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Genetic variability and population divergence of *Rhododendron platypodum* Diels in China in the context of conservation

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Genetic diversity in endangered species is of special significance in the face of escalating global climate change and alarming biodiversity declines. *Rhododendron platypodum* Diels, an endangered species endemic to China, is distinguished by its restricted geographical range. This study aimed to explore genetic diversity and differentiation among its populations, gathering samples from all four distribution sites: Jinfo Mountain (JFM), Zhaoyun Mountain (ZYM), Baima Mountain (BMM), and Mao'er Mountain (MEM). We employed 18 pairs of Simple Sequence Repeat (SSR) primers to ascertain the genetic diversity and structural characteristics of these samples and further utilized 19 phenotypic data points to corroborate the differentiation observed among the populations. These primers detected 52 alleles, with the average number of observed alleles (N_a) being 2.89, the average number of effective alleles (N_e) being 2.12, the average observed heterozygosity (H_o) being 0.57, and the expected heterozygosity (H_e) being 0.50. This array of data demonstrates the efficacy of the primers in reflecting *R. platypodum*'s genetic diversity. SSR-based genetic analysis of the populations yielded H_o , H_e , and Shannon index (I) values ranging from 0.47 to 0.65, 0.36 to 0.46, and 0.53 to 0.69, respectively. Notably, the ZYM population emerged as the most genetically diverse. Further analysis, incorporating molecular variance, principal component analysis, UPGMA cluster analysis, and structure analysis, highlighted significant genetic differentiation between the Chongqing (BMM, JFM, ZYM) and Guangxi (MEM) populations. Morphological data analysis corroborated these findings. Additionally, marked genetic and morphological distinctions were evident among the three Chongqing populations (BMM, JFM, and ZYM). This suggests that, despite the observed regional differentiation, *R. platypodum*'s overall genetic diversity is relatively constrained compared to other species within the *Rhododendron* genus. Consequently, *R. platypodum* conservation hinges critically on preserving its genetic diversity and protecting its distinct populations.

KEYWORDS

biodiversity protection, plant conservation, endangered plant species, simple sequence repeat, genetic structure, phenotype differentiation

1 Introduction

Conserving endangered plant species is crucial to the survival of biological diversity and ecosystem stability (Torres-Díaz et al., 2021; Chen et al., 2022; Flores-Argüelles et al., 2022; Harrington et al., 2023; Singh et al., 2023). *Rhododendron platypodum* Diels is a rare and endangered plant species that has a limited distribution and is one of those species that requires urgent attention (Ma et al., 2021). Human activities continue to affect natural habitats (Arif et al., 2023; Boast et al., 2023) and understanding the genetic diversity and population differentiation of endangered plants such as *R. platypodum* becomes essential for effective conservation strategies. Scholars intended to provide an overview of the importance of genetic diversity (Planchuelo et al., 2020). Other researchers (Ma et al., 2021) have also delved into the ecological and conservation aspects of *R. platypodum*, with the primary objective of comprehending its genetic composition.

Adaptability, resilience, and the long-term survival of species are inextricably linked to genetic diversity within populations (Jiajia et al., 2023). Populations can withstand environmental changes, diseases, and other stressors if their gene pool is diverse. The evolution of new traits and adaptations is also facilitated by genetic variation (Ye et al., 2020). Loss of genetic diversity can lead to species being less able to adapt to changes in their environment, making them more vulnerable to extinction consequently (Johnson and Molano-Flores, 2023; Naz et al., 2024). Therefore, assessing the genetic diversity of endangered species is essential for developing effective conservation measures. *R. platypodum* is commonly known as the Flat Pod *Rhododendron* and is an endemic species that is found only in certain regions of the world (Ma et al., 2021). It has distinct ecological characteristics. Human disturbances such as habitat fragmentation, deforestation, and land development are often the cause of it thriving in disturbed habitats (He et al., 2023). It is particularly vulnerable to the impacts of these anthropogenic activities due to its limited distribution and habitat requirements (Zheng et al., 2023). It is well known for its ornamental value, but habitat loss and overexploitation have threatened the species' survival.

It is estimated that there are approximately 850 *Rhododendron* species in the world, most of which grow in high-altitude subtropical areas. In China, *Rhododendron* forms distribution centers in Yunnan, Sichuan, and Xizang provinces (Wang et al., 2017; Yu et al., 2017). *Rhododendron* plants are recognized as the queen of flowers because of their large flowers, unique shapes, and vibrant colors. This makes them valuable for conservation and development (Mao and Gogoi, 2012; Shen et al., 2015). Genetic diversity can be effectively conserved for endangered plants by understanding its distribution within and between populations (Kwiecinska-Poppe et al., 2020; Zizhuo et al., 2022). The diversity of morphological traits can be used to reveal variations in phenotypic traits among populations within a distribution area. This is an important aspect of genetic diversity research (Zhao et al., 2012). The use of simple sequence repeat (SSR) markers for analyzing genetic diversity can provide more direct evidence. The genetic diversity of several *Rhododendron* species has been studied so far, including *R. vialii* (Yang et al., 2020), *R. molle* (Zhang et al., 2021), and *R. dauricum* (DePristo et al., 2011).

R. platypodum is an evergreen shrub. The plant grows on rocks or in dense forests between the elevations of 1820 and 2,130 m above sea level (Hong, 1983). This species was previously listed as vulnerable on the Red List of *Rhododendrons* (Gibbs et al., 2011) and the International Union for Conservation of Nature (IUCN) (Ma et al.,

2021). The species *R. platypodum* has been classified as endangered after a reassessment of its endangerment (Zizhuo et al., 2022). *R. platypodum* has very limited resources, insufficient competitiveness, a narrow distribution range, and weak renewable resources (Huang et al., 2020; Zizhuo et al., 2022). At present, this species is only known to occur on Jinfo Mountain (JFM), Zhaoyun Mountain (ZYM), and Baima Mountain (BMM) in Chongqing, and Maoer Mountain (MEM) in Guangxi, China. Research has previously characterized the MEM population in terms of conservation, but the population is now uniformly classified as having *R. Platypodum* in it (Gibbs et al., 2011). Nevertheless, high-intensity human disturbance has adversely affected *Rhododendron* survival and reproduction, indicating the need for immediate protection and research.

Therefore, we selected four populations of *R. platypodum* in this study from JFM, ZYM, and BMM in Chongqing and MEM in Guangxi. Furthermore, morphological characteristics and SSR markers were analyzed to determine genetic diversity and population phenotypic structure. In addition, a comparative analysis of populations was carried out simultaneously. This study assesses the genetic diversity and population differentiation of *R. platypodum* to inform conservation strategies for its long-term survival. Specifically, the study aims to: (Chen et al., 2022) evaluate genetic diversity within and among *R. platypodum* populations using molecular markers; (Torres-Díaz et al., 2021) investigate the patterns of population differentiation and gene flow between different populations of *R. platypodum*; and (Harrington et al., 2023) identify potential genetic bottlenecks or inbreeding effects that might limit the species' genetic diversity. Conservation strategies for endangered species such as *R. platypodum* require understanding genetic diversity and population differentiation. It is vitally needed to understand the genetic makeup of this species to preserve its genetic variability, enhance its resilience, and ultimately prevent its extinction. This study provides valuable insights into the phylogenetic status of *R. platypodum* and provides a foundation for future conservation action.

2 Materials and methods

2.1 Materials

The collection of materials was systematically executed across all *R. platypodum* natural distribution sites, including JFM, ZYM, BMM, and MEM, from April to May 2020 (Figure 1). Each distribution site underwent meticulous sampling, adhering to a minimum distance criterion of 15 meters. This distance was determined based on the spatial distribution of individual plants within the respective sites. Due to the inherent challenges posed by the limited population of *R. platypodum* and the arduous accessibility associated with plants growing on cliff rock walls, a total of 61 samples were acquired from these sites, with each site ranging between 8 and 20, as delineated in Table 1. The selection process for molecular analysis necessitated the procurement of three to five leaves from healthy adult plants. These leaves were meticulously inspected to ensure pest and disease freedom. These collected leaves were subsequently preserved using silica gel after being thoroughly dried. In tandem with the molecular analysis, morphological assessment required the collection of branches and inflorescences. These botanical components were promptly sealed within zip-lock bags and stored within an icebox, ensuring their preservation and integrity for subsequent measurements.

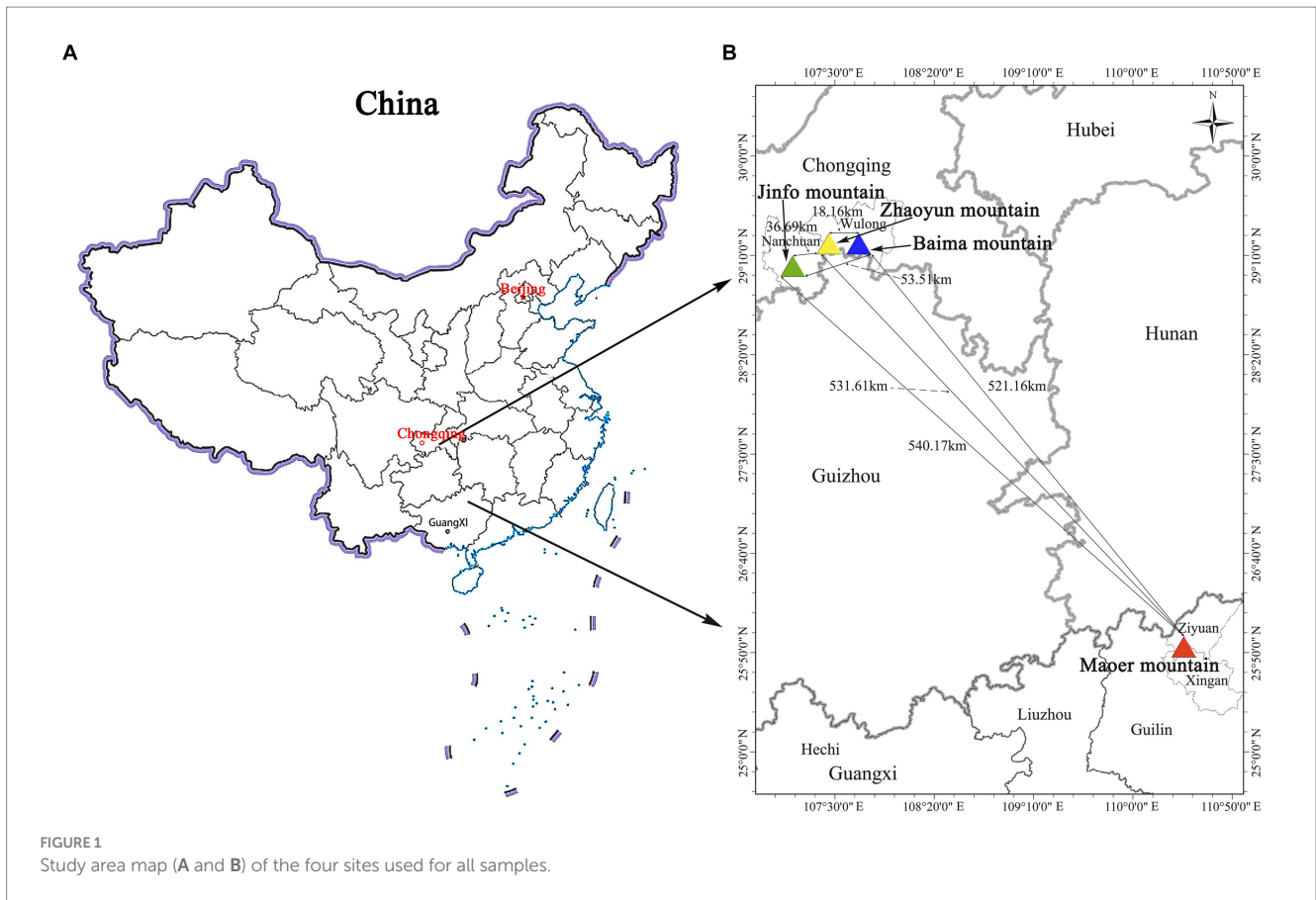


FIGURE 1 Study area map (A and B) of the four sites used for all samples.

TABLE 1 The sampling information for this study includes the sampling site location, population number, population distribution range, and biotope.

Population code	Sampling sites	Number of samples	Latitude and longitude	Altitude (m)	Population area (ha)	Biotope
JFM	Jinfo Mountain, Chongqing	20	N29°01'18.88" E107°11'11.18"	2041–2,130	7.8	Ridge, cliff
ZYM	Zhaoyun Mountain, Chongqing	20	N29°11'52.87" E107°27'32.06"	1733–1882	22.1	Ridge, cliff
BMM	Baima Mountain, Chongqing	8	N29°12'28.21" E107°36'52.36"	1704–1749	0.1	Ridge, cliff
MEM	Maoer Mountain, Guangxi	13	N25°51'51.17" E110°25'04.42"	2020~2095	0.9	Ridge, slope

2.2 Total DNA extraction and screening of simple sequence repeat (SSR) primers

The total DNA from each sample was extracted using the CTAB method (Jinlu et al., 2013), and the concentration of each sample was determined using Nanodrop. OD260/280 ratios were used to determine the quality of DNA.

In accordance with the published SSR study on *Rhododendron* sect. *Ponticum* G. Don (Jinlu et al., 2013; Kaiyun et al., 2020), 68 pairs of SSR primers were synthesized, and 18 pairs were selected for the experiment (Table 2). We used a reaction volume of 10 µL for the PCR amplification. As part of this system, 0.2 µL DNA template, 0.1 µL Taq enzyme, 0.2 µL dNTP mixture, and 0.5 µL of forward and reverse primers were used.

We carried out the following reaction procedures: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 100 s for 35 cycles, and finally extension at 72°C for 10 min. Electrophoresis of PCR products was performed on an 8% polyacrylamide gel for 30–40 min at 350 V. The gel was stained with a 2% silver nitrate solution for 10–15 min before being photographed.

2.3 SSR-based analysis of genetic diversity and genetic structure

PCR amplification and genotyping of all samples were performed according to the steps, using SSR primers for PCR amplification and

TABLE 2 Simple sequence repeat (SSR) primer sequences used in the present study.

Primer No.	Primer sequences		Repeat Motif	T _m (°C)
	Forward primers	Reverse primers		
A1	CCCCGACCGTTCCTAAAT	ATGACGATGTCGAGCTGGTC	(CT) 8	61
A2	GGCCTCCTTAGGGTTCCA	CCTTTGGCAACGAAACAGGG	(TC) 8	62
A13	AGGGAGAAGGAGAACGGTT	AACGGGACAGAGAAAAACCT	(GGA) 5	60
A16	CAGAGCTGGACTGAGTGAGC	GAACAATCGACTGGGACCGT	(CT) 9	59
A19	GGCTGCCTCTCTGAAACTT	GGTATACGTCAGTCCACC	(GAG) 5	58
A20	ATACAAGCCCAACTCCGGTG	CGCCTCGAAGAACCAATCT	(TC) 7	62
A21	CCTTGCTCCATTGGGCAT	TCAGAGCTTGCTTGGCTTGA	(GTC) 5	62
A26	GTTATCAGCGCTCCCCTCTC	ACACGAACCCAAACCATGA	(TG) 6	61
A39	CAATCAAGGGGCTACCATGT	CGAAAGTTGGTGGTATCCGT	(AGG) 6	57
A40	AGTGGACAGTGAGTACACC	TCGGATGAATTGCGTTGTAA	(TGT) 5	60
A42	CCTGAATCCATCCTGTCCTG	GCTGAGGGATCACCAGACAT	(GA) 8	56
A44	GGAAGTTTCGGCAGCAGTAG	CCTTCTCCAACTCCCTTTC	(GGT) 5	56
A53	AACCAGTACAGGACGCCAAG	CTCCCGAGAAGATCAAGCAG	(GCG) 5	56
A55	CCTCCTCTCGCATCTTCTC	CACTGCCATCTCTCACTCCA	(TCG) 5	56
A56	AAACCTCTCGCTCTTCCC	GGTGTGGTCTTCATGGTTT	(CT) 7	56
A58	GGTGGATCAGAAGGGACTGA	ACATGAAGATCATGGGCGAT	(CTT) 5	55
A64	ATGGATCGTCTGGACGAAG	AAGGCCACTAGAAGAAGCCC	(CT) 8	60
A67	GGGGTAGGGGATACTTTGA	GTCGACGACTTTGGTCCAGT	(GCT) 6	55

development. All samples produced effective PCR bands. Utilizing the software tools POPGENE 32 (Yeh et al., 1997) and GenAIEx ver. 6.5 (Peakall and Smouse, 2006), an array of crucial genetic parameters were computed, encompassing the count of observed alleles (N_a), the tally of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), the Shannon index (I), the inbreeding coefficient (F_{is}), and Nei's genetic distance. Additionally, the polymorphic information content (PIC) was ascertained using Cervus 3.0.7, and the assessment of adherence to Hardy–Weinberg equilibrium (HWE) was conducted (Slate et al., 2000). This multifaceted analysis contributes to a comprehensive exploration of the genetic dynamics under consideration. The genetic differentiation coefficient (F_{st}) and gene flow (Nm) were calculated using GenAIEx version 6.0 (Peakall and Smouse, 2006). Analysis of molecular variance (AMOVA), principal component analysis (PCA), and the Mantel test were also conducted using this software. The UPGMA clustering trees were drawn with MEGA using Nei's genetic distance value (Tamura et al., 2021). We used the Bayesian clustering method in structure (Gilbert et al., 2012) to analyze *R. platypodum*'s genetic structure. We adopted an independent allele frequency mixing model. The number of classes (K) was set to 1–10, the length of the burn-in period and MCMC were both set to 100,000, and each K value was run ten times.

2.4 Differentiation of phenotypic traits

Plant phenotypic characteristics encompass the external manifestations of their underlying genetic traits, which serve as crucial indicators in the examination of genetic differentiation. In line with the morphological classification criteria pertinent to the genus

Rhododendron, a total of 19 morphological indices were meticulously selected for evaluation. These encompassed 6 indices related to leaf morphology, 2 indices related to inflorescence characteristics, and 11 indices pertaining to flower morphology. To amass comprehensive leaf morphological data, each sampled individual was tasked with measuring a minimum of 15 leaves derived from 5 distinct branches. In parallel, the assessment of inflorescence morphology required the measurement of a minimum of 5 inflorescences, while flower morphology data necessitated the measurement of at least 5 flowers from more than 5 distinct inflorescences. Statistical analysis was conducted utilizing univariate covariance analysis (ANOVA) through the use of SPSS 20.0. Multiple comparisons were subsequently performed employing the Tukey method to discern significant differences among various populations. Finally, PCA was employed as an instrumental tool to ascertain phenotypic differentiation across diverse population groups.

3 Results

3.1 SSR primer polymorphism

We employed 18 SSR primer pairs to discern a total of 52 alleles within the cohort of 61 samples. Among these alleles, there was N_a of 2.89, N_e of 2.12 [range = 1.07 (A67)–2.76 (A20)], H_o of 0.57 [range = 0.07 (A67)–1.00 (A20)], and H_e of 0.50 [range = 0.06 (A67)–0.64 (A20)]. The PIC range was 0.06 to 0.57. Overall, seven loci (A19, A20, A26, A40, A53, A58, and A64) displayed high polymorphism (PIC > 0.5), while two loci (A67 and A13) showed low polymorphism (PIC < 0.25). In addition, other loci displayed medium polymorphism (0.25 < PIC < 0.5). Out of these 18 loci, 7 showed consistency with HWE (Table 3).

3.2 Analysis of genetic diversity

Across all populations, N_a was higher than N_e . In particular, N_a and N_e were maximized for the JFM and ZYM populations, respectively (Table 4). The values of H_o , H_e , and I for the different populations studied ranged from 0.47 to 0.65, 0.36 to 0.46, and 0.52 to 0.69, respectively. All three parameters were maximized in the ZYM population. Furthermore, it is noteworthy that the F_{is} value exhibited consistent negative trend across all populations under study. This observation signifies the persistence of a high level of heterozygosity within each population. This implies that the individuals comprising these populations do not stem from inbreeding processes.

3.3 Genetic differentiation and genetic structure

Genetic variation among populations using SSR-based AMOVA was 14% and among individuals within populations was 86% (Table 5). As indicated by the results of interpopulation F_{st} and gene flow (Nm) (Table 6), the genetic differentiation coefficients between the four populations ranged from 0.05 to 0.19, while gene flow ranged from 1.10 to 5.13. JFM and ZYM populations displayed the lowest differentiation coefficients and the highest frequency of gene exchanges. There was the greatest differentiation coefficient among the BMM and MEM populations, with the least amount of gene exchange.

PCA results determined that the first and second PCA axes accounted for 23.54 and 14.59% of the total variation, respectively

(Figure 2). On the first principal axis, the MEM population was separated from the JFM, ZYM, and BMM populations. On the second principal axis, the three populations from Chongqing appeared separated to some extent. MEM and JFM, ZYM, and BMM populations were distinguished by UPGMA clustering trees (Figure 3). The JFM population from Chongqing first gathered with the ZYM population before gathering with the BMM population. In all the populations tested by the Mantel test, genetic distance positively correlated with geographic distance ($R^2=0.606, p=0.068$) (Figure 4). This suggests that geographical isolation contributes to variation among populations.

The structure analysis revealed that if $K=2$, ΔK becomes the largest, suggesting that “2” is the optimal number of clusters (Figure 5). It is evident that the populations of Chongqing and Guangxi are distinct when $K=2$. When $K=3$, the JFM and BMM populations are separated, and the genetic components of the ZYM population show heterozygosity with those of the JFM and BMM populations. The four populations are all separated when $K=4$, despite the presence of a small amount of genetic heterozygosity between them.

3.4 Analysis of phenotypic differentiation

Figures 6, 7 depict the examination of genetic differentiation, gene flow, and phenotypic traits within *R. platypodum* populations, which is further elaborated in Table 7. In the JFM population, leaf length and width were significantly wider than those in the other populations ($p < 0.01$). Petiole lengths in the JFM population were significantly longer than those in the ZYM population ($p < 0.01$) but were similar in the

TABLE 3 Genetic diversity indexes of different SSR loci in *R. platypodum* for all samples.

Locus	Number of alleles observed (N_a)	Number of effective alleles (N_e)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Polymorphic information content (PIC)	Hardy–Weinberg equilibrium (HWE)
A1	5	2.04	0.56	0.51	0.47	NS
A2	2	1.99	0.64	0.50	0.37	NS
A13	3	1.09	0.08	0.08	0.08	ND
A16	2	1.91	0.79	0.48	0.36	***
A19	3	2.60	0.38	0.62	0.54	***
A20	4	2.76	1.00	0.64	0.57	***
A21	2	2.00	0.95	0.50	0.38	***
A26	3	2.54	0.48	0.61	0.54	NS
A39	2	2.00	0.97	0.50	0.38	***
A40	3	2.63	0.38	0.63	0.55	NS
A42	3	2.05	0.74	0.52	0.41	**
A44	2	1.98	0.25	0.50	0.37	**
A53	3	2.60	0.52	0.62	0.55	NS
A55	3	2.01	0.90	0.51	0.38	***
A56	2	1.82	0.59	0.46	0.35	NS
A58	5	2.56	0.16	0.61	0.55	***
A64	3	2.50	0.75	0.61	0.52	NS
A67	2	1.07	0.07	0.06	0.06	ND
Mean	2.89	2.12	0.57	0.50	0.41	

TABLE 4 Genetic diversity indexes of *R. platypodum* for different populations.

Population	Number of samples	Average number of alleles observed (N_a)	Average effective alleles observed (N_e)	Observed heterozygosity (H_o)	Average expected heterozygosity (H_e)	Shannon index (I)	Inbreeding coefficient (F_{is})
JFM	20	2.50	1.87	0.52	0.41	0.65	-0.22
MEM	13	2.28	1.89	0.57	0.42	0.64	-0.31
ZYM	20	2.28	1.96	0.65	0.46	0.69	-0.43
BMM	8	1.94	1.68	0.47	0.36	0.53	-0.29

TABLE 5 Analysis of molecular variance within/among populations of *R. platypodum*.

Source of variation	DOF	Sum of squares	Percentage of variation (%)
Inter-population	3	81.84	14%
Intra-population	118	460.55	86%
Total	121	542.38	100%

TABLE 6 Genetic differentiation (F_{st} , above diagonal) and gene flow (Nm , below diagonal) of four *R. platypodum* populations.

	JFM	MEM	ZYM	BMM
JFM	---	0.13	0.05	0.12
MEM	1.69	---	0.11	0.19
ZYM	5.13	2.03	---	0.08
BMM	1.86	1.10	2.73	---

BMM and MEM populations. The petiole width ($p < 0.01$) was consistent across different populations, although it was maximized in the JFM population and minimized in the MEM population. The aspect ratio of the petioles of the MEM population was considerably greater than that of other populations ($p < 0.01$). MEM populations exhibited the most lateral veins, whereas ZYM populations exhibited the fewest lateral veins. In the MEM population, traits such as floral part size, flower number, corolla length, corolla lobe depth, style length, and radius of the stigma were significantly smaller than those in the other populations ($p < 0.01$). Compared to the other populations, the MEM population had a significantly longer pedicel, calyx, and radius of the ovary ($p < 0.05$).

PCA performed using the phenotypic data revealed that the JFM, ZYM, and BMM populations are highly similar, indicating that they are significantly different from the MEM population (Figure 8).

4 Discussion

4.1 Genetic diversity of *Rhododendron platypodum*

In comparison with the other *R. platypodum* populations, the ZYM population exhibits the highest genetic diversity (Table 4). In contrast, the BMM population exhibits the lowest genetic diversity. Genetic diversity was similar between the JFM and MEM populations. ZYM plants are widely distributed and contain many plants (Table 1). This population of plants may be characterized by rich genetic

diversity due to the conditions under which genetic information is exchanged between individuals. Although the BMM population has a small size and a narrow distribution, its low genetic diversity might be due to its small size and narrow distribution.

H_e of the population samples in this study ranged from 0.35 to 0.41 (average = 0.41). Similarly, *R. platypodum* had a lower N_e than *R. protistum* ($H_e = 0.60$) (Ma et al., 2013), *R. simsii* ($H_e = 0.75$) (Li et al., 2022), *R. ripense* ($H_e = 0.80$) (Tan et al., 2009), and *R. dauricum* ($H_e = 0.57$) (DePristo et al., 2011). Comparative species belong to the same genus as *R. platypodum*. However, the N_e of *R. platypodum* was significantly higher than that of *R. rufohirtum* ($H_e = 0.28$) (Kondo et al., 2009). The H_e values of cosmopolitan and stenochoric species were 0.62 and 0.42, respectively (Hsieh et al., 2013), and *R. platypodum* was found to be genetically similar to stenochoric species.

The F_{is} of all populations involved was negative, indicating no inbreeding. In other species of the genus, like *Rhododendron longipedicellatum* (Li et al., 2018), *R. protistum* (Zizhuo et al., 2022), *R. rex* (Li et al., 2022), *R. dauricum* (DePristo et al., 2011), and *R. molle* (Zhang et al., 2021), the situation was similar. This may be attributed to *Rhododendron* growth and breeding habits (Keller and Waller, 2002; Stoeckel et al., 2006). *Rhododendron* plants are perennial woody plants that have a long lifespan. *R. platypodum* exhibits mainly outbreeding and partial self-breeding (Ma et al., 2021), which suggests that the current populations are probably descended from ancestral populations with a rich genetic history.

Various levels of genetic variation have been identified within and among *R. platypodum* populations. The genetic diversity of populations inhabiting relatively undisturbed and less fragmented habitats may exhibit a markedly higher degree when compared to populations residing in fragmented or degraded environments. This observation aligns seamlessly with the prevailing theory positing that habitat fragmentation tends to curtail gene flow while concurrently elevating genetic isolation (Hira et al., 2023; Jiajia et al., 2023). This ultimately results in a decrease in genetic diversity within small and isolated populations. Prioritizing conservation efforts requires identifying populations with high genetic diversity, since these populations can serve as sources of genetic material for the recovery of species (Garza et al., 2020).

4.2 Genetic differentiation of *Rhododendron platypodum*

Plant populations exhibit a comprehensive genetic structure that reflects their evolutionary history. Therefore, it is imperative to formulate conservation strategies for endangered species based on

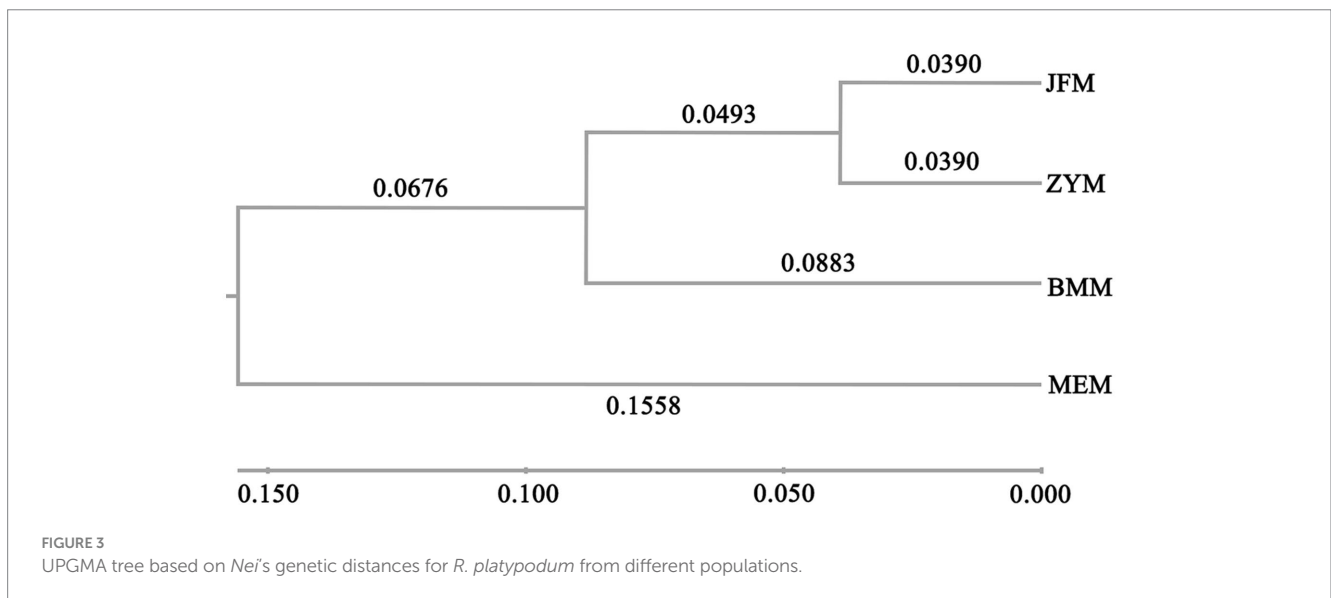
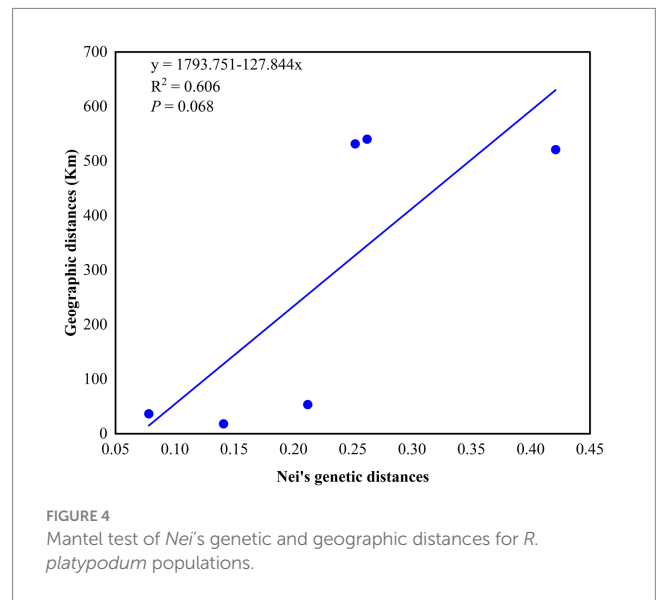
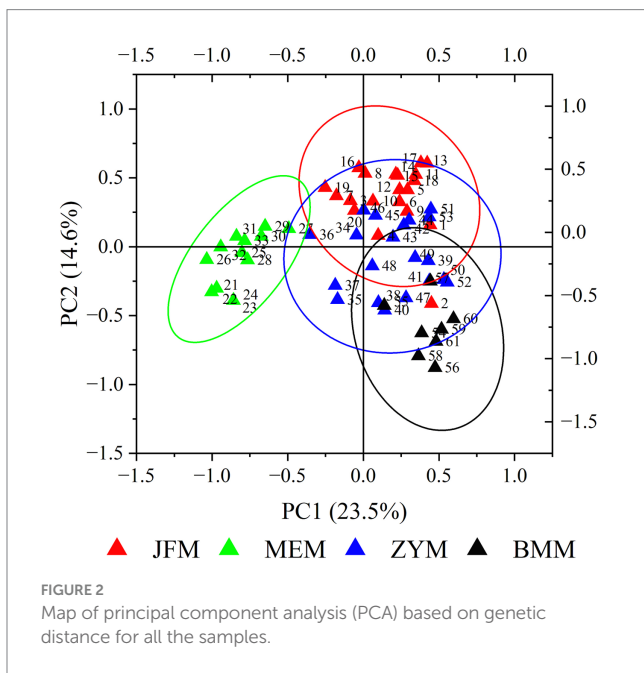
these data. AMOVA based on SSR revealed that 86% of the total genetic variation was accounted for by intra-population genetic variation, whereas 14% was accounted for by inter-population genetic variation; these findings align with other studies (Zhao et al., 2012; Shen et al., 2015; Yu et al., 2017; Yang et al., 2020; Ma et al., 2021; Zhang et al., 2021). Moreover, our study's $K=2$ structure analysis in genetic differentiation (Figure 5) sheds light on systematic relationships in the *Rhododendron* genus, as previously reported by other researchers (Milne, 2004; Wilson, 2016).

F_{st} provides important information about population genetic structure. In this study, the three Chongqing populations and the Guangxi population exhibited F_{st} within a range of 0.11–0.19 (Table 6), indicating that there was significant genetic differentiation between the Chongqing population and the Guangxi population. Further confirmation of this differentiation was achieved through

PCA and UPGMA cluster analysis. Structure analysis revealed significant differences between the Chongqing and Guangxi populations. Similar results were found in the morphological analysis of the data. This indicated that the Guangxi population had shorter leaves, narrower petioles, and smaller inflorescences and flowers. This is also one of the reasons for the controversy surrounding the MEM population classification.

All three Chongqing populations showed F_{st} values between 0.05 and 0.12, which indicates only a small to moderate degree of genetic differentiation. It was found that when $K=3$, the genetic components of the ZYM population were mixed with those of the JFM and BMM populations. It is evident that they differ morphologically in terms of leaf length, leaf width, and ovary length (Table 7; Figure 7).

R. platypodum population differentiation patterns provide insight into connectivity between different populations of this species. Keeping populations healthy and preventing inbreeding requires an understanding of gene flow and barriers to gene exchange. A high



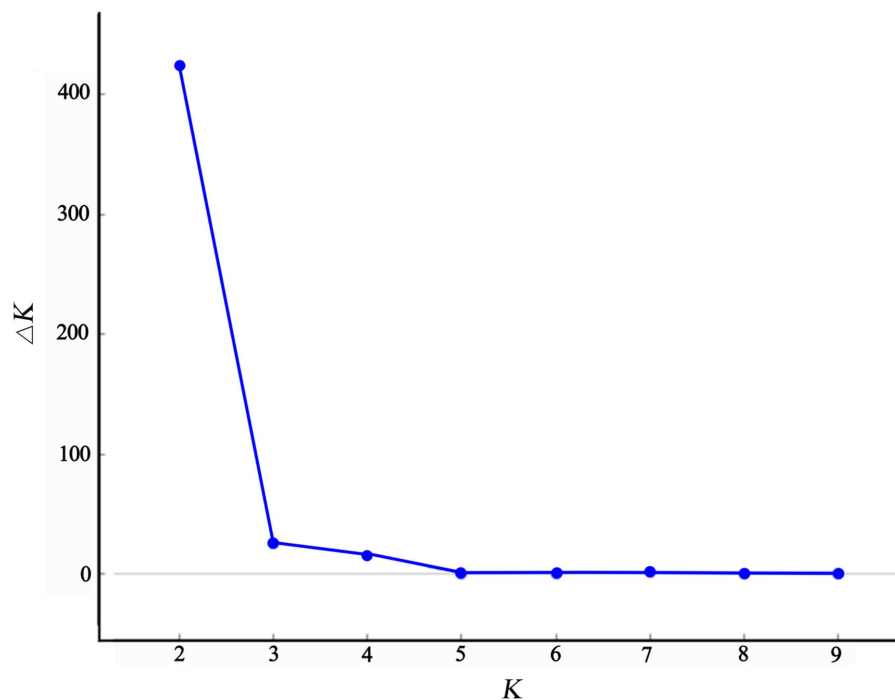


FIGURE 5
Relations of the number of to-be-determined groups K and estimated ΔK .

degree of genetic differentiation between populations may indicate limited gene flow. This may be caused by geographical barriers, habitat fragmentation, or both (Zheng et al., 2022; Arif et al., 2024). The identification of restricted gene flow areas can assist in implementing strategies to enhance connectivity between populations, such as the creation of habitat corridors and the promotion of natural pollination vectors.

4.3 Recommendations on *Rhododendron platypodum* protection

R. platypodum, with its limited resource base and confined distribution area, presents an unusual ecological context. This study reveals substantial differentiation between the Guangxi and Chongqing populations of the broad-stalked *R. platypodum*, along with discernible genetic and morphological variations among the three populations in Chongqing. These revelations underscore the pivotal significance of each population in preserving the genetic diversity and structural integrity of the wide-stalked *R. platypodum*. Presently, the JFM, BMM, and MEM populations are all situated within designated nature reserves. JFM and MEM have received particular attention as priority hotspots for safeguarding *Rhododendron* plant diversity in China, as evidenced by previous studies (Zhao et al., 2012; Shen et al., 2015; Yu et al., 2017; Yang et al., 2020; Ma et al., 2021; Zhang et al., 2021). Consequently, they have been subjected to increased scrutiny and more comprehensive protection measures. Notably, the ZYM population stands out with its substantial number of individuals, abundant seedlings, and extensive geographical spread. These

factors collectively contribute to the highest genetic diversity observed. This population occupies a central role in the overall genetic structure of *R. platypodum* and is therefore endowed with the utmost conservation value. Nevertheless, it is imperative to acknowledge that, despite its ecological significance, the ZYM population currently experiences a gap in protection efforts. Therefore, it is imperative to enhance and fortify conservation measures to ensure the enduring survival and prosperity of this vital genetic reservoir.

This study has direct implications for *R. platypodum* conservation. The survival of the species depends on a multifaceted approach (Tsukamoto et al., 2021; Liu et al., 2022; Abbasi et al., 2023; Cao et al., 2023; Rauf et al., 2023; Naz et al., 2024): (Chen et al., 2022) Restoration efforts should aim to improve the quality and connectivity of habitats to facilitate healthy populations and gene flow (Torres-Díaz et al., 2021). *R. platypodum* is a valuable species in the ecosystem, and conservation efforts can be bolstered by raising awareness of its importance and role within it (Harrington et al., 2023). Protecting the habitats of the species and enforcing regulations against illegal collection and destruction of those habitats is vital (Singh et al., 2023). Genetic monitoring and research must occur continuously in order to determine the extent of changes in genetic diversity and adjust conservation strategies as necessary.

5 Conclusion

The present study employed a set of 18 SSR primers to examine 61 samples drawn from four distinct populations located

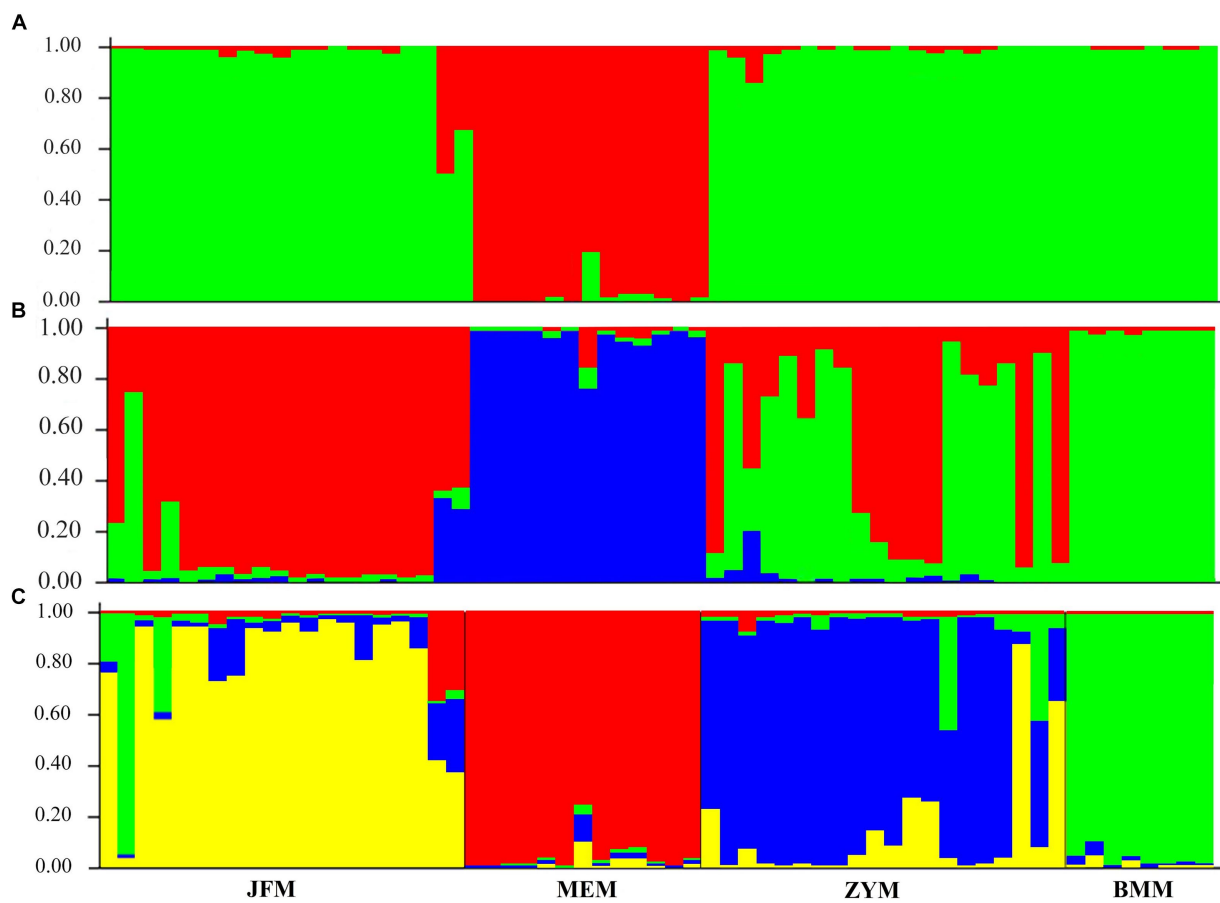


FIGURE 6 Genetic structure for different K values with the STRUCTURE program for 4 populations of *R. platypodum*. (A) K = 2, (B) K = 3, (C) K = 4.

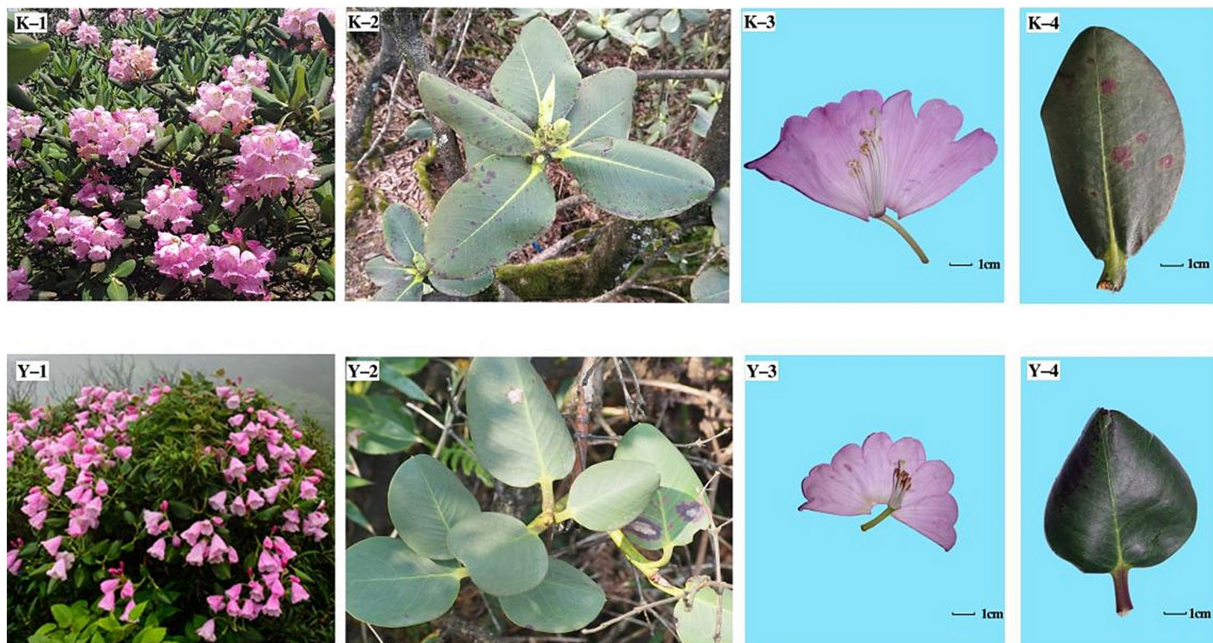
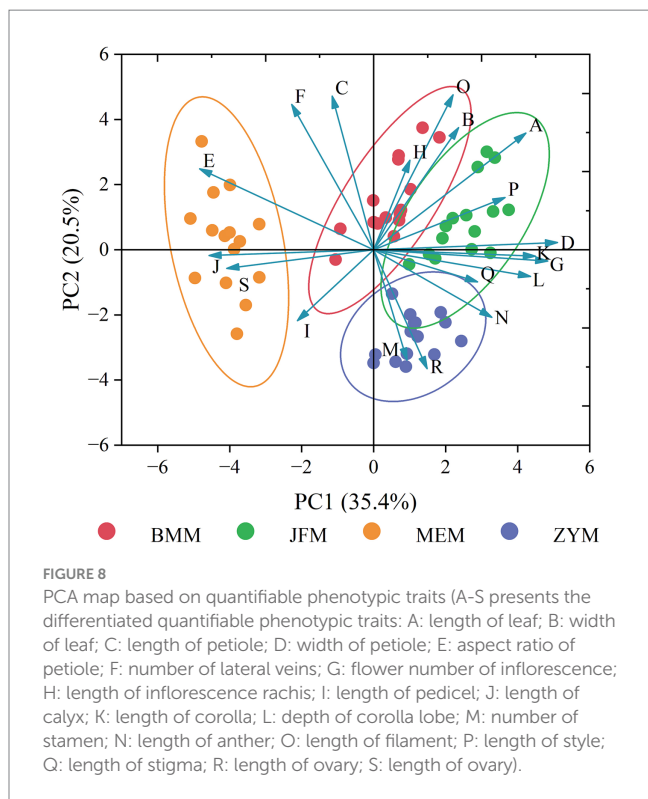


FIGURE 7 Phenotypes of the ZYM and MEM populations. K-1, K-2, K-3, and K-4 indicate the inflorescence, phyllo-taxy, and flower and leaf morphologies of *R. platypodum* (ZYM), respectively; Y-1, Y-2, Y-3, and Y-4 indicate the inflorescence, phyllo-taxy, and flower and leaf morphologies of *R. platypodum* (MEM), respectively.

TABLE 7 Phenotypic traits of four *R. platypodum* populations.

	Traits	JFM	ZYM	BMM	MEM
Leaf	Length of leaf (A)	141.09 ± 11.19 ^a	82.26 ± 15.76 ^b	113.24 ± 18.10 ^c	70.31 ± 9.97 ^d
	Width of leaf (B)	82.71 ± 15.45 ^a	57.59 ± 9.96 ^b	64.44 ± 11.39 ^c	63.55 ± 9.61 ^c
	Length of petiole (C)	22.21 ± 6.4 ^a	14.19 ± 3.7 ^b	20.89 ± 4.11 ^a	22.91 ± 6.47 ^a
	Width of petiole (D)	12.7 ± 1.68 ^a	9.58 ± 1.43 ^b	7.36 ± 1.89 ^c	5.15 ± 0.55 ^d
	Aspect ratio of petiole (E)	1.85 ± 0.57 ^a	1.50 ± 0.43 ^b	2.96 ± 0.71 ^c	4.45 ± 1.17 ^d
	Number of lateral veins (F)	13.63 ± 1.49 ^a	11.41 ± 1.33 ^b	14.95 ± 2.29 ^c	15.53 ± 2.84 ^c
Flower	Flower number of inflorescence (G)	14.93 ± 1.95 ^a	15.07 ± 2.02 ^a	15.27 ± 1.84 ^a	6.6 ± 1.2 ^b
	Length of inflorescence rachis (H)	56.76 ± 2.87 ^a	53.66 ± 2.75 ^a	56.19 ± 2.73 ^a	54.81 ± 5.13 ^a
	Length of pedicel (I)	34.08 ± 2.41 ^a	34.83 ± 2.59 ^a	31.89 ± 4.02 ^b	36.35 ± 3.89 ^b
	length of calyx (J)	2.05 ± 0.11 ^a	2.11 ± 0.22 ^a	2.06 ± 0.14 ^a	2.61 ± 0.37 ^b
	Length of corolla (K)	46.18 ± 5.54 ^a	43.53 ± 7.30 ^b	44.17 ± 4.54 ^{ab}	35.1 ± 3.52 ^c
	Depth of corolla lobe (L)	14.60 ± 2.07 ^a	14.29 ± 2.44 ^a	13.87 ± 1.71 ^a	11.41 ± 0.86 ^b
	Number of stamen (M)	14.44 ± 1.09 ^{abd}	14.77 ± 0.74 ^b	13.72 ± 1.17 ^c	14.16 ± 1.07 ^d
	Length of anther (N)	3.62 ± 0.27 ^a	3.6 ± 0.3 ^a	3.17 ± 0.38 ^b	3.16 ± 0.24 ^b
	Length of filament (O)	31.54 ± 2.11 ^a	23.79 ± 3.67 ^b	29.88 ± 4.59 ^c	26.08 ± 3.42 ^d
	Length of style (P)	35.81 ± 1.9 ^a	34.33 ± 2.7 ^b	37.49 ± 3.07 ^c	30.48 ± 3.48 ^d
	Radium of stigma (Q)	3.56 ± 0.45 ^a	3.92 ± 0.65 ^b	3.47 ± 0.78 ^a	3.09 ± 0.22 ^c
	Length of ovary (R)	5.87 ± 0.38 ^a	6.23 ± 0.64 ^b	4.95 ± 0.61 ^c	5.48 ± 0.47 ^d
	Radium of ovary (S)	3.91 ± 0.33 ^a	4.24 ± 0.69 ^b	4.11 ± 0.68 ^{ab}	4.95 ± 0.44 ^c

Different superscript letters on the same line mean significant difference ($P < 0.05$), while the same letters mean no significant difference.



comparison to most *Rhododendron* species documented, and it closely resembles the patterns of endemic species. Corroborating the genetic analysis, the outcomes of PCA, UPGMA cluster analysis, and structure analysis consistently delineated the separation of *R. platypodum* into two discrete groups: the Chongqing group and the Guangxi group. Importantly, these groups exhibited substantial genetic differentiation between them. Furthermore, the phenotypic data analysis, comprising 19 distinct categories, aligned harmoniously with the genetic structure. Notably, the Chongqing and Guangxi groups displayed noteworthy disparities in various traits, encompassing leaf length, petiole width, inflorescence size, and flower size. Moreover, within the Chongqing group, significant distinctions were observed among different populations, particularly in terms of leaf length, width, and ovary size. The comprehensive findings of this study hold significant implications for *R. platypodum* conservation. It underscores the necessity of intensifying protection efforts, particularly in the case of the Zhaoyun Mountain population, which has the highest genetic diversity among the populations under scrutiny. This study thus serves as a valuable reference point for future conservation strategies. It reinforces the urgency of safeguarding genetic diversity within the Zhaoyun Mountain population.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

LM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. LL: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. WF: Investigation, Methodology, Visualization, Writing – original draft. ZD: Investigation, Software, Visualization, Writing – original draft. YL: Investigation, Validation, Visualization, Writing – original draft. CW: Formal Analysis, Investigation, Project administration, Writing – original draft. YX: Investigation, Project administration, Writing – original draft. ML: Validation, Visualization, Writing – review & editing. MA: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HW: Conceptualization, Data curation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

LL was employed by China Forestry Group Co., Ltd. LL, WF, ZD, and YL were employed by Chongqing Forestry Investment Development Co. Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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