Genetic Diversity and Genetic Structure of Wild Pinellia ternata in China (Postprint)

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Abstract

Quaternary climate fluctuations and geographic and environmental isolation have profoundly influenced the genetic diversity, genetic structure, and geographic distribution patterns of modern plants. This study employed molecular phylogeographic approaches to analyze three chloroplast fragments (psbK-psbI, atpF-atpH, and trnL-F) from 212 individuals across 19 populations of the medicinal plant Pinellia ternata, aiming to investigate its genetic diversity, genetic structure, patterns and causes of geographic distribution, and population historical dynamics. The results showed: (1) The total haplotype diversity (Hd) of Pinellia ternata was 0.882, and the total nucleotide diversity (π) was 1.23 $\times$ 10^{-3}, indicating high genetic diversity at the species level. (2) Analysis of Molecular Variance (AMOVA) revealed that genetic variation in Pinellia ternata occurred primarily among populations, with significant genetic differentiation (FST = 0.909, P < 0.001) and low within-population genetic diversity (HS = 0.134); the coefficient of genetic differentiation among populations, NST = 0.913, was greater than GST = 0.855 (0.01 < P < 0.05), indicating a distinct phylogeographic structure of chloroplast haplotypes. (3) Neutrality test results showed that Tajima's D, Fu & Li's D, and Fu and Li's F* values were all non-significant positive values, Fu's Fs value was a non-significant negative value, and the mismatch distribution curve was bimodal, indicating that Pinellia ternata populations as a whole have not experienced expansion events. (4) The geographic distribution of haplotypes revealed that the southwestern region and the central-eastern region harbored higher haplotype diversity and contained private haplotypes, suggesting that glacial refugia existed in these two regions during the Quaternary glacial period. In summary, through the analysis of three chloroplast genes in Pinellia ternata from different regions, this study elucidated its genetic diversity, genetic structure, and geographic distribution patterns, and also provided scientific recommendations and conservation strategies for the molecular screening and protection of superior germplasm of Pinellia ternata.

Full Text

Preamble

Chinese Title: 中国野生半夏的遗传多样性和遗传结构研究

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Abstract: Quaternary climate fluctuations and geographic and environmental isolation have profoundly influenced the genetic diversity, genetic structure, and geographic distribution patterns of modern plants. This study employed molecular phylogeographic methods to analyze three chloroplast fragments (psbK-psbI, atpF-atpH, and trnL-F) from 212 individuals across 19 populations of the medicinal plant *Pinellia ternata*. We investigated its genetic diversity, genetic structure, geographic distribution patterns and their underlying causes, and explored its population historical dynamics. The results showed: (1) The total haplotype diversity (Hd) of *P. ternata* was 0.882, and total nucleotide diversity (π) was 1.23×10^{-3}, indicating high genetic diversity at the species level. (2) Analysis of Molecular Variance (AMOVA) revealed that genetic variation occurred primarily among populations, with significant genetic differentiation (FST = 0.909, $P < 0.001$) and low within-population genetic diversity (HS = 0.134). The genetic differentiation coefficient among populations was NST = 0.913 > GST = 0.855 ($0.01 < P < 0.05$), demonstrating a clear phylogeographic structure of chloroplast haplotypes. (3) Neutrality tests showed that Tajima's D, Fu & Li's D, and Fu and Li's F* values were all non-significant positive values, while Fu's Fs was a non-significant negative value, and mismatch analysis curves showed a bimodal pattern, indicating that *P. ternata* populations have not experienced expansion events. (4) Haplotype geographic distribution revealed high haplotype diversity and the presence of private haplotypes in southwestern and central-eastern regions, suggesting that these two areas served as glacial refugia during the Quaternary glaciation. In summary, analysis of three chloroplast genes in *P. ternata* from different regions clarifies its genetic diversity, genetic structure, and geographic distribution patterns, and provides scientific recommendations and conservation strategies for molecular screening and protection of superior germplasm resources.

Keywords: *Pinellia ternata*, 地理分布格局, 遗传多样性, 遗传结构, 避难所

English Title: Genetic Diversity and Genetic Structure of Wild *Pinellia ternata* (Araceae) in China

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Abstract: Global climate change, particularly Quaternary climate fluctuations and environmental isolation, has profoundly impacted the genetic diversity, genetic structure, geographical distribution patterns, and population historical dynamics of modern plants. We employed molecular phylogeographic methods to analyze three chloroplast non-coding fragments (psbK-psbI, atpF-atpH, and trnL-F) from 212 individuals across 19 populations of *Pinellia ternata* (Araceae), a perennial medicinal herb, to investigate its genetic diversity, genetic structure, geographical distribution patterns and their underlying causes, as well as its population historical dynamics. The results revealed: (1) The total haplotype diversity (Hd) of *P. ternata* was 0.882, and total nucleotide diversity (π) was 1.23×10^{-3}, indicating high genetic diversity at the species level. (2) Analysis of Molecular Variance (AMOVA) showed that genetic variation occurred primarily among populations (89.27%), with significant genetic differentiation (FST = 0.909, P < 0.001) and low within-population genetic diversity (HS = 0.134). The genetic differentiation coefficients among populations were GST = 0.855 and NST = 0.913, respectively, with NST > GST (0.01 < P < 0.05), demonstrating a clear phylogeographic structure of chloroplast haplotypes. (3) Neutrality tests revealed that Tajima's D, Fu & Li's D, and Fu and Li's F* values were all non-significant positive values, while Fu's Fs was a non-significant negative value. Mismatch analysis curves exhibited a bimodal pattern, indicating that *P. ternata* populations have not experienced expansion events. (4) Haplotype geographic distribution showed high haplotype diversity and the presence of private haplotypes in southwestern and central-eastern regions, suggesting these areas served as glacial refugia during the Quaternary ice age. In summary, analysis of three chloroplast genes in *P. ternata* from different regions clarifies its genetic diversity, genetic structure, and geographical distribution patterns, and provides scientific recommendations and conservation strategies for molecular screening and protection of superior germplasm resources.

Keywords: *Pinellia ternata*, geographical distribution pattern, genetic diversity, genetic structure, glacial refugia

Introduction

Global climate change, particularly Quaternary climate fluctuations and environmental changes, has profoundly influenced the genetic diversity, genetic structure, and geographic distribution patterns of modern plants (Hewitt, 1996, 2004; Bennett & Provan, 2008). In Europe and North America, extensive phylogeographic studies have revealed the genetic structure and evolutionary history of extant plant populations (Hickerson et al., 2010; Qiu et al., 2011; Liu et al., 2012; Ye et al., 2017). During glacial periods, extensive continen-

tal glaciers forced thermophilic plants at high latitudes to retreat southward, forming "glacial refugia" (Hewitt, 2004). Conversely, during interglacial or postglacial periods, warming temperatures and glacial retreat allowed plants from refugia to migrate back to higher latitudes (Hewitt, 1996, 2004; Shafer et al., 2010).

However, the impacts of global climate change and environmental fluctuations on the genetic diversity, genetic structure, phylogeographic distribution patterns, and population demographic history of Chinese plants differ markedly from those in Europe and North America (Qiu et al., 2011; Liu et al., 2012; Meng et al., 2015; Wang et al., 2017), manifesting in several aspects: (1) Due to the absence of large-scale glaciation in China, plants underwent range contractions during glacial periods when temperatures dropped, resulting in multiple glacial refugia; (2) During interglacial or postglacial periods, warming climate enabled individuals surviving in glacial refugia to expand their ranges; and (3) The uplift of the Qinghai-Tibet Plateau caused mountain and river isolation, monsoon-induced alternation between wet and dry conditions, aridity, and Quaternary glacial-interglacial cycles, all of which were major factors shaping plant phylogeographic patterns. However, these studies have primarily focused on the Qinghai-Tibet Plateau, southwestern China, the Qinling Mountains and adjacent regions, and have mainly examined woody plants, with relatively few similar investigations conducted in other regions or on herbaceous species.

Plant chloroplast DNA (cpDNA) is a circular double-stranded DNA molecule that, compared to nuclear genomic DNA, features small molecular weight, multiple copies, and simple structure. Moreover, recombination rarely occurs among different cpDNA fragments, resulting in high genetic differentiation and low gene flow in plant population studies, which enables clearer elucidation of species' genetic variation and phylogeographic patterns (Avise et al., 2000; Sudhir et al., 2016). Consequently, an increasing number of studies have employed multiple chloroplast genes to investigate plant genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics (Zhang, 2022).

Pinellia ternata (Thunb.) Breit. is a perennial herbaceous plant in the family Araceae, also known as Shoutian, Diwen, or colloquially as Mayutou, Tianluoxing, and Wuxincai. It is a commonly used traditional Chinese medicine that grows in moist, warm, shaded, and loose sandy soils below 2,500 m elevation, and is endemic to East Asia. Wild *P. ternata* is distributed across all provinces in China except Inner Mongolia, Qinghai, Xinjiang, and Tibet (Li et al., 2004; Zhang, 2007). Due to geographic distance, population and phenotypic differentiation have occurred over time, affecting its genetic diversity and genetic structure. Meanwhile, extensive commercial exploitation and lack of conservation measures have led to a sharp decline in wild *P. ternata* resources, necessitating collection and genetic background analysis of wild germplasm. As the utilization of *P. ternata* continues to expand, related research has proliferated, though primarily focusing on chemical composition, toxicity, and pharmacological effects

(Li et al., 2021).

Few scholars have investigated the phylogeny and evolutionary relationships of *P. ternata*. For instance, Zhao and Li (2016) used combined matK+rbcL sequences to analyze phylogenetic relationships within *Pinellia*, demonstrating that the matK+rbcL combination could serve as a DNA barcode for species identification. Zhang (2007) employed ITS sequences to analyze sequence variations among *P. ternata* from different regions of China and their correlation with geographic distribution and external morphology, revealing that rDNA variation was associated with geographic distribution. Pan et al. (2021) used ITS sequences to reveal population genetic structure and genetic diversity in *P. ternata*. Zhang et al. (2021) utilized selected SSR primers to analyze genetic diversity among different populations. Zheng et al. (2013) analyzed chloroplast non-coding regions (psbK-psbI and atpF-atpH) in *P. ternata* and related species, identifying abundant variable sites. However, comprehensive studies on genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics based on multiple chloroplast genes remain lacking. As a herbaceous species with wide distribution and strong ecological adaptability, spanning different climate zones and multiple biodiversity hotspots, *P. ternata* represents an ideal material for investigating genetic structure, phylogeographic distribution patterns, and population historical dynamics in herbaceous and widespread species. This study employs plant phylogeographic methods based on three chloroplast genes (psbK-psbI, atpF-atpH, and trnL-F) to analyze 212 wild samples from 19 natural populations across 15 provinces in eastern, central, northwestern, and southwestern China. We aim to explore the genetic diversity, genetic structure, phylogeographic distribution patterns, and population historical dynamics of this medicinal plant, elucidate genetic variation among and within populations, determine haplotype geographic distribution patterns, infer glacial refugia during the Quaternary glaciation, and propose scientific recommendations and conservation strategies for molecular screening and protection of superior germplasm resources. This research provides important references for conservation, utilization, and molecular-assisted breeding of *P. ternata* resources, while also offering theoretical foundations for further exploring the evolution of Chinese flora and the formation of species diversity.

Materials and Methods

1.1 Experimental Materials

Pinellia ternata materials were collected and preserved from 2015 to 2020, covering 15 provinces including Guizhou, Zhejiang, Gansu, and Sichuan, spanning northern and southern China and representing natural geographic distribution zones in eastern, central, northwestern, and southwestern regions. A total of 212 individuals from 19 natural populations were collected, with location information measured using Global Positioning System (Table 1). Fresh leaves of *P. ternata* were collected in the field and immediately dried with silica gel for total DNA extraction. Voucher specimens were deposited in the Herbarium of

the College of Life Sciences, Guizhou University.

1.2 DNA Extraction and Amplification Sequencing

Total DNA was extracted from silica gel-dried leaves using a novel plant genomic DNA extraction kit (spin column type). Primers were selected based on previously published *P. ternata* sequences and related reports (Zheng et al., 2013), targeting three chloroplast gene fragments: psbK-psbI, atpF-atpH, and trnL-F. Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Primer sequences and PCR amplification protocols are listed in Table 2 . The PCR amplification reaction system was 25 L, containing 12.5 L of 2× Taq PCR MasterMix, 8.5 L ddH_2O, 1 L forward primer, 1 L reverse primer, and 2 L DNA template. Total DNA and PCR products were detected using 1% agarose gel electrophoresis. After electrophoresis, gels were placed in a gel imaging system for observation, and qualified products were sent to Sangon Biotech (Shanghai) Co., Ltd. for purification and sequencing.

1.3 Data Analysis

Sequences were aligned using MEGA7.0 software (Sudhir et al., 2016), with erroneous bases corrected and sequences manually adjusted to remove primer regions at both ends. PhyloSuite software (Zhang et al., 2020) was used for sequence assembly. DNASP6.0 software was employed to count haplotype numbers and calculate haplotype diversity (Hd), nucleotide diversity (π), Tajima's D, Fu and Li's F*, Fu & Li's D, and Fu's Fs values for each population, and to conduct mismatch analysis to detect expansion events (Tajima, 1989; Fu, 1997). PERMUTCpSSR 2.0 was used to calculate total genetic diversity (HT), average within-population genetic diversity (HS), and genetic differentiation coefficients (GST and NST). Network software was used to construct haplotype network diagrams (Bandelt et al., 1999). AMOVA analysis in the Arlequin software package (Lu, 2018) was used to calculate population genetic structure and detect genetic variation within and among populations, with genetic differentiation coefficient (FST) and gene flow (Nm) calculated to further reveal the degree of population differentiation. ArcGIS 10.2 software was used to map the geographic distribution of population haplotypes.

Results

2.1 Sequence Variation and Haplotype Diversity

The combined psbK-psbI+atpF-atpH+trnL-F sequences were successfully sequenced for 212 individuals. The aligned and corrected sequence length was 1,947 bp, containing 11 variable sites with a G+C content of 31.80%. These three sequence fragments were submitted to GenBank under accession numbers OL310546–OL310559, OL310532–OL310545, and OL310560–OL310573, respectively. As shown in Table 3 , only populations from Zhaotong City, Yunnan Province (C6) in southwestern China; Guiyang City, Guizhou Province (C14);

Mianyang City, Sichuan Province (C17); Chizhou City, Anhui Province (C7) in eastern China; and Yiwu City, Zhejiang Province (C13) harbored multiple haplotypes, while all other populations possessed only a single haplotype. DNASP software analysis revealed that the total haplotype diversity (Hd) of *P. ternata* was 0.882, and total nucleotide diversity (π) was 1.23×10^{-3}. The highest haplotype diversity (0.5333) was observed in Zhaotong City, Yunnan (C6) and Mianyang City, Sichuan (C17), followed by Chizhou City, Anhui (C7) and Yiwu City, Zhejiang (C13). Overall, populations in southwestern and eastern China exhibited relatively high genetic diversity levels.

2.2 Haplotype Distribution

DNASP software was used to detect haplotypes from the combined psbK-psbI+atpF-atpH+trnL-F sequences (excluding insertions and deletions), identifying a total of 14 haplotypes (H1–H14). Haplotype H10 was the most frequent, occurring in 53 individuals, and together with H2 showed the widest distribution range. Haplotype H10 was distributed across Yichang City, Hubei (C2); Liping County, Qiandongnan, Guizhou (C3); Tianshui City, Gansu (C4); Zhaotong City, Yunnan (C6); and Chizhou City, Anhui (C7). Haplotype H2 was found in Yiwu City, Zhejiang (C13); Shangluo City, Shaanxi (C15); Shijiazhuang City, Hebei (C16); Mianyang City, Sichuan (C17); and Shangrao City, Jiangxi (C19). Except for haplotypes H1, H2, H10, and H11, which occurred in multiple populations, all other haplotypes were private. Haplotype H3 was exclusive to Yiwu City, Zhejiang (C13); haplotypes H4 and H5 only in Guiyang City, Guizhou (C14); haplotype H6 only in Mianyang City, Sichuan (C17); private haplotype H7 only in Dandong City, Liaoning (C1); private haplotype H8 only in Qingdao City, Shandong (C10); haplotype H9 only in Guyuan City, Ningxia (C18); haplotype H12 only in Chizhou City, Anhui (C7); private haplotype H13 only in Nantong City, Jiangsu (C8); and private haplotype H14 only in Kaiyang County, Guizhou (C9). Chizhou City (C7) harbored the greatest number of haplotype types (H10, H11, and H12). Haplotype network analysis of *P. ternata* chloroplast genes showed that other haplotypes were derived from H10 at the center, suggesting that H10 represents the ancestral haplotype (Figure 1 [Figure 1: see original paper]).

The pie chart shows the frequency of haplotypes in each population.

2.3 Mismatch Analysis and Neutrality Tests

DNASP software was used to conduct neutrality tests and mismatch analysis on the sequences. Neutrality tests were non-significant. The conservative Tajima's D statistic was 0.67883 ($P > 0.10$); Fu and Li's D* was 1.38007 ($0.10 > P > 0.05$); Fu and Li's F* was 1.34421 ($P > 0.10$); and Fu's Fs was -1.304. The positive Tajima's D value suggests that *P. ternata* populations may have experienced a bottleneck effect. The mismatch distribution graph exhibited a bimodal pattern, with expected values contradicting observed values, violating the population expansion model. Neutrality test results also detected no signals

of population expansion, indicating a stable population history without overall expansion events (Figure 3 [Figure 3: see original paper]).

The green line represents the distributions expected for expanding and the red line represents the observed mismatch distribution.

2.4 Genetic Diversity and Population Genetic Structure

PERMUT analysis revealed total genetic diversity (HT) of 0.882 and average within-population genetic diversity (HS) of 0.134. Genetic differentiation coefficients among populations were GST = 0.855 and NST = 0.913, with NST > GST ($0.01 < P < 0.05$), indicating that at the population level, different haplotypes with close phylogenetic relationships occurred within the same population, and chloroplast haplotypes exhibited a clear molecular phylogeographic structure.

Molecular variance analysis (AMOVA) showed that genetic variation occurred primarily among populations, accounting for 89.27% of total variation in the 19 *P. ternata* populations, while only 10.73% of variation originated within populations (Table 4). The genetic differentiation coefficient FST was 0.909 ($P < 0.001$), with significance tested through 1,000 permutations. FST > 0.25 indicates that genetic differentiation among *P. ternata* populations has reached a significant level, with obvious isolation. Assuming drift-migration equilibrium for fragment variation, the average gene flow value (Nm) among populations at the species level was estimated as 0.02 based on FST values, indicating extremely low gene flow among *P. ternata* populations.

Discussion

3.1 Genetic Diversity

Population genetic diversity is closely associated with living environments, with numerous environmental factors directly or indirectly influencing genetic diversity (Jiang, 2017). Generally, widespread species exhibit higher genetic diversity than narrowly distributed species (Hamrick, 1992). *P. ternata* has a broad distribution range, with different genetic and morphological variations existing across different geographic environments. During our field surveys, we observed different ecotypes of *P. ternata* showing variation in plant size, leaf shape, and spathe characteristics, which aligns with our experimental data. Analysis of three cpDNA fragments from 212 individuals across 19 natural populations revealed total haplotype diversity of Hd = 0.882 and nucleotide diversity of $\pi = 1.23 \times 10^{-3}$, which exceeds the average chloroplast genetic variation of 0.67 reported for 170 species by Petit et al. (2005). Zhang (2021) used SSR molecular markers to study 17 *P. ternata* populations and found an average Nei's gene diversity index (h) of 1.03, while Pan et al. (2021) analyzed 20 populations using ITS sequences and obtained haplotype diversity (Hd) of 0.8596. Our results are consistent with these findings, all indicating high genetic diversity at the

species level in *P. ternata*. This outcome arises from two main factors. First is natural selection by the environment. Research shows that species distribution range is closely correlated with genetic diversity. Complex topography and diverse climate changes in different distribution areas or habitats create geographic isolation, leading to substantial differentiation among populations in morphology, physiology, genetics, and ecological habits (Wang et al., 2011; Pan et al., 2021). As a widespread species, *P. ternata* likely harbors different genetic and morphological variations across diverse geographic environments, which we observed during sampling as different ecotypes varying in plant size, leaf shape, and spathe characteristics, consistent with our experimental data. Second is gene mutation. Studies have shown that *P. ternata* possesses both sexual and asexual reproductive systems, with asexual reproduction being dominant due to highly sterile male gametes (Wang et al., 2000). In plants with difficult sexual reproduction, gene mutation becomes the primary source of genetic variation (Wang et al., 2011), a characteristic that may contribute to high genetic diversity among populations. Additionally, *P. ternata* possesses a complex gene pool that may have accumulated rich chloroplast genetic variation throughout its long evolutionary history and generational turnover, providing abundant material basis for gene mutation.

3.2 Geographic Distribution Pattern and Population History

The geographic distribution patterns and genetic structure of plant populations are influenced by numerous factors including geological and historical climate changes, habitat heterogeneity, and the degree of seed-mediated gene exchange (Liu et al., 2021). Our study revealed a clear phylogeographic structure in *P. ternata* ($NST = 0.913 > GST = 0.855$, $0.01 < P < 0.05$) and significant genetic differentiation among populations ($FST = 0.909$, $P < 0.001$), indicating infrequent gene exchange among populations and significant geographic isolation or environmental heterogeneity. Based on FST values, the average gene flow (Nm) among populations at the species level was estimated as 0.02. When $Nm < 1$, gene flow is insufficient to counteract population differentiation caused by genetic drift within populations (Slatkin & Montgomery, 1985). Therefore, we infer that geographic isolation (or environmental heterogeneity) and genetic drift are the primary factors driving genetic differentiation among *P. ternata* populations. Low gene flow among populations stems from two main reasons: (1) Reproductive mode (seed propagation, tuber propagation, and bulbil propagation) (Zhang et al., 2016). Bulbils are particularly important for *P. ternata* reproduction, with their size and number closely related to tuber yield (Zhang et al., 2013). Bulbils, daughter tubers, and fruits remain near maternal plants, lacking effective dispersal mechanisms and making *P. ternata* a poor colonizer (Gu & Guo, 1990), resulting in low gene flow and high genetic differentiation among populations. (2) Geographic isolation and environmental factors have fragmented *P. ternata* habitats, interrupting or reducing gene exchange among different populations or regional groups. Neutrality test results for Tajima's D and Fu's Fs were non-significant, indicating that *P. ternata* populations con-

form to a neutral evolution model. The bimodal mismatch distribution, with expected values contradicting observed values, suggests that *P. ternata* has not experienced recent large-scale expansion, contrary to the expansion event proposed by Pan et al. (2021). This discrepancy may relate to the maternal inheritance pattern of chloroplast DNA, where gene flow occurs primarily through seed dispersal (Hu & Li, 2002), whereas nuclear genes are biparentally inherited with both seed and pollen flow contributing to inter-population gene flow. Compared to pollen dispersal, seed dispersal is more limited, leading to inconsistent results.

Haplotype geographic distribution revealed multiple relatively isolated distribution zones, each containing its own private and major haplotypes. Research indicates that haplotypes located centrally in network structures, with high frequency and wide geographic distribution, are generally more ancient (Freeland et al., 2012). In this study, haplotype H10 showed high frequency, wide distribution, and central network position, suggesting it represents the most ancestral haplotype. Chizhou City, Anhui (C7) not only possessed high haplotype diversity but also the greatest number of haplotype types, consistent with the conclusion that *P. ternata* originated in eastern China and spread along the Yangtze River to surrounding areas (Li, 1996). Fourteen of the 19 populations contained only a single haplotype, likely related to the degree of within-population variation and reproductive mode. For *P. ternata*, which reproduces primarily through vegetative propagation, a population likely contains only one genotype, i.e., a single clone. Glacial refugia typically refer to areas that provided survival domains for plants during glacial periods, particularly the Last Glacial Maximum, when climate and topographic conditions underwent dramatic changes, forcing species to undergo large-scale geographic relocation (Haffer, 1969). According to glacial refuge theory, regions with high genetic diversity, ancient haplotypes, and numerous endemic haplotypes may represent potential refugia for a species during glacial periods (Favre et al., 2010). Since Zhaotong City, Yunnan (C6); Guiyang City, Guizhou (C14); and Mianyang City, Sichuan (C17) in southwestern China, as well as Chizhou City, Anhui (C7) and Yiwu City, Zhejiang (C13) in eastern China all exhibited high genetic diversity and contained both ancient and private haplotypes, *P. ternata* likely possessed at least two or more glacial refugia during the Quaternary glaciation. We hypothesize that refugia existed in central-eastern and southwestern China during the Quaternary ice age, consistent with our research group's findings on *Allium macrostemon* (Mo et al., 2019; Shi et al., 2021), likely due to their similar distribution and ecological habits.

This study investigated genetic diversity, phylogeographic patterns, and population historical dynamics of *P. ternata*, with sampling covering 15 provinces including Guizhou, Zhejiang, Gansu, and Sichuan, spanning northern and southern China and representing natural geographic distributions in eastern, central, northwestern, and southwestern regions, thereby capturing the entire geographic distribution of this plant in China. Although we did not collect materials from Japan or the Korean Peninsula, our conclusions show similarities and consis-

tency with our research group's previous phylogeographic and genetic diversity studies on herbaceous plants with similar distributions to *P. ternata*, such as *Allium macrostemon* and *Bupleurum longiradiatum*, as well as previous research on phylogeographic patterns of *Ginkgo biloba* (Fan, 2014) and *Liquidambar formosana* (Sun, 2017) distributed across southwestern China and central-eastern regions. Therefore, our sampling strategy was feasible and our conclusions are reliable.

3.3 Molecular Screening and Conservation Strategies for Medicinal *P. ternata*

Pinellia ternata tubers are used medicinally for drying dampness, resolving phlegm, reversing adverse flow of stomach qi, arresting vomiting, and dispersing lumps, making it one of the most commonly used traditional Chinese medicines. Clinically, it is widely used for anti-tumor, anti-fertility, anti-spasmodic, lipid-regulating, expectorant, anti-inflammatory, and coronary heart disease treatments (Wang et al., 2012). In recent years, excessive use of chemical fertilizers and pesticides (such as herbicides) in farmland, coupled with unregulated harvesting, has led to continuous exploitation of farmland and wastelands, destroying wild *P. ternata* habitats and causing wild resources to become increasingly depleted. Additionally, domestic and international demand for *P. ternata* continues to grow, particularly due to global warming and increasing respiratory diseases, further expanding market demand. Therefore, breeding superior germplasm or developing new varieties has become an urgent practical need for *P. ternata* production development. Furthermore, continuous cropping obstacles have caused severe diseases and pests, reduced product quality, decreased yields, increased prices, and resulted in few (or no) new varieties (An et al., 2018). Very few *P. ternata* varieties with red tubers exhibit characteristics such as high yield and high resistance, yet no complete breeding method has been established (Zhang et al., 2021). Studies have shown that *P. ternata* is sensitive to direct sunlight, with moderate shading promoting growth and development while excessive shading leads to poor growth or even death (Chang, 2022). High temperatures ($ $32°C) or low winter temperatures can both cause seedling fall or dormancy in *P. ternata*, affecting yield (Zhang et al., 2004). Therefore, breeding directions focus on tolerance to intense light and high temperature, low temperature and damp shade, and continuous cropping, aiming to extract superior new germplasm from *P. ternata* resources. Breeding samples were screened for superior lines through various indicators including biological characteristics comparison, resistance identification, yield analysis, and reproductive trait studies. Subsequently, based on field performance of foundation materials in resource nurseries, superior target lines were selected using several main indicators as target values: growth vigor, leaf number per plant, disease resistance, bulbil position and production capacity, tuber shape, and productivity. Chang (2022) associated yield traits with cytological characteristics in *P. ternata*, finding that germplasm with smaller genomes seemed more conducive to bulbil formation and underground tuber proliferation. At the molecular level,

our study identified that Zhaotong City, Yunnan (C6); Guiyang City, Guizhou (C14); Mianyang City, Sichuan (C17); Chizhou City, Anhui (C7); and Yiwu City, Zhejiang (C13) all possess high genetic diversity, representing distribution areas of wild superior germplasm.

In addition to breeding superior varieties or developing new cultivars of *P. ternata*, conservation measures should be implemented for its wild resources, with in situ or ex situ conservation applied to areas with high genetic diversity. Due to its wide distribution but small wild populations, ex situ conservation should be the primary approach for *P. ternata*, such as establishing germplasm resource nurseries (banks) for this medicinal plant, with priority given to protecting populations with high genetic diversity. Populations possessing unique haplotypes, including Dandong, Liaoning (C1); Chizhou, Anhui (C7); Nantong, Jiangsu (C8); Kaiyang, Guizhou (C9); Qingdao, Shandong (C10); Yiwu, Zhejiang (C13); Guiyang, Guizhou (C14); Mianyang, Sichuan (C17); and Guyuan, Ningxia (C18), should also receive priority protection. Meanwhile, when developing ex situ conservation strategies, individuals with special traits should be included in the conservation scope as much as possible.

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