

Conservation recommendations

The results of this study demonstrated that the genetic diversity of *M. insignis* was low. In addition to ensuring that the population was able to self, the number of individuals was also an important factor due to the continuous reduction of anthropogenic activities. It is, therefore, necessary to improve the investigation of the plant resources and appropriately conserve the native environment of the large-scale population and the ex situ conservation of the small-scale population. The conservation of species does not only provide conservation for a single group but for different groups or different genotype groups. In regard to multi-population species, the screening of excellent germplasm resources should not only improve the conservation and management efficiency of germplasm resources but also reduce costs (Miao et al., 2016). However, the genetic information of only two *M. insignis* populations was investigated. The sampling range in future studies should be expanded to accurately screen out excellent germplasm resources. In addition, a one-way mixing phenomenon was identified between the two species. These two species should avoid proximity in ex situ conservation to ensure the purity of genetic information for the species. Due to the geographical distance between the two species, this gene mixing phenomenon can not only explain the possible evolutionary relationship between the two species but also

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more importantly, shows that there may be an *M. longipedunculata* population in Caowang Mountain, Leye County, Baise City (China). Therefore, the species investigation in this area should be accelerated and the corresponding conservation should be rapidly established. The inability to rapidly expand the population is due to the self-pollination mating strategy. Therefore, the primary task should be to conserve the wild seedlings in situ or ex situ and expand the population size based on maintaining the excellent characteristics of the female parent via tissue culture, cuttings, and other asexual reproduction methods, to provide a solid material basis for future introduction and breeding.

Two ex situ conservation populations with high genetic diversity can therefore be utilized for breeding improved varieties. Although the ex situ conservation duration of *M. longipedunculata* was relatively short, the degree of differentiation between *M. longipedunculata* and wild populations has gradually increased. Therefore, to ensure the adaptability of the species to the native habitat and the success of the subsequent introduction and regression experiments, it is necessary to identify the genetic diversity of the ex situ conservation population and increase the gene exchange between the ex situ and the wild populations in time via artificial pollination and other breeding methods.

Conclusions

In this study, Hyper-seq method was used to sequence a total of 58 samples from two wild populations of *M. insignis* as well as one wild population and two ex situ conservation populations of *M. longipedunculata*. A total of 6,596 high-quality SNP loci were detected. The genetic diversity, population genetic structure, and phylogenetic tree of the two populations were constructed. The results showed that the genetic diversity of the widely distributed *M. insignis* was lower than that of the narrowly distributed *M. longipedunculata*. This may be related to the significant anthropogenic destruction of *M. insignis* habitat. In addition, although the *M. longipedunculata* population was small, high genetic diversity was still maintained. The results of this study demonstrate that the individuals were primarily outcrossing, which may be the reason for this phenomenon. The genetic diversity of the two ex situ conservation populations was higher than that of their wild populations, suggesting that the ex situ conservation can effectively conserve the genetic diversity of *M. longipedunculata* and this population can be used for future breeding. In addition, the results of the population genetic structure of the two populations demonstrated mixed genetic components between the two populations. This material could be preferred for future cross-breeding testing. Since only two populations of *M. insignis* were selected for genetic diversity investigation, the results were not comprehensive enough. Therefore, the sampling range should be expanded in the future to screen out high-quality germplasm resources and accelerate the conservation of these species.

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Conflict of interests. The authors declare no competing interests.

Data availability. Raw sequencing data are available at NCBI with the SRA accession number of PRJNA1037033
(https://dataview.ncbi.nlm.nih.gov/object/PRJNA1037033?reviewer=pjnht5rev90an34aft2ojnjfti).

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